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Molecular Cloning of a Member of the Gene Family That Encodes pMGA, a Hemagglutinin of *Mycoplasma gallisepticum*

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A hemagglutinin with an M_r of 67,000 (pMGA) from *Mycoplasma gallisepticum* S6 was purified by using monoclonal antibody affinity chromatography. Purified pMGA was treated with a number of enzymes, the resultant peptides were purified, and their amino acid sequence was determined by using an Applied Biosystems (model 471A) protein sequencer. The DNA sequence encoding two peptides was used to dictate the sequences of synthetic oligonucleotides which were used to screen a library of *Eco*RI-cut *M. gallisepticum* DNA in pUC18. A clone reactive to both probes was isolated and found to contain a recombinant insert of 10 kb. The clone was mapped by using restriction endonucleases and fragments subcloned into pUC18 for DNA sequencing. Analysis of part of the DNA sequence revealed an open reading frame containing 1,941 nucleotides which encoded 647 amino acids. The amino terminus was preceded by a putative leader sequence of 25 amino acids. A promoter region preceding the putative start codon GUG was also located. This gene would encode a mature protein of 67,660 Da. There were a number of differences between the predicted amino acid sequence and that determined by direct peptide sequencing. Also, two tryptic peptides of pMGA were not found in the DNA sequence. This suggested that the cloned gene did not encode pMGA but did encode a homolog (pMGA1.2). Furthermore, downstream of pMGA1.2 was a region of DNA encoding a leader sequence followed by an amino acid sequence with high homology to that encoded by the pMGA1.2 gene. The presence within *M. gallisepticum* of a family of pMGA genes is inferred from the DNA sequence and Southern transfer data. A possible role for this gene family in immune evasion is discussed.

Recent work in this laboratory has resulted in the molecular characterization of an adhesin molecule, pMGA, from the avian respiratory pathogen *Mycoplasma gallisepticum* (12). This molecule was shown by selective trypsinization experiments and by specific blockade by pMGA-specific monoclonal antibodies to be a hemagglutinin of *M. gallisepticum* (12). Although pMGA elicits a strong immune response in fowls infected with pathogenic *M. gallisepticum*, the organism persists and has been isolated up to 1 year after infection (9). If such infected fowls are challenged with a different pathogenic strain of *M. gallisepticum*, colonization of the host with the challenge strain is minimal (8) but the original strain persists. Variations in the molecular weight of immunostained bands between three separate strains of *M. gallisepticum* were observed by Western blotting (immunoblotting) by using pMGA-specific monoclonal antibodies (12).

Studies of the adhesin molecules of the human respiratory pathogen *Mycoplasma pneumoniae* have implicated a plasma membrane molecule, P1, with an approximate M_r of 170,000. The adhesin function of P1 was demonstrated by trypsinization experiments in which P1 was selectively digested from the cell surface and by specific monoclonal antibody blockade of P1; both treatments reduced *M. pneumoniae* attachment to epithelial cells in tracheal organ cultures (3, 7). The gene encoding P1 has been molecularly cloned, and the amino acid sequence has been analyzed (5, 20). Examination of the P1 gene from two separate *M. pneumoniae* strains revealed a number of sequence differences (19). Ruland et al. (17) observed that parts of the P1 gene were repeated up to 10 times within the *M. pneumoniae* genome. These observations together with descriptions of antigenic variation in other mycoplasma species including the variable lipoproteins of *Mycoplasma hyorhinis* (16) suggest that these organisms may utilize antigenic variation as a means of evading immune responses.

As part of a study on the role of pMGA in the pathogenesis of respiratory disease by *M. gallisepticum*, we decided to isolate the pMGA gene and thereby to evaluate the molecular topology of the protein it encodes. We present data herein which establish that a family of pMGA-like genes exists within the *M. gallisepticum* genome and that two members of the gene family contain structural attributes consistent with the possibility that both genes are transcriptionally and translationally competent. Despite this, the pMGA variant expressed by *M. gallisepticum* S6 grown under conditions used in this laboratory is encoded by neither of these genes. We conclude that a selection mechanism which results in the expression of a single pMGA gene from a repertoire must exist. We consider the possible significance of this finding to the persistence of *M. gallisepticum* strains in the host and the resistance of the host to further infection with other strains.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *M. gallisepticum* S6 was initially filter cloned five times and passaged in broth medium (1) supplemented with 10% swine serum (23). Protein and DNA were extracted from *M. gallisepticum* S6 grown from ampoules of passage 313. *Escherichia coli* DH5α (kindly donated by H. Nagesha, University of Melbourne) was grown (with shaking) at 37°C in Luria broth (LB)

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containing 50 μg of ampicillin per ml or on LB agar also containing 50 μg of ampicillin per ml.

Isolation of mycoplasma DNA. Mycoplasma cells were grown till late log phase and harvested by centrifugation (20,000 × *g* for 30 min). The cells were resuspended in 0.1 M phosphate buffer–0.33 M NaCl (pH 7.4) and spun at 20,000 × *g* for 5 min. This step was repeated once. The pellet was resuspended in 10 mM Tris–10 mM EDTA–10 mM NaCl (pH 8.0)–HCl, and the cells were lysed by the addition of sodium dodecyl sulfate (SDS) (25%, 0.02 volume). Proteinase K (100 μg/ml; Boehringer Mannheim) was added to the lysate, and the mixture was incubated at 37°C overnight. To the solution was added 1 μg of RNase A (Sigma) per ml, and the mixture was incubated at 37°C for an additional 30 min. The mixture was extracted with an equal volume of phenol equilibrated with 0.1 M Tris (pH 8.0)-HCl, once with phenol-chloroform (1:1), and once with chloroform-isoamyl alcohol (24:1). Sodium acetate (2.5 M, pH 5, 0.1 volume) was added to the solution, and the DNA was precipitated with ethanol.

Enzymatic digest of pMGA. Approximately 1 mg of pMGA was affinity purified by using a monoclonal antibody affinity column as described previously (12). The material was first dialyzed against 1 mM ammonia to remove salt and then subjected to reduction and carboxamidomethylation with iodoacetamide. Digestions were conducted with cyanogen bromide or in separate experiments with the enzyme trypsin (Worthington) or endoproteinase-glu-c (Boehringer Mannheim). For CNBr digestions, lyophilized pMGA samples (100 to 1,000 μg) were dissolved in 70% (vol/vol) formic acid containing 25 mg of CNBr (Merck) per ml. Digests were incubated for 16 to 20 h, and volatile reagents were removed by vacuum centrifugation. Enzyme digestions were performed at an enzyme/substrate ratio of 5% (wt/wt) and a final protein concentration of 1 mg/ml by using NH_4HCO_3 as the digestion buffer.

Peptide isolation and purification. CNBr digests were subjected to SDS-polyacrylamide gel electrophoresis by using a Tricine buffer system to facilitate the resolution of low-molecular-weight fragments (18). The gels were subjected to electrophoretic transfer to polyvinyldifluoride membranes (Millipore) and stained with Coomassie brilliant blue, and peptide zones were excised for protein sequence analysis.

Digests with proteolytic enzymes were subjected to reversed-phase fast protein liquid chromatography (FPLC) essentially as described previously (13). Briefly, each digest was chromatographed by using a C18 column (Pharmacia, Pep RPC), and the elution of peptide fractions was monitored by measuring A_{214}; fractions were collected manually and rotary evaporated to dryness, and appropriate samples were rechromatographed on the same column. The initial fractionation was conducted with 10 mM formic acid (pH 4.0)–NaOH as a buffer, and the secondary fractionation was conducted with unbuffered 0.1% (vol/vol) trifluoroacetic acid (Applied Biosystems, Inc. [ABI]). Linear acetonitrile gradients were used to elute peptides (0 to 90% acetonitrile, delivered at 1 ml/min over 90 min). Purified peptides were then sequenced.

Edman degradation. The automated Edman degradation was performed with an ABI model 471A protein sequencer equipped with a Brownlee Laboratories microgradient delivery system to conduct chromatographic identification of phenylthiohydantoin (PTH) amino acids.

Preparation of synthetic oligonucleotide probes. The nucleotide sequences predicted for peptides T3 and C7 (see Table 1) were used in constructing 23- and 20-mer degenerate oligonucleotides, respectively. The resultant oligonucleotides were produced on a PCR MATE (ABI), cleaved from the solid support, and purified by passage over an OPC column (ABI).

Construction of mycoplasma genomic DNA library. *Eco*RI (Boehringer Mannheim)-restricted genomic mycoplasma DNA fragments were cloned into an *Eco*RI-cut pUC18 vector. Recombinant plasmids were transformed into *E. coli* DH5α cells by methods described by Maniatis et al. (11).

Screening of recombinant library. Screening of the recombinant library for probe-reactive DNA was conducted by using standard techniques detailed by Maniatis et al. (11). Briefly, duplicate lifts were made of recombinant *E. coli* colonies grown on agar by using Hybond-N (Amersham) membranes. Membranes were incubated in prehybridization buffer containing 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM Na_3PO_4, and 1 mM EDTA [pH 7.7]), 0.5% SDS, 0.2% nonfat skim-milk powder, 1× Denhardt solution, and 100 μg of single-stranded salmon sperm DNA (Sigma) per ml at 42°C for 4 h. Oligonucleotide probes were 5′ labeled with (γ-^{32}P)ATP (Amersham) by utilizing Boehringer Mannheim polynucleotide kinase; unincorporated label was removed by using a G-50 (Pharmacia) spin column as described by Maniatis et al. (11). Labeled oligonucleotide probes were allowed to hybridize with individual membranes at 42°C overnight. The membranes were washed twice in 6× SSPE containing 0.5% SDS for 10 min each time at 60°C. The membranes were blotted dry and then exposed to XAR-5 film (Eastman Kodak), and the film was developed.

Southern blot hybridization. Genomic DNA of *M. gallisepticum* S6 was digested to completion with DNA restriction enzymes *Hin*dIII and *Eco*RI (Pharmacia). The digested DNA together with ^{32}P-labeled λ DNA markers (Bresa) was subjected to electrophoresis in 0.7% agar gel and blotted onto Hybond-N membranes as described before. Hybridization of oligonucleotide probes to membranes was carried out under the same conditions described previously. Cloned mycoplasma DNA was purified by using Prep-A-Gene (Boehringer Mannheim) as described below, radiolabeled, and used as probes in Southern hybridization. The radioactively labeled clone inserts were hybridized to individual membranes overnight at 55°C, and the membranes were finally washed in 0.1× SSPE with 0.5% SDS for 10 min each at 66°C.

Subcloning of mycoplasma DNA insert for DNA sequencing. Recombinant clones reactive to both oligonucleotide probes were isolated and grown in LB at 37°C overnight with shaking. Plasmid DNA was extracted by the method of Maniatis et al. (11), and a limited restriction map of the recombinant DNA was ascertained. The DNA insert was digested by an appropriate DNA restriction enzyme and subjected to electrophoresis, and the resultant fragments were isolated from the gel. The excised fragments were purified by using Prep-A-Gene (Boehringer Mannheim) as described in the manufacturer's instructions, cloned into suitably digested pUC18, and used to transform *E. coli* DH5α cells. Cells were plated on Luria agar containing the chromagen 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal; Boehringer Mannheim). White colonies carrying the correct recombinant DNA insert were grown overnight in LB, and the plasmid DNA was harvested and purified for subsequent DNA sequencing by using Prep-A-Gene.

DNA sequencing. The nucleotide sequences of the cloned DNA fragments were determined by the dideoxy-chain termination method with T7 DNA polymerase (Promega) as described in the manufacturer's instructions. The majority of the sequencing was done by utilizing synthetic oligonucle-

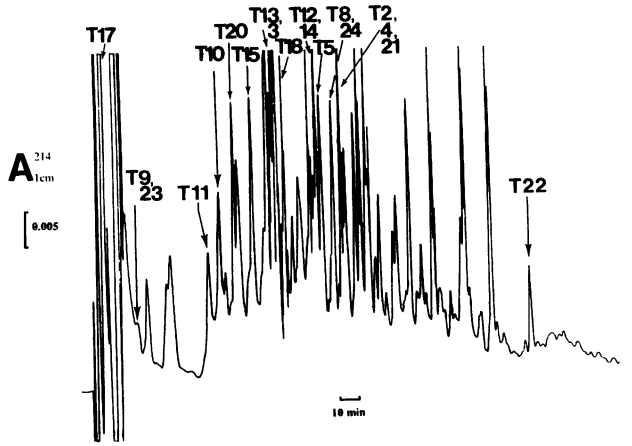

FIG. 1. Reversed-phase FPLC of a pMGA tryptic peptide digest. Arrows indicate fractions from which tryptic peptides were isolated for further purification and sequence analysis. Peptide designations refer to the sequences in Table 1.

otide primers complementary to regions 5′ and 3′ of the inserted DNA of pUC18. Synthetic oligonucleotides based on complementary regions of previously sequenced DNA were used as primers to initiate and extend DNA sequencing.

Computer analysis of the cloned fragments. The DNA sequence was analyzed by using the University of Wisconsin Genetics Computer Group DNA program package. The amino acid sequence was analyzed by using the FASTA program with the algorithms of Pearson and Lipman (15).

Nucleotide sequence accession number. The accession number for the mycoplasma nucleotide sequence is M83178.

TABLE 1. Peptide amino acid sequences

Peptide[a]	Amino acid sequence
C1	GLTTIFD...
T2	NLGLYVDYK
T3	QTFDEQHAELVK
T4	TATLAPYADAQY...
T5	MHLSGLYDAGK
G6	DLEPVEGDPLTAGA...
C7	ANTKIVEAIKDEVLNPQK
T8	AQPANYSFVGYK
T9	TIFTNSDEPR
T10	YTLE...
T11	LVNTSDQMK
T12	LGLEYK
T13	TPTVNDINVAK
T14	IEFSVPAEK
T15	LSSSPNNWNK
C16	VPKNNVQKFPLANNTSN...
T17	GVFASGVGSDLK
T18	FSTGDMSSNNVTVK
C19	SSNNVTVKQLFTGNLT...
T20	TFDTSATTESTR
T21	TLTLVEGLNK
T22	IVVSGTTENIGAP...
T23	LPDSFG...
T24	VEVF-GD...

[a] CNBr (C), endoproteinase-glu-C (G), and tryptic (T) peptide sequences were obtained as described in the text.

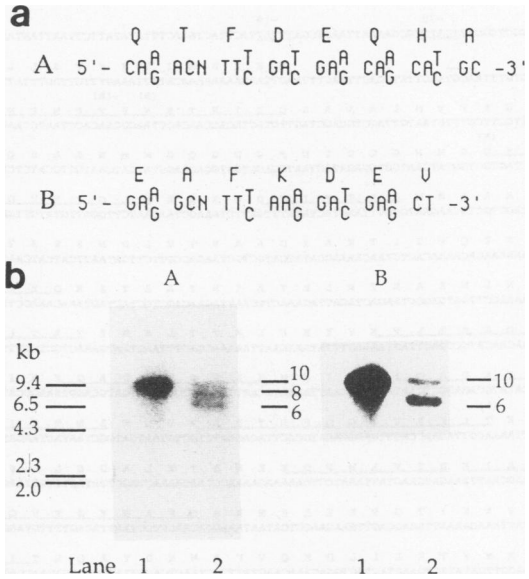

FIG. 2. (a) PMGA-specific oligonucleotides. The oligonucleotide probes A and B were assembled by using an ABI PCR MATE for amino acid analysis of peptides T3 and C7, respectively. The symbol N denotes the simultaneous addition of all four nucleotides at the positions shown. (b) Reactivity of pMGA oligonucleotides with *M. gallisepticum* DNA. pUC18/pMGA and genomic DNA of *M. gallisepticum* were digested with *Eco*RI and subjected to Southern transfer by using oligonucleotide probes A and B (panel a) for detection of complementary sequences. Lanes 1 and 2 of both panels A and B are *Eco*RI digests of pUC18/pMGA DNA and *M. gallisepticum* genomic DNA, respectively. Probes A and B were hybridized to panels A and B, respectively.

RESULTS

Partial amino acid sequence analysis of pMGA. The pMGA protein was purified by using monoclonal antibody affinity chromatography as described previously (12), and samples of pMGA were subjected to proteolytic and chemical digestion. Figure 1 shows the results of reversed-phase FPLC on the tryptic digest of pMGA and the fractions which were subsequently used to isolate the tryptic peptides sequenced in this study (Table 1).

Cloning of a gene encoding a number of pMGA peptides. Synthetic oligonucleotides 23 and 20 nucleotides in length were assembled; their nucleotide sequences are based on the amino acid sequences of peptides T3 and C7 (Fig. 2a). Both oligonucleotides were radiolabeled and hybridized to *Eco*RI-cut genomic DNA of *M. gallisepticum* in a Southern trans-

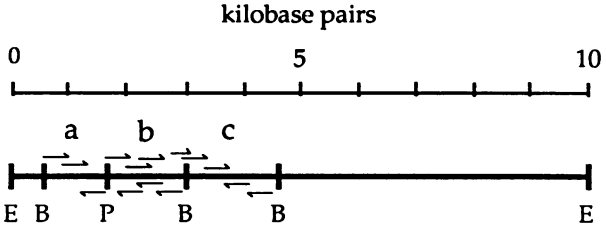

FIG. 3. Endonuclease restriction map of probe-reactive *M. gallisepticum* DNA insert. Fragments a, b, and c were subcloned into pUC18 for DNA sequencing. The sequencing strategy is depicted by the arrows. Abbreviations: E, *Eco*RI; B, *Bgl*II; P, *Pst*I.

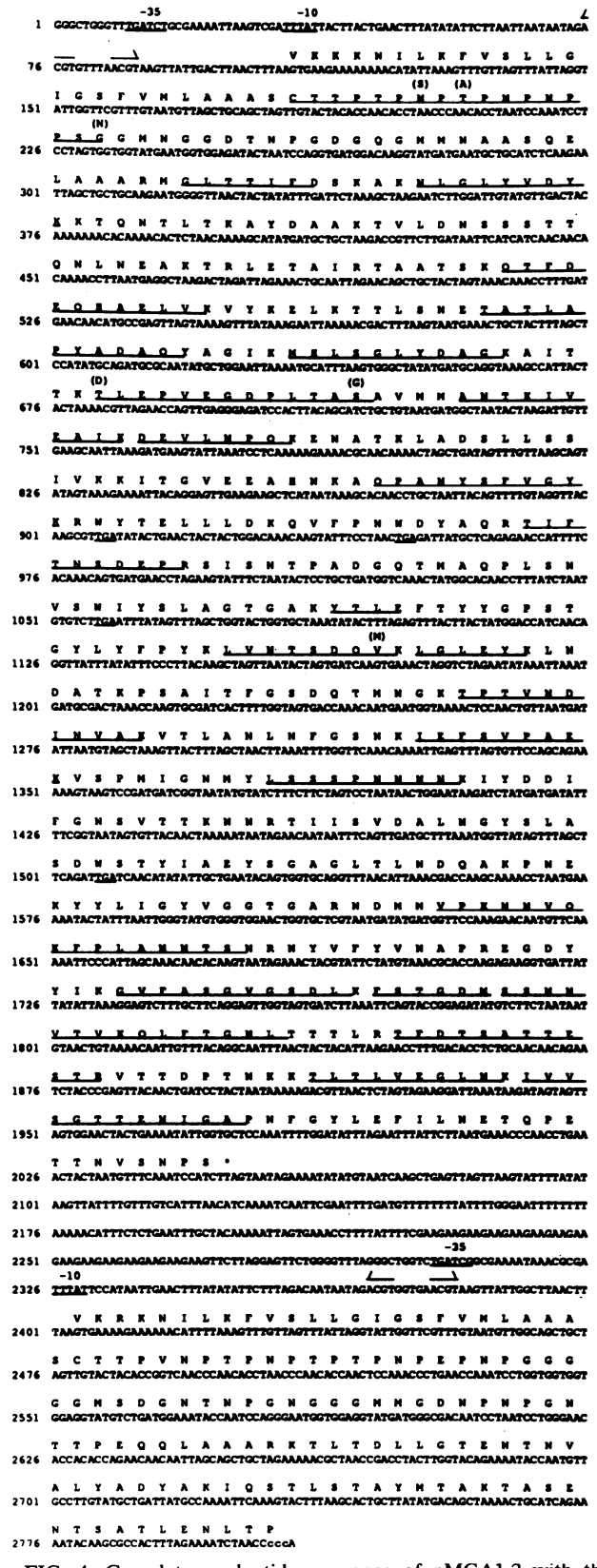

FIG. 4. Complete nucleotide sequence of pMGA1.2 with the partial sequence of pMGA1.3 shown above. The deduced amino acid sequence is shown above the first nucleotide of the codon. The amino acid sequences from pMGA (Table 1) are underlined, and differences are shown in parentheses above the corresponding amino acid. It has been reported that *M. gallisepticum*-like *Mycoplasma capricolum* uses the TGA codon for tryptophan instead of a termination signal (6). The TGA codons for tryptophan in pMGA1.2 are underlined. The proposed promoter regions preceding transcription starts of pMGA1.2 and pMGA1.3 are underlined and labeled −35 and −10. Stem-loop structures are indicated by arrows found at nucleotide positions 81 and 2374. The restriction enzyme positions used for subcloning are as follows: *Pst*I, 177; *Bgl*II, 1409; and *Bgl*II, 2803.

fer. Figure 2b shows that the 23-mer (panel A, lane 2) probe hybridized to 10-, 8-, and 6-kb bands in Southern transfer and the 20-mer (panel B, lane 2) probe hybridized to 10- and 6-kb bands. A genomic library of *Eco*RI-cut *M. gallisepticum* DNA was constructed in pUC18, and recombinant colonies were screened for their ability to hybridize to both of the radiolabeled oligonucleotide probes, A and B. One doubly reactive clone was isolated and found to contain a 10-kb insert of mycoplasma DNA reactive with both oligonucleotide probes (Fig. 2b, lanes 1). The DNA insert was subjected to cleavage by using a limited number of restriction enzymes, and the deduced restriction map is presented in Fig. 3. Three separate fragments were subcloned into pUC18 and designated a, b, and c (Fig. 3). The clones were subjected to DNA sequencing, and, where necessary, oligonucleotide primers were prepared to continue DNA sequencing.

Nucleotide and amino acid sequences. The partial DNA sequence of the 10-kb insert and its corresponding amino acid sequences are presented in Fig. 4. The DNA sequence is divided into two putative genes, pMGA1.2 and pMGA1.3. The pMGA1.2 gene begins at nucleotide position 109 with the start codon GTG and ends at position 2050 with the stop codon TAG. A 16-amino-acid sequence (beginning at nucleotide position 184) of pMGA1.2 has 87.5% homology (by the LFASTA program [15]) to the amino-terminal sequence of pMGA (the plasma membrane protein expressed and affinity purified from cultured *M. gallisepticum* S6 cells). The pMGA1.2 gene would encode a precursor protein of 647 amino acids (70.257 kDa), including a signal peptide of 25 amino acids. The start codon beginning at nucleotide position 109 is followed by three basic residues and then a stretch of 21 amino acids primarily hydrophobic in nature. The mature protein would contain 622 amino acids with a molecular mass of 67.66 kDa. Within pMGA1.2 are found 22 of the peptide sequences shown in Table 1; their positions are underlined in Fig. 4. Differences in amino acids of sequenced peptides and their respective DNA sequences are indicated (Fig. 4) by letters in parentheses. Note that peptides T23 and T24 of Table 1 or any identifiable sequence homologies are not found in the encoded amino acid sequence of pMGA1.2. Residues flanking the amino-terminal cysteine residue A-A-S-C show good agreement with the consensus sequence proposed for the bacterial signal peptidase II, which specifically cleaves the signal peptides of lipoproteins (21). The second putative gene (pMGA1.3) lies 354 nucleotides downstream of pMGA1.2 (Fig. 4). From the DNA sequence presented so far, the amino acid sequence of pMGA1.3 exhibits 46% homology (15) with pMGA1.2.

The signal peptide and amino-terminal sequences of pMGA1.3 and pMGA1.2 and the amino-terminal sequence of pMGA are shown in Fig. 5. The postulated signal peptide sequences of pMGA1.2 and pMGA1.3 are identical except for the conservative replacement of arginine for lysine at the

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pMGA                              NH2 -  CTTPTPSPTPNPNPPSN
pMGA1.2     VKKKNILKFVSLLGIGSFVMLAAAS CTTPTPNPTPNPNPPSG
pMGA1.3     VKRKNILKFVSLLGIGSFVMLAAAS CTTPV NPTPNPTPTNP
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FIG. 5. Signal peptide and amino-terminal sequences of pMGA1.3 and pMGA1.2 and the amino-terminal sequence of pMGA. Differences in the amino-terminal sequences are underlined.

second amino acid position of the signal sequence (Fig. 5). Differences in the amino-terminal sequences are underlined, with pMGA1.2 and pMGA1.3 differing by 3 and 6 amino acids, respectively, from pMGA. No sequence homology of any significance to any other protein sequence was found when computer searches of the standard protein data bases were used.

Preceding the translational start of pMGA1.2 is a putative promoter region consisting of a −35 region and a −10 region separated by 17 nucleotides (Fig. 4). A similar region preceding the predicted translational start of pMGA1.3 also occurs. Between the putative promoter region and the predicted translational start of pMGA1.2 and pMGA1.3, an imperfect palindrome exists (Fig. 4, arrows) with the potential to form a base-paired short loop.

Southern blot analysis of the *M. gallisepticum* genome by using pMGA1.2 and pMGA1.3 as probes. To enumerate those genes related in sequence to pMGA, we used subclones b and c (Fig. 3) to probe *M. gallisepticum* genomic DNA. Lanes 1 and lanes 2 of Fig. 6 contain genomic DNA of *M. gallisepticum* digested with *Eco*RI and *Hin*dIII, respectively. Figure 6A was probed with fragment b (containing 65% of the amino-terminal end of the pMGA1.2 gene). As expected, fragment b in lane 1 containing the *Eco*RI digest detected a 10-kb band (self) but also hybridized to bands of 18.5, and 6 kb. The *Hin*dIII digest in lane 2 probed with fragment b contained a 4.6-kb band (self) and, in addition, species of 18.5, 2.6, and 2 kb. Figure 6B was probed with fragment c containing 35% of the pMGA1.2 gene, a short segment of the pMGA1.3 gene, and the entire intervening region. Fragment c detected *Eco*RI bands of 10 (self), 18.5, 13, 6, and 2.7 kb. In lane 2 containing the *Hin*dIII digest, fragment c detected species of 4.6 (self), 18.5, 9.4, and 6.7 kb.

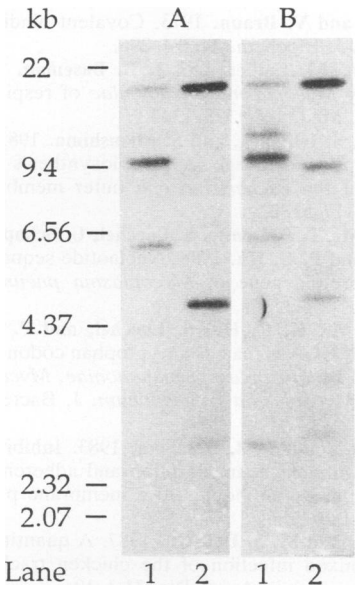

FIG. 6. Southern blot of genomic *M. gallisepticum* DNA digested with *Eco*RI and *Hin*dIII (lanes 1 and lanes 2, respectively) and probed with radiolabeled clones b (panel A) and c (panel B) as described in the text.

DISCUSSION

We reported previously that *M. gallisepticum* expressed a major plasma membrane protein, pMGA, that was shown to be involved in the agglutination of fowl erythrocytes. The primary aim of this study was to obtain the complete amino acid sequence of *M. gallisepticum* pMGA. The pMGA amino acid sequence could then be used to predict a molecular topology of the protein and to perform computer-assisted homology studies which might enable an ancestral sequence relationship between pMGA and other proteins of known structure and function to be established. These aspects are discussed below.

In this work, two oligonucleotides based on the amino acid sequences of selected pMGA peptides were constructed, radiolabeled, and used to detect the pMGA gene in a library of mycoplasma DNA in pUC18. A mycoplasma DNA fragment (approximately 10 kb) was cloned, partially sequenced, and shown to possess the coding potential for at least one complete pMGA-like protein (Fig. 4, pMGA1.2). Within this 10-kb fragment, a second pMGA-like sequence (pMGA1.3) begins shortly after the pMGA1.2 sequence terminates. Differences between the amino acid sequence of pMGA peptides and that predicted by the pMGA1.2 DNA sequence indicate that the pMGA1.2 gene does not code for pMGA but does so for a closely related variant. This conclusion was extended by the detection of multiple DNA species when cloned fragments b and c were used to probe restriction digests of *M. gallisepticum* DNA (Fig. 6). The hybridization of both fragments to common bands suggests that each band could contain at least one complete copy of a pMGA1.2-like DNA sequence. On the basis of as-yet-incomplete data, the pMGA1.3 gene seems to be less closely related to either pMGA or pMGA1.2 than the sequences of the last two are to one another. Differences were observed between the Southern blot binding patterns of cloned fragments and those of the oligonucleotide probes. Clone b contains the 23-mer oligonucleotide sequence used in cloning and would be expected to hybridize with an 8-kb *Eco*RI fragment in lane 1 of Fig. 6. The failure of clone b to hybridize with an 8-kb *Eco*RI fragment could be attributed to the use of high-stringency washing together with low overall homology between clone b and the *Eco*RI fragment. The failure of the 20-mer oligonucleotide probe to bind an 8-kb *Eco*RI fragment in Fig. 2b and the success of the 23-mer probe in doing so (Fig. 2a) may be due to the complete absence of this sequence in the 8-kb DNA fragment or the highly variable nature of the complementary region of DNA in the gene.

It was initially anticipated that knowledge of the complete amino acid sequence of pMGA might reveal at least the nature and location of the anchor structure which must attach the protein to the *M. gallisepticum* plasma membrane. The gene which encodes pMGA has not been sequenced in this study, but the closely related pMGA1.2 gene has been. This gene contains a typical hydrophobic leader or signal sequence immediately after the start codon. The putative leader sequence of pMGA1.2 (residues 5 to 26) is by far the most hydrophobic segment of the protein, and no internal

segment approaches it with respect to overall hydrophobicity. Specifically, no segment which might correspond to a hydrophobic stop transfer sequence occurs within the pMGA1.2 gene.

It is possible that pMGA1.2 is a lipoprotein. The protein sequence flanking the amino-terminal cysteine, A-A-(−1)S-(+1)C, may constitute a signal peptidase II cleavage site where postsynthetic events subsequent to the proteolytic cleavage process may lead to the addition of a diacyl glyceryl group to the cysteine sulfhydryl of the amino-terminal cysteine residue and of a fatty acyl group to its α-amino group (2). These fatty acyl groups could provide an amino-terminal anchor for the pMGA1.2 protein. However, affinity-purified pMGA is amenable to Edman degradation and therefore cannot possess an acyl group linked to its amino-terminal α-amino group (4). The possibility that pMGA is a lipoprotein has not been formally resolved, and the method used in membrane anchorage must await more experimental work.

It was appropriate to search for putative defects in the control regions of the pMGA1.2 and pMGA1.3 genes to seek an explanation for the fact that they are probably not expressed at the protein level in cultured *M. gallisepticum* S6 cells. Both genes contain sequence motifs similar to those expected for transcriptional promoter regions (Fig. 4). Specifically, 70 nucleotides upstream to the GUG start codon of pMGA1.2 is located a putative Pribnow box, a requirement for the initiation of transcription in bacterial cells. Between the Pribnow box and the translational start, as described in Fig. 4, a palindrome with the potential to form a base-paired short loop exists. The significance of this loop is unclear, but it is reminiscent in structure of rho-independent transcription stops which consist of eight-nucleotide base-paired stems followed downstream by a short run of pyrimidines. Whether this structure plays a role in the transcriptional regulation of pMGA1.2 is not yet clear.

No convincing Shine-Dalgarno sequence occurs in the segment of either pMGA1.2 or pMGA1.3 upstream of the translation initiators. The absence of a Shine-Dalgarno sequence from the pMGA1.2 gene may have important ramifications for the regulation of expression of pMGA-like genes. It should be noted, however, that the *tuf* gene of *Mycoplasma genitalium* has been reported to utilize an alternative sequence motif to initiate translation of mRNA molecules (10).

In summary, the work presented in this article makes it clear that a repertoire of pMGA-like genes exists in the *M. gallisepticum* genome, as established by the ability of two nonoverlapping fragments of the pMGA1.2 or pMGA1.3 locus to identify the same *Hin*dIII and *Eco*RI fragments by Southern transfer of *M. gallisepticum* DNA. One possible reason for the existence of multiple pMGA-like genes may be to provide opportunities for *M. gallisepticum* to alter its surface antigens in response to an immunological attack by its host in the course of an infection. How such alterations in pMGA expression might occur as a consequence of immune attack is as yet unclear. It is obviously important in this regard to molecularly clone the pMGA gene which is actually expressed upon the surface of a clone of cultured *M. gallisepticum* cells and to seek a reason for its selective expression. It is intriguing that the three strains of *M. gallisepticum* we have already examined have a related but different pMGA molecule. One possibility to account for this finding is that rapid mutational divergence has occurred between different *M. gallisepticum* field isolates. Alternatively, these three strains may differ primarily in the pMGA variant gene expressed from a repertoire which all three strains possess. Work is currently in progress to distinguish these two alternatives. The variability in the pMGA structure expressed in different strains of *M. gallisepticum* and the existence of multiple pMGA-related genes have ample precedent in other bacterial cells and specifically in other *Mycoplasma* species. The P1 gene of *M. pneumoniae* or at least segments of it are reported to occur repeatedly within the genome (17). Phase variation within *M. hyorhinis* is due to the expression of multiple, related size variants of lipoproteins (Vlps) (16). The high frequency at which Vlps alter within *M. hyorhinis* populations may contribute an important technique of adaptive immune evasion for this organism. Another species, *Mycoplasma hominis*, expresses extensive strain-to-strain antigenic heterogeneity with respect to a 94-kDa polypeptide as defined by strain-specific reactivity patterns with monoclonal antibodies (14). Similar heterogeneity of a 150-kDa surface protein has been reported between strains of *Mycoplasma pulmonis* by using serological and biochemical techniques (22). The molecular mechanisms which underlie the ability of mycoplasma species to vary their surface antigens are not yet clear nor in any case has variability been definitively linked to immune evasion.

Further studies on the genes responsible for encoding the pMGA proteins of *M. gallisepticum* should, at the very least, contribute to an understanding of whether pMGA protein variation is due to gene switching events, and if it is, they should provide an understanding of how these switching events take place.

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