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Regulation of the *Porphyromonas gingivalis fimA* (Fimbrillin) Gene

HUA XIE,[1]* WHASUN O. CHUNG,[2] YOONSUK PARK,[2] AND RICHARD J. LAMONT[2]

School of Dentistry, Meharry Medical College, Nashville, Tennessee,[1] and Department of Oral Biology, University of Washington, Seattle, Washington[2]

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In common with many bacterial virulence genes, the fimbrillin (*fimA*) gene of *Porphyromonas gingivalis* is modulated in response to environmental fluctuation. The *trans*-acting components that comprise the regulatory system for transcriptional activity of the *fimA* gene in *P. gingivalis* were investigated. Three major proteins were found to bind to the upstream region of the *fimA* promoter. One of these proteins was fimbrillin itself, and the other two were a major arginine protease (Rgp) and lysine protease (Kgp). Production of these proteins was necessary for maximal *fimA* transcription. An exogenous *fimA* promoter-*lacZ* reporter was inactive when introduced into a strain of *P. gingivalis* carrying a mutation in the indigenous *fimA* gene. Furthermore, *fimA* mRNA levels were significantly decreased in *rgp* and *kgp* mutant strains. These data indicate that *P. gingivalis* has evolved multiple levels of control of fimbrial gene expression to enhance its survival in hostile environments.

Periodontal diseases are a group of chronic inflammatory infections that affect millions of people worldwide and cause destruction of the periodontal tissues, eventually leading to exfoliation of the teeth. Periodontal diseases ensue following the establishment of a mixed microbial subgingival biofilm, and foremost among these pathogenic agents is the gram-negative anaerobe *Porphyromonas gingivalis* (25). A number of virulence factors contribute to the pathogenicity of *P. gingivalis*. These include proteolytic enzymes that degrade host tissue and inactivate immune effector molecules; hemagglutinins that target the cells towards hemin, a requisite iron source; and fimbriae that are required for attachment to oral surfaces such as epithelial cells and to antecedent plaque bacteria and for invasion of epithelial cells (4, 9, 11, 28). As the oral cavity is a continuously changing environment, successful colonizers such as *P. gingivalis* have the ability to sense and respond to environmental conditions. Regulation of proteases, hemagglutinins, superoxide dismutase, and fimbriae has been documented, primarily at the transcriptional level, although it has also been reported at the posttranscriptional level (2, 9, 11, 26). However, although regulation of virulence genes is known to occur, the nature of the regulatory proteins and pathways in *P. gingivalis* has not been defined.

To gain an understanding of the regulatory networks in *P. gingivalis*, we are studying the expression of fimbrillin, the monomeric subunit of the *P. gingivalis* major fimbriae. Previous work has shown that expression of the gene encoding fimbrillin (*fimA*) and subsequent fimbria-dependent phenotypic activity are tightly regulated by environmental cues (30). Expression of the *fimA* gene is maximal at a relatively lower temperature and higher hemin concentration compared to normal growth conditions. In contrast, serum and salivary molecules inhibit *fimA* promoter activity. More-detailed site-specific mutagenesis experiments suggested that the *fimA* upstream region has a σ^{70}-recognized RNA polymerase binding site along with an upstream element and a site determining positive regulation (31). Thus, we have proposed that *trans*-acting element(s) are involved in positive regulation of expression of the *fimA* gene in *P. gingivalis* by interacting with the *fimA* promoter region. The objective of this study was, therefore, to identify the proteins involved in regulation of the *fimA* gene. The results indicate that *fimA* expression is regulated by fimbrillin itself and by two major proteases of *P. gingivalis*.

* Corresponding author. Mailing address: School of Dentistry, Meharry Medical College, Nashville, TN 37208. Phone: (615) 327-5981. Fax: (615) 327-2989. E-mail: hxie@mail.mmc.edu.

MATERIALS AND METHODS

Bacterial stains, vectors, and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. *P. gingivalis* strains were grown in Trypticase soy broth (Becton Dickinson) or on 1.5% Trypticase soy broth agar plates, supplemented with yeast extract (1 mg/ml; Difco), hemin (5 μg/ml), and menadione (1 μg/ml), at 37°C in an anaerobe chamber (85% N_2, 10% H_2, 5% CO_2). Antibiotics—erythromycin (20 μg/ml) and gentamicin (100 μg/ml)—were used where appropriate. *Escherichia coli* DH5α was used as the host strain for recombinant plasmids and grown in L broth with antibiotics—ampicillin (100 μg/ml), kanamycin (50 μg/ml), trimethoprim (200 μg/ml), and tetracycline (10 μg/ml) when necessary.

Purification of sequence-specific DNA binding proteins. To purify *P. gingivalis* proteins binding to the regulatory sequences upstream from the *fimA* promoter, we utilized biomagnetic beads (Dynal, Oslo, Norway) according to the manufacturer's instructions. Briefly, the *P. gingivalis* 33277 *fimA* promoter region with a deletion of the RNA polymerase −35 binding site (31) was amplified by PCR. The template was plasmid MP58, which carries the mutated *fimA* promoter (31). The primers used were 5′-GGAATTCCGACGCTATATGCAAGACAA-3′ and 5′-TGTAACGGGTTCTGCCTCGT-3′, which was biotinylated at the 5′ end to facilitate binding to streptavidin-coated magnetic (M-280) beads. PCR mixtures contained 10 pmol of template DNA, 30 pmol of each primer, 1.5 mM of $MgCl_2$, a 10 mM concentration of each deoxynucleoside triphosphate (dNTP) and 5 U of *Taq* DNA polymerase (Gibco-BRL). The amplification was performed in a thermal cycler (Techne) at 94°C for 45 s, 42°C for 1 min, and 72°C for 1 min for a total 30 cycles, followed by 10 min of elongation at 72°C. The PCR product was 166 bp (designated F_{166}) and was immobilized on magnetic beads through a streptavidin-biotin interaction. A cell extract of *P. gingivalis* was prepared from the cells grown at 37°C to late log phase and disrupted by sonication. The extract was partially purified on a DEAE-cellulose column (Pharmacia Biotech) prior to reaction with F_{166}-coated beads. The reaction mixture was separated in a magnetic field and washed, and specific DNA binding proteins were eluted with 1 M NaCl. Finally, the DNA binding proteins were resuspended in 10 mM Tris buffer by passing through CentriSpin-20 (Princeton Separations, Inc), and visualized by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels (7) stained with Coomassie blue. As a control, beads were coated with *fimA* promoter region from which the regulatory sequences were deleted. Template plasmid pMP591 was amplified by PCR with primers MP150 (31), 5′-GCTATGGTGTTGTTGGGTTGCATATTCA-3′, and biotin- 5′-TGTAACGGGTTCTGCCTCGT-3′. The PCR product was 103 bp (designated F_{103}) and contains deletions from position −240 to −90, position −85 to −71, and position −64 to −48.

Recombinant protein, antisera, and immunoblotting. Recombinant fimbrillin (rFim) was produced by PCR amplification of the *fimA* coding sequences from *P. gingivalis* 33277 chromosomal DNA (6) and cloning into the pET30 expression system (Novagen). Following induction in *E. coli*, rFim was purified by chromatography over a Ni^{2+} metal chelation resin and elution with imidazole. Upstream vector-derived sequences were then removed by cleavage with enterokinase. rFim is full-length mature fimbrillin without the leader amino acid sequence.

TABLE 1. Bacterial strains and plasmids used and constructed in this study

Strain or plasmid	Relevant characteristic(s)[a]	Source or reference
Strains		
P. gingivalis 33277	Type strain from ATCC	This laboratory
P. gingivalis UPF	Derivative of 33277, *fimA*$_p$::*lacZ* gene, Em^r	31
P. gingivalis MPF35A	Derivative of *P. gingivalis* 33277, *mpf35*::*fimA*, *fimA*$_p$::*lacZ* gene, Em^r	This study
P. gingivalis MP150A	Derivative of *P. gingivalis* 33277, *mp150*::*fimA*, *fimA*$_p$::*lacZ* gene, Em^r	This study
P. gingivalis MPS10A	Derivative of *P. gingivalis* 33277, *mps10*::*fimA*, *fimA*$_p$::*lacZ* gene, Em^r	This study
P. gingivalis YPF1	FimA-deficient mutant of 33277, Em^r	13
P. gingivalis YPP1	RgpA-deficient mutant of 33277, Em^r	23
P. gingivalis YPP2	Kgp-deficient mutant of 33277, Em^r	23
E. coli DH5α	*endA1 hsdR17 supE44 thi-1 recA gyrA96 relA1* Δ(*lacZYA-argF*) *U169*λ-ϕ80 *dlacZ* ΔM15, recipient for recombinant plasmids	Gibco-BRL
Plasmids		
pJRD215	Wide-host-range cosmid vector, Km^r Sm^r Mob$^+$, unable to replicate in *P. gingivalis*	22
Tn*4351*	Contains a 3.8-kb *Eco*RI fragment from pBF4, Tc^r expressed in *E. coli*, Em^r expressed in *P. gingivalis*	24
R751	IncP plasmid used to mobilize vectors from *E. coli* to *Bacteroides* recipient Tp^r Tra$^+$	22
pMP58	*fimA*$_p$::*lacZ* gene in pUC19 with a 16-bp deletion from −23 to −8 (*mp*58::*lacZ*), Am^r	31
pMPF35	*mpf35*::*lacZ* gene in pJRD215, three-base change from TTG to CCA at position −46 to −44, Km^r	31
pMP150	*mp150*::*lacZ* gene in pJRD215, 150-bp deletion from −240 to −90, Km^r	31
pMPS10	*mps10*::*lacZ* gene in pJRD215, three-base change from TAA to GCC at position −11 to −9, Km^r	31
pMP591	*mp150*::*lacZ* gene in pJRD215, 15-bp deletion from −85 to −71 and 17-bp deletion from −64 to −48, Km^r	31

[a] Abbreviations: Km^r, Sm^r, Tc^r, Em^r, and Tp^r, resistance to kanamycin, streptomycin, tetracycline, erythromycin, and trimethoprim, respectively; Mob$^+$, can be mobilized; Tra$^+$, capable of self transfer; *fimA*$_p$, *fimA* promoter.

Monospecific rabbit antibodies to rFim were produced by Covance Inc., Princeton, N.J. For blotting, proteins were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. Antibodies to rFim or *P. gingivalis* cells (1:10,000) were used as the probe with peroxidase-conjugated secondary antibodies (1:3,000). Antigen-antibody binding was developed with 0.05% diaminobenzidine tetrahydrochloride.

Mobility shift DNA-binding assay. Mobility shift DNA-binding assays were conducted by using the Bandshift kit (Pharmacia Biotech), as previously described (31). The F_{166} PCR product containing the *fimA* upstream region was used as probe. Briefly, the DNA fragment was labeled with [α-^{32}P]dATP (3,000 μCi/mmol; NEN) using the Klenow fragment. For the protein-DNA reaction, 1 μg of ^{32}P-labeled DNA and 1 μg of protein were mixed and incubated at room temperature for 20 min. The mixture was then loaded onto a 5% nondenaturing polyacrylamide gel and electrophoresed in 0.5 Tris-borate-EDTA buffer at 10 V/cm. Finally, the gel was dried and exposed to X-ray film at −70°C.

Construction of *fimA* mutant strains. Site-specific mutations in the *fimA* upstream region were generated by a unique-site elimination mutagenesis kit (Pharmacia Biotech) as described in detail previously (31). Three mutant alleles of the *fimA* promoter were created and fused with a *lacZ* gene. One mutation (MPF35) was located in the −35 RNA polymerase binding site (−46 to −44), with a 3-bp substitution (TTG to CAA), and the second (MP150) carried a deletion from position −240 to −90, a region in which we have detected positive regulatory sequences (31). The third (MPS10) was a 3-bp substitution (TAA to GCC) at position −11 to −9, a region that is not involved in promotion of *fimA* transcription. The mutant alleles were then conjugated into *P. gingivalis* 33277 and integrated to the chromosomal DNA by a single crossover (Fig. 1). The

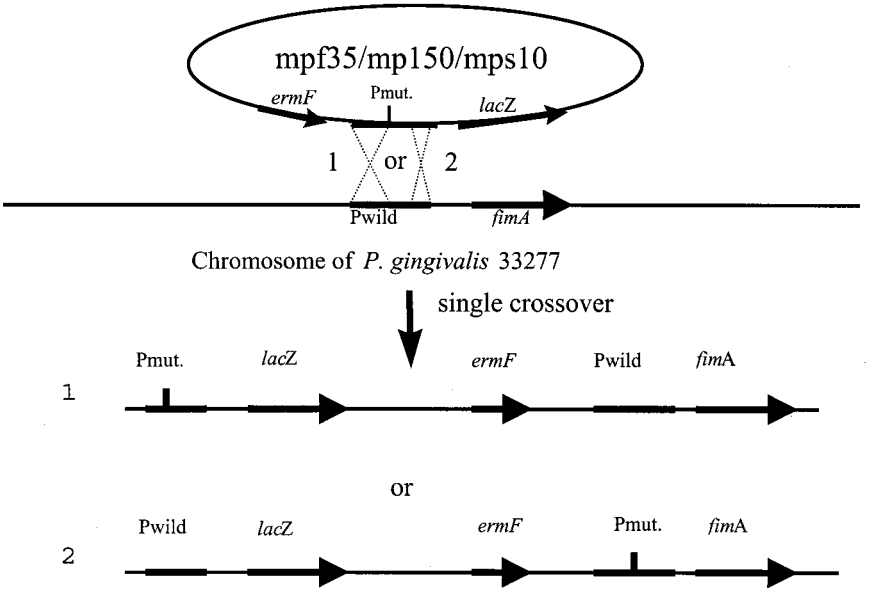

FIG. 1. Introduction of the mutated *fimA* promoter into *P. gingivalis* chromosomal DNA. Two genomic configurations can occur after a single crossover between plasmid MPF35 MPF150, or MPS10 and chromosomal DNA. In configuration 1, the crossover occurs upstream of the mutation. In configuration 2, the recombination occurs downstream of the mutation. Pmut. and Pwild represent the mutated and wild-type *fimA* promoter, respectively; *ermF* is an erythromycin resistance gene. Configuration 2 was selected by examination by PCR with primer pairs designed for Pmut. and *fimA* and for Pwild and *lacZ*.

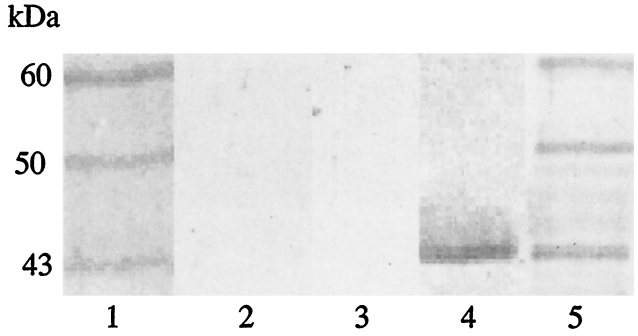

FIG. 2. Proteins binding to the *fimA* upstream region. Proteins were eluted from magnetic beads conjugated with F_{166} (lanes 1, 3, 4, and 5) or with F_{103} (lane 2) and separated by SDS-PAGE. Coomassie-stained proteins from strains 33277 are shown in lanes 1 and 2. Lane 3 contains a Coomassie stain of strain YPF1 (FimA deficient). Lanes 4 and 5 are immunoblots of 33277 eluted proteins, developed with antifimbrillin or anti-*P. gingivalis* antibodies, respectively. Sizes are indicated.

authenticity of the mutated sequence was verified by DNA sequencing and PCR analysis with specific pairs of primers for each mutation (31).

RT-PCR. The level of *fimA* mRNA expression was tested in the *rgpA* and *kgp* null mutants YPP1 and YPP2 (23). The oligonucleotides for the *fimA* gene were as follows: $fimA_1$, 5′-AATCGTGCTTTTGGAGTTGG-3′; $fimA_2$, 5′-ACCAAC GAGAACCCACTCAG-3′. Reverse transcription was performed in the presence of 2 μg of total RNA, 50 ng of reverse primer, 50 U of reverse transcriptase (RT) (Ambion), 13 U of RNase inhibitor, a 10 mM concentration of each dNTP, and 1× RT buffer. The reaction was carried out at 72°C for 2 min and then at 48°C for 1 h. Controls without RT were included in all experiments. The resulting cDNA was amplified, with each 100 μl of PCR mixture containing 1× PCR buffer, 3 μl of cDNA, 1.5 mM $MgCl_2$, a 10 mM concentration of each dNTP, 100 ng of each primer, and 2.5 U of *Taq* DNA polymerase. The amplification conditions were denaturation at 96°C for 30 s, annealing at 45°C for 30 s, and elongation at 72°C for 2 min. The expected size of the *fimA* PCR product was 1 kbp. As a control, an unrelated gene (an open reading frame demonstrating homology to the *luxS* gene) was also amplified.

Protein sequencing. The DNA binding proteins eluted from magnetic beads were separated by SDS–10% PAGE. Following visualization with Coomassie blue, proteins were excised from the gel. Tryptic digestion followed by high-pressure liquid chromatography separation and Edman degradation were performed at the Biotechnology Center at the Fred Hutchinson Cancer Research Center, Seattle, Wash.

β-Galactosidase assays. Expression of the *lacZ* gene under control of the *fimA* promoter was measured by the standard spectrophotometric β-galactosidase assay with *o*-nitrophenyl-β-D-galactopyranoside as the substrate, as described by Miller (17). *P. gingivalis* strains were recovered from late log phase and tested at an optical density at 600 nm of 0.4 to 0.6.

RESULTS

Interaction between *P. gingivalis* proteins and the *fimA* promoter. It is well documented that regulatory proteins can bind to specific sites in the upstream regions of genes, and this, in turn, influences the function of RNA polymerase (3). To search for regulatory proteins involved in *fimA* expression, we used a magnetic bead-bound DNA fragment of the *fimA* upstream region (F_{166}) as bait for potential DNA-binding proteins from a cell extract of *P. gingivalis* 33277. After elution from the *fimA* promoter and visualization by SDS-PAGE, three major *fimA* binding proteins were observed, with molecular masses of approximately 60, 50, and 43 kDa (Fig. 2, lane 1). None of these proteins were recovered after incubation of the cell extract with magnetic beads conjugated with F_{103}, the *fimA* upstream region from which the regulatory sequences were deleted (Fig. 2, lane 2). Furthermore, extract from the *fimA* null mutant, YPF1, did not bind to F_{166} (Fig. 2, lane 3). Western blot analysis with anti-FimA antibody indicated that the 43-kDa band was fimbrillin (Fig. 2, lane 4). Antibodies to *P. gingivalis* cells revealed additional fainter bands in the 45- to 49-kDa region (Fig. 2, lane 5).

The identity of the major binding proteins was further investigated by amino acid sequencing. Three trypsin-digested peptide fragments from the 43-kDa band matched the fimbrillin amino acid sequence (Fig. 3A). Furthermore, a peptide derived from the 60-kDa peptide demonstrated 100% homology to the arginine-specific cysteine protease, Rgp (Fig. 3C). Thus, this molecule could be either RgpB or a posttranslational processed fragment of RgpA (4). Four of the peptides

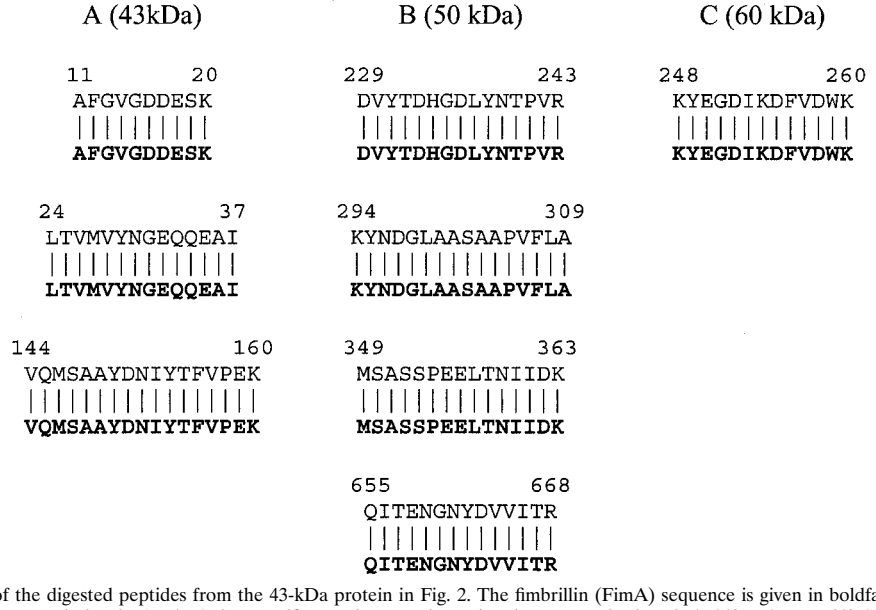

FIG. 3. (A) Sequences of the digested peptides from the 43-kDa protein in Fig. 2. The fimbrillin (FimA) sequence is given in boldface letters. (B) Sequences of the peptides from the 50-kDa protein in Fig. 2. The lysine-specific cysteine proteinase (Kgp) sequence is given in boldface letters. (C) Sequence of the peptide from the 60-kDa protein in Fig. 2. The Gingipain R2 (RgpB) sequence is given in boldface letters. Numbers represent amino acid residues from sequences deposited in GenBank (accession numbers: FimA, BAA86887; Kgp, AAC26523; RgpB, A55426).

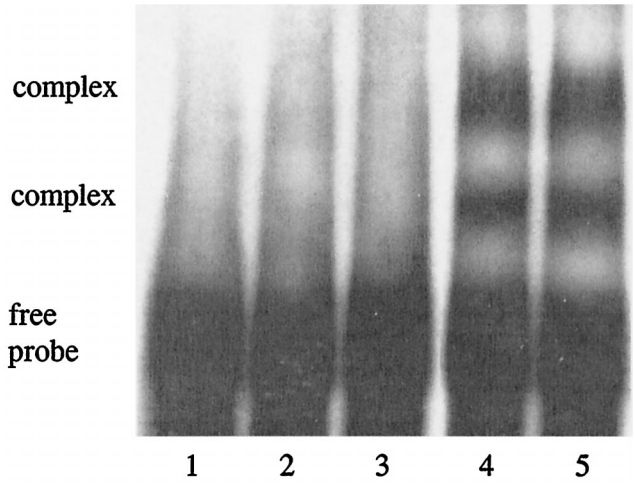

FIG. 4. Mobility shift DNA binding assay. Lane 1, *fimA* upstream fragment (F_{166}) only; lane 2, *fimA* promoter fragment and 1 μg of purified fimbrillin; lane 3, *fimA* promoter, the 33277 proteins eluted from magnetic beads, and 250 pmol of unlabeled F_{166}; lane 4, *fimA* promoter and the 33277 eluted proteins from magnetic beads; lane 5, *fimA* promoter, the 33277 eluted proteins from magnetic beads, and 3 μg of calf thymus DNA.

from the 50-kDa protein were 100% homologous to the lysine-specific cysteine protease, Kgp (Fig. 3B).

Mobility shift. To test the ability of FimA to bind directly to the *fimA* promoter region, a bandshift experiment was performed (Fig. 4). Proteins eluted from the magnetic beads interacted with the *fimA* promoter, resulting in the formation of DNA-protein complex that halted movement of the DNA fragment (F_{166}) (Fig. 4, lane 4). Mobility shift occurred in the presence of unrelated DNA (Fig. 4, lane 5), but was competitively inhibited by excess unlabeled F_{166} (Fig. 4, lane 3). The bandshift profile indicated more than one mobility change, which was probably due to instability of the multiple protein-DNA complex during the experimental process. Purified fimbrillin alone failed to associate with the *fimA* promoter (Fig. 4, lane 2), suggesting that fimbrillin may not bind to its own promoter directly but may associate with the promoter through other regulatory proteins. Proteases could function in this capacity; however, this appears unlikely, as in the absence of FimA the proteases could not be recovered from F_{166} (Fig. 2). Minor binding proteins visualized by Western blotting with whole *P. gingivalis* antibodies (Fig. 2) are also potential candidates, and work is under way to resolve this issue.

Effect of *fimA* mutations on *fimA* promoter activity. Based on the evidence provided by the binding assays, we predicted that fimbrillin serves as a regulatory protein in its own expression. To further test this hypothesis, we generated *P. gingivalis* strains containing a *fimA* promoter-*lacZ* reporter chromosomal fusion along with a mutation in the indigenous *fimA* promoter. Thus, these strains contain two *fimA* promoters, with one driving the *fimA* structural gene and the other promoting transcription of the *lacZ* gene. Insertion of the *fimA*::*lacZ* construct does not alter expression of *fimA* mRNA or levels of FimA protein (31). The resulting strains, MPF35A and MP150A, contain mutations at the −35 region (3-bp substitution) and the upstream regulatory sequence (150-bp deletion), respectively. These strains allow us to examine the influence of loss of production of FimA protein on *fimA* promoter activity (as reported by *lacZ* activity). RT-PCR confirmed both that *fimA* mRNA is either not produced (MPF35A) or significantly reduced (MP150A) and that there was no transcriptional read-through from the *ermF* gene (not shown). As shown in Table 2, *lacZ* activity decreased by around 40% in strain MP150A and to background levels in MPF35A. As an additional control, a mutation was introduced into a region upstream of the *fimA* structural gene and distal to the −10 site (MPS10A). This mutation does not affect promoter activity (31), and the resulting strain possessed wild-type levels of *fimA* mRNA and did not have reduced β-galactosidase activity (Table 2).

Transcription of *fimA* in *rgp* and *kgp* mutants. As the data indicated that the major cysteine proteases of *P. gingivalis* are also involved in regulation of *fimA* expression, loss of protease expression should cause downregulation of *fimA*. Thus, we examined *fimA* mRNA levels in *P. gingivalis* strains YPP1 (which contains an insertional inactivation of the *rgpA* gene) and YPP2 (which contains an insertional inactivation of the *kgp* gene) (23) by RT-PCR (Fig. 5). Expression of *fimA* mRNA was significantly reduced in these mutants compared to wild type. In contrast there were no differences in expression of a *luxS*-like gene that was identified by a homology search of the *P. gingivalis* genome database (http://www.tigr.org).

DISCUSSION

It is becoming increasingly apparent that fine control over the expression of bacterial virulence determinants is required for successful bacterial colonization and disease progression. Such control can be the outcome of complex and regulatory networks that sense the prevailing environmental conditions and transduce information to modulate gene expression (16). *P. gingivalis* is well adapted to life in the subgingival environment and regulates gene expression in response to orally relevant parameters (2, 9, 11, 26). However, the signal transduction pathways and regulatory mechanisms in this strict anaerobe are not well understood. Evidence provided here suggests that several well-known proteins of *P. gingivalis* are members of a *fimA* regulatory system. These include fimbrillin itself, along with an arginine-specific cysteine protease (Rgp) and a lysine-specific cysteine protease (Kgp). These proteins could bind to, and cause a mobility shift of, the *fimA* upstream region. Site-specific mutagenesis also showed that the wild-type *fimA* promoter was maximally activated only when the *fimA* structural gene was fully expressed. Indeed, a 3-bp replacement in the −35 region of *fimA* alone was sufficient to repress transcription from the intact *fimA* promoter in a reconstituted *lacZ* gene fusion system. Furthermore, transcriptional activity of *fimA* was

TABLE 2. Effect of *fimA* promoter mutations ($fimA_{MP}$) on the expression of *fimA*::*lacZ* fusion genes

P. gingivalis strain	Promoter[a]	Fusion	β-Galactosidase level[b]
UPF	$fimA_{WT}$	$fimA_{WT}$::*lacZ*	260 ± 9
MP150A	$fimA_{MP150}$	$fimA_{WT}$::*lacZ*	158 ± 10
MPF35A	$fimA_{MPF35}$	$fimA_{WT}$::*lacZ*	17 ± 1
MPS10A	$fimA_{MPS10}$	$fimA_{WT}$::*lacZ*	300 ± 16

[a] $fimA_{WT}$ represents wild-type promoter; $fimA_{MP150}$ represents the *fimA* promoter with a 150-bp deletion of positive regulatory sequence spanning position −240 to −90 of the *fimA* gene; $fimA_{MPF35}$ represents the *fimA* promoter with a substitution mutation, from TTG to CCA, at position −46 to −44, affecting the −35 RNA polymerase binding site; $fimA_{MPS10}$ represents the *fimA* promoter with a substitution mutation, from TAA to GCC, at position −11 to −9, which does not affect promoter activity.

[b] Miller units obtained from *P. gingivalis* grown at 37°C. Means ± standard deviations are indicated ($n = 3$).

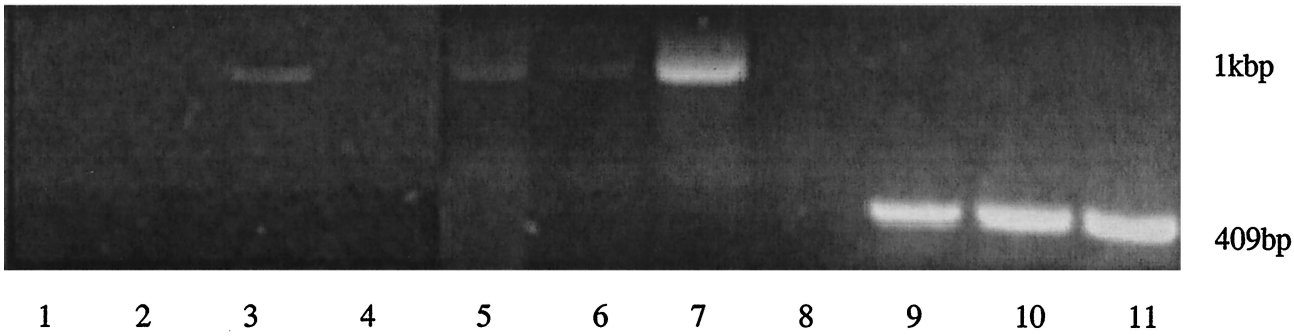

FIG. 5. RT-PCR of *fimA* mRNA in YPP1 and YPP2 protease-deficient mutants. Lanes 1, 5, and 9, YPP1; lanes 2, 6, and 10, YPP2; lanes 3, 7, and 11, 33277; lanes 4 and 8, negative controls without RT. Lanes 1 to 4, cDNA amplified for 30 cycles with *fimA* primers; lanes 5 to 8, amplified for 40 cycles with *fimA* primers; lanes 9 to 11, amplified for 35 cycles with control primers for a *luxS*-like gene.

significantly reduced in mutants lacking expression of either RgpA or Kgp. Thus, the presence of all three of these proteins appears to be necessary for maximal expression of *fimA*. Whether these proteins assemble prior to or after DNA association remains to be determined, and it is possible that the observed participation of the proteolytic enzymes is a consequence of their preexisting association with prefimbrillin in a processing capacity. In either event a role for additional direct DNA-binding proteins is suggested. Work is currently under way to identify these molecules.

The phenomenon of autoregulation is well established for several bacterial genes. Liberek and Georgopoulos (12) reported that in *E. coli*, the heat shock genes, *dnak*, *dnaJ*, and *grpE*, were negatively autoregulated, and mutations in any one of these genes could lead to their own constitutive expression. In some studies the mechanisms of gene autoregulation were revealed. The simplest autoregulation pathway involves the gene products serving as activators or repressors by binding to the promoter region of the same gene (5). An alternative pathway involves autoregulation at the translational level, whereby the protein can bind to its own mRNA (15). Autoregulation at a posttranscriptional level is unlikely in *P. gingivalis*, since an operon fusion was used in our experimental system. Autoregulation affords bacteria an additional level of genetic control and thus facilitates fine-tuning of protein expression. In the case of *P. gingivalis* fimbrillin, this may be important in optimizing protein levels during the transition from colonization to initiation of disease. Fimbriae are essential mediators of adherence and invasion and are thus necessary for colonization of the organism (1, 8, 9, 10, 29). However, fimbriae are also potent inducers of immune cell function (19, 20) and hence may be detrimental to the organism when it engages in a more intimate interaction with periodontal tissues. The ability to amplify down- and upregulation signals may thus provide *P. gingivalis* with a selective advantage in mixed microbial oral biofilms.

P. gingivalis is an asaccharolytic organism and utilizes a number of proteolytic enzymes to provide peptides for growth. These proteinases also have a variety of effects with relevance to pathogenicity, including tissue destruction and inactivation of effector molecules of the immune system (4, 28). Previous studies have also reported a role for proteases in the fimbriation of *P. gingivalis*. Mutants that are defective in Rgp production possess very few fimbriae on their cell surfaces (18). These observations have been explained on the basis of posttranslational processing, since proteases can cleave the N-terminal amino acid (leader peptide) of FimA protein in vitro (21). However, reports vary on whether fimbriation is affected in single *rgpA* or *kgp* mutants, or whether a double *rgpAB* mutation is required to reduce fimbrillin production. For example, Nakayama et al. (18) showed that an *rgpA-rgpB* double mutant possessed very few fimbriae on the cell surface, whereas fimbriation was normal in an *rgpA* single mutant. In contrast, Tokuda et al. (27) demonstrated that an *rgpA* single mutant did not express detectable levels of the FimA protein as determined by Western blotting, nor were fimbriae visible following electron microscopy of the cells. Moreover, the *rgpA* single mutant in the latter study had reduced expression of *fimA* mRNA. Our observations are more consistent with those of Tokuda et al. (27) and demonstrate that individual proteases can play a direct role in fimbrillin production at the transcriptional level. Linkage of fimbrillin expression to proteolytic activity may allow coordination of activity of a number of the molecules that drive the pathophysiology of the organism.

The fimbriae of *P. gingivalis* contribute significantly to virulence. Indeed, fimbria-deficient mutant cells are less pathogenic in animal models (14) and are unable to form biofilms (Y. Park, J. W. Costerton, G. S. Cook, D. R. Demuth, and R. J. Lamont, Abstr. 77th Meet. Int. Assoc. Dent. Res., abstr. 2514, 1999). *P. gingivalis* fimbriae do not exhibit homology to other gram-negative fimbriae, either in terms of sequence or chromosomal arrangement, and may represent a unique class of fimbrial structure (6). An understanding of the mechanism of transcriptional control of fimbria production should provide important insights into the basis of the pathogenicity of this important periodontal pathogen.

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