Regulation of mga Transcription in the Group A Streptococcus:
Specific Binding of Mga within Its Own Promoter and Evidence
for a Negative Regulator

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Transcription of mga, encoding the multiple virulence gene regulator of the group A streptococcus, is
positively autoregulated. This regulation requires a DNA region (Pmga) that contains both a promoter
proximal to mga (P2) and a promoter located further upstream (P1). To determine if Mga has a direct role in
this process, its ability to bind to specific sequences within Pmga was tested. A purified fusion of Mga to the
C-terminal end of maltose-binding protein (MBP-Mga), encoded by malE-mga, was shown previously to bind
to the promoter regions of mga-regulated genes, including scpA and emm. We report here that MBP-Mga can
function in vivo to regulate emm and mga. Electrophoretic mobility shift assays and DNase I footprinting
were used to demonstrate specific binding of MBP-Mga to two ca. 59-bp binding sites in Pmga centered around
bases −108 and −180 from the major P2 start of transcription. Mga binding sites from Pemm and PscpA were
shown to compete for binding at the two Pmga sites, suggesting that the same domain of Mga interacts at all
of these promoter targets. Deletion of the distal Pmga binding site (site I) in vivo resulted in loss of
Mga-dependent transcription from the P2 start. However, the same lesion resulted in an increase in P1
transcription that was independent of Mga. This suggests the existence of a repressor of mga transcription with
a binding site overlapping those of Mga.

The group A streptococcus (GAS), Streptococcus pyogenes, is
a human pathogen that elicits a wide array of supplicative
diseases of the skin and throat, including pharyngitis and impetigo. In some cases, GAS infections can lead to serious
nonsuppurative sequelae such as rheumatic fever and glomeru-
lonephritis (4). Serious invasive streptococcal diseases, such as
necrotizing fasciitis, myositis, and streptococcal toxic shock
syndrome, are characterized by infections within normally ster-
ile sites of the human host and can often lead to death in
typically healthy individuals (2). The ability of GAS to adapt
and grow within the different niches of the human host that it
encounters during the course of these infections suggests an
environmentally responsive control of its virulence determi-
nants.

Mga is a multiple-gene regulator of GAS that responds to
environmental signals by activating transcription of several vir-
ulence genes. These include the genes encoding the antiphago-
cytic M protein (emm), Csa peptidase (scpA), and in strains
where these are present, M-like proteins (e.g., fcrA, enn, and
sph) and serum opacity factor (sof) (5, 8, 10, 17, 24, 28). Mga
functions as a DNA-binding protein that interacts directly with
sequences in the promoters of two Mga-regulated genes, emm
and scpA (22). Binding of Mga occurs at a 45-bp site within
each of the two promoters centered 52 bp upstream of the start
of transcription, which may allow Mga to interact with RNA
polymerase and stimulate transcription initiation (22).

Mga is also required for its own positive autoregulation, and
this requires 473 bp of DNA located directly upstream of its
coding sequence (25). This extended Pmga region contains two
distinct promoters: a distal start of transcription, P1, and a start
site located proximal to mga, P2 (12, 25). Studies on an M type
6 GAS strain have determined that the P2 promoter represents
the major start site for mga transcription. It exhibits about
10-fold more activity than the P1 promoter (25). The use of
dual promoters (P1 and P2) within Pmga has been demon-
strated for other GAS serotypes as well (e.g., M types 12 and
49) and most likely represents a general mechanism of mga
expression (3, 27). Currently, very little is known about the
specifics of mga regulation and the role of Mga in this process.

Many bacterial transcription factors regulate their own ex-
pression by binding directly to their own promoters (14, 19).
To aid in the identification of other Mga-regulated promoters
in GAS, a consensus Mga binding sequence was derived from
Pemm and PscpA binding studies (22). Inspection of the Pmga
region revealed no significant sequence homology with the
consensus Mga binding element found upstream of Pemm and
PscpA. This suggests that Mga does not bind directly to Pmga
as it does to the other promoters. However, the Mga protein
possesses two distinct N-terminal domains that show predicted
helix-turn-helix DNA-binding motifs (26). Therefore, it is pos-
sible that Mga binding to Pmga involves different residues of
the protein than those that bind to Pemm and PscpA. To
investigate a possible direct role of Mga in autoregulation of
mga expression, the ability of Mga to bind specifically within
the Pmga regulatory region was investigated.

MATERIALS AND METHODS
Bacterial strains and media. S. pyogenes JRS4 is a streptococcin-resistant
derivative of serotype M6 strain D471 (33). GAS strains JRS14 (mga-1) (8) and
JRSS19 (mga-10) (23) are Mga derivatives of JRS4. The pVIT GAS strain
RTG229 was derived from JRS4 and contains the transposon Tn916 (12).
Escherichia coli DH5α (hldR17 recA1 gyrA endA1 relA1) was used as the host
for plasmid constructions. E. coli was grown in Luria-Bertani broth (32), and
S. pyogenes was grown in Todd-Hewitt medium supplemented with 0.2% yeast
extract. Antibiotics were used at the following concentrations: ampicillin at 100 µg/ml for E. coli, chloramphenicol at 30 µg/ml for E. coli, erythromycin at 750 µg/ml for E. coli and 0.1 µg/ml for S. pyogenes, kanamycin at 50 µg/ml for E. coli

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and 300 μg/ml for S. pyogenes, and spectinomycin at 100 μg/ml for E. coli and S. pyogenes.

**DNA manipulations.** Plasmid DNA was isolated from E. coli using the Wizard Maxiprep and Miniprep systems (Promega). Genomic DNA was isolated from GAS by the method of Chassy (9). DNA fragments were isolated from agarose gels by electroelution (20). Colony hybridization was performed with the DIG DNA labeling and detection system (Boehringer Mannheim) using DIG-labeled DNA probes. DNA sequencing was done by the method of Sanger et al. (31) using Sequenase (Bethesda Research Laboratories). PCR for cloning and promoter probe was performed using Phusion high-fidelity DNA polymerase (Stratagene). PCR for diagnostic assays was performed using Taq DNA polymerase (Sigma).

**Construction of the Pmga-malte-mga plasmid pJRS2019.** To place the maltE-mga fusion allele of pJRS2016 (22) downstream of the mga gene, 698 bp of DNA upstream of the mga gene was amplified using the template pJRS180 (26) and the mutagenic primers Pmga-HIII and Pmga-Pst (Table 1) to introduce HindIII (5') and PstI (3') restriction sites, respectively. The resulting Pmga fragment was inserted into HindIII-PstI-digested plasmid pBluescript II KS1 using the gel extraction recovery system (Qiagen). Recombinant plasmid was transformed into E. coli strain DH5α (17) and transformed colonies were screened for the introduction of the HindIII fragment by colony hybridization with a DIG-labeled DNA probe specific for the HindIII fragment. The positive colonies were picked and plasmid DNA was isolated. The HindIII-PstI fragment of plasmid pBluescript II KS1 was purified over an amylose column (New England Biochemicals [NEB]). Protein concentrations were determined using the Bio-Rad protein assay kit.

**Expression and purification of MBP-Mga from E. coli.** MBP-Mga was purified from E. coli as described previously (22). Briefly, cultures of E. coli SA2817 containing pJRS2016 (Ptau-malte-mga) were grown to 30°C in Luria-Bertani broth supplemented with ampicillin (100 μg/ml) and expression of the protein was induced by addition of 6 mM IPTG (isopropyl- β-D-thiogalactopyranoside). Cells were harvested by passage through a prechilled French pressure cell, and MBP-Mga was purified over an amylose column (New England Biochemicals [NEB]). Protein concentrations were determined using the Bio-Rad protein assay kit.

**Mobility shift assays.** Promoter probes were generated by PCR amplification using the plasmid template pJRS180 (26) and primers described in Table 1. PCR fragments were end labeled with [γ-32P]ATP using T4 polynucleotide kinase (NEB). Labeled fragments were excised from a 3% polyacrylamide gel, extracted by crush-and-soak elution, and purified using the QIAquick PCR purification system (Qiagen).

**Mobility shift assays.** Shift assays were performed as described previously (22) in a total reaction volume of 25 μl containing 12 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 1 mM EDTA, 0.6 mM dithiothreitol, 60 mM KCl, 5 mM MgCl2, and 40 ng of poly(dI-dC) per μl. A constant amount of labeled promoter probe DNA (ca. 20 to 40 pM) and various concentrations of MBP-Mga (10 to 320 nM) were used in each reaction. Competition experiments were performed by inclusion of cold probes prior to protein addition. Gels were processed as described previously (22). Gels were dried under vacuum at 80°C for 1 h and autoradiographed using phosphor exposure screens. Images were then scanned at 176-μm resolution using a Molecular Dynamics PhosphorImager 445SI or

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**TABLE 1. PCR primers used in this study**

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**recA**

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* a. The sequences in boldface are introduced restriction sites.
Storm 660, and the resulting data were analyzed with the ImageQuaNT software (version 4.2a).

DNase I protection assays. Substrate fragments for nucleosome protection were prepared in two different ways. (i) PCR-amplified promoter fragment 23–15 was amplified from pJRS180 (26) using the primers OYR23 and OYL15 (Table 1). The product was cloned by blunt-end ligation into the HincII site of pNBE193 (NEB) to produce pJRS303, and the orientation of the insert was verified by PCR analysis. A strand-specific 3' -labeled insert from pJRS303 was produced using the Klenow fragment of DNA polymerase as described elsewhere (18). (ii) Strand-specific labeled PCR-derived promoter fragments were generated using either a sense or an antisense primer that had been end labeled as described above. Labeled promoter probes were purified through polyacrylamide gels as described above.

DNase I protection assays were performed as described previously in a total reaction volume of 100 μl (22). Various amounts of unlabeled competitor DNAs were added, when appropriate, prior to addition of protein. Reactions were stopped by addition of DNase I inhibitor solution (750 mM ammonium acetate, 75 mM EDTA, 33 μg of trNA per ml), concentrated by ethanol precipitation, and separated on a 6% sequencing gel. The method of Maxam and Gilbert (21) was used to sequence the labeled fragments. Gels were processed as described above.

Construction of pVIT GAS strains to study Pmv deletions. The pVIT system (7) was used as described previously (12) with some modifications (described below). In this system, designed for in vivo analysis of activity of Pmg in single copy in GAS, a small region of Tn916 is included in a pVIT vector (vectors for integration into Tn916) which can then integrate into a single copy of Tn916 in the chromosome of GAS strain RTG229. Following the procedures of Geint et al. (12), a fragment of Pmg upstream or downstream of a promoterless gene (cat) was inserted between the PvuII and HindIII sites of the pBluescript plasmid (Stratagene). The cat-12 allele was created by inverse PCR on pJRS228 using the diverging primers Mga-s1d and Mga-s1dL (Table 1), resulting in a deletion of 63 bp of GAS DNA including Mga binding site I and the introduction of an EcoRI restriction site. The inverse PCR product was digested with EcoRI and religated to create pMga-s1d. The 410-bp BamHI-HindIII fragment from pMga-s1d was cloned into BamHI-HindIII-digested pVT164 to produce pJRS5301. The plasmid pJRS578 contains a 139-bp PCR fragment including only the Pmg2 promoter amplified from GAS strain D471 (serotype M6) with its native promoter, with an E. coli promoter. It has been presented by the fusion protein MBP-Mga and native Pmg promoter (25). A 485-bp PCR fragment containing the minimal Pmg regulatory region (25) amplified from pJRS530 using primers Pmga-Bam and Pmga-Xho (Table 1) and cloned into pBluescript KI-Str (Stratagene) to produce pJRS2019 (Ma).

RESULTS

The MBP-Mga fusion protein activates transcription in vivo. Previous studies showed that a fusion of the E. coli maltose-binding protein to the amino terminus of Mga (MBP-Mga) bound specifically to the promoters of Mga-regulated genes from the serotype M6 GAS strain JRS4 (22). However, the ability of MBP-Mga to function in vivo to autoregulate and activate other Mga-regulated genes in a GAS strain lacking wild-type Mga was never demonstrated.

The plasmid pJRS2050 (1), containing the wild-type Mga gene from GAS strain D471 (serotype M6) with its native promoter, has been shown previously to complement several mga mutations in different GAS strains (1, 23). To test the in vivo activity of MBP-Mga, JRS14, a GAS of serotype M6 containing a Tn916 insertion within the mga promoter region (mga1) that results in loss of Mga activity (6), was used. A promoterless malE-mga allele, encoding MBP-Mga, was cloned downstream of the native mga regulatory region (Pmga region) in the vector pLZ12-Spec to produce pJRS2019 (Materials and Methods), which was introduced by electroporation into JRS14. Total RNA was isolated at mid-exponential phase and assayed by hybridization for transcripts from mga, emm, and, as a control for equal loading of gels, recA.

Compared to the recA control, no transcripts for mga or emm were detectable in JRS14 containing pLZ12-Spec, the vector without an insert (Fig. 1). As previously observed (1), JRS14/pJRS2050, which contains the wild-type mga allele under the control of its own promoter, produced both mga and emm transcripts (Fig. 1). JRS14/pJRS2019 (carrying Pmga-malE-mga) also produced both mga and emm transcripts (Fig. 1). This is consistent with the previous finding that MBP-Mga positively autoregulates its own expression. However, unexpectedly, pJRS2019 resulted in more mga transcript than did pJRS2050 and less emm transcript (Fig. 1). Thus, it appears that the ability of MBP-Mga to positively autoregulate mga transcription is actually enhanced slightly over that of Mga. The unexpected decrease in transcripts of the Mga-regulated gene emm could be a consequence of either less-efficient activation by the fusion protein MBP-Mga or the overexpression of this protein.

Mga binds to specific upstream sequences of mga. In the M6 GAS strain JRS4, a 473-bp region directly upstream of mga, which includes the distal P1 and the proximal P2 starts of transcription, is required for maximal mga expression (25). To begin a study on the autoregulation of mga in GAS, the ability of Mga to bind to sequences within this Pmga region was determined. Six overlapping promoter probes encompassing this region were amplified by PCR, end labeled with [γ-32P]ATP, and tested for binding to the MBP-Mga fusion protein by electrophoretic mobility shift assay.

Increasing amounts of MBP-Mga resulted in decreased mo-
bility of four overlapping Pmga region probes: 22-1, 23-15, 2-2, and 1-2 (Fig. 2 and data not shown). Addition of the respective unlabeled probe to each reaction mixture prevented formation of the complexed species, whereas addition of a nonspecific unlabeled probe did not (data not shown). No detectable effect on the mobility of the flanking probes 22-14 and 3-12 was ever observed, even with protein concentrations of up to 350 nM, indicating that MBP-Mga does not bind to these sequences.

DNase I protection assays define two MBP-Mga binding sites upstream of the mga P2 promoter. To identify the sequences within the Pmga region that interact with MBP-Mga, protection of this region from DNase I digestion was determined in the presence of the fusion protein. Probe 23-15 (Fig. 2) was labeled on the sense strand and incubated with increasing amounts of MBP-Mga (Materials and Methods). Two regions were protected from DNase I digestion; these define a distal binding site, I, of ca. 61 bp and a proximal binding site, II, of ca. 58 bp (Fig. 3A and B). DNase I analysis of probe 23-15 labeled on the antisense strand protected regions almost identical to those described above and did not reveal any additional binding sites (Fig. 4). Additional DNase I analysis of probes 22-1, 2-2, and 1-2 (Fig. 2) verified the results reported above (data not shown). Since probes 22-1 and 1-2 contain only a single binding site, these data indicate that Mga can bind either site I or II in the absence of the other site.

The sequences in site I protected by MBP-Mga extend from base -153 to base -211 upstream of the mga open reading frame. This places the center of the site at base -185 from the P2 transcript start (Fig. 3B). Binding of MBP-Mga in this region suggests that Mga acts directly to activate the major P2 transcriptional start of mga and thus to positively autoregulate its expression.

Addition of unlabeled probe 23-15 inhibited the binding to both protected regions, while probes 22-14 and 3-12, which did not show binding to MBP-Mga by mobility shift assays (Fig. 2), also failed to prevent protection in DNase I assays (Fig. 3A and 4). DNase I footprint analysis also showed that MBP-Mga occupies both sites I and II simultaneously, even at the lowest protein concentration used (Fig. 3A and 4). These data demonstrate that binding of MBP-Mga is specific at binding sites I
and II and that MBP-Mga appears to have similar affinities for both of the sites within the Pmga region.

Comparison of Mga binding to different Mga-regulated promoters. Mga has been shown to bind directly to the promoters of the Mga-activated genes *emm* (Pemm) and *scpA* (PscpA) (22), in addition to binding to the Pmga region. Since the consensus sequence recognized by Mga at Pemm and PscpA is not apparent at Pmga, it seemed possible that different domains of the 62-kDa Mga protein are involved in binding to these promoters. To investigate this, competition DNase I protection assays were performed. The Pmga promoter probe 23-15 (Fig. 2) was labeled on the sense strand (Materials and Methods). Labeled probe (2 pM) was incubated with increasing amounts of MBP-Mga as follows: lane 1, probe alone; lanes 2 and 5, probe plus DNase I; lane 3, probe plus 120 nM MBP-Mga and DNase I; lane 4, probe plus 240 nM MBP-Mga and DNase I. Unlabeled competitor sequences (40 nM) were added prior to addition of MBP-Mga as follows: lane 6, lane 4 reaction mixture plus 22-14; lane 7, lane 4 reaction mixture plus 23-15; lane 8, lane 4 reaction mixture plus 3-12. Thick vertical bars designate positions of Mga binding sites I and II. The *DraI* restriction site is shown; nucleotide positions are relative to the start of *mga* translation (see panel B). Base pair positions are shown on the left. (B) Location of Mga binding sites within Pmga. Shown is 478 bp of sequence upstream of the *mga* coding region (thick arrow). The *P1* and *P2* starts of transcription (thin arrows) were determined by primer extension (this study) and differ slightly from those reported by Okada et al. (25). The −10 and −35 hexamers for both starts are designated by overlines. Nucleotides protected from DNase I by MBP-Mga binding are shaded, while those hypersensitive to DNase I digestion are indicated by asterisks. The region deleted in *mga-12* GAS strain JRS5301 is shown by the bracket below the sequence. The *DraI* restriction site corresponding to the *mga-1* Tn916 insertion site (8) is indicated. The VL1, VL2, and VR sites are described in Fig. 5. Nucleotides are numbered relative to the start of *mga* translation (+1).
of the Mga protein binds to its own promoter and to other Mga-regulated promoters.

**Mga binding site I is involved in autoregulation by Mga.** Since Mga binds in vitro within the Pmga promoter region, Mga may function directly in autoregulation in vivo. To test this, a deletion of Mga binding site I \((mga-12)\) was constructed and assayed for its effect on transcription from the Pmga promoter. The pVIT system was used to provide a single chromosomal copy of the wild-type or mutant form of Pmga transcribing the reporter \(cat\) gene, while Mga protein is supplied from its unlinked normal chromosomal location (Materials and Methods).

Four pVIT-derived strains were compared (Fig. 5): strain JRS529, containing the integrated pVIT164 vector with no
Pmga insert driving cat transcription; strain JRS530, which has the wild-type Pmga region cloned in front of cat; strain JRS5301, which contains a 64-bp deletion that removes Mga binding site I (mga-12) cloned upstream of cat; and strain JRS578, which contains a deletion of the DNA upstream of P2 (including the P1 promoter and both Mga binding sites) cloned upstream of cat.

Specific initiation of transcription from the P1 and P2 mga promoters was determined for each strain by extension of two different primers, of which one was specific for cat (cat86-PE; Fig. 6A and Table 1), to assay transcription at the pVIT locus, and one was specific for mga (Pmga2-PE; Fig. 6A and Table 1), to check transcription from the wild-type mga locus. From the mga-specific primer, all four strains produced a strong P2 product and a barely detectable P1 product (Fig. 5, mga-specific, and 6B). This is in agreement with published data on Pmga (25, 27) and provides an internal control for direct comparison of transcription at the pVIT locus.

As expected, JRS529 (promoterless cat) produced no detectable cat-specific Pmga promoter transcripts from its pVIT locus (Fig. 6B) and JRS530 (wild-type Pmga-cat) generated a cat-specific Pmga profile almost identical to the wild-type Pmga pattern described above (Fig. 5 and 6B). The JRS578 strain, which lacks both Mga binding sites, as well as P1 (mga-21), showed no detectable P2 activity from the cat-specific primer (Fig. 5 and 6B). This supports previous observations (3, 12, 25) suggesting that P2 transcription requires sequences upstream of the deletion in this strain.

At the pVIT locus of JRS5301, which has a deletion in Mga binding site I (mga-12), there was a complete loss of cat-specific P2 initiation and a corresponding strong increase in the level of cat-specific P1-derived transcripts (Fig. 5 and 6B). These data suggest that the region encompassing Mga binding site I is important for both the activation of the downstream P2 promoter and repression of the upstream transcript initiated at P1. However, the overall effect of the loss of Mga binding site I on expression of mga was not obvious since the amount of cat transcript (assayed by hybridization) in JRS5301 (mga-12) was almost the same as that in wild-type strain JRS530 (Fig. 5 and 6C).

Mga binding at site I is required for P2 activation. To determine whether the requirement for binding site I for transcription from P2 is dependent on Mga, the wild-type mga gene was insertionally inactivated in the pVIT strain JRS530 to produce JRS1530 (Materials and Methods). As expected, no mga-specific transcript was detectable by hybridization in JRS1530 (data not shown). In the absence of Mga in strain JRS1530, which has an intact Pmga region at the pVIT locus, no cat-specific P2 product was detected in primer extension studies (Fig. 5; data not shown). Since this transcript was
present in JRS530, which differs from JRS1530 only by having an intact mga gene, this supports the interpretation from mga-12 binding site I deletion strain JRS5301 (above) that Mga binding is needed to activate transcription from the P2 promoter.

Repression of P1 requires binding site I but not Mga. At the pVIT locus of JRS5301, which has a deletion in binding site I, there is a strong increase in the amount of cat-specific P1 transcript compared to JRS530, which has an intact Pmga region (Fig. 5 and 6B). Although there is no detectable cat-specific transcription from P2 in JRS5301 (see above), the total amount of cat transcript in this strain was almost the same as for wild-type strain JRS530 (Fig. 5 and 6C). Thus, it appears that binding site I is needed not only for activation of the P2 promoter but also for repression of the P1 promoter in the Pmga region.

To determine whether this repression is dependent on Mga, the wild-type mga gene was insertionally inactivated in strain JRS529 to produce JRS1529 and in strain JRS5301 to produce JRS15301. P1 in strain JRS1530, the Mga- strand, still produces a detectable mga-specific P1 transcript, as shown by primer extension analysis (data not shown). Therefore, transcription from P1 does not appear to require Mga.

Surprisingly, the absence of Mga had no effect on cat-specific P1 promoter activity in strain JRS15301, compared with its Mga+ parent, strain JRS5301 (Fig. 5; data not shown). Both JRS530 and JRS1530 produce barely detectable amounts of a cat-specific transcript that initiates at P1, while JRS5301 and JRS15301, with binding site I deleted, produce significantly higher amounts of cat-specific P1 transcripts. Therefore, it can be deduced that repression of the P1 promoter requires binding site I (see above) and that this repression does not involve Mga. These data suggest the existence of a protein other than Mga that binds at Mga binding site I to inhibit transcription from the P1 promoter.

**DISCUSSION**

Mga binds to two sites within Pmga in vitro. Expression of mga is positively autoregulated, and this control requires the entire 473-bp regulatory region (Pmga) located upstream of mga (12, 25). Because Mga is a DNA-binding protein that binds within the promoters of the Mga-regulated emm and scpA genes, it seemed possible that autoregulation by Mga was also direct. To address this, an MBP-Mga fusion protein was used in DNase I footprinting and gel retardation experiments to assay specific Mga binding within the Pmga region. The Pmga region contains two promoters, a major one located proximal to the coding region (P2) and one located further upstream that produces much less transcript (Fig. 3A and 4). We have shown here that Mga binds to two 59-bp sites upstream of the major P2 transcriptional start site within Pmga (sites I and II). Mga also appears to bind to both sites with equal affinity since a DNA fragment containing only site II (fragment 1-2) competed equally well for binding of MBP-Mga to both sites on separate DNA fragments.

It is anticipated from these in vitro binding studies that in strain JRS5301, which has binding site I deleted, Mga will still be bound to site II. With Mga bound there, it might be expected to repress transcription initiating from upstream promoter P1. However, this does not occur (Fig. 5 and 6). This suggests either that Mga binds weakly to a single site and is displaced during transcription or that binding in vivo differs from that in vitro, perhaps due to supercoiling.

No transcription initiating from P2 was detected in JRS5301, although Mga should be bound at site II and would be expected to activate this nearby downstream promoter. This suggests the possibility that Mga must occupy both binding sites I and II to activate P2, perhaps to achieve an appropriate polymerization state for association with RNA polymerase (RNAP).

**What defines an Mga recognition site within different promoters?** Although Mga has two helix-turn-helix motifs in its amino-terminal end that may be directly involved in protein-DNA interactions, DNA fragments containing the Pemm or the PscpA binding sites competed for MBP-Mga binding in DNase I footprinting experiments at both Pmga sites even though the sites were on different DNA fragments. Thus, Mga may use the same domain to interact with the sites found in Pemm, Pemn, and PscpA. Alternatively, Mga may utilize a separate domain to bind to Pemn and PscpA, producing a conformational change that would affect binding to Pmga.

If a single domain of Mga interacts with all three sites, binding might involve recognition of similar DNA sequences in all cases. However, the two regions within Pmga protected by MBP-Mga show very little DNA sequence similarity to the consensus Mga binding site previously derived from the Pemm and PscpA promoters (22). Using sequence alignment, the four individual Mga binding sites were found to have less than 50% sequence similarity to each other and attempts to generate a more accurate consensus using the four known sites were unsuccessful. It should be remembered that DNase I footprinting analysis of regions protected by a protein are likely to include many nucleotides that are not directly contacted by that protein. This may be even more likely with larger proteins such as the 62-kDa Mga protein, which is at least twice as large as most bacterial DNA-binding proteins. Furthermore, the high AT content of the sequence in the regions bound by Mga makes it even more difficult to identify a possible recognition consensus in the absence of experimental knowledge of the nucleotides contacted.

Both of the Pmga binding sites that are protected by Mga are much larger than the sites protected within Pemn and PscpA. MBP-Mga protects a 45-bp region upstream of emm and scpA, while the two Mga-protected regions within Pmga are both 59 bp long. Since the competition experiments indicated that the
A.

**cat-specific transcripts**

B.

C.

**mga-specific transcripts**
same domain of Mga is involved in the DNA-protein interactions at all four sites, the disparity in size may reflect the number of Mga molecules that are bound at the different sites. Some bacterial regulators bind to their DNA target sequences as homomultimeric species, such as dimers or tetramers, and it seems possible that a larger Mga species is required for binding in the Pmga region than in Pemm or PscpA.

**Distances of the Mga binding sites from their target promoters.** Direct interaction between a regulator protein and RNAP bound at a promoter is the primary mode of activation of genes in bacteria (13, 14, 30). Transcriptional activators of bacteria bind upstream of the promoter that they regulate in RNAP bound at a promoter is the primary mode of activation complex and thus activate transcription (16, 30). For both in the Pmga region than in Pemm or PscpA.

Some bacterial regulators bind to their DNA target sequences as homomultimeric species, such as dimers or tetramers, and it seems possible that a larger Mga species is required for binding in the Pmga region than in Pemm or PscpA. The greater distance of these sites from P2 within Pmga (over 100 bp upstream) suggests that Mga functions by a different mechanism of activation in this promoter than that which it utilizes at Pemm and PscpA.

Several different models may be suggested to explain the ability of Mga to activate the P2 promoter from distant sites. Bacterial transcriptional activators that use remote sites often interact with promoters that use RNAP associated with alternate sigma factors (14). Although this may be the case for Mga activation of P2, the presence of alternative sigma factors in GAS has yet to be investigated. Alternatively, Mga may interact with an additional factor that binds to a site adjacent to RNAP at the P2 promoter. An example of this can be found in E. coli, where some activator proteins bound at remote sites interact with the global regulator Crp, which binds close to the promoter to activate transcription (14). Finally, an additional Mga binding site located proximal to P2 may exist. In this case, DNA looping might cause Mga to interact with RNAP. However, such a third site must exhibit a lower affinity for Mga than the two defined here since no additional sites were detected by DNase I analysis at the levels of protein used in our study.

**Evidence of a repressor of P1 activity.** Even though the role of the P1 promoter in determining appropriate mga expression remains unclear, the observation that P1 activity is regulated suggests that it is important. If synthesis of the P1 repressor is itself regulated, it may allow P1 to respond differently to environmental signals than P2. This will enable the organism to be attuned more sensitively to environmental changes. In agreement with this, transcription from the P1 promoter does not appear to require Mga (Fig. 5 and 6). Although a previous study reported that P1 was dependent on Mga for activity (3), those results were based on a spontaneous mutant of an M12 GAS strain that may differ from the strain used here.

Expression of mga is positively autoregulated, which implies that some form of negative regulation exists to limit exponentially increasing Mga synthesis. We have provided the first suggestion of such a negative element in the M6 strain studied. Deletion of the sequences containing Mga binding site I resulted in an increase in transcriptional initiation from the P1 promoter, even in the absence of Mga. This suggests that this lesion removes a cis-acting element necessary for repression of transcription from the P1 start site. Thus, while deletion of site I, which is upstream of P2, prevents Mga-dependent activation of the P2 promoter, it also leads to derepression of P1 transcription (Fig. 5 and 6). This might occur by binding of a classical repressor that blocks RNA polymerase from progressing through site I, which is 80 bp downstream of P1. Although Podbielski et al. recently identified a global negative regulator that may affect mga expression in an M type 49 GAS strain (29), they reported that this gene is absent from the M6 strain used in this study. Thus, the negative regulator implied by our work remains to be discovered.

The possible involvement of a second trans-acting molecule in the regulation of mga expression indicates that control of mga is more complex than originally thought. Since the recognition site for this new regulator overlaps a binding site for Mga, one could imagine that these two factors might compete for access to these sites. This would create a new level of regulation that may involve both binding affinities and protein-protein interactions. Since all of the different strains studied so far (3, 25, 27) are similar in Pmga architecture, our current and subsequent findings may be applicable to other serotypes of GAS and thus provide a general theme of mga regulation. Only with further investigation of mga regulation will we be able to propose a more detailed model of this intricate process that will lead us to a better understanding of this important human pathogen.

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