Transcriptional Regulation of Type 4 Pilin Genes and the Site-Specific Recombinase Gene, \textit{piv}, in \textit{Moraxella lacunata} and \textit{Moraxella bovis}

DEBORAH WELLNER HEINRICH AND ANNA C. GLASGOW*

Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia 30322

Received 6 June 1997/Accepted 23 September 1997

\textit{Moraxella lacunata} and \textit{Moraxella bovis} use type 4 pili to adhere to epithelial tissues of the cornea and conjunctiva. Primer extension analyses were used to map the transcriptional start sites for the genes encoding the major pilin subunits (\textit{tfpQ/I}) and the DNA invertase (\textit{piv}), which determines pilin type expression. \textit{tfpQ/I} transcription starts at a $\sigma^54$-dependent promoter (\textit{tfpQ/I}p$_2$) and, under certain growth conditions, this transcription is accompanied by weaker upstream transcription that starts at a potential $\sigma^{54}$-dependent promoter (\textit{tfpQ/I}p$_1$). \textit{piv} is expressed in both \textit{M. lacunata} and \textit{M. bovis} from a putative $\sigma^{54}$-dependent promoter (\textit{pipv}) under all conditions assayed. $\sigma^{54}$-dependent promoters require activators in order to initiate transcription; therefore, it is likely that \textit{tfpQ/I}p$_1$ is also regulated by an activator in \textit{Moraxella}. Primer extension assays with RNA isolated from \textit{Escherichia coli} containing the subcloned pilin inversion region from \textit{M. lacunata} showed that \textit{pipv} is used for the expression of \textit{piv}; however, \textit{tfpQ/I}p$_2$ is not used for the transcription of \textit{tfpQ/I}. Transcription from \textit{tfpQ/I}p$_2$ was activated in \textit{E. coli} when the sensor (PilS) and response regulator (PilR) proteins of type 4 pilin transcription in \textit{Pseudomonas aeruginosa} were expressed from a plasmid. These results suggest that the expression of the type 4 pilin in \textit{M. lacunata} and \textit{M. bovis} is regulated not only by a site-specific DNA inversion system but also by a regulatory system which is functionally analogous to the PilS-PilR two-component system of \textit{P. aeruginosa}.

\textit{Moraxella lacunata} and \textit{Moraxella bovis} are gram-negative human and bovine pathogens, respectively, that primarily infect the conjunctiva and cornea (3, 15, 29, 32). Attachment to the epithelial tissues of these areas is mediated by type 4 pili (28). Other bacteria that express type 4 pili include \textit{Neisseria gonorrhoeae}, \textit{Neisseria meningitidis}, \textit{Vibrio cholerae}, \textit{Aeromonas hydrophila}, and \textit{Dichelobacter nodosus} (10, 13, 23, 25, 37). In bacteria expressing type 4 pili, the major pilin subunit is expressed as a pre-pilin protein with an unusually short 6-aminoc-acid leader sequence followed by a predominantly hydrophobic domain (35). The hydrophobic domain and the leader sequence are required to deliver the protein to an ATP-dependent, Sec-independent secretory pathway (34). During the process of secretion, a pre-pilin peptidase cleaves the leader sequence and methylates the first amino acid of the mature protein, creating N-methylphenylalanine (36). Thus, type 4 pili have also been classified as McPhe pili.

\textit{M. bovis} exhibits antigenic and phase variations of its type 4 pili, alternately expressing Q- or I-type pili and switching between P$^+$ (piliated) and P$^-$ (nonpiliated) at a frequency as high as 10$^{-4}$ per cell per generation (21). \textit{M. bovis} cells expressing Q-type pili are more efficient at establishing infection than those expressing I-type pili (27, 29), and nonpiliated \textit{M. bovis} cells cannot establish infection (15). Therefore, pili are important virulence factors. The genes encoding the major pilin subunits of \textit{M. bovis} are located on an invertible segment of DNA (Fig. 1) that allows the alternate expression of \textit{tfpQ} or \textit{tfpI} (type four pilin) (12). The shared amino terminus is encoded outside the invertible segment, and the genes for the carboxy termini of the Q- and I-type pilin proteins are oriented in opposite directions within the invertible segment. The orientation of the invertible DNA determines which pilin gene will be transcribed. An additional open reading frame (\textit{tfpB}) is found within the invertible segment; the function of \textit{tfpB} is as yet unknown (12). The \textit{piv} gene, located immediately adjacent to the invertible segment, encodes the putative site-specific DNA invertase (19, 20). The pilin genes of \textit{M. lacunata} share considerable homology with the pilin genes of \textit{M. bovis} and are identically arranged (26). However, the \textit{M. lacunata} \textit{tfp} gene contains a 19-bp perfect tandem repeat near the inversion junction site that creates a frameshift, resulting in a shortened, nonfunctional pilin gene product. Thus, \textit{M. lacunata} phase variation is an on/off switch of Q-type pilin expression.

In \textit{M. lacunata} and \textit{M. bovis}, the \textit{tfpQ/I} pilin genes and \textit{piv} have potential $\sigma^{54}$-dependent promoters, based on sequence inspection. $\sigma^{54}$ is an alternative sigma factor, encoded by \textit{rpoN}, that interacts with core RNA polymerase and binds to the consensus sequence -27TGGCAC-N$_5$-TTGCA-11 (24). Unlike that in $\sigma^{32}$-dependent promoters, the spacing between the highly conserved GG and GC dinucleotides of $\sigma^{54}$-dependent promoters is invariant (24). The $\sigma^{54}$-RNA polymerase holoenzyme (Er$^{54}$) requires an activator to initiate transcription. The activator catalyzes ATP-dependent isomerization of a closed complex between Er$^{54}$ and the promoter to an open complex that is transcriptionally competent (39, 40).

If \textit{tfpQ/I} and/or \textit{piv} uses a $\sigma^{54}$-dependent promoter, it is likely that there is regulation of type 4 pilin expression beyond the control mediated by DNA inversion, i.e., at the level of initiation of transcription. As with many $\sigma^{54}$-dependent promoters, the activation of transcription may be controlled by a two-component regulatory system. For example, in \textit{P. aeruginosa} a two-component regulatory system controls the initiation...
of transcription from the α54-dependent promoter of the type 4 pilin gene, pilA. The sensor of this two-component system, PilS, is a histidine kinase which autophosphorylates in response to an as-yet-unidentified environmental signal and subsequently transfers the phosphate to PilR. The phosphorylated PilR can then interact with Es54 and activate pilA transcription (14). PilR interacts with a transcriptional enhancer sequence upstream of the α54-dependent promoter which contains the sequence 5′C/GTGTC3′ repeated at the four PilR binding sites (16). This enhancer region also has homology to the recognition sequence of NifA (5′TGT-N11-ACA3′), which is an activator of α54-dependent transcription of the nitrogen fixation genes in Klebsiella pneumoniae (16). The potential α54-dependent promoter for tfpQ/I has the repeated consensus sequence for PilR DNA binding and the overlapping NifA recognition sequence found in the pilA enhancer sequence, suggesting a possible similarity in the regulation of these type 4 pilin genes.

To address the regulation of tfpQ/I and piv, we used primer extension assays to map the promoters for these genes in M. lacunata and M. bovis. We also mapped the transcriptional start sites from a subclone of the M. lacunata pilin inversion region in Escherichia coli. Analyses of transcription in ρpoN mutant or wild-type E. coli strains in the presence of the PilS and PilR proteins of P. aeruginosa further defined transcriptional regulation of the type 4 pilin genes.

(A preliminary report of these findings was presented at the 97th General Meeting of the American Society for Microbiology, Miami Beach, Fla., May 1997.)

MATERIALS AND METHODS

Strains and plasmids. The strains and plasmids used in this study are listed in Table 1. pAG1206 was created by inserting the BamHI/HindIII fragment of PB300 (7), containing the P. aeruginosa pilS and pilR genes, into the BamHI/HindIII sites of pMPM-K3 (22), carrying the p15A origin of replication and a pBluescript multicloning site.

Media and growth conditions. E. coli was grown on Luria-Bertani (LB) agar plates or in LB media (31) at 37°C with the appropriate antibiotics at the following concentrations: ampicillin, 100 μg/ml; kanamycin, 10 μg/ml; and chloramphenicol, 30 μg/ml. M. lacunata and M. bovis were grown on sheep blood agar plates (BBL Microbiology Systems, Cockeysville, Md.), heart infusion agar with rabbit blood (BBL), GC agar plates (11), or chocolate agar plates (Difco Laboratories, Detroit, Mich.) or in GC broth (11). All cultures were grown at 37°C unless otherwise indicated.

RNA isolation. Bacterial cultures were scraped with a sterile loop and resuspended in 20 mM Tris-HCl (pH 7.6)–10 mM NaCl–1 mM EDTA–0.5% sodium dodecyl sulfate. Liquid cultures (5 ml) were centrifuged in a Beckman J2-21 centrifuge with a Beckman JA20 rotor for 5 min at 4,000 × g and resuspended

FIG. 1. Schematic representation of the type 4 pilin DNA inversion regions of M. lacunata and M. bovis. The parentheses denote the invertible segment of DNA. The boxed sequence is that of the tfpQ/I promoter region in M. lacunata. An arrow marks the start of tfpQ/I transcription. The potential binding site for the RNA polymerase holoenzyme that uses α54 (Es54) is marked with two bold lines. The highly conserved −24 GO and −12 GC motifs of the α54-dependent promoters are underlined. The predicted start of tfpQ/I transcription is marked with an asterisk. The tfpQ/I promoter is indicated with an inverted caret, and the potential −10 and −35 sequences are underlined. The sequences similar to the P. aeruginosa PilR binding sites are denoted with plus signs, and the sequence similar to the NifA recognition sequence is underlined with a broken line. The piv-2 and p-pilin primers used in the primer extension assays are indicated.
**RESULTS**

**Mapping of the tfpQ/I and piv promoters.** The potential \(\sigma^{34}\)-dependent promoter sequence, located approximately 100 bp from the translational start site of the tfpQ/I gene, differs from the consensus \(\sigma^{34}\) binding site sequence by only one base (in bold) (5\(^{\text{TGGTAC-N}_{10}-\text{ATGCA3}}\)) and retains the invariant spacing between the highly conserved GC and GC dinucleotide residues. The potential \(\sigma^{34}\)-dependent promoter sequence for the piv gene also retains the 10-base spacing between the GG and GC dinucleotide residues. The potential \(\sigma^{34}\)-dependent promoter sequence for the piv gene also retains the 10-base spacing between the GG and GC dinucleotide residues.

**TABLE 1. Strains and plasmids**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype or description</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>endA1 hisD17 supE44 thi-1 recA1 gyrA96 relA1 Δ(lacZΔ2YF-argF)</td>
<td>Bethesda Research Laboratories</td>
</tr>
<tr>
<td>DH5a</td>
<td>U109 h-&lt;880 ΔlacZΔ15</td>
<td></td>
</tr>
<tr>
<td>YMC10</td>
<td>endA1 thi-1 hisD17 supE44 ΔlacU169 hsdRK</td>
<td>Steve Lory; 2</td>
</tr>
<tr>
<td>THI</td>
<td>M15ΔpoSn</td>
<td>Steve Lory; 2</td>
</tr>
<tr>
<td>DPH29.1</td>
<td>DH5ΔpMD1</td>
<td>Laboratory strain</td>
</tr>
<tr>
<td>DWH15</td>
<td>DH5ΔpMDL1/pAG1206</td>
<td>This study</td>
</tr>
<tr>
<td>DWH16</td>
<td>DH5ΔpMDL1/pMPM-K3</td>
<td>This study</td>
</tr>
<tr>
<td>DWH17</td>
<td>YMC10ΔpMDL1</td>
<td>This study</td>
</tr>
<tr>
<td>DWH18</td>
<td>THI/pMDL1</td>
<td>This study</td>
</tr>
<tr>
<td>DWH19</td>
<td>YMC10ΔpMDL1/pMPM-K3</td>
<td>This study</td>
</tr>
<tr>
<td>DWH20</td>
<td>THI/pMDL1/pMPM-K3</td>
<td>This study</td>
</tr>
<tr>
<td>DWH21</td>
<td>YMC10ΔpMDL1/pAG1206</td>
<td>This study</td>
</tr>
<tr>
<td>DWH22</td>
<td>THI/pMDL1/pAG1206</td>
<td>This study</td>
</tr>
<tr>
<td>Moraxella lacunata</td>
<td>ATCC 17956</td>
<td>20</td>
</tr>
<tr>
<td>F9223</td>
<td>Wild-type laboratory strain</td>
<td>CDC, Atlanta, Ga.</td>
</tr>
<tr>
<td>Moraxella bovis</td>
<td>Tifton</td>
<td>Carl F. Marrs</td>
</tr>
<tr>
<td>Plasmids</td>
<td>6 kb of M. lacunata DNA in pBluescript from a λZap (Stratagene) library, containing the pilin inversion region (Fig. 1); Amp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>20</td>
</tr>
<tr>
<td>pJB300</td>
<td>P. aeruginosa pilS pilR in pBluescript; Amp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Steve Lory; 7</td>
</tr>
<tr>
<td>pMPM-K3</td>
<td>Cloning vector with p15A ori and pBluescript multicloning site; Kan&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Matthias Mayer; 22</td>
</tr>
<tr>
<td>pAG1206</td>
<td>BamHI/HindIII segment of pB300 containing the pilS pilR genes in the BamHI/HindIII sites of pMPM-K3; Kan&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pBluescript</td>
<td>Cloning vector; Amp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Stratagene, La Jolla, Calif.</td>
</tr>
</tbody>
</table>

* CDC, Centers for Disease Control and Prevention.
CA3’); however, it diverges from the consensus sequence in the two nucleotides (in bold) before the GC doublet (from TT to GA), which have been shown to be critical contacts for σ^54 binding (8, 9). Thus, this sequence is predicted to be a low-affinity σ^54 binding site. Primer extension assays were used to determine the 5’ end of the transcripts for tfpQ/I and piv with the primers shown in Fig. 1. Piliated colonies, which were distinguished by colony morphology (5), were picked for RNA isolation. Piliated colonies have a corroding morphology with a raised translucent center, and nonpiliated colonies are flat and opaque. TEM and transformation assays for natural competence were used to confirm that these morphologies corresponded to the state of piliation. Indeed, the corroding colonies displayed bundled pili in TEM and were capable of acquiring a chromosomal marker (Smr) in the transformation assays. Flat, opaque colonies were nonpiliated in TEM and not naturally competent (data not shown). The absence of the tfpQ/I promoter was also noted for M. lacunata ATCC 17956 and M. bovis Tifton grown on heart infusion agar with rabbit blood, GC medium, or chocolate agar (data not shown).

FIG. 2. Primer extension assays mapping the start sites for the tfpQ/I and piv transcripts. Primer extension assays were performed with M. lacunata and M. bovis RNA as described in Materials and Methods. (A) Primer extension assays with RNA from M. lacunata and M. bovis grown on sheep blood agar plates and with the p-pilin primer to detect tfpQ/I transcripts. The bands marked S1 (start 1) and S2 (start 2) correspond to the start of the transcripts initiated at tfpQ/Ip_1 and tfpQ/Ip_2, respectively. (B) Primer extension assays with RNA from M. lacunata and M. bovis grown on either GC agar plates (GC) or chocolate agar plates (CAP) and with the piv-2 primer to detect piv transcripts. The bands marked Spiv correspond to the start of the transcripts initiated at prop. The primers used for the primer extension assays were also used for the DNA sequence reactions for tfpQ/I and piv from pMxL1.
The start site for the piv transcript was also determined by primer extension assays with RNA isolated from *M. lacunata* and *M. bovis* grown on GC agar and chocolate agar plates as indicated in Fig. 2B. In each case, the 5' end of the transcripts initiated at a putative σ54-dependent promoter 111 bp upstream of the start site for the translation of piv. The apparent absence of initiation of transcription from the potential σ54-dependent promoter for piv is consistent with the prediction, based on chemical interference and base substitution DNA binding assays, that this promoter sequence is a low-affinity σ54 and Eσ54 binding site (8, 9). However, since σ54-dependent transcription is often regulated by environmental signals, it is still possible that this promoter is used under conditions that were not tested.

Primer extension assays were also performed with RNA isolated from *E. coli* DH5α carrying plasmid pMXL1, which contains the *M. lacunata* inversion region and exhibits inversion in *E. coli* (20). Figure 3 shows that the tfpQ/I transcripts did not map to the tfpQ/Ip2 promoter sequence, which is utilized in *M. lacunata* and *M. bovis*. Instead, we detected transcripts which may correspond to processed versions or early terminations of primer extension from the tfpQ/Ip1 promoter in *M. lacunata* and *M. bovis*. It is also possible that these transcripts correspond to additional promoters used in *E. coli*; however, there were no identifiable promoter sequences associated with the 5' ends of the transcripts. Since transcription from a σ54-dependent promoter is dependent on an activator protein, it is likely that *E. coli* lacks a homolog of the *M. lacunata* tfpQ/I activator protein and therefore that Eσ54 cannot initiate transcription from tfpQ/Ip2.

Primer extension assays for the piv transcript performed with RNA isolated from *E. coli* containing pMXL1 revealed that the σ70-dependent promoter pivp, which is used in *M. lacunata* and *M. bovis*, is also used in *E. coli* (18a).

**P. aeruginosa PilS-PilR can activate tfpQ/Ip2 transcription in *E. coli***. In the pilA transcriptional enhancer sequence of *P. aeruginosa*, four binding sites for the PilR activator are centered approximately 100 bp upstream of the Eσ54 binding site (16). Approximately 200 bp upstream of tfpQ/Ip2, there is significant sequence similarity with the PilR binding sites of the pilA enhancer sequence (Fig. 1). To determine if transcription from tfpQ/Ip2 could be activated in *E. coli* by PilR, primer extension assays were performed with RNA isolated from an *E. coli* strain containing pMXL1 and a plasmid expressing pilS and pilR from plac (pAG1206). The pilR translation product in *E. coli* is full length, but the pilS product is missing its transmembrane domain due to initiation of translation at an internal TTG methionine codon instead of the GTG codon used in *P. aeruginosa* (7). Although the activity of this truncated form of PilS was not determined in *E. coli*, Boyd and Lory (6) have shown that an identically truncated gene product from a 5' deletion of pilS (PilS-cyt), which is cytoplasmically located, retains its histidine kinase activity in *P. aeruginosa*. Therefore, PilS expressed from pAG1206 may also be an active histidine kinase. In the primer extension assays (Fig. 3), when PilS-PilR was expressed from pAG1206, transcripts initiating at the tfpQ/Ip2 promoter were detected (Fig. 3). tfpQ/Ip2 transcripts were not observed when the vector alone was cotransformed with pMXL1.

**PilS-PilR-dependent tfpQ/I transcription in *E. coli** requires the rpoN gene product, σ54**. To verify that PilR-stimulated tfpQ/I transcription from tfpQ/Ip2 (Fig. 3) was dependent on Fig. 3. PilS-PilR activation of tfpQ/Ip2 transcription in *E. coli*. Primer extension assays were performed with RNA expressed from pMXL1 in the presence (pilS and pilR expressed from pAG1206) or absence (parent expression vector or no additional plasmid present) of PilS-PilR in *E. coli*. The p-pilin primer was used for the extensions and the sequencing of pMXL1. The bands marked S1 and S2 correspond to the start of the transcripts initiated at tfpQ/Ip1 and tfpQ/Ip2, respectively.
σ54, pMxL1 and pAG1206 were transformed into the rpoN mutant E. coli strain TH1 and its parent strain, YMC10. RNA from the resulting strains (DWH17 to DWH22) was used in primer extension assays for tfpQ/Ip transcription. As a control for RNA levels, primer extension assays were performed with the same RNA preparations to measure β-lactamase transcription from plasmid pMxL1. Figure 4 shows that PilS-PilR did not activate transcription from tfpQ/Ip2 in the absence of σ54. As was seen with DH5α (Fig. 3), no tfpQ/Ip2 transcripts were detected in the rpoN+ E. coli parent strain (YMC10) unless PilS-PilR was expressed. Primer extension assays with RNA from rpoN mutant and wild-type strains containing the vector pMPM-K3 and pMxL1 revealed no tfpQ/Ip2 transcripts; the primer extension products that were present may have represented early termination products of transcription from tfpQ/Ip1. Interestingly, in both rpoN mutant and wild-type strains containing PilS-PilR, it appeared that the presence of pAG1206 downregulated tfpQ/Ip1 transcription.

In Figure 5, the relative amounts of transcription from tfpQ/Ip1 and tfpQ/Ip2, determined from the primer extension assays in Figure 4, are shown normalized for RNA concentrations with the corresponding β-lactamase primer extension assays. This comparison of transcript levels indicated that transcription from tfpQ/Ip1 was decreased in strains in which PilS-PilR was present. Three of the potential PilR binding sites in the region upstream of tfpQ/Ip2 overlap tfpQ/Ip1 (Fig. 1); therefore, binding of PilR to the enhancer-like sequence for tfpQ/Ip2 may repress transcription from tfpQ/Ip1. A similar pattern of repression and activation by an activator protein regulates transcription from the σ70- and σ54-dependent promoters of the glnA (glutamine synthetase) gene in E. coli and Salmonella typhimurium (33; see Discussion). Figure 5 also shows that transcription from the tfpQ/Ip2 promoter was detected at significant levels only in the strain in which RpoN and PilS-PilR (pAG1206) were present.

Effect of growth conditions on tfpQ/I transcription. In an attempt to identify environmental factors that would influence tfpQ/I transcription, RNA was isolated from M. lacunata ATCC 17956 grown under a variety of conditions. To mimic conditions of the eye, bacteria were grown in 257 mM NaCl (1) and compared with bacteria grown in either 86 mM NaCl or no NaCl. The iron concentration was also reduced 10-fold, since there are lactoferrins in tears that limit iron accessibility (38). A low-glucose environment was created by reducing D-glucose from 22 to 5.6 mM, which was previously shown to decrease piliation in N. gonorrhoeae (18). The effects of 10% human AB serum and growth temperature (25 versus 37°C) were also assayed. Under all conditions tested, there were no significant changes in the levels of tfpQ/I transcription (data not shown).

DISCUSSION

In order to adjust to changing conditions in the progression of infection, many pathogenic bacteria regulate the expression of virulence factors in response to environmental signals and/or use programmed genetic alterations which provide selective advantages to a subgroup of the infecting population. Pili are important virulence factors for many bacteria, mediating adherence to host cells, defining tissue tropism, and providing a means of evading the host immune system by antigenic variation of the major pilin subunits. It has been demonstrated that type 4 pili are essential virulence factors for a number of
was mapped by primer extension assays to a binding site for the PilR activator protein from the blood, and chocolate agar (data not shown). When the activation of \( \text{tfpQ/I} \) was not used for \( \text{tfpQ/I} \) transcription levels were quantitated from the primer extension assays and \( \text{tfpQ/I} \) activation of \( \text{tfpQ/I} \) is active at low levels in a nitrogen-rich environment and is dependent on \( \sigma^70 \). The \( \text{tfpQ/I} \) promoter is activated at high levels when nitrogen is limited and is \( \sigma^74 \)-dependent. The response regulator protein (NtrC) activates transcription from \( \text{glnAp} \) when it is phosphorylated by the histidine kinase (NtrB) in response to a complex signal transduction system regulating nitrogen utilization. When phosphorylated NtrC binds the transcriptional enhancer sequence to activate \( \text{glnAp} \), transcription, it binds two sites overlapping the \(-35 \) sequence and transcriptional start site of \( \text{glnAp} \), thus repressing \( \text{glnAp} \) transcription. This similarity of the organization of the transcriptional regulatory elements for \( \text{tfpQ/I} \) and \( \text{glnA} \) is striking and suggests future directions for characterization of the regulation of \( \text{tfpQ/I} \) in \( \text{M. lacunata} \) and \( \text{M. bovis} \).

In summary, our results indicate that piv and \( \text{tfpQ/I} \) are not coordinately regulated. Although piv is transcribed from a \( \sigma^70 \)-dependent promoter, \( \text{tfpQ/I} \) is transcribed from a \( \sigma^74 \)-dependent promoter. With all other genes whose transcription is dependent on \( \sigma^74 \), \( \text{tfpQ/I} \) transcription from the \( \sigma^54 \)-dependent promoter requires an activator protein. Thus, the expression of the type 4 pili in \( \text{M. lacunata} \) and \( \text{M. bovis} \) is controlled by both a site-specific DNA inversion system that specifies which pilin gene will be transcribed (\( \text{tfpQ} \) or \( \text{tfpI} \)) and a regulatory system that controls the initiation of transcription of \( \text{tfpQ/I} \).

ACKNOWLEDGMENTS

We thank Steve Lory, Carl F. Marrs, Matthias Mayer, and the Centers for Disease Control, Atlanta, Ga., for providing plasmids and bacterial strains. We also thank Steve Lory and Timothy Hoover for helpful discussion and input. Finally, we thank June Scott, Timothy Hoover, and Charles P. Moran for critical review of the manuscript.

This work was supported by National Science Foundation Presidential Young Investigator award MCB-9396003 to A.C.G.

REFERENCES

7. Boyd, J. M., T. Koga, and S. Lory. 1994. Identification and characterization of pathogenic bacteria, including \( \text{M. bovis} \) (28, 35). Results from in vivo infections of calf eyes with \( \text{M. bovis} \) suggest that not only are type 4 pili essential for initiation of infection, but also switching between Q-type and I-type pili is important for the progression of disease (29). This switching is controlled by Piv-mediated site-specific DNA inversion of the chromosomal DNA segment containing the carboxyl-terminal regions of the Q- and I-type pilin genes (20, 21). Transcription of the recombinase gene, piv, and/or transcription of the pilin genes, \( \text{tfpQ/I} \), may provide another level of type 4 pilus regulation in \( \text{M. lacunata} \) and \( \text{M. bovis} \). The primary \( \text{tfpQ/I} \) transcript in \( \text{M. bovis} \) and \( \text{M. lacunata} \) was mapped by primer extension assays to a \( \sigma^54 \)-dependent promoter (\( \text{tfpQ/I} \)). When RNA was isolated from bacteria grown on sheep blood agar plates, this transcript was accompanied by a second, weaker transcript that initiated at an upstream putative \( \sigma^74 \)-dependent promoter (\( \text{tfpQ/I} \)). This \( \text{tfpQ/I} \) transcript was not seen under any other growth conditions, including growth on GC media, heart infusion agar with rabbit blood, and chocolate agar (data not shown). When the \( \text{M. lacunata} \) pilin genes were expressed in \( \text{E. coli} \), the \( \text{tfpQ/I} \) promoter was not used for \( \text{tfpQ/I} \) transcription, although transcripts that corresponded to the \( \text{tfpQ/I} \) promoter seen in \( \text{M. lacunata} \) were detected. The lack of a \( \text{tfpQ/I} \) transcript corresponding to the \( \text{tfpQ/I} \) promoter suggests that there are no \( \text{E. coli} \) homologs for the activator required for the \( \sigma^54 \)-dependent \( \text{tfpQ/I} \) transcription in \( \text{M. lacunata} \). Examination of the \( \text{tfpQ/I} \) promoter region revealed several potential binding sites for the PilR activator protein from the \( \text{P. aeruginosa} \) type 4 pilin system. In \( \text{E. coli} \), the \( \text{P. aeruginosa} \) sensor and activator proteins, PilS and PilR, were able to substitute for the missing \( \text{M. lacunata} \) factors and activate transcription from the \( \text{tfpQ/I} \) promoter. This activated transcription was abolished in an \( \text{rpoN} \) mutant \( \text{E. coli} \) strain, demonstrating that transcription is actually dependent on \( \sigma^54 \).

Another possible role for a PilR homolog regulating \( \text{tfpQ/I} \) transcription in \( \text{Moraxella} \) was indicated by the primer extension assays, which revealed a decreased level of \( \text{tfpQ/I} \) transcription in both wild-type and \( \text{rpoN} \) mutant \( \text{E. coli} \) strains in the presence of PilS-PilR. This result suggests that in \( \text{M. bovis} \) and \( \text{M. lacunata} \), the protein which serves as an activator for \( \text{tfpQ/I} \) may also block transcription from \( \text{tfpQ/I} \) when bound to a transcriptional enhancer sequence. This dual function as a repressor and an activator for a response regulator protein is seen in the regulation of transcription of the glnA \( \text{ntrBC} \) operon in \( \text{E. coli} \) and \( \text{S. typhimurium} \) (for a review, see reference 33). The expression of \( \text{glnA} \) is regulated from two promoters, designated \( \text{glnAp} \), and \( \text{glnAp} \), \( \text{glnAp} \), located 114 bases upstream of \( \text{glnAp} \), is active at low levels in a nitrogen-rich environment and is dependent on \( \sigma^70 \). The \( \text{glnAp} \) promoter is activated at high levels when nitrogen is limited and is \( \sigma^74 \)-dependent. The response regulator protein (NtrC) activates transcription from \( \text{glnAp} \) when it is phosphorylated by the histidine kinase (NtrB) in response to a complex signal transduction system regulating nitrogen utilization. When phosphorylated NtrC binds the transcriptional enhancer sequence to activate \( \text{glnAp} \), transcription, it binds two sites overlapping the \(-35 \) sequence and transcriptional start site of \( \text{glnAp} \), thus repressing \( \text{glnAp} \) transcription. This similarity of the organization of the transcriptional regulatory elements for \( \text{tfpQ/I} \) and \( \text{glnA} \) is striking and suggests future directions for characterization of the regulation of \( \text{tfpQ/I} \) in \( \text{M. lacunata} \) and \( \text{M. bovis} \).

FIG. 5. Comparison of levels of \( \text{tfpQ/I} \) and \( \text{tfpQ/I} \) promoters in \( \text{E. coli} \). \( \text{tfpQ/I} \) transcription levels were quantitated from the primer extension assays shown in Fig. 4, normalized for RNA concentrations with the \( \beta \)-lactamase primers in both wild-type and \( \text{rpoN} \) mutant \( \text{E. coli} \) strains in the presence and absence of PilS-PilR were compared.

Downloaded from http://jb.asm.org on November 7, 2017 by guest