Genome Size of *Mycoplasma genitalium*

CHUNG J. SU AND JOEL B. BASEMAN*

Department of Microbiology, The University of Texas Health Science Center, 7703 Floyd Curl Drive,
San Antonio, Texas 78284-7758

Received 19 March 1990/Accepted 29 May 1990

The genome size of *Mycoplasma genitalium* was determined by using restriction enzymes that infrequently cut its DNA. The calculated value of 577 to 590 kilobases is one-fourth smaller than the genome of *Mycoplasma pneumoniae*, which is considered among the smallest genomes of self-replicating organisms.

Mycoplasma genitalium is a newly discovered species isolated in 1980 from the urethras of males with nongonococcal urethritis (22, 23). Recently, it was also identified in the throat swab specimens of pneumonia patients as a coisolate of *Mycoplasma pneumoniae* (2). Morphological, biochemical, serological, and genetic studies indicate that these two mycoplasma species share common biological features. Both organisms require cholesterol, ferment glucose, exhibit immunologic cross-reactivity, and are flask shaped with a distinct tiplike organelle (12, 22, 23). The major cytoadhesin, a 170-kilodalton (kDa) protein in *M. pneumoniae* (1, 19), and a corresponding 140-kDa protein in *M. genitalium* (5, 16) concentrate at the tip (1, 7, 8, 9), share epitopes (15), and possess homologous sequences at the DNA and protein levels (5, 6). However, differences between these two mycoplasmas have also been observed. Electrophoretic patterns of digested genomic DNA from each species indicate genetic heterogeneity (3). The G+C content of the 170-kDa adhesin gene of *M. pneumoniae* is 53.5%, while that of the 140-kDa adhesin gene of *M. genitalium* is 40% (5). Comparisons of codon usage between the two mycoplasmas reveal a preferential usage of A- and T-rich codons by *M. genitalium* (5). Furthermore, these organisms differ dramatically in their acquisition of human lactoferrin, as reflected in the saturable and specific binding of lactoferrin by *M. pneumoniae* and the total absence of such binding by *M. genitalium* (20).

Mycoplasmas are considered the smallest free-living organisms and have a circular double-stranded DNA with a molecular size of about 500 megadaltons (13, 14, 16). Their genomes are also relatively low in guanine and cytosine (G+C) content, and *M. pneumoniae* and *M. genitalium* have G+C levels of 40 and 32%, respectively (5).

The genome of *M. pneumoniae* has been selected as a model for studying the minimal amount of genetic information required for a self-replicating biological system (13). Wenzel and Herrmann (24) screened an *M. pneumoniae* cosmid library, analyzed 32 overlapping cosmid clones covering a continuous DNA stretch of 720 kilobases (kb), and estimated the total genomic length of *M. pneumoniae* to be about 800 kb. Other physical mapping approaches such as the use of infrequent-cutting restriction enzymes and pulse-field gel electrophoresis analysis have provided similar estimates (D. C. Krause and C. B. Mawn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, G31, p. 153). These data are comparable with earlier results based on DNA renaturation kinetics and electron microscopy (14, 16).

Because of the biological-chemical similarities and virulence potential of *M. pneumoniae* and *M. genitalium*, we examined the genome structure of *M. genitalium* G37. To find infrequent-cutting restriction enzymes, we digested 8 μ g of *M. genitalium* chromosomal DNA with various restriction enzymes and then separated the DNA by electrophoresis on 0.75% agarose gel. This technique also allowed the accurate size estimate of small DNA fragments. Figure 1 presents selected restriction enzyme digestion patterns representing the *M. genitalium* genome. *Bam*HI generated fragments mostly in the 8- to 50-kb range. *Eco*RI digested more often, and fragments ranged from 2.5 to 30 kb. *Hind*III generated small fragments, all below 9.5 kb. In contrast, three other enzymes with GC-rich recognition sequences, *Sac*I, *Sac*II, and *Sma*I, all cut *M. genitalium* DNA infrequently. *Sac*I generated at least two small fragments of 3.5 and 9.5 kb, with the rest of the fragments in the high-molecular-weight range. *Sac*II produced two small fragments of 5 and 12.2 kb and several high-molecular-weight bands. All fragments generated by *Sma*I were larger than 20 kb.

On the basis of the initial screening results, restriction enzymes *Apa*I, *Mlu*I, *Nar*I, *Nru*I, *Sac*I, *Sal*I, and *Sma*I that cut the *M. genitalium* DNA infrequently were used to analyze the genome size. *M. genitalium* was grown in 32-oz (950-ml) glass prescription bottles containing 70 ml of SP4 medium (21) at 37°C for 3 days before harvesting (15). Cells were scraped from the glass, pelleted by centrifugation, washed twice with phosphate-buffered saline buffer (10 mM sodium phosphate [pH 7.2]–0.1 M NaCl), and frozen at –70°C. The methods of Jackson and Cook (11) were used to prepare agarose-encapsulated intact genomic DNA. Mycoplasma pellets from 12 bottles were suspended in 0.8 ml of phosphate-buffered saline buffer and warmed to 37°C. Then, 0.4 ml of 1.6% agarose (SeaKem low-gelling-temperature agarose; FMC Corp., Rockland, Maine) prewarmed at 42°C was added and mixed, 0.4 ml of prewarmed mineral oil was added, and the mixture was vortexed for 30 s at top speed and chilled in ice for 5 min to allow the agarose to gel. The mycoplasma-containing agarose beads were washed extensively to remove mineral oil and unencapsulated mycoplasmas. The beads were suspended in 0.25 M EDTA (pH 8.0)–1% sodium dodecyl sulfate and digested with 2 mg of protease K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml at 50°C for 24 h. Then, the beads were washed extensively with buffer (10 mM Tris hydrochloride (pH 8.0)–10 mM EDTA) containing 2 mM of phenylmethylsulfonyl fluoride, rinsed three times with the same buffer, and kept at 4°C. Agarose-encapsulated *M. pneumoniae* organisms were prepared similarly for comparative purposes.

* Corresponding author.

FIG. 1. Initial screening of infrequent-cutting restriction enzymes of *M. genitalium* DNA (8 μ g) digested and separated on 0.75% agarose gel. Lane 1, *Hind*III-digested λ phage DNA as molecular size marker; lane 2, *Bam*HI; lane 3, *Eco*RI; lane 4, *Hind*III; lane 5, *Sac*I; lane 6, *Sac*II; lane 7, *Sma*I. Numbers at left are the molecular sizes in kilobases.

Restriction enzymes were purchased either from Bethesda Research Laboratories, Gaithersburg, Md. or from New England BioLabs, Inc., Beverly, Mass. DNA-containing agarose beads were equilibrated with appropriate buffers, digested with various restriction enzymes, and separated on 0.8% agarose gels in 0.5 $\times$ TBE buffer (1 $\times$ TBE is 90 mM Tris–45 mM boric acid–0.5 mM EDTA). A programmable

FIG. 2. Restriction fragments of *M. genitalium* genome separated by inverse field electrophoresis. Lanes 1 and 11, λ phage concatamers used as molecular size standards; lane 2, other molecular weight DNA markers (Bethesda Research Laboratories); lane 3, *Apal*; lane 4, *Mlu*I; lane 5, *Nar*I; lane 6, *Nru*I; lane 7, *Sac*I; lane 8, *Sac*II; lane 9, *Sall*; lane 10, *Sma*I.

TABLE 1. Apparent sizes of restriction fragments from DNA of *M. genitalium* G37

Restriction endonuclease	Estimated fragment sizes (kb)	Total kb
<i>Apal</i>	500, 65, 18	583
<i>Mlu</i> I	180, 120 $\times$ 2, 60, 35, 25, 16, 15, 7, 3.5	581.5
<i>Nar</i> I	310, 140 $\times$ 2	590
<i>Nru</i> I	320, 120, 70, 35, 22, 17	584
<i>Sac</i> I	185, 175, 85, 38, 35, 24, 22, 9.5, 3.5	577
<i>Sac</i> II	460, 110, 12.2, 5	587.2
<i>Sall</i>	510, 80	590
<i>Sma</i> I	150, 125 $\times$ 2, 75, 38, 30, 22 $\times$ 2	587

power inverter (PPI-200; M.J. Research Inc., Cambridge, Mass.) with a preset program was used to regulate electrophoresis. Gels were run at room temperature at 7 V/cm for 20 to 24 h with buffer recirculation. High-molecular-weight DNA markers and megabase DNA standards were purchased from Bethesda Research Laboratories. λ DNA concatamers were prepared by ligating λ bacteriophage DNA with T4 DNA ligase (4). Figure 2 shows DNA band patterns after digestion with selected restriction enzymes and separation by using preset program 4 of the power inverter. Under these experimental conditions, the program permitted clear separation of DNA fragments from 10 to 200 kb. Fragments larger than 200 kb required program 5 or 7 to obtain correct molecular sizing (data not shown). *Apal* cut the *M. genitalium* genome into 3 pieces of 500, 65, and 18 kb, indicating a total genome size of about 583 kb. *Mlu*I cut the genome into 10 pieces, and *Nar*I cut the genome into 3 pieces. The results of the analysis are summarized in Table 1. Thus, from the size of restriction fragments generated by different enzymes, we calculated the genome of *M. genitalium* to be about 577 to 590 kb (4×10^8 daltons). Using similar methodology and restriction enzymes *Rsr*II, *Apal*, *Nar*I, and *Not*I, we calculated the genome of *M. pneumoniae* to be about 780 kb (data not shown), which is consistent with earlier reports (14, 16; Krause and Mawn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989). Therefore, the *M. genitalium* genome is one-fourth smaller than that of *M. pneumoniae* and is at the low end of the minimal genome size for a free-living organism (13, 14). The difference in genome size between *M. pneumoniae* and *M. genitalium* could be explained in part by the existence of multiple copies of many repeated sequences in *M. pneumoniae* (17, 25). Some of these repeated gene families share extensive homology with the major cytoadhesin gene (P1) of *M. pneumoniae*. In contrast, the corresponding *M. genitalium* cytoadhesin gene (140-kDa protein) has many fewer copies of repeat sequences (unpublished results). These data further support the distinct species designation of *M. genitalium* and its uniqueness as a human pathogen.

This research was supported in part by Public Health Service grant AI 27873 from the National Institute of Allergy and Infectious Diseases.

We thank Rose Garza for her secretarial assistance.

LITERATURE CITED

- Baseman, J. B., R. M. Cole, D. C. Krause, and D. K. Leith. 1982. Molecular basis for cytoadsorption of *Mycoplasma pneumoniae*. *J. Bacteriol.* 151:1514–1522.
- Baseman, J. B., S. F. Dallo, J. G. Tully, and D. L. Rose. 1988. Isolation and characterization of *Mycoplasma genitalium* strains from the human respiratory tract. *J. Clin. Microbiol.* 26:2266–2269.

3. Baseman, J. B., K. L. Daly, L. B. Trevino, and D. L. Drouillard. 1984. Distinctions among pathogenic human mycoplasmas. *Isr. J. Med. Sci.* **20**:866–869.
4. Carle, G. F., and M. V. Olson. 1984. Separation of chromosomal DNA molecules from yeast by orthogonal-field-alternation gel electrophoresis. *Nucleic Acids Res.* **12**:5647–5664.
5. Dallo, S. F., A. Chavoya, C. J. Su, and J. B. Baseman. 1989. DNA and protein sequence homologies between the adhesins of *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. *Infect. Immun.* **57**:1059–1065.
6. Dallo, S. F., J. R. Horton, C. J. Su, and J. B. Baseman. 1989. Homologous regions shared by adhesin genes of *Mycoplasma pneumoniae* and *Mycoplasma genitalium*. *Microb. Pathog.* **6**: 69–73.
7. Feldner, J., U. Gobel, and W. Bredt. 1982. *Mycoplasma pneumoniae* adhesin localized to tip structure by monoclonal antibody. *Nature (London)* **298**:765–767.
8. Hu, P. C., R. M. Cole, Y. S. Huang, T. A. Graham, D. E. Gardner, A. M. Collier, and W. A. Clyde. 1982. *Mycoplasma pneumoniae* infection: role of a surface protein in the attachment organelle. *Science* **216**:313–315.
9. Hu, P. C., V. Schaper, A. M. Collier, W. A. Clyde, M. Horikawa, Y. S. Huang, and M. F. Barile. 1987. A *Mycoplasma genitalium* protein resembling the *Mycoplasma pneumoniae* attachment protein. *Infect. Immun.* **55**:1126–1131.
10. Inamine, J. M., T. P. Denny, S. Loechel, U. Shaper, C. H. Huang, K. F. Bott, and P. C. Hu. 1988. Nucleotide sequence of the P1 attachment-protein gene of *Mycoplasma pneumoniae*. *Gene* **64**:217–229.
11. Jackson, D. A., and P. R. Cook. 1985. A general method for preparing chromatin containing intact DNA. *EMBO J.* **4**:913–918.
12. Lind, K., B. O. Lindhardt, H. J. Schütten, J. Blom, and C. Christiansen. 1984. Serological cross-reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. *J. Clin. Microbiol.* **20**:1036–1043.
13. Morowitz, H. J. 1967. Biological self-replicating systems. *Prog. Theor. Biol.* **1**:35–58.
14. Morowitz, H. J., and D. C. Wallace. 1973. Genome size and life cycle of the mycoplasmas. *Ann. N.Y. Acad. Sci.* **225**:63–73.
15. Morrison-Plummer, J., A. Lazzell, and J. B. Baseman. 1987. Shared epitopes between *Mycoplasma pneumoniae* major adhesin protein P1 and a 140-kilodalton protein of *Mycoplasma genitalium*. *Infect. Immun.* **55**:49–56.
16. Razin, S. 1985. Molecular biology and genetics of mycoplasmas (*Mollicutes*). *Microbiol. Rev.* **49**:419–455.
17. Su, C. J., A. Chavoya, and J. B. Baseman. 1988. Regions of *Mycoplasma pneumoniae* cytoadhesin P1 structural gene exist as multiple copies. *Infect. Immun.* **56**:3157–3161.
18. Su, C. J., A. Chavoya, and J. B. Baseman. 1989. Spontaneous mutation results in loss of the cytoadhesin (P1) of *Mycoplasma pneumoniae*. *Infect. Immun.* **57**:3237–3239.
19. Su, C. J., V. V. Tryon, and J. B. Baseman. 1987. Cloning and sequence analysis of cytoadhesin P1 gene from *Mycoplasma pneumoniae*. *Infect. Immun.* **55**:3023–3029.
20. Tryon, V. V., and J. B. Baseman. 1987. The acquisition of human lactoferrin by *Mycoplasma pneumoniae*. *Microb. Pathog.* **3**:437–443.
21. Tully, J. G., D. L. Rose, R. F. Whitcomb, and R. P. Wenzel. 1979. Enhanced isolation of *Mycoplasma pneumoniae* from throat washings with a newly modified culture medium. *J. Infect. Dis.* **139**:478–482.
22. Tully, J. G., D. Taylor-Robinson, R. M. Cole, and D. L. Rose. 1981. A newly discovered mycoplasma in the human urogenital tract. *Lancet* **i**:1288–1291.
23. Tully, J. G., D. Taylor-Robinson, R. M. Cole, and J. M. Bove. 1983. *Mycoplasma genitalium*, a new species from the human urogenital tract. *Int. J. Syst. Bacteriol.* **33**:387–396.
24. Wenzel, R., and R. Herrmann. 1988. Physical mapping of the *Mycoplasma pneumoniae* genome. *Nucleic Acids Res.* **16**: 8323–8336.
25. Wenzel, R., and R. Herrmann. 1988. Repeated DNA sequences in *Mycoplasma pneumoniae*. *Nucleic Acids Res.* **16**:8337–8350.