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Mry, a *trans*-Acting Positive Regulator of the M Protein Gene of *Streptococcus pyogenes* with Similarity to the Receptor Proteins of Two-Component Regulatory Systems

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In the *Streptococcus pyogenes* M6 strain D471, an insertion of the conjugative transposon Tn*916* into a region 2 kb upstream of the promoter of *emm6* (the structural gene for the M protein) rendered the strain M negative (M. G. Caparon and J. R. Scott, Proc. Natl. Acad. Sci. USA 84:8677–8681, 1987). In the present work, we show that this insertion mutation, *mry-1*, is 244 bp upstream of an open reading frame encoding a protein we call Mry. This protein is visible on a gel after transcription and translation in vitro. We have developed a technique for complementation analysis in *S. pyogenes* and have used it to show that the wild-type *mry* gene is dominant to two mutant alleles. This dominance indicates that Mry acts in *trans* as a positive regulator of the *emm6* gene. The translated DNA sequence of *mry* has two regions of similarity to the motif common to the receptor protein of two-component regulatory systems. In addition, the N terminus of Mry has two regions resembling a helix-turn-helix motif. Mry does not appear to be a global regulator of virulence determinants in the group A streptococcus because there is no effect of the *mry-1* mutation on production of the hyaluronic acid capsule or streptokinase.

The group A streptococci (*Streptococcus pyogenes*) are important human pathogens that cause impetigo, which is sometimes followed by acute glomerulonephritis, and pharyngitis, which in some individuals leads to rheumatic fever. Rheumatic fever remains an important disease in less-developed parts of the world and appears to be on the rise in the United States. In addition, *S. pyogenes* also causes the recently recognized disease referred to as toxic streptococcal syndrome which is often lethal (3). Thus, a better understanding of the pathogenesis of this organism is being sought.

The major virulence factor of the group A streptococcus is considered to be the fibrillar surface protein called the M protein, which protects the bacterium from phagocytosis in the human host. This dimeric coiled-coil molecule is highly variable both in length and in sequence; over 80 different antigenic types of M protein are currently defined. The primary immune response to infection by *S. pyogenes* involves generation of antibodies that recognize epitopes present on M protein. Antibodies that can neutralize the antiphagocytic properties of this molecule (opsonic antibodies) are protective against further infection by these streptococci. However, this protection appears to be type specific so that an individual immune to streptococci of one serological M type is not protected from infection by organisms expressing a different M type.

Other factors that have been proposed as important for virulence of group A streptococci include the hyaluronic acid capsule, which may help protect the organisms from phagocytosis (42, 43), the plasminogen-activating streptokinases, which may aid in dissemination of the streptococci in tissue (21), the immunoglobulin G Fc receptor protein (37), which may sequester opsonic immunoglobulin G antibodies, the C5a peptidase that can prevent polymorphonuclear leukocytes from recognizing chemotactic signals (41), the protease that may be involved in invasive infections (4, 5), and the pyrogenic exotoxins important in scarlet fever and implicated in toxic streptococcal syndrome (3, 35, 40).

The antigenic variability of the M protein and its antigenic similarity to heart tissue are major problems for the development of an antistreptococcal vaccine. Because of this, our research has focused not just on the structure-function relationships of the M protein but also on trying to gain an understanding of the regulation of its production. To this end, we have isolated a mutant of the M6 *S. pyogenes* strain D471 generated by insertion of the conjugative transposon Tn*916* about 2 kb upstream of the promoter of *emm6*, the structural gene for the M6 protein. This insertion reduces the amount of M protein expressed by about 50-fold by reducing transcription of *emm6*.1, so we named this insertion mutation *mry-1* (M protein RNA yield) (6). If this insertion is in a gene encoding a diffusible positive regulator of *emm6*, the wild type should be dominant to the *mry-1* mutation. To test this, a complementation test must be performed. A major problem in studying gene regulation in the group A streptococcus has been the paucity of techniques for genetic analysis. Therefore, for this work, we have had to develop an approach to allow the generation of a partial diploid required for complementation studies.

In an M12 group A streptococcal strain, the occurrence of several spontaneous deletions upstream of the structural gene for the M protein was reported by Cleary's group (34). These deletions appear to render the strains M12 negative. Because they also reduce production of C5a peptidase, these deletions have been named *virR* (33, 34). We wished to clarify the relationship between the *virR* mutants and *mry-1* and to gain a better understanding of the mode of action of the *mry-1* insertion mutation.

In this work, we demonstrate that *mry-1* is in a site that regulates production of a protein we call Mry, which positively regulates transcription of the structural gene for M6.

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The previously characterized deletions (33) are within the open reading frame (ORF) for this protein. Because Mry has no detectable effect on capsule production or on streptokinase production, it does not appear to be a global regulator of virulence determinants. Thus, we retain its original name. In addition, we find that its sequence suggests that it may be the receptor component of a two-component signal transduction system (1, 14, 24, 36).

MATERIALS AND METHODS

Bacterial strains. *Escherichia coli* DH5α (*recA1 gyrA96*) was used for molecular cloning experiments. *S. pyogenes* JRS4 (*emm*6.1) is a spontaneous streptomycin-resistant derivative of strain D471, and strain JRS14 was constructed by transposition of Tn*916* into strain D471 (6).

Media. *E. coli* DH5α was grown in Luria broth (32) or tryptone (32), and *S. pyogenes* strains were grown in Todd-Hewitt broth supplemented with 0.2% yeast extract (THY) (11). Antibiotics were used at the following concentrations: ampicillin (Ap) at 50 μg/ml, chloramphenicol (Cm) at 10 μg/ml for *E. coli* and 5 μg/ml for *S. pyogenes*, kanamycin (Km) at 25 μg/ml for *E. coli* and 200 μg/ml for *S. pyogenes*, and streptomycin at 1,000 μg/ml for *S. pyogenes*.

Recombinant DNA techniques. Plasmid DNA was prepared from *E. coli* by alkaline lysis followed by two rounds of equilibrium centrifugation through ethidium bromide-cesium chloride gradients. Chromosomal DNA from *S. pyogenes* was prepared as described by Chassy (8), following growth in THY medium supplemented with 20 mM glycine. Restriction endonucleases and ligases were used according to the recommendations of the manufacturers.

Plasmid constructions. The vector used for plasmid constructions was pLZ12 (7a) in which a multiple cloning site had been inserted into the Km gene of the promiscuous plasmid pNZ12 (10). Plasmid pJRS1006.5 is a cosmid clone containing the *mry* locus and the *emm*6 gene from *S. pyogenes* D471 (7). Plasmid pJRS1036.2 contains the ca. 3-kb *Bam*HI-*Xba*I fragment of pJRS1006.5 cloned into pLZ12. Plasmid pJRS180 was created by inserting the blunted *Bam*HI-*Hin*dIII fragment of pJRS1006.5 at the blunted *Bam*HI site of pLZ12.

The omega-Km2 element was constructed as follows. A 1.6-kb *Cla*I fragment of plasmid pAT21-1 containing the *aphA-3* kanamycin resistance gene from Tn*1545* (39) was blunted and inserted into the blunted *Sal*I fragment of pUC4K (23) to generate pUC4-21K. The 1.6-kb *Hin*cII fragment of this plasmid carrying *aphA-3* was used to replace the streptomycin/spectinomycin resistance gene of the omega element. To do this, the omega element (12) was digested with *Bss*hII and *Sph*I, the ends were blunted, and the Km fragment was inserted to form the omega-Km2 element. Plasmid pJRS182 was generated by inserting the 1.9-kb *Hin*cII fragment containing the omega-Km2 element into the blunted *Sst*I site of pJRS180. The omega-Km2 element is flanked by DNA sequences encoding signals for termination of transcription and translation. A *Bam*HI fragment of pUC4-21K containing the *aphA-3* gene was cloned into the *Bam*HI site of pLZ12 to construct plasmid pLZ12-21K.

Electroporation of JRS14. Cells were grown overnight at 37°C in THY medium containing 20 mM glycine. On the following day, the overnight culture was diluted 20-fold in the same medium and incubated at 37°C until it reached an optical density (at 600 nm) of 0.2. Cells were centrifuged at 8,800 × *g* for 10 min at 10°C. The pellet was washed twice with electroporation solution (cold 15% glycerol). Cells were resuspended in 0.5 ml of electroporation solution and incubated on ice for 10 min. Electroporation was carried out by using a Bio-Rad gene pulser and pulse controller system set at 1.75 kV, 200 Ω, and 25 μF. A 1-μg amount of DNA was added to 0.2 ml of cells, and the cells were placed in an electroporation cuvette (0.2 cm). After being electroporated, the cells were transferred to plastic tubes containing 10 ml of THY and the cultures were incubated at 37°C for 90 min, pelleted at 8,800 × *g*, washed once in 10 ml of THY, resuspended in 1 ml of THY, and plated on THY agar containing appropriate antibiotics.

Western blot (immunoblot) analysis. M protein was extracted from streptococcal cells with bacteriophage lysin, separated by sodium dodecyl sulfate (SDS)-12.5% polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and assayed with monoclonal antibody 10B6 as described previously (6).

Nucleotide sequence. Sequencing of both strands of the 2-kb fragment containing the *mry* gene was carried out with plasmid DNA by use of the Sanger protocol (31) as modified for supercoiled templates by Zagursky et al. (44) and using T7 DNA polymerase (Sequenase; U.S. Biochemicals). Homology searches were performed on the GenBank DNA database by using the FASTP algorithm (27). Individual sequences were compared by using the University of Wisconsin GCG package (9).

Nucleotide sequence accession number. The sequence data has been assigned GenBank accession number M58461.

RESULTS

The *mry* gene acts in *trans*. The *mry-1* mutation in *S. pyogenes* JRS14 is an insertion of Tn*916* about 2 kb upstream of the *emm*6 gene. This results in a dramatic decrease in transcription of *emm*6 (6). To determine whether the insertion is in a gene encoding a diffusible regulator of *emm*6, complementation was assayed.

For this purpose, we devised a system for the introduction of genes into *S. pyogenes* that would result in the production of a stable partial diploid. For a vector, we used the plasmid pLZ12, which is based on the naturally promiscuous pSH71 replicon (10) originally isolated in *Lactococcus lactis* (formerly *Streptococcus lactis*). Plasmid pLZ12 is capable of replication in *E. coli* as well as in many streptococcal species. Two different chromosomal fragments representing the wild-type *S. pyogenes* strain D471 were cloned into this plasmid for complementation analysis (Fig. 1). Plasmid pJRS1036.2 contains a 3-kb *Bam*HI-*Xba*I fragment from the region located upstream of the *emm*6 promoter, and pJRS180 has a larger insert, the 4.6-kb *Bam*HI-*Hin*dIII fragment of D471 that includes about two-thirds of the *emm*6 coding sequence (Fig. 1).

We developed an electroporation technique to allow the introduction of these plasmids into JRS14, the *S. pyogenes* strain with the *mry-1* mutation. The transformants were kept under constant selection for the plasmid marker, Cm. To assay complementation, Western blots of lysin extracts of the streptococcal strains were reacted with monoclonal antibody 10B6, which identifies M6 protein (20) (Fig. 2). Lane 1 shows the M protein of the wild-type control strain JRS4 (indicated by an arrow), and lane 2 contains an extract made from JRS75, a derivative of JRS4 from which the *emm*6 structural gene was deleted by in vitro manipulation followed by allele replacement (26) (Fig. 2). The extract from the *mry-1* mutant, JRS14, shows no detectable M protein

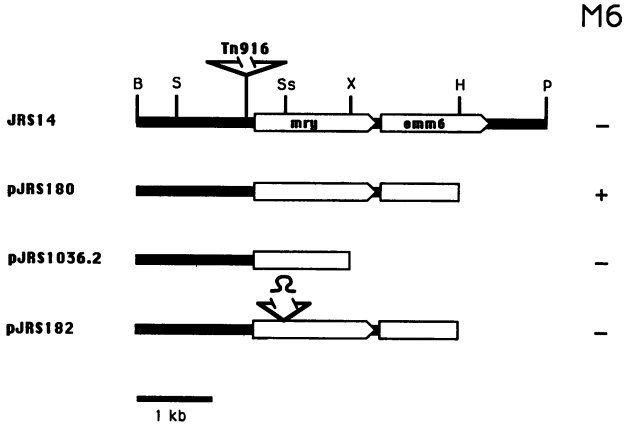

FIG. 1. Map of the region affecting M protein production and complementation of the *mry-1* mutation in JRS14. The top thick line represents the chromosome of the *S. pyogenes* strain JRS14, and the three lines beneath it indicate the streptococcal DNA inserted into pLZ12. Abbreviations for restriction endonuclease sites: B, *Bam*HI; S, *Sal*I; Ss, *Sst*I; X, *Xba*I; H, *Hin*dIII; P, *Pst*I. The open triangles above JRS14 and pJRS182 indicate the sites of insertion of Tn*916* and the omega-Km2 fragment, respectively. The inserted fragments are not drawn to the same scale as the rest of the figure. Boxes represent the location of indicated genes, and arrowheads show the direction of their transcription. The column labeled M6 indicates M6 expression as detected in Western blots.

(Fig. 2, lane 3), and the M protein is not restored when the vector alone is present (Fig. 2, lane 4). However, the presence of pJRS180 in JRS14 causes production of M protein (Fig. 2, lane 5), indicating that the fragment in this plasmid complements the *mry* mutation. Thus, a *trans*-acting product is produced from this region and acts to turn on expression of *emm*6. The amount of M6 in JRS14 harboring pJRS180 appears to be greater than that in the wild-type strain JRS4. That the gene dosage of *mry* is higher from the multicopy plasmid than from the chromosome suggests that the amount of Mry is limiting for the amount of M6 produced.

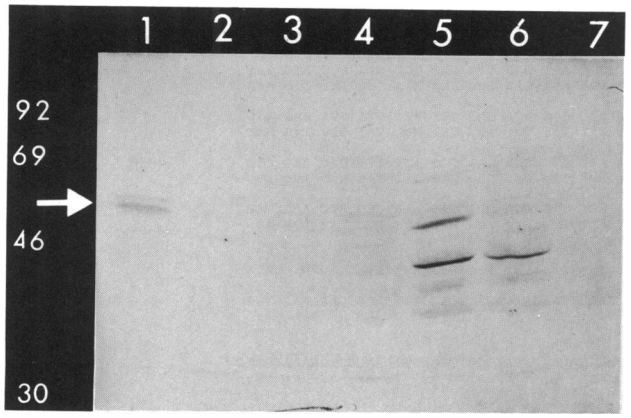

FIG. 2. Western blot of lysin extracts of *S. pyogenes* reacted with monoclonal antibody 10B6. Extracts are as follows: lane 1, JRS4; lane 2, JRS75; lane 3, JRS14; lane 4, JRS14(pLZ12-21K); lane 5, JRS14(pJRS180); lane 6, JRS14(pJRS182); lane 7, JRS14 (pJRS1036.2). The molecular sizes (in kilodaltons) of standard proteins (Rainbow protein molecular size markers; Amersham) are indicated on the left side of the panel. The arrow shows the location of the M6 protein.

In addition to the *mry* region upstream of *emm*6, plasmid pJRS180 contains the N-terminal part of the structural gene for M6 up to the *Hin*dIII site (Fig. 1). In lane 5, in addition to the full-length M6 protein produced from the chromosomal gene, the N-terminal fragment produced from the plasmid is also visible. This fragment has a molecular size of about 46 kDa, as predicted from the DNA sequence, and appears to be more plentiful than the full-length protein encoded on the chromosome. As expected from an M protein lacking the anchor and attachment regions of the C-terminal segment of the molecule, the N-terminal M protein fragment was found in a higher concentration in the supernatant than in the cell extract (data not shown).

The smaller upstream DNA fragment present in pJRS1036.2 does not complement the *mry-1* mutation in JRS14 to produce M protein (Fig. 2, lane 7). This indicates that the *mry* region required in *trans* for M protein production includes DNA located between the *Xba*I site and *emm*6.1 (Fig. 1).

The sequence of the *mry* region. The sequence of a 2,079-bp DNA fragment of pJRS180 upstream of the *Xba*I site was determined. Analysis of this sequence indicates that there is an ORF of 1,590 bp (starting at base 1 in Fig. 3) located 244 bp downstream of the Tn*916* insertion site in JRS14. The predicted molecular size of the 530-amino-acid product is 62 kDa, and its estimated pI is 6.47. An analysis of the features of the predicted protein sequence is reserved for the Discussion.

The identified ORF is responsible for complementation of *mry-1*. The *mry-1* Tn*916* insertion did not interrupt this ORF. Therefore, it was necessary to determine whether this ORF is, in fact, responsible for complementation of the *mry-1* allele for production of M protein. To determine this, we inserted a DNA fragment that has transcriptional and translational stops in all reading frames (omega-Km2) into a site (*Sst*I) near the N terminus of this ORF in plasmid pJRS180 to create plasmid pJRS182 (Fig. 1). This insertion mutation is designated *mry-2*. Unlike pJRS180, this plasmid does not restore production of the chromosomally encoded full-length M protein to strain JRS14 (Fig. 2, lane 6). However, the N-terminal fragment of the M protein produced from the high-copy-number plasmid is detectable, possibly because in this construct it may be expressed independently of *mry* (Fig. 2, lane 5). This demonstrates that the ORF shown in Fig. 3 is actually responsible for complementation of the *mry-1* mutation and therefore represents the *mry* structural gene.

Mry protein product. The DNA sequence of *mry* predicts that it encodes a protein of 62 kDa. There is one protein species of about this size (69 kDa) produced by in vitro transcription-translation of plasmid pJRS180 (Fig. 4, lane 3) but not of the insertion mutation *mry-1* (Fig. 4, lane 4) or of the deletion mutant *mry-2* (Fig. 4, lane 2). Possible reasons for weak expression of the Mry protein are discussed below.

The 52-kDa polypeptide made from pJRS1036.2 is probably the truncated Mry product expected from the deletion of the carboxy-terminal part of the *mry* gene (Fig. 4, lane 2). In pJRS182, the insertion of the omega-Km2 fragment at the *Sst*I site (Fig. 1) should have interrupted the ORF at residue 113 to give a truncated protein with a molecular size of about 13 kDa. The high background in this part of the gel precludes visualization of this band on lane 4 of Fig. 4.

The ca. 55-kDa proteins in lanes 3 and 4 may correspond to the product of the *emm*6 segment fused to the Km gene of

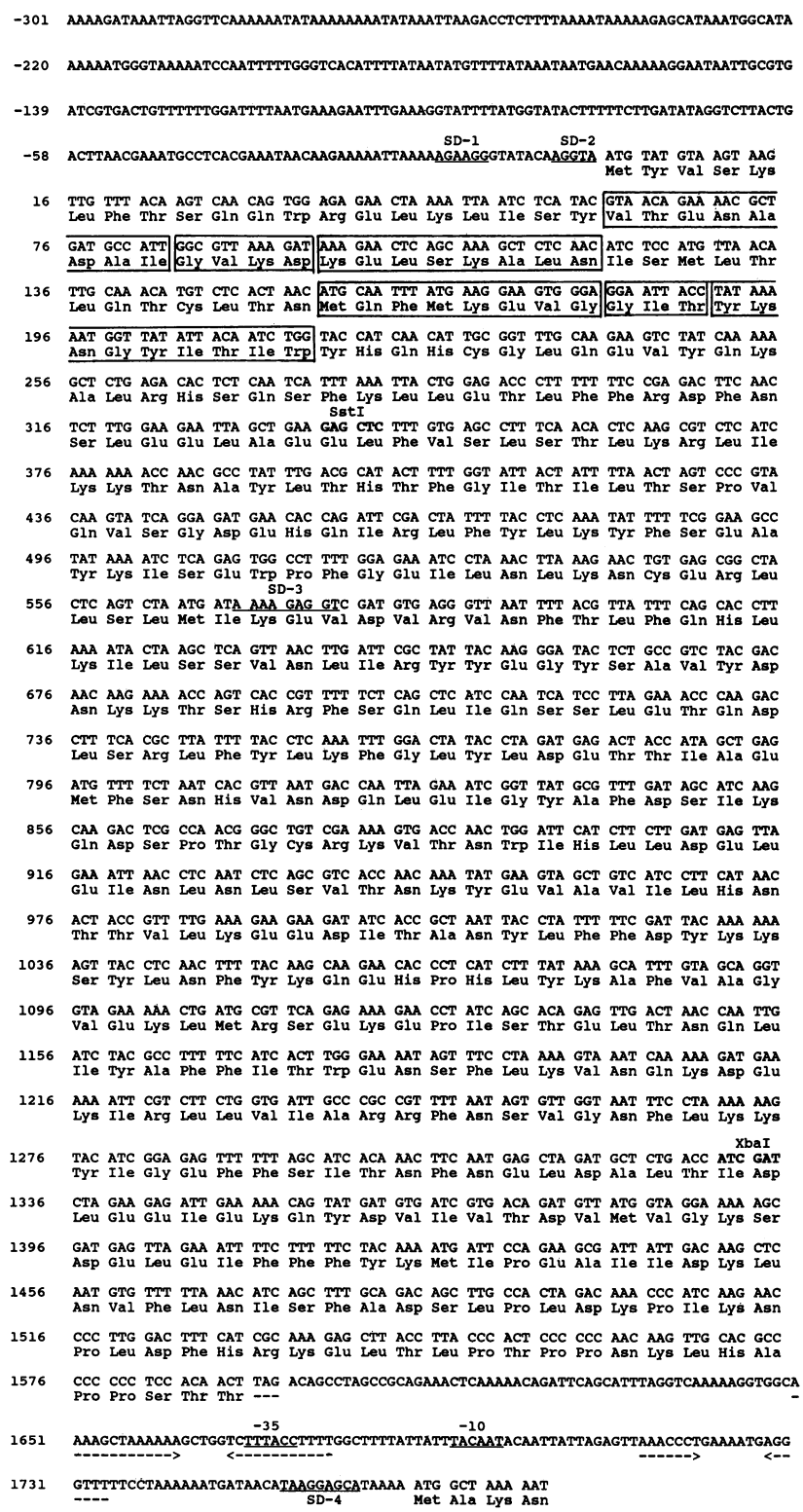

FIG. 3. Sequence of the 2,079-bp DNA fragment encoding Mry and adjacent regions. The first base in the ATG codon that serves as the start of translation of Mry is designated 1. The three predicted ribosome binding sequences, SD-1, SD-2, and SD-3, centered at positions −14, −1, and 574, respectively, are indicated above the sequence. The locations of the sites for the restriction endonucleases *Sst*I and *Xba*I are indicated above the respective recognition sequences. The arrows at positions 1650 to 1661, 1668 to 1679, 1713 to 1720, and 1728 to 1734 indicate inverted repeats that may function as signals for termination of transcription of the Mry message. The −35 and −10 regions of the primary promoter used for *emm*6 transcription (17) are also shown. The ribosome binding site (SD-4) centered at position 1758 and required for translation of the M6 protein is also depicted. Translation of the M6 protein starts at nucleotide 1768.

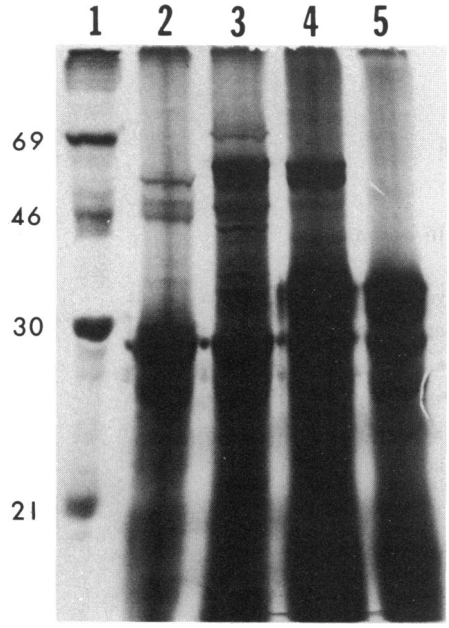

FIG. 4. In vitro transcription-translation of *mry* DNA in an *E. coli* extract. The [^{35}S]methionine-labeled products of in vitro transcription and translation were electrophoresed through an SDS-12.5% polyacrylamide gel. Lane 1 contains ^{14}C-labeled standard proteins (Amersham). Templates for the reactions were as follows: lane 2, pJRS1036.2; lane 3, pJRS180; lane 4, pJRS182; lane 5, pLZ12-21K. The molecular sizes (in kilodaltons) of the protein standards are indicated on the right side of the figure.

pLZ12 in pJRS1036.2 and pJRS180. From the DNA sequence, this fusion protein should have a molecular size of 45 kDa.

There are several protein bands with molecular sizes near 46 kDa in lanes 2 through 4 (Fig. 3). In lanes 2 and 3 there are two bands that could be generated by premature cessation of translation of *mry* or by degradation of the Mry protein. The larger of the two additional bands present in lane 3 could be the translated product of the *mry* gene, if the putative third internal ribosomal binding site was used (Fig. 3; and see Discussion), while the smaller could be a degradation product of it. The two bands present in lane 4 could be degradation products of the M6 protein fused to the Km gene.

Plasmids pJRS182 and pLZ12-21K also produced a ca. 35-kDa protein (more clearly visible in shorter exposures of the autoradiogram), which is the size expected from the *aphA-3* gene product present in these constructs. The ca. 28-kDa protein band in lanes 1 to 4 is probably the *cat* gene product expected from the chloramphenicol resistance vector.

DISCUSSION

Because the M protein is a major virulence factor of *S. pyogenes*, a serious human pathogen, it is important to understand the mechanism by which its production is regulated. A locus, called *mry*, which was defined by a Tn*916* insertion in the genome of the M6 *S. pyogenes* strain D471, reduces production of M protein RNA by about 50-fold (6). The insertion was found to be located about 2 kb upstream of the promoter for *emm6*. To determine whether the Tn*916* insertion affects a gene encoding a diffusible product, we developed a complementation system for use in *S. pyogenes*. We optimized an electroporation system to deliver the promiscuous plasmid pLZ12, which we found to be maintained in this organism with sufficient stability to provide a partial diploid whose phenotype can be tested.

We identified a fragment of the chromosome of the wild-type *S. pyogenes* strain D471 that complements the *mry-1* Tn*916* insertion mutation in JRS14 to produce M6 protein. The sequence of the complementing fragment, which lies upstream of the *emm6* gene, contains an ORF encoding a protein, Mry, with a predicted molecular size of 62 kDa. Since the *mry-1* Tn*916* insertion mutation lies 244 bases upstream of this ORF, we needed to determine whether the ORF was actually responsible for complementing *mry-1*. When pJRS1036.2, which does not contain the complete Mry ORF, was introduced into JRS14 containing the *mry-1* mutation, no complementation was obtained. As a second test, the omega fragment, which possesses transcriptional and translational stop codons in all reading frames, was inserted into the putative *mry* gene to construct *mry-2*. As expected, the *mry-2* DNA fragment in pJRS182 also does not complement the *mry-1* mutation. This demonstrates that the Mry ORF is required for complementation of the *mry-1* mutation and indicates that the insertion mutation reduces expression of *emm6* by preventing production of the Mry protein.

The *mry-1* insertion mutation, originally used to identify *mry*, does not lie within an ORF (data not shown). Instead, the insertion appears to disrupt a *cis*-acting upstream region required for expression of this protein. This required region may be the promoter for Mry, but, since this area is very AT rich, it is difficult to predict the location of the promoter on the basis of DNA sequence alone.

The gene encoding the 69-kDa Mry polypeptide is preceded by two putative ribosome binding sites (Fig. 3, SD-1 and SD-2). Neither one is likely to be used very efficiently. SD-1 has a calculated free energy of interaction of −7.4 kcal/mol (1 cal = 4.184 J) (38), which is less favorable than the average for *E. coli* (−11.6 kcal/mol) (22). Also, the last base of SD-1 is 12 bases from the proposed ATG start codon, well above the average of 7 bases found in *E. coli* (13). SD-2 has a calculated free energy of interaction of −12.4 kcal/mol, which is more favorable than the average for *E. coli* but less than the average for gram-positive genes (−17 kcal/mol) (22). However, there are no bases between this sequence and the putative ATG start codon. This suggests that SD-1 is more likely than SD-2 to be used for translation of the *mry* message in *E. coli* but that it is used very inefficiently. We believe that this inefficiency accounts for the very small amount of Mry product seen after in vitro transcription and translation of the *mry* gene in an *E. coli* extract (Fig. 4).

There is a third potential ribosome binding site (Fig. 3, SD-3) centered at nucleotide 574 with a calculated free energy of interaction of −10.1 kcal/mol. SD-3 is 4 bases upstream of a GTG codon (bases 583 to 585) which could serve as the start of translation for a protein with a predicted molecular size of 50 kDa. A protein of this size is seen after in vitro transcription and translation of pJRS180 (Fig. 4, lane 3). If SD-3 is utilized, the carboxy-terminal deletion of Mry in pJRS1036.2 should result in a product whose molecular size is predicted to be 30 kDa, which is also observed in the in vitro experiment (Fig. 4, lane 2). Thus, we conclude that this translational start site also appears to be used at least in *E. coli* extracts.

In Northern blots (RNA blots), the *emm6* transcript is the size expected for a monocistronic RNA (6) and primer extension experiments located the 5′ end of the predominant *emm6* transcript 63 bases from the ATG start codon of the M

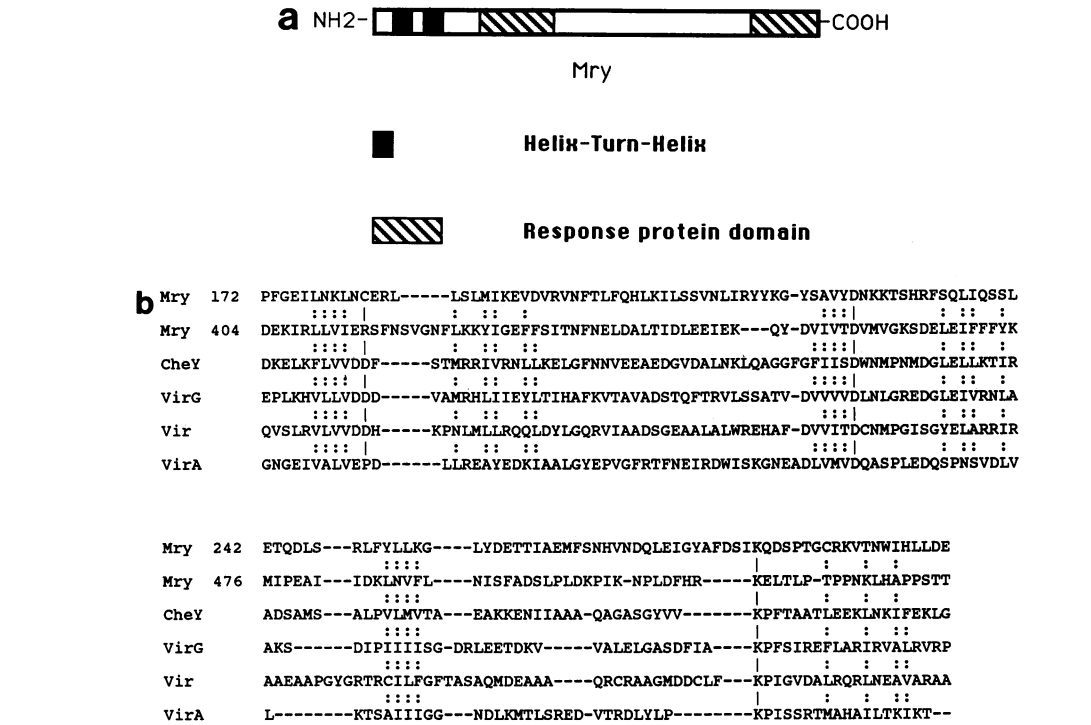

FIG. 5. Two-component regulator motifs in Mry protein. (A) The two black boxes represent the two regions homologous to the helix-turn-helix domains, while the two cross-hatched boxes indicate the regions homologous to the family of regulatory proteins of the two-component regulatory systems. The locations of the amino and carboxy termini of Mry are indicated by NH_2 and COOH, respectively. (B) The two Mry regions possessing homology to the conserved amino terminal regions of the regulatory proteins that are members of the signal transducing system are shown at the top. The bottom sequences were taken from Stock et al. (36). The numbers on the left of the Mry peptides indicate their location on the Mry molecule. Colons (:) indicate conserved hydrophobic residues, while vertical lines (|) link the highly conserved amino acids. Gaps introduced to improve alignment are indicated by a dashed line (– – –) in the sequence. (C) The two Mry regions (residues 21 to 40 and 53 to 72) homologous to the helix-turn-helix motifs of several regulatory proteins are indicated on the upper portion of the figure. The sequences on the lower portion were taken from Harrison and Aggarwal (16). The boxes enclose the amino acids conserved among these proteins.

protein (17). It is unlikely that this transcript results from processing of a longer transcript that begins at the promoter for *mry*, since complementation of the *mry-2* mutation in trans indicates that, for expression of *emm*6, transcription need not be continuous from the *mry* promoter. Thus, we expect to find a transcriptional terminator between the coding sequence for Mry and that for M6.

Downstream of the termination codon of *mry* (bases 1714 to 1735) is a 7-base inverted repeat sequence (Fig. 3) which may terminate transcription. The calculated free energy of formation of a stem-loop structure involving this sequence (38) is −10.8 kcal/mol, which is less favorable than for the terminator present at the end of the *emm*6 sequence (−22 kcal/mol) (18). There is another potential terminator located at bp 1651 to 1680 which has a calculated free energy of structure formation of −6.3 kcal/mol. Neither of these

terminators resembles an efficient *E. coli* terminator structure, and it seems possible that both are used inefficiently.

Because the *emm6* promoter lies between the end of the coding sequence of *mry* and the first proposed end of the *mry* transcript (Fig. 3), antitermination must occur at this site to produce the M protein if this terminator is active. It is possible that the Mry protein modulates the efficiency of transcription termination at this site to allow transcription of the *emm6* gene.

Our results indicate that the Mry protein is required for expression of *emm6* in strain D471. If a gene homologous to *mry* exists in all group A streptococcal strains, the Mry product might serve as a potential target for prophylaxis of streptococcal diseases. A *Hin*dIII-*Xba*I DNA fragment containing *mry* and about 2-kb upstream sequences from strain D471 hybridized to DNA from six of six group A streptococcal strains of different M types. In four of these strains, the size of the homologous chromosomal *Hin*dIII fragment was the same as that in D471 and the other two were within about 500 bp of this size. This is consistent with the possibility that all group A streptococcal strains have an *mry* gene.

A search of the GenBank database revealed that sequences upstream of two other M protein genes, *emm12* (30) and *emm1* (15), were highly homologous to Mry. Only partial sequences corresponding to the *mry* region of the M1 and M12 strains are available, but assuming that a few errors exist in these partial sequences, the predicted polypeptides are nearly identical (93% for the M12 strain and 95% for the M1 strain) to Mry from D471. Thus, all existing evidence suggests that an *mry* gene very similar in sequence to the one described above occurs in *S. pyogenes* strains of different M types. To determine whether Mry proteins are functionally equivalent and widely distributed among *S. pyogenes* strains, we are crossing the *mry-2* omega-Km2 insertion allele into group A strains representing different M types.

In many pathogenic organisms, a single *trans*-acting factor often regulates several virulence determinants. Because M12 strains with reduced production both of M12 and of the C5a peptidase contained deletions upstream of *emm12*, Simpson et al. (33) have named this region *vir*. Our sequence analysis indicates that this *vir* region lies within the *mry* gene, so it seemed possible that the Mry protein is needed for the production of other potential virulence factors. However, when *S. pyogenes* JRS14 (containing the *mry-1* insertion mutation) was compared with JRS4, its parent, no differences in the amount of hyaluronic acid capsular material (26a) or streptokinase (12a) were detected. Thus, while Mry may regulate several virulence determinants, it does not appear to be a global regulator of all other possible virulence genes of group A streptococci.

In many bacterial pathogens, the synthesis of virulence factors is influenced by the environment. This adaptation to environmental changes is often accomplished by transduction of environmental signals into changes in protein conformation by a two-component signal transducing system (1, 14, 24, 36). In such a system, the first component is a membrane-spanning sensor protein which, in response to an environmental change, transmits a signal (usually by phosphorylation) to the second component or receptor protein. The latter regulates transcription of genes under environmental control. Examples of these systems used by bacterial pathogens include the VirA (sensor) (19) and VirG (receptor) (29) proteins of *Agrobacterium tumefaciens*, ToxR of *Vibrio cholerae*, which has both a sensor and a receptor motif separated by a region that spans the cytoplasmic membrane (25), the *vir* region of *Bordetella pertussis*, which encodes three products with homology to the signal transducing proteins (2), and the AgrA protein of *Staphylococcus aureus*, which shows homology with the receptor components (28, 36).

The sensor components recognize a conserved motif at or near the amino terminus of the receptor protein (36). The translated sequence of *mry* shows two regions that resemble this conserved receptor protein domain. However, neither of these motifs is located at the N terminus of Mry (Fig. 5a): one is located between residues 172 and 300, and the second is located between residues 404 to 528 (Fig. 5b). Near their C terminus, response proteins often include a helix-turn-helix DNA binding domain used in regulating transcription of the operons they control. There are two potential DNA binding domains in Mry (Fig. 5c). However, again in contrast to other receptor proteins, both motifs are localized in the amino-terminal region (Fig. 5a). No other receptor-type proteins with two of each type of domain have been described at this time. It is possible that each receptor domain of Mry responds to a different group of environmental signals and that each DNA-binding domain regulates a different subset of genes.

The structure of Mry strongly suggests that it is capable of responding to environmental change. Consistent with this, we have recently found (5a) that transcription of the *emm6* gene is temperature dependent. We are currently testing the role of Mry in temperature regulation and are searching for a possible sensor protein that would serve to transduce the environmental signal and alter the Mry protein which in turn could pass on the response to the *emm* gene.

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