INFECTION AND IMMUNITY, Oct. 1989, p. 3237–3239
0019-9567/89/103237-03$02.00/0

Vol. 57, No. 10

Spontaneous Mutation Results in Loss of the Cytadhesin (P1) of *Mycoplasma pneumoniae*

CHUNG-JEY SU, ARTURO CHAVOYA, AND JOEL B. BASEMAN*

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

Received 11 April 1989/Accepted 10 July 1989

The cytadhesin (P1) structural gene of a spontaneous mutant of *Mycoplasma pneumoniae* which displayed a P1-negative phenotype was analyzed. An extra adenine was discovered in a stretch of normally seven adenines near the N-terminal region of the mutant P1 structural gene. The frameshift mutation resulted in the early termination of protein translation. Possible causes of the mutation are discussed.

Mycoplasma pneumoniae is a procaryotic pathogen of the human respiratory tract and the causative agent of primary atypical pneumoniae (4). Its adherence to respiratory epithelial cells is mediated by a 170-kilodalton protein adhesin, designated P1, which densely clusters at the tip organelle of virulent organisms (1, 5, 6). Antibodies reactive against P1 block the attachment of *M. pneumoniae* to host cells (9), and *M. pneumoniae* mutants lacking P1 lose cytadherence and virulence capabilities (10).

In our previous study of the *M. pneumoniae* proteins associated with cytadherence (10), spontaneous hemadsorption-negative (HA^-) mutants were isolated at a high frequency (0.7%) from a single colony of the virulent hemadsorption-positive (HA^+) strain M129-B16 of *M. pneumoniae*. Protein profiles of these mutants were analyzed by one- and two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and one class of mutants (class IV) which lost the ability to synthesize P1 and several other proteins (proteins A, B, and C; molecular weights, 72,000, 85,000, and 37,000, respectively) was isolated.

Recently, we cloned and sequenced the entire P1 structural gene and flanking regions of virulent HA^+ *M. pneumoniae* (18). To further understand the control of P1 gene expression as well as the possible relationship between P1 and proteins A, B, and C, we analyzed the P1 gene of an HA^-, P1-deficient class IV mutant.

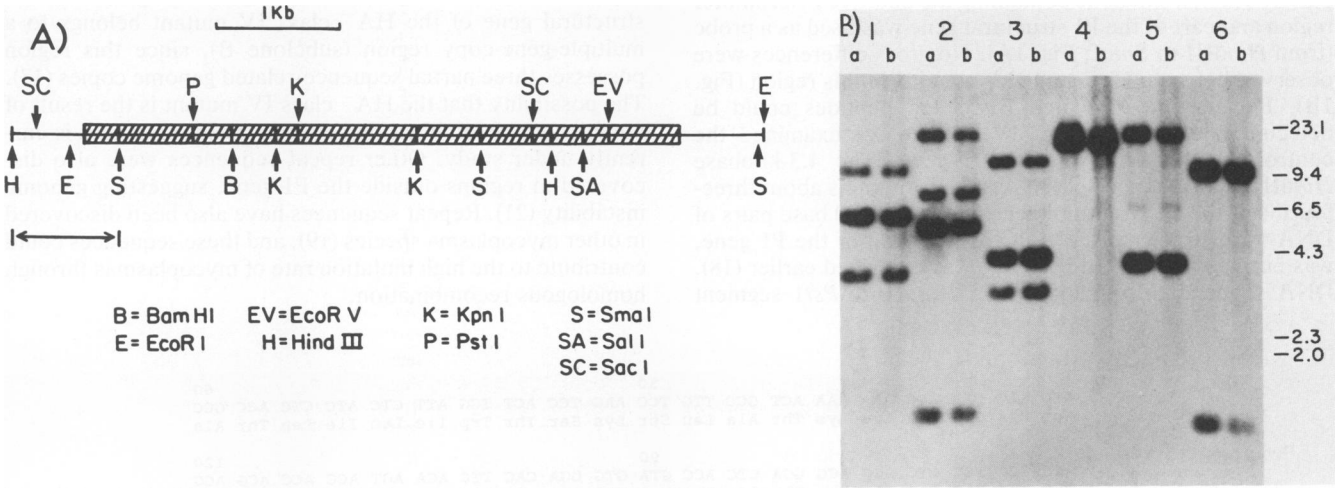

FIG. 1. (A) Restriction endonuclease map of the P1 gene. ▨, Structural gene of P1; |↔|, *Hin*dIII-*Sma*I subclone used in the Southern blot analysis. Kb, Kilobases. (B) Southern blot analysis of the P1 gene from virulent HA^+ *M. pneumoniae* (lanes a) and the HA^- class IV mutant (lanes b). DNA from each organism was isolated and digested to completion with various restriction enzymes: 1, *Bam*HI; 2, *Eco*RI; 3, *Hin*dIII; 4, *Pst*I; 5, *Sac*I; and 6, *Sma*I. Digested DNA was fractionated by agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized to the radioactive probe (*Hin*dIII-*Sma*I subclone) as previously described (17). Numbers on the right indicate the positions of molecular weight standards in kilobases.

Southern blot analysis was performed to detect whether gene rearrangements, such as insertion or deletion of DNA, had occurred. Both HA^+ and HA^- *M. pneumoniae* strains were grown in Edward medium for 3 days and harvested as previously described (13). DNA was extracted from cell pellets, digested with appropriate restriction enzymes, separated by agarose gel electrophoresis, and transferred to nitrocellulose filters according to standard protocols (11, 17).

* Corresponding author.

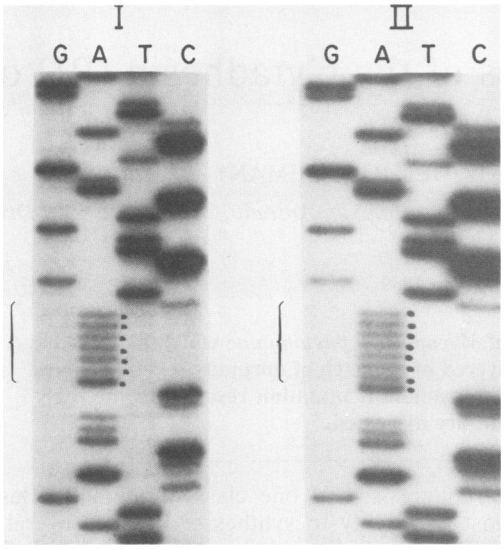

FIG. 2. Comparative DNA sequences of the P1 gene demonstrating the mutation. I, DNA sequence of virulent HA^+ *M. pneumoniae*; II, corresponding sequence of the HA^- class IV mutant. Braces and dots highlight the seven adenines in the HA^+ strain and the eight adenines in the HA^- mutant.

Because the P1 gene contains numerous regions of repeated sequences and therefore hybridizes to multiple parts of the *M. pneumoniae* genome, producing complex hybridization patterns (17), a subclone containing the putative P1 promoter region and part of the P1 structural gene was used as a probe (from *Hin*dIII to *Sma*I; Fig. 1A). No gross differences were observed between HA^+ and HA^- strains in this region (Fig. 1B). Because no truncated P1-related peptides could be detected in the HA^- class IV mutant, we examined the control region of the mutant P1 gene. The 4.3-kilobase *Hin*dIII-digested piece of DNA, which contains about three-fourths of the P1 structural gene and about 400 base pairs of DNA 5′ upstream to the AUG start codon of the P1 gene, was purified and cloned into pUC9 as described earlier (18). DNA sequences from the 5′-end *Hin*dIII-to-*Pst*1 segment were determined by subcloning appropriate pieces of DNA into M13 phage and employing the dideoxy chain termination method (12, 14). A single base insertion of adenine into a stretch of seven adenines was detected in the class IV mutant at the position corresponding to nucleotides 13 to 18 of the P1 gene, in contrast to the HA^+ P1 sequence (Fig. 2). This insertion resulted in a frameshift causing the premature termination of the P1 sequence at position 138, where a serine codon (AGC) changed to a termination codon (TAG) (Fig. 3) (18). This spontaneous single base insertion is probably the result of in vivo DNA polymerase slippage at the reiterated bases during DNA replication (16). This type of mutation has been observed in the *Escherichia coli lac I* gene, in which a run of five A · T base pairs accounts for 70% of the frameshift mutations (15). Similar mechanisms have been reported in the *Neisseria gonorrhoeae* pilin gene, in which spontaneous insertions or deletions occur predominantly in a stretch of eight G · C base pairs, resulting in pilin-negative phenotypes (2, 8).

Our results demonstrate that one base insertion of adenine into a stretch of seven adenines in the normal P1 structural gene results in the phenotypic loss of P1 in the HA^- class IV mutant. However, our results do not explain the high rate of spontaneous mutation observed in *M. pneumoniae* (10). Other reports have also described a high mutation rate in mycoplasma species (20). During our analysis of the P1 structural gene (17), we subcloned the P1 gene and flanking sequences into 14 subclones. These subclones were used to establish that two-thirds of the P1 gene exists as multiple copies throughout the genome. The mutated region of the P1 structural gene of the HA^- class IV mutant belongs to a multiple-gene-copy region (subclone B), since this region possesses three partial sequence-related genome copies (17). The possibility that the HA^- class IV mutant is the result of homologous recombination between these sequences is currently under study. Other repeat sequences were also discovered in regions outside the P1 gene, suggesting genome instability (21). Repeat sequences have also been discovered in other mycoplasma species (19), and these sequences could contribute to the high mutation rate of mycoplasmas through homologous recombination.

```
                                                30                                  60
ATG CAC CAA ACC AAA AAA ACT GCC TTG TCC AAG TCC ACT TGG ATT CTC ATC CTC ACC GCC
Met His Gln Thr Lys Lys Thr Ala Leu Ser Lys Ser Thr Trp Ile Leu Ile Leu Thr Ala

                                                90                                 120
I   ACC GCC TCC CTC GCG ACG GGA CTC ACC GTA GTG GGA CAC TTC ACA AGT ACC ACC ACG ACG
    Thr Ala Ser Leu Ala Thr Gly Leu Thr Val Val Gly His Phe Thr Ser Thr Thr Thr Thr

    CTC AAG CGC CAG CAA TTT AGC
    Leu Lys Arg Gln Gln Phe Ser


                                   *            30                                  60
ATG CAC CAA ACC AAA AAA AAC TGC CTT GTC CAA GTC CAC TTG GAT TCT CAT CCT CAC CGC
Met His Gln Thr Lys Lys Asn Cys Leu Val Gln Val His Leu Asp Ser His Pro His Arg

                                              ?90                                  120
II  CAC CGC CTC CCT CGC GAC GGG ACT CAC CGT AGT GGG ACA CTT CAC AAG TAC CAC CAC GAC
    His Arg Leu Pro Arg Asp Gly Thr His Arg Ser Gly Thr Leu His Lys Tyr His His Asp

    GCT CAA GCG CCA GCA ATT TAG
    Ala Gln Ala Pro Ala Ile End
```

FIG. 3. DNA sequences and predicted amino acid sequences of the P1 gene in HA^+ (I) and HA^- (II) strains. The P1 gene in HA^+ *M. pneumoniae* starts from the ATG (Met) codon and has a continuous open reading frame for 1,627 amino acids (18). In the HA^- mutant an extra adenine (asterisk) inserted at the beginning of the P1 structural gene results in a frameshift mutation which creates a stop codon (TAG) 120 base pairs downstream.

Of the 22 HA^- mutants isolated (10), four belong to a group designated class III which lacks proteins A, B, and C but retains P1 (10). From our unpublished observations as well as from other data (7), the P1 gene probably exists in a multiple-gene operon. The polarity of the HA^- class IV mutant might explain the loss of all four proteins (P1, A, B, and C) simultaneously if the genes coding for proteins A, B, and C are located in the same operon.

Woese et al. (22) developed an evolutionary tree for mycoplasmas based on the T_1 RNase-digested oligonucleotide catalogs of their 16S rRNAs. They observed that eubacterial 16S rRNA contains highly conserved sequences that appear as a subset of every eubacterial oligonucleotide catalog. In the mycoplasma group a relatively high proportion of these conserved sequences are absent (22). From these data and the observations that mycoplasma DNA polymerases have the same fidelity as *E. coli* polymerase 1 but lack proofreading function (3), they concluded that mycoplasmas have high mutation rates (22). The existence of many repeated sequences reported by us and others (17, 21) may further contribute to genome instability in mycoplasmas. We are currently examining the link between high mutation rates and multiple-gene-copy regions in *M. pneumoniae* and its impact on virulence.

We thank Rose Garza and Pat Hart for secretarial assistance.

This work was supported by Public Health Service grant AI 18540 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Baseman, J. B., R. M. Cole, D. C. Krause, and D. K. Leith.** 1982. Molecular basis for cytadsorption of *Mycoplasma pneumoniae*. J. Bacteriol. **151**:1514–1522.
2. **Bergström, S., K. Robbins, J. M. Koomey, and J. Swanson.** 1986. Piliation control mechanisms in *Neisseria gonorrhoeae*. Proc. Natl. Acad. Sci. USA **83**:3890–3894.
3. **Boxer, L. M., and D. Korn.** 1979. Structural and enzymological characterization of the homogeneous deoxyribonucleic acid polymerase from *Mycoplasma orale*. Biochemistry **18**:4742–4749.
4. **Chanock, R. M., L. Hayflick, and M. F. Barile.** 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Natl. Acad. Sci. USA **48**:41–49.
5. **Feldner, J., U. Gobel, and W. Bredt.** 1982. *Mycoplasma pneumoniae* adhesin localized to tip structure by monoclonal antibody. Nature (London) **298**:765–767.
6. **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.
7. **Inamine, J. M., T. P. Denny, S. Loechel, U. Schaper, 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.
8. **Koomey, M., E. C. Gotschlich, K. Robbins, S. Bergström, and J. Swanson.** 1987. Effects of *rec*A mutations on pilus antigenic variation and phase transitions in *Neisseria gonorrhoeae*. Genetics **117**:391–398.
9. **Krause, D. C., and J. B. Baseman.** 1983. Inhibition of *Mycoplasma pneumoniae* hemadsorption and adherence to respiratory epithelium by antibodies to a membrane protein. Infect. Immun. **39**:1180–1186.
10. **Krause, D. C., D. K. Leith, R. M. Wilson, and J. B. Baseman.** 1982. Identification of *Mycoplasma pneumoniae* proteins associated with hemadsorption and virulence. Infect. Immun. **35**: 809–817.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.
13. **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. Immuno. **55**:49–56.
14. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with a chain-terminating inhibitor. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
15. **Schaaper, R. M., and R. L. Dunn.** 1987. Spectra of spontaneous mutations in *Escherichia coli* strains defective in mismatch correction: the nature of *in vivo* DNA replication errors. Proc. Natl. Acad. Sci. USA **84**:6220–6224.
16. **Streisinger, G., and J. E. Owen.** 1985. Mechanisms of spontaneous and induced frameshift mutation in bacteriophage T4. Genetics **109**:633–659.
17. **Su, C. J., A. Chavoya, and J. B. Baseman.** 1988. Regions of *Mycoplasma pneumoniae* cytadhesin P1 structural gene exist as multiple copies. Infect. Immun. **56**:3157–3161.
18. **Su, C. J., V. V. Tryon, and J. B. Baseman.** 1987. Cloning and sequence analysis of cytadhesin P1 gene from *Mycoplasma pneumoniae*. Infect. Immun. **55**:3023–3029.
19. **Taylor, M. A., R. V. Ferrell, K. S. Wise, and M. A. McIntosh.** 1988. Reiterated DNA sequences defining genomic diversity within the species *Mycoplasma hyorhinis*. Mol. Microbiol. **2**:665–672.
20. **Watson, H. L., L. S. McDaniel, D. K. Blalock, M. T. Fallon, and G. H. Cassell.** 1988. Heterogeneity among strains and a high rate of variation within strains of a major surface antigen of *Mycoplasma pulmonis*. Infect. Immun. **56**:1358–1363.
21. **Wenzel, R., and R. Herrmann.** 1988. Repetitive DNA sequences in *Mycoplasma pneumoniae*. Nucleic Acids Res. **16**:8337–8350.
22. **Woese, C. R., J. Maniloff, and L. B. Zablen.** 1980. Phylogenetic analysis of the mycoplasmas. Proc. Natl. Acad. Sci. USA **77**:494–498.