Phase Variations of the *Mycoplasma penetrans* Main Surface Lipoprotein Increase Antigenic Diversity

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*Mycoplasma penetrans* is a recently identified mycoplasma, isolated from urine samples collected from human immunodeficiency virus (HIV)-infected patients. Its presence is significantly associated with HIV infection. The major antigen recognized during natural and experimental infections is an abundant P35 lipoprotein which, upon extraction, segregates in the Triton X-114 detergent phase and is the basis of *M. penetrans*-specific serological assays. We report here that the P35 antigen undergoes spontaneous and reversible phase variation at high frequency, leading to heterogeneous populations of mycoplasmas, even when derived from a clonal lineage. This variation was found to be determined at the transcription level, and although this property is not unique among the members of the class *Mollicutes*, the mechanism by which it occurs in *M. penetrans* differs from those previously described for other *Mycoplasma* species. Indeed, the P35 phase variation was due neither to a P35 gene rearrangement nor to point mutations within the gene itself or its promoter. The P35 phase variation in the different variants obtained was concomitant with modifications in the pattern of other expressed lipoproteins, probably due to regulated expression of selected members of a gene family which was found to potentially encode similar lipoproteins. *M. penetrans* variants could be selected on the basis of their lack of colony immunoreactivity with a polyclonal antiserum against a Triton X-114 extract, strongly suggesting that the mechanisms involved in altering surface antigen expression might allow evasion of the humoral immune response of the infected host.

The complete sequences of the genomes of *Mycoplasma genitalium* (15) and *M. pneumoniae* (19) are now available and confirm that mollicutes (trivial name, mycoplasmas) are the self-replicating organisms with the smallest genomes (≥580 kbp [5]). They lack the genes for numerous common biosynthetic pathways (38–40), reflecting the parasitic lifestyle of these microorganisms. This parasitism and the limited biosynthetic capacity of the mycoplasmas demand efficient membrane transporters for the acquisition of precursors for metabolism. Since mycoplasmas have no outer membrane or cell wall, such transporters are necessarily located in their single plasma membrane, and due to the many growth requirements of these microorganisms, they must be able to transport many different precursors. In addition to having this critical role in cell physiology, the mycoplasma membrane components are also directly exposed to the host immune system. Mycoplasma infections are chronic, and the microorganisms must therefore have strategies for withstanding the damage caused by this immune vigilance. A number of studies have shown that several species of mycoplasmas are able to modify their surface antigens at a high frequency. This plasticity may contribute to evasion from the host humoral immune response (for reviews, see references 10 and 55). Most of these variable surface antigens are lipoproteins, and lipoproteins are known to be immunogenic, mainly through their acylated N-terminal structures (7, 34, 41). A prominent feature of many, if not all, mycoplasmas is the large number of lipoproteins in their membrane (54), which is consistent with the numerous putative lipoprotein genes identified in the genome sequences (20). Other than the few lipoproteins which have been suggested to be part of ABC transporters (11, 15, 19, 48), the physiological role of these lipoproteins remains unknown. The mycoplasmas thus must cope with the problem of having most of their cell surface covered with lipoproteins which, being highly immunogenic, are prone to antibody recognition. Being preferential targets for immune systems, mycoplasma lipoproteins are considered to be potentially important for the development of serological assays and vaccine preparations.

*Mycoplasma penetrans* is a newly identified species, isolated from humans, with a unique morphology characterized by an elongated flask shape. This isolate penetrates eucaryotic cells (hence its name), possesses cytopathic effects in vitro (16, 27), and kills a large proportion of chicken embryos experimentally infected via the yolk sac (18). *M. penetrans* was initially isolated from the urine of a patient suffering from a primary antiphospholipid syndrome suggests that *M. penetrans* may be pathogenic, at least under certain circum-

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stances (55a). Since isolation of \textit{M. penetrans} from clinical samples is extremely painstaking (28, 29), the detection of infection by this mycoplasma relies almost exclusively on serological assays. These assays are based on an antigenic preparation consisting of a Triton X-114 (TX-114) extract of the mycoplasma (52). This extract contains two major polypeptides with apparent molecular masses of 38 kDa (P38) and 35 kDa (P35). We previously showed that P35 is the most abundant protein in the \textit{M. penetrans} membrane and that both P35 and P38 are acetylated (13). The gene encoding P35 was isolated and found to be located on a second open reading frame encoding a putative lipoprotein of 30.9 kDa showing significant similarity with P35 (13). The P35 polypeptide is the earliest and the major antigen recognized in infected patients and in experimentally infected animals (17, 35, 52).

Here we report the ability of \textit{M. penetrans} to modulate its major surface antigen, P35, by phase variation. Although this property is not unique among the members of the class \textit{Molllicutes}, the mechanism by which this variation occurs in \textit{M. penetrans} seems to differ from those previously described in other \textit{Mycoplasma} species.

### MATERIALS AND METHODS

**Mycoplasma strain and culture conditions.** \textit{Mycoplasma} GTU-54-6A1, initially isolated by S.-C. Lo (Armed Forces Institute of Pathology, Bethesda, Md.), was kindly provided by J. Talip (National Institute of Allergy and Infectious Diseases, Frederick, Md.) and was cultured in liquid broth or on 1% Noble agar (Difco, Detroit, Mich.) in SP-4 medium containing 10% fetal calf serum (50).

**Antibodies.** Murine P35-specific monoclonal antibody (MAb) 7 (immunglobulin G1 [IgG1] isotype) was established by T. Sasaki. The specificity of MAb 7 for P35 was established in another study (35). A rabbit hyperimmune serum (polyclonal antibody [PAb]) 2 was raised against a whole \textit{Mycoplasma} TX-114 extract and reacts predominantly with the P30, P35, and P38 polypeptides (35). For use in colony immunoblotting, the serum was diluted in phosphate-buffered saline (PBS)-0.1% bovine serum albumin (BSA); for use in Western immunoblotting, it was diluted in the same buffer containing 0.1% Tween 20.

**Transmission electron microscopy.** To locate the P35 polypeptide at the mycoplasma cell surface, we used an immunogold labeling technique whereby whole cells were labeled with anti-P35 MAb 7 at 0.03 mg/ml, essentially as described by Le Gall et al. (22). Briefly, the \textit{M. penetrans} cells were washed once in PBS and resuspended in PBS. Nickel grids previously coated with a Formvar carbon film were placed on the mycoplasma suspension for 3 min. The cells were fixed for 20 min with 2% glutaraldehyde in 0.1 M cacodylate buffer, heated at 60°C for 15 min, and collected in duplicate on a GeneScreen Plus membrane (NEN, Boston, Mass.) by vacuum (Minifold II; Stackonics, Las Vegas, Nevada). The grids were laid onto PBS–10 mM NH4Cl for 10 min, then on PBS–1% BSA for 10 min, and then on a PBS–0.5% BSA–0.1% gelatin solution containing 10 μg of MAb 7 per ml for 1 h at room temperature. After five washes in PBS–0.5% BSA–0.1% gelatin, the grids were incubated on a 1:25 dilution of the gold-conjugated secondary antibodies (anti-mouse IgG and IgM antibodies conjugated to 10-nm-diameter gold particles from Amersham International plc., Amersham, Bucks., United Kingdom) in PBS–0.5% BSA–0.1% gelatin. The grids were negatively stained with 1% phosphotungstic acid, and examined with a JEOL EX 1200 electron microscope operating at an accelerating voltage of 80 kV.

**Mycoplasma protein analysis.** TX-114 phase partitioning of cellular antigens was performed by using Fasta software (version 3.06) in-
GGTCTTCCACCAACGCCAGCA-3') was designed from a phylogenetically conserved region of the atpD gene and used as a control. The atpD gene encodes the ATP synthase F1 β subunit, and the ATP/10 primer was determined by alignment of the atpD genes from M. pneumoniae, M. genitalium, and M. gallinarum from a phylogenetically conserved region of the gene encoding the β subunit of the ATP synthase F1. The specificities of the oligonucleotide probes OP15, OP30, OMP12-1 and ATP/10 were verified by Southern blot experiments (data not shown). The patterns obtained were consistent with the known restriction maps (OP35, OP30, and OMP12-1) or with the presence of a single gene in the genome (ATP/10).

Blots were hybridized at 37°C for 16 h in a buffer obtained by dissolving a hybridization buffer tablet (Amer sham International) in 10 ml of distilled water as recommended by the manufacturer. After incubation, the membrane was washed at room temperature for 10 min with 2× SSPE (1× SSPE is 0.15 M NaCl, 10 mM NaH2PO4H2O, and 1 mM EDTA [pH 7.4]), 10 min with 2× SSPE–2% SDS, and 10 min with 0.1× SSPE. Blots were exposed to BioMax Films (Eastman Kodak).

Nucleotide sequence accession numbers. The GenBank/EMBL accession numbers for DNA sequences of IMP12, IMP13, and IMP14 are AJ006067, AJ006068, and AJ006069, respectively.

RESULTS

The M. penetrans P35 antigen undergoes high-frequency phase variations. By immunoelectron microscopy, P35 is exclusively detected on the mycoplasma cell surface, including on the tip structure (Fig. 1A), which is believed to mediate cytoadherence (16, 27). However, analysis of several electron microscope fields indicated that a small proportion (about 1.5%) of the cells were not labeled with MAb 7 (Fig. 1A). Thus, in a given lineage, not all of the mycoplasma cells appear to express P35-specific immunoreactivity on the surface. This phenomenon was further evaluated by colony immunoblotting (Fig. 1B to D); using reactivity to MAb 7 as a marker for P35 expression, a small percentage of the colonies were found to be P35− (Fig. 1B). A clonal lineage, selected for its P35+ phenotype, was found to switch to the P35+ phenotype with a calculated frequency of about 2 × 10−3 per cell per generation. Numerous M. penetrans colonies exhibited a mixed phenotype, with only some sectors recognized by MAb 7 (Fig. 1C), consistent with a similar high switching frequency of expression during colony growth. The heterogeneity of colonies for surface antigen expression was also evaluated by using antibodies exhibiting a broader specificity. M. penetrans colonies were immunolabeled with a rabbit antisem raised against the whole M. penetrans TX-114 antigen extract (PAb 2). Some of the colonies were still not found to immunoreact (Fig. 1D). The frequencies of P35+ colonies and of colonies showing a mixed phenotype were about 4 × 10−3 and 1.5 × 10−2 per cell per generation, respectively.

M. penetrans variants nonreactive with MAb 7 (P35− phenotype) were isolated and subcultured. Most of the colonies from these variants were P35−, but a few bound MAb 7 (Fig. 1E), indicating a reversion to the original P35+ phenotype and demonstrating the reversibility of the P35− ↔ P35+ phenotype switching.

Various clonal variants were chosen for further analysis: P35+ variants selected with either MAb 7 (M4 and M6) or PAb 2 (P1, P2, and P4) and the corresponding P35− revertants (RM41 derived from M4; RM61 and RM62 derived from M6). The phenotypic stability of each clonal lineage (P35+ or P35−) was tested. After three in vitro passages, the relative proportions of the P35− and P35+ colonies were unchanged, indicating that under these in vitro conditions, the phenotype was stable (data not shown).

Analysis of the protein content of the different M. penetrans clonal variants. To evaluate if the variable P35 expression at the mycoplasma cell surface was due to a true P35 phase variation (on−off), or to switching between masked and unmasked P35, TX-114 extracts from selected clonal variants (M4, M6, P1, P2, and P4) were analyzed by SDS-PAGE (Fig. 2A). We confirmed our previous finding (13) that P35 is the main antigen in the M. penetrans TX-114 extract (Fig. 2). The protein profiles from the selected clonal variants differed from that of the type strain. No P35 was detected in the M4 extract. The M6 and P1 extract profiles, although differing from that of M4, were similar to each other: the major band was an antigen with an apparent molecular mass of 38 kDa, and there was only a faint band at the P35 position. Finally, two other profiles were obtained with the two other clonal variants (P2 and P4). Although P2 and P4 were obtained from colonies which did not react with PAb 2, examination of the protein profile (Fig. 2A) and Western blot analysis (data not shown) indicated that P35 was still produced in these variants.

The protein profiles of the M4 and M6 TX-114 extracts were found to differ from those of their corresponding revertants (RM4, RM61, and RM62) (Fig. 2B). This observation was confirmed by immunoblotting (Fig. 2B). Whereas MAb 7 revealed only a faint band in the M4 protein profile, most probably resulting from the low percentage of P35− revertants in this clonal lineage (Fig. 2B), the corresponding RM4 revertant exhibited an intense P35 band. A similar result was obtained with a whole-cell lysate used in place of the TX-114 extract for this variant (data not shown). For the other variant (M6), there was a MAb-reacting polypeptide, although the intensity of the signal was lower than those obtained with either the type strain or its revertants RM61 and RM62. The higher immunoreactivity of P35 in M6 extracts than in M4 extracts (Fig. 2B) may be correlated with the difference in the frequency of reversion in a culture. Indeed, immunoelectron microscopy observation of the MAb 7 reactivity of cells from M4 and M6 cultures indicated proportions of revertants of 1 and 12%, respectively (data not shown). Western blotting with PAb 2 confirmed the lack of P35 in M4 and indicated that other antigens in the TX-114 extract were nevertheless recognized by antibodies in this antisem (Fig. 2B). These results indicate that P35 expression undergoes phase variation.

P35 phase variation is not associated with chromosomal rearrangement. To determine whether large-scale DNA rearrangements were associated with P35 phase variation, restriction digests of the genomic DNA preparations from P35+ and P35− clonal populations were compared (Fig. 3). Three endonucleases were chosen for their different restriction sites in the HpaI 2.9-kbp fragment, encompassing the p35 gene: BclI has a single site within the p35 gene. MspI releases a DNA fragment overlapping most of this gene, and HpaI was used to clone the fragment. Genomic Southern blotting was performed either with the entire pMP11 or with the isolated p35 gene without its 5′-signal sequence-encoding fragment as the probe (Fig. 3B). There were no detectable differences in the restriction patterns obtained with the genomic DNAs from the type strain and the variant M4. Thus, large-scale DNA rearrangements (transposition, inversion, or duplication) of the p35 gene do not appear to be associated with P35 phase variation. In addition, the obtained profiles strongly suggested that there is only a single copy of the p35 gene in the M. penetrans genome.

To confirm the Southern blotting results, the lengths of two regions were determined by PCR: the p35 gene by using primers P35/6 and P35/7, and the region located immediately upstream from this gene by using primers P35/1 and P35/2 (Fig. 3A). These primers sets allowed the amplification of a 1,080-bp DNA fragment (with primers P35/6 and P35/7) and of a 499-bp DNA fragment (with primers P35/6 and P35/7) (Fig. 3C). The sizes of the fragments amplified from the type strain were the same as the sizes of those amplified from the selected variants (M4, M6, P1, P2, and P4).
P35 phase variation is not associated with mutations within the \( p35 \) promoter or within the \( p35 \) gene itself. Although the \( p35 \) gene was previously identified (13), the transcription initiation site remained to be determined. It was identified by a primer extension assay (Fig. 4A), 132 bp upstream from the proposed translation initiation site. Potential \( -10 \) and \( -35 \) boxes were deduced (Fig. 4B). Interestingly, two direct repeat sequences were found within this promoter region.

To identify any point mutations either within this promoter region or within the \( p35 \) gene, the sequence from nt 1 to 1840
indicated. The positions of P35 and P38 are indicated.

The revertants were obtained from the variants on the basis of their P35 phenotype established by colony immunoblotting with MAb 7. Similar amounts of protein were TX-114 phase-fractionated proteins from the PAb 2, as indicated. After electrophoresis, the proteins were stained with Coomassie blue. The position of P35 is indicated by an arrow. (B) SDS-PAGE analysis of strain (T) and derived variants (M4, M6, P1, P2, and P4). These variants were selected from the type strain on the basis of colony immunoblotting with MAb 7 or with PAb 2, as indicated. After electrophoresis, the proteins were stained with Coomassie blue or by immunoblotting with MAb 7 or the polyclonal antiserum, as indicated. The positions of P35 and P38 are indicated.

in IMP11 was PCR amplified from the type strain and from the variant M4 and determined in its entirety. There was perfect identity between the determined sequences, which clearly indicated that there was no point mutation associated with P35 phase variation in M4. The absence of point mutations was also confirmed for other variants (P1, P2, P4, and M6) by sequencing the region from nt 20 to 500 in IMP11.

The p35 gene belongs to a family of related lipoprotein genes. The organization of the p30 and p35 genes within IMP11 suggested that they might belong to a family of related genes encoding lipoproteins (13). In particular, there was a striking identity of the 5′ ends of these two genes, which we used to develop a strategy to amplify a putative lipoprotein gene located upstream from IMP11 on the *M. penetrans* genome. A forward primer (P38/1) was derived from the conserved region of the p30 and p35 genes, and the reverse primer (P38/2) corresponded to a sequence located upstream from the p35 gene promoter. Using this strategy, we obtained several amplicons, and three were sequenced. All carried potential lipoprotein genes. The sequence from one of the selected plasmids (pMP14), as hypothesized, overlapped the 5′ end of IMP11 and was found to encompass part of the gene encoding a putative lipoprotein with a deduced molecular mass of 36 kDa for its mature product (pepIMP14 [Fig. 5]). Putative lipoprotein genes were also found on two other plasmids obtained (pMP12 and pMP13). The genomic positions of the regions inserted in pMP12 and pMP13 are not known. Sequence analysis indicated that they were both PCR amplified from the single P38/1 primer. In agreement with this finding, two divergent and partially overlapping open reading frames, encoding putative lipoproteins, were found to reside on the pMP12 insert (Fig. 5). These related (some being putative) genes, designated mpl genes, share perfect identity in the region corresponding to a lipoprotein signal sequence. The different polypeptides deduced from the mpl genes share a certain degree of similarity. Multiple alignments clearly indicate almost perfect identity of their N termini, including their signal sequences (Fig. 5). With the notable exception of pepIMP12 and pepIMP13, which share a high degree of identity, more diversity in the primary structures of these polypeptides was found in regions closer to their C termini. The sizes of the deduced mature polypeptides are between 10.6 kDa (pep2IMP12) and 36.8 kDa (pepIMP14); it should also be noted that the size of pepIMP13 is not known because its gene on IMP13 is truncated. We also cannot exclude the possibility that other genes in the *M. penetrans* genome encode related Mpls. Finally, the search for similarity with the mpl-encoded polypeptides in databases did not reveal any high score of identity.

P35 phase variation is conditioned by transcription of the p35 gene. To determine whether the P35 phase variation was associated with corresponding modifications in the production (or stability) of its mRNA, RNA blotting experiments were performed. To compare lipoprotein gene expression, 1 μg of total RNA from each *M. penetrans* variants was blotted on the membrane. In addition, to control for total mRNA amounts, an mRNA for a gene conserved among mollicutes was also specifically detected. The selected gene, *atpD*, which encodes the β subunit of the ATP synthase F1, was chosen because its expression was presumed to be the same in the different *M. penetrans* variants. The intensities of the hybridization signal with the *atpD*-specific probe were similar for the variants, except for RM61 and P4, for which the amount of detected *atpD* transcript was lower (Fig. 6). Taking this variation into account, we found that the p35 gene was expressed to a high level in strain GTU and to a lower level in the RM4, RM61, RM62, P2, and P4 variants. p35 gene expression was lowest in the M4, M6, and P1 variants, in accordance with the low amount of P35 detected in these variants by Western blotting. Thus, P35 phase variation is determined by variations of the level of p35 transcription.

The expression of two other lipoprotein genes (*p30* [encoding P30] and *IMP12-I* [encoding pep1IMP12]) was also studied in the same experiment. Interestingly, *p30* gene expression was found particularly low in GTU and M6. The expression of *IMP12-I* was more similar among the different variants (Fig. 6).

**DISCUSSION**

This study demonstrated that P35, the *M. penetrans* major surface antigen, undergoes high-frequency phase variation at a rate similar to that described for other mycoplasma variable lipoproteins (for a review, see reference 10). Sequence analysis of the gene encoding P35 and flanking sequences reveals that this gene is located upstream from a second open reading frame (ORF2) encoding a putative lipoprotein of 30.9 kDa (P30) with significant similarity with P35 (13). In addition, we have identified other related genes, designated mpl genes,
which potentially encode lipoproteins. A remarkable feature of the deduced lipoproteins is that they all have identical signal sequences. Although the mature moieties of these polypeptides differ, they are nevertheless homologous. At least three of these genes (the gene encoding pepIMP14, p35, and p30) are clustered in the M. penetrans genome. A similar organization of genes encoding variable lipoproteins has been previously described for M. bovis (1, 31), M. hyorhinis (43, 56), M. fermentans (47, 48), M. gallisepticum (32), and M. pulmonis (2, 46). Analysis of the protein patterns obtained with the TX-114 extract from the different M. penetrans variants clearly indicated that the P35 phase variation was associated with modifications of the expression of the other lipoproteins; this was also partially confirmed by the results of RNA slot blotting. However, the amount of p30 mRNA in the different variants could not be correlated with that of p35 transcript, suggesting that the expression of these two genes is not coordinated. The heterogeneity in the protein patterns among the M. penetrans variants was remarkable and highlighted the multiple possible combinations in the repertoire of lipoproteins expressed at a given time.

The role of P35 in M. penetrans physiology is not known. It is striking that such an abundant protein is nonessential: under the electron microscope, the different variants exhibited similar morphologies and the capsule layer identified recently by us was not affected by the absence of P35 expression (data not shown). In addition, the rates of in vitro growth of the variants were similar. Since two-component sensory elements have not been found in the genomes of M. pneumoniae and M. genitalium (20), it is possible that some of the mycoplasma lipoproteins act as sensory elements. A similar hypothesis has been formulated for the lipoprotein OspA from the phylogenetically unrelated Borrelia burgdorferi, whose genome shows some similarities with those of M. pneumoniae and M. genitalium (14).

FIG. 3. Analysis of genomic DNA from the M. penetrans type strain and derived variants. (A) Restriction map of pMP11, including the P35-encoding region. The positions of restriction sites of the three enzymes used in the Southern blot analysis are indicated. Positions of the PCR primers used for the analysis of two regions are shown below the map by arrowheads. (B) Southern blot analysis of DNA from the type strain (T) and the P35 variant (M4). As indicated, two probes were used: pMP11 (left) and the isolated p35 gene without its sequence encoding fragment (right). The positions and sizes of the major hybridizing DNA fragments are indicated. (C) Comparison of the sizes of fragments amplified by PCR from two genomic DNA of the type strain (T) and from five P35 variants (M4, M6, P1, P2, and P4). A DNA fragment encompassing the p35 gene (size of the amplicon, 1,080 bp) was PCR amplified with the PCR primers P35/6 and P35/7, and another upstream from this gene (size of the amplicon, 499 bp) was amplified with PCR primers P35/1 and P35/2. In both ethidium bromide stained-agarose gels, the DNA marker (M) was a 100-bp DNA ladder.

FIG. 4. Identification of the transcription start site of the p35 gene and analysis of the deduced promoter region. (A) Autoradiogram of a polyacrylamide gel used to analyze the size of the DNA obtained by reverse transcription. Because the radioactive signals obtained with the sequencing ladder and the primer extension did not have similar intensities, the figure is a composite of two exposure times. The position of transcription start site of the p35 gene is shown by an arrow. E designates the results from primer extension. (B) Deduced promoter region for the p35 gene. Numbers above the sequence correspond to the nucleotide numbering in IMP11. The transcription start site and the −35 and −10 boxes are in black boxes. The P35-encoding region is boxed, and the first nucleotide is in a black box with a number indicating its position with reference to the transcription start site. Two inverted repeated sequences are underlined with arrows.
FIG. 5. Analysis of members of the mpet gene family. (A) Genetic organization of mpet genes. The isolation and sequencing of IMP11 have previously been reported (13). In this study, the sequence upstream from IMP11 was determined (IMP14). Two other independent M. penetrans DNA regions (IMP12 and IMP13) whose genomic location relative to IMP11 is unknown were PCR amplified, isolated, and sequenced. The shaded boxes indicate identical regions encoding putative signal sequences. (B) Multiple alignment of P35-related polypeptides, obtained by using Clustal W software. The boxed amino acids are common to at least four of the six sequences. Boxes indicate identical residues (bold letters) or conservative changes.
The identification of colonies which did not immunoreact with PAb 2 was striking because it suggested that either the complete set of expressed surface proteins can change or newly expressed surface polypeptide(s) provided a mask for those proteins recognized by PAb 2. Analysis of the TX-114 extracts from the variants P2 and P4 favored the second possibility because P35 continued to be present in these variants. This result is of particular importance, as it may reveal the putative role of these antigenic variations in the escape from the immune response. A heterogeneous reaction pattern of mycoplasma colonies to a polyclonal antiserum was previously described for another species, M. bovis (44). In addition, there is a growing body of evidence that mycoplasma surface antigenic variation may provide a protective mechanism against the damage caused by the immune response. First, it was shown that the repertoire of variable antigens is changed after an experimentally induced in

In contrast to other variable mycoplasma lipoproteins, we observed phase variation but no size variation of P35. Size variation has been associated with the presence of repetitive elements in the C terminus of the gene encoding the variable protein. The p35 gene has no such repeated elements. Size variation has been described, however, for surface antigens in Ureaplasma urealyticum, M. hyorhinis, M. pulmonis, and M. bovis (for a review, see reference 12). The genetic mechanisms underlying the mycoplasma lipoprotein phase variations have been elucidated in a few cases: DNA rearrangements such as that described for the M. pulmonis Vsa antigens (2, 46), point mutations either within the promoter region (56) or within the gene itself (48, 57), and masking/unmasking of epitopes (44). In M. hyorhinis, the Vlp antigen phase variations are due to random size modifications of the poly(A) box within the vlp gene promoter (56). We have identified the promoter of the p35 gene and found that it presents some interesting features. The distance between the sites for initiation of transcription and translation is 132 bp, which is similar to that described for the promoter of M. hyorhinis Vlp (56). Although the sequence between the −35 box and the translation start codon has an A+T content of 84%, there is no poly(A) tract as was found between the −35 and −10 boxes of M. hyorhinis Vlp genes (56). The size variation of this repetitive tract was shown to be associated with phase variation of these lipoproteins (56). However, such an AT-rich region upstream from the transcription initiation site suggests DNA bending (51) due to the binding of regulatory proteins (36). This possibility is also supported by the presence of two dyad symmetries in the vicinity of the promoter. Since the P35 phase variation was not associated with DNA rearrangement or with mutation within the p35 gene or its promoter, we suggest that the regulated expression of p35 could be due to an as yet unidentified regulatory protein(s).

The demonstration of P35 phase variation has implications for the development and use of M. penetrans serological assays. Indeed, current enzyme-linked immunosorbent assays are based on a P35-containing TX-114 extract (17, 52). However, it is possible that P35 expression is necessary for the early stages of mycoplasma infection, and in this case we would expect to detect P35-specific antibodies in every M. penetrans-infected host. This possibility is supported by experimental infections. Alternatively, P35 may not be essential, in which case testing for anti P35 antibodies would be unsatisfactory. Our current estimates of the incidence of M. penetrans cases of infection, which is difficult to document because of the fastidious growth of the organism from clinical sites, may therefore be underestimated.

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O.N. and I.C. equally contributed to this work.

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ADDENDUM IN PROOF

During the time that the present paper was being reviewed, an outstanding review on different aspects of mycoplasma biology, including antigen variations, was published (S. Razin, D. Yogev, and Y. Naot, Microbiol. Mol. Biol. Rev. 62:1094–1156, 1998). In addition, in another recently published paper (G. Pyrowolakis, D. Hofmann, and R., Herrmann, J. Biol. Chem. 273:24792–24796, 1998), a mycoplasma lipoprotein was found to have a key role in the cell metabolism as the subunit b of the FfF₁-type ATPase.

REFERENCES


