Essential Role for the *gtfA* Gene Encoding a Putative Glycosyltransferase in the Adherence of *Porphyromonas gingivalis*

Masahiro Narimatsu,* Yuichiro Noiri, Shousaku Itoh, Nobuo Noguchi,
Takashi Kawahara, and Shigeyuki Ebisu

*Department of Restorative Dentistry and Endodontology, Osaka University Graduate
School of Dentistry, Suita, Osaka 565-0871, Japan*

***Porphyromonas gingivalis*, an oral bacterium, might play a role in the pathogenesis or progression of adult periodontitis. In this study, we isolated from *P. gingivalis* a putative glycosyltransferase gene, designated *gtfA*, which had a consensus domain for glycosyltransferase in its N terminus. GtfA consisted of 248 amino acids and its predicted molecular mass was 28 kDa; however, as the molecular mass of endogenous GtfA protein was around 40 kDa, this suggested that GtfA had undergone some posttranslational modifications. To reveal the role of the *gtfA* gene in *P. gingivalis*, we established *gtfA*-deficient strains by allelic replacement. Morphologically, *gtfA*-deficient *P. gingivalis* lacked mature fimbriae. *gtfA*-deficient *P. gingivalis* also showed a very low ability for autoaggregation, and its ability to attach to epithelial cells was severely impaired. Thus, the results indicate that the *gtfA* gene is required for *P. gingivalis* autoaggregation as well as attachment to epithelial cells. These results suggest that GtfA might have an important role in the pathogenicity of *P. gingivalis* by regulating adhesion.**

Oral bacterial communities, called biofilms, are composed of numerous genetically distinct types of bacteria that exist close to host surfaces such as enamel or oral epithelia (14). For example, more than 300 species of bacteria may exist in the subgingival area (18), which suggests the complexity of oral bacterial communities. Among the flora, several anaerobes are considered to be associated with human periodontitis. In particular, *Porphyromonas gingivalis*, a black-pigmented bacterium, has been implicated as playing an important role in the pathogenesis of periodontitis (19). *P. gingivalis* possesses a variety of virulence factors, such as secreted proteases, that can act directly on periodontal tissues. These can induce the degradation of host tissues and the activation of host proenzymes (18), and the virulence of these proteases has been revealed by using mutant strains (7, 28, 29). In addition, there are other factors that might have indirect effects by regulating host reactions. For example, *P. gingivalis* lipid A, a bioactive center of lipopolysaccharide, activates toll-like receptor 4- and MyD88-dependent pathway (26). Another study has shown that *P. gingivalis* lipopolysaccharide also activates toll-like receptor 2 (12). Thus, recent intensive studies have revealed the roles of these potent virulent factors.

Another major virulence factor of *P. gingivalis* is its ability to interact with a variety of surfaces (18). *P. gingivalis* can interact with various microbial cells (10, 11, 13, 17), saliva components (2), host epithelial cells (6), and extracellular matrices (22).

Furthermore, *P. gingivalis* can enter epithelial cells (16); this ability might play an important part in the penetration of periodontal tissues. *P. gingivalis* is detected throughout human periodontal pockets (25). At the bottom of the periodontal pocket, the so-called plaque-free zone, *P. gingivalis* adheres to root surfaces and is covered with a glycocalyx-like structure (24). Recent studies have also indicated that *P. gingivalis* has the ability to form biofilms in vitro on various surfaces (4, 20). We believe that this might be an important aspect of the pathogenicity of *P. gingivalis*.

In several bacteria, sugar metabolites or glycoconjugates can act as bacterial adhesins, and glycosyltransferases participate in the synthesis of these products as well as in the adherence of bacteria to other surfaces. For example, it is well known that an open reading frame (ORF) in the *ica* locus, involved in the synthesis of polysaccharide intercellular adhesin, exhibits *N*-acetylglucosaminyltransferase activity in *Staphylococcus epidermidis* (9). In *Escherichia coli* K-12, *rfaG*, encoding a glycosyltransferase, is required for adhesion to polystyrene plates as well as for lipopolysaccharide synthesis (8). Thus, glycosyltransferases might have important roles in bacterial adhesion, but little is known about their role in *P. gingivalis*.

In this study, we isolated a putative glycosyltransferase gene, designated *gtfA*. The GtfA protein consists of 248 amino acids and has a glycosyltransferase domain at its N terminus. By homologous recombination, we established *gtfA*-deficient mutants, which lacked mature fimbriae and showed reduced abilities for autoaggregation and attachment to epithelial cells. In addition, the ability to attach to extracellular matrices, including type I collagen, was impaired in the *gtfA*-deficient mutants. Thus, the *gtfA* gene regulates attachment ability and might be involved in colonization of oral surfaces.

* Corresponding author. Mailing address: Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, 1-8, Yamada-oka, Suita, Osaka 565-0871, Japan. Phone: 81-6-6879-2927. Fax: 81-6-6879-2927. E-mail: narimatu@dent.osaka-u.ac.jp.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *P. gingivalis* 381 was cultured anaerobically (90% N_2, 5% CO_2, 5% H_2) at 37°C in GAM broth (Nissui Pharmaceutical, Tokyo, Japan) or on 5% sheep blood agar plates (Trypticase soy agar; Becton Dickinson) supplemented with hemin (5 μg/ml) and menadione (1 μg/ml). *Escherichia coli* DH5α and BL21 were cultured in Luria-Bertani (LB) broth or on LB agar plates containing ampicillin (100 μg/ml) at 37°C.

Isolation of the *gtfA* gene. For isolation of putative glycosyltransferase genes, the *P. gingivalis* genomic database was searched with the BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). As a query, we used pfam00535, a consensus sequence of type 2 glycosyltransferases, which transfer sugar from nucleotide-sugar conjugates to various substrates (3). We designated one of the genes encoding a putative glycosyltransferase *gtfA*. We found that the locus name for the *gtfA* ORF was PG0750 in the TIGR database (http://www.tigr.org). For cloning of genomic fragments containing the ORF for *gtfA* from *P. gingivalis* 381, PCR was performed with specific primers 5′-GCC TCT TTG TGC CGG TAT CGA C-3′ and 5′-TTT GTA GGA CTT TGT GAC CCG G-3′, based on the genomic sequence from *P. gingivalis* W83. The PCR product was subcloned into the pGEM-T easy vector (Promega) and designated pGEM-T-GtfA.

Generation of *gtfA*-deficient *P. gingivalis*. For construction of a targeting vector, an *ermF-ermAM* cassette encoding an erythromycin resistance gene was excised from pYKP009 with SacI and PstI (15). Then, the fragment was blunted by T4 DNA polymerase and inserted into the EcoRV site of pGEM-T-GtfA, resulting in pGEM-T-GtfA-ermF-ermAM, which was linearized with SacI, and 2 μg of the linearized vector was introduced into *P. gingivalis* by electroporation as described previously (7). After electroporation, cells were incubated in GAM broth for 16 h, then plated onto 5% sheep blood agar plates containing erythromycin (10 μg/ml), and incubated for 7 days. Erythromycin-resistant colonies were inoculated into GAM broth, and genomic DNA was prepared from these clones. NcoI-digested DNA was subjected to Southern blotting to isolate the clones that had undergone homologous recombination, and these were designated *P. gingivalis* RE1. Two independent clones were used for the following experiments to confirm the phenotype. A digoxigenin-labeled probe was made with the EcoRI fragment of pGEM-T-GtfA and a digoxigenin High Prime kit (Roche) according to the manufacturer's recommendations. The hybridized probe was detected with an alkaline phosphatase-conjugated antidigoxigenin antibody and CSPD (Roche).

Preparation of antiserum against GtfA and Western blotting. For construction of an expression vector that expresses GtfA protein with a 6x His tag in its N terminus, a fragment containing the ORF for GtfA was amplified by PCR with pGEM-T-GtfA as the template. The sequences of the primers used were 5′-GA*G GAT CC*G AAG AGA ACA AAC CTG TTT TTT CGA-3′ and 5′-TTT GTA GGA CTT TGT GAC CCG G-3′; the BamHI site is in italics. The PCR product was subcloned into pGEM-T-Easy, and then the BamHI-SalI fragment was ligated into the BamHI and SalI sites of pQE80L (Qiagen, Hilden, Germany), resulting in pQE80L-GtfA. *E. coli* BL21 was used as the host strain for pQE80L-GtfA, and the 6x His-tagged GtfA protein was purified with Ni-nitrilotriacetic acid-agarose (Qiagen) under denaturing conditions. After dialysis with phosphate-buffered saline (PBS), 6x His-tagged GtfA protein was used as the antigen to obtain polyclonal antiserum from rabbits. Immunization was performed at Medical & Biological Laboratories Co., Ltd., Nagoya, Japan.

For Western blotting, 10^8 *P. gingivalis* cells were suspended in Laemmli's sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer and boiled for 5 min. The eluted proteins were separated on a 4 to 20% gradient SDS-polyacrylamide gel. After transfer to polyvinylidene difluoride membranes (Immobilon P; Millipore), they were incubated with 1:2,000-diluted anti-GtfA antiserum or 1:2,000-diluted antifimbria antiserum, which recognizes FimA (30), at 4°C overnight. Membranes were washed with Tris-buffered saline containing 0.1% Tween 20 and then incubated with 1:10,000-diluted horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Dako, Glostrup, Denmark). The immune complexes were visualized by a chemiluminescence system (ECL; Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), and representative data are shown.

Transmission electron microscopy. *P. gingivalis* cells were washed three times with PBS and fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, followed by embedding in 3% agar. After postfixation by osmium tetroxide and dehydration, specimens were embedded in LR White (EM Polysciences, Warrington, Pa.). Thin sections were contrasted with uranyl acetate and lead citrate. Images were obtained with a Hitachi 7100 electron microscope at 75 kV.


```
gtfA        1 MEENKPVFSIITVCYCARDIIAKTAHSVLSQTYPNIEYIVVDGASPDNTAEVVRSLS-SD  59
pfam00535   1 --------SVIIPTYNEEKYLEETLESLLNQTYLNFEIIVVDDGSTDGTVEILEEYAKKD  52
                      *  *   *  * * *** **** * **  ***  *         *

gtfA       60 --IRLIS-EPDKGLYDAMNKGLRMALGDYVWFLNAGDLLPSDDLVEAIANGIKSSPMGLP 116
pfam00535  53 PRIRVIRLEENLGKAAARNAGLKHATGDYILFLDADDEVAP-DWLEKLLELLEKN--G-A 108
              ** *  *    *  * *  *** ** * *    *        *

gtfA      117 DVLYGDTMLIDATGKEKGLRRLRPPFELQWRSFADGMLVCHQAFIAKRLIAPPYNMKYRF 176
pfam00535 109 DVVIGSRVAIFGETRLDG-RALRMELLLLLGKLGARSLG-LKVLF---LIGSN-AL-YR- 160
              **  *   * *    * * *  *   *        **   **

gtfA      177 SADFDWCIRILQAARNIVNTEMILVHSLDDRGGITYKNHKASLKERFLIMKHYYGSRKSI 236
pfam00535 161 REALE---ELF------------------------------------------------ 168

gtfA      237 MKHLSFFFRRKR                                                 248
pfam00535 168 ------------                                                 168
```

FIG. 1. Amino acid sequences of GtfA and pfam00535, a consensus sequence for glycosyltransferases. Alignment of GtfA from *P. gingivalis* W83 (248 amino acids) and pfam00535 (168 amino acids) is shown. Asterisks indicate identical residues.

Autoaggregation of *P. gingivalis*. Autoaggregation assays of *P. gingivalis* cells were performed as previously described (29), except that a different wavelength was used to monitor the optical densities.

Briefly, *P. gingivalis* cells in the early to mid-log phase were collected by centrifugation, washed three times with PBS, and then resuspended in PBS to an optical density (OD) of 1.0 at 550 nm. The OD of each suspension was monitored for autoaggregation. Three independent experiments were performed, and representative data are shown.

Attachment to epithelial cells and extracellular matrices. HEp-2 epithelial cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum in a humidified atmosphere with 5% CO_2. HEp-2 cells were plated in 24-well plates (10^5 cells per well) and allowed to attach for 24 h. *P. gingivalis* cells were washed three times with PBS and resuspended in PBS at a density of 10^7 or 10^6 cells/ml. Prior to seeding of *P. gingivalis* cells, the culture medium of the HEp-2 cells was sucked up and washed three times with PBS. One milliliter of *P. gingivalis* suspensions was seeded on the HEp-2 cells and incubated for 90 min. After the incubation, nonadherent *P. gingivalis* cells were removed by washing three times with PBS. HEp-2 cells were lysed with 1 ml of sterile water, and attached or internalized *P. gingivalis* cells were recovered. One-hundred-microliter aliquots of *P. gingivalis* cell suspensions were plated onto 5% sheep blood agar plates, followed by colony counting. The averages and standard deviations of the CFU from five wells were calculated, and representative data from three independent experiments are shown.

To evaluate the ability to attach to extracellular matrices, 96-well plates coated with type I collagen, laminin, and fibronectin were used (Biocoat; Becton Dickinson). One-hundred-microliter aliquots of *P. gingivalis* suspensions in PBS (10^8 cells/ml) were plated into wells and incubated for 3 h. Nonadherent cells were removed by washing three times with PBS, and adherent cells were stained with 0.1% crystal violet. After being washed with distilled water, the plates were dried and the OD at 570 nm was measured. Averages and standard deviations of the ODs for five wells were calculated, and representative data from three independent experiments are shown.

RT-PCR. Total RNA was purified from bacterial culture in mid-log phase. cDNA was synthesized by use of random primers and ReverTra Ace (Nacalai Tesque, Kyoto, Japan). To detect *fimA* expression, we used specific primers for type I *fimA*, because *P. gingivalis* 381 possesses the type I *fimA* gene (1). The sequences of the primers used were as follows: type I *fimA*, 5′-CTG TGT GTT TAT GGC AAA CTT C-3′ and 5′-AAC CCC GCT CCC TGT ATT CCG A-3′; and 16S rRNA, 5′-TGT AGA TGA CTG ATG GTG AAA ACC-3′ and 5′-ACG TCA TCC CCA CCT TCC TC-3′ (1). The PCR conditions are available on request.

RESULTS

Isolation of a putative glycosyltransferase gene from *P. gingivalis*. To isolate a putative glycosyltransferase gene(s) from *P. gingivalis*, we carried out a BLAST search with the consensus amino acid sequence of glycosyltransferases as the query. We obtained some ORFs which had a consensus domain for glycosyltransferase from a genomic database for *P. gingivalis* W83. We designated one of the ORFs as *gtfA* (Fig. 1). The product
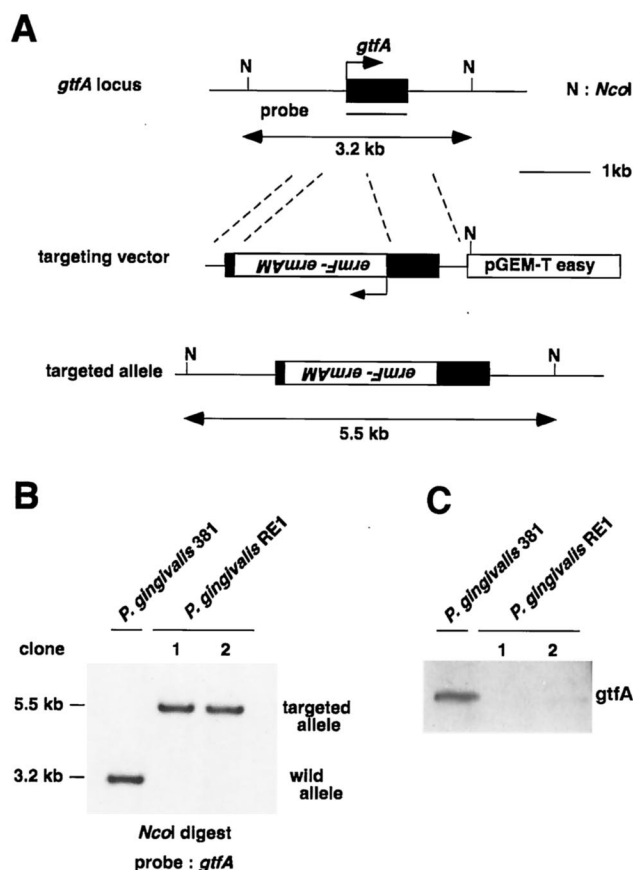
FIG. 2. Targeted disruption of *gtfA* locus. (A) Structures of the *gtfA* locus, targeting vector, and targeted allele. N, NcoI. (B) Southern blot analysis of *P. gingivalis* clones after selection with erythromycin. NcoI-digested DNA was probed with the *gtfA* ORF. The 3.2-kb wild-type fragment and the 5.5-kb targeted fragment are indicated. (C) Western blotting with anti-GtfA antiserum. Whole extracts from *P. gingivalis* 381 and *P. gingivalis* RE1 were immunoblotted with rabbit anti-GtfA polyclonal antiserum.

FIG. 3. Transmission electron microscopic image and expression of fimA in *gtfA*-deficient mutant. (A) Images of *P. gingivalis* 381 (left panel) and *P. gingivalis* RE1 (right panel) are shown. Bar, 100 nm. Arrowheads indicate fimbriae of *P. gingivalis* 381. (B) Detection of FimA protein by Western blotting. (C) Detection of *fimA* mRNA expression by RT-PCR.

GtfA consisted of 248 amino acids and had a consensus domain for glycosyltransferase at its N terminus. The glycosyltransferase domain in the N terminus showed 30% identity to pfam00535, and its product had a predicted molecular mass of approximately 28kDa. We isolated the *gtfA* gene from *P. gingivalis* 381 by genomic PCR, which indicated that the *gtfA* gene was also conserved in *P. gingivalis* 381. *gtfA* showed the highest identity to TIGR locus BT2947, an ORF for a putative glycosyltransferase in *Bacteroides thetaiotaomicron* (48%), although the function of the ORF remains unknown. From the BLAST search of the *E. coli* genome database, GtfA showed the highest identity to WcaE (25% identity), a putative glycosyltransferase that might be involved in extracellular polysaccharide synthesis in *E. coli* (27).

Generation of *gtfA*-deficient mutants. To reveal the role of the *gtfA* gene, we developed *gtfA*-deficient *P. gingivalis* by homologous recombination (Fig. 2A). The targeting vector was constructed by insertion of an erythromycin resistance cassette into the *gtfA* ORF. After electroporation of the targeting vector, erythromycin-resistant clones were obtained. Following the first screening of homologous recombinants by PCR (data
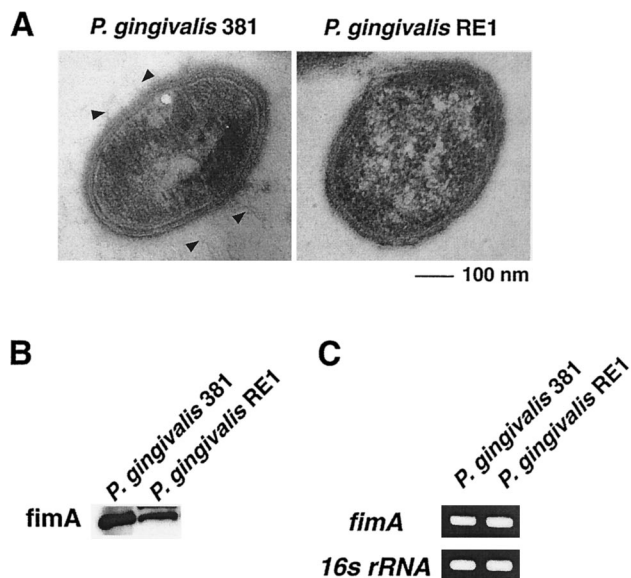
not shown), we performed Southern blot analysis to identify clones that had undergone the correct recombination. In this analysis, homologous recombinants showed a 5.5-kb NcoI fragment due to an insertion of erythromycin resistant cassette, compared to the 3.2-kb NcoI fragment of the wild-type strain (Fig. 2B).

The homologous recombinants, designated *P. gingivalis* RE1 and *P. gingivalis* 381, were subjected to Western blotting with anti-GtfA antiserum (Fig. 2C). The molecular mass of the endogenous GtfA protein was approximately 40 kDa, suggesting that GtfA had undergone some posttranslational modifications. GtfA protein could not be detected in *P. gingivalis* RE1, indicating that the *gtfA* gene was correctly disrupted in *P. gingivalis* RE1. The appearance or pigmentation of the colonies of *P. gingivalis* RE1 was similar to that of the wild type, and its growth was slightly slower than that of the wild type (data not shown). These results suggested that *gtfA* is not essential for cell survival, cell growth, or pigmentation.

We observed the morphology of *P. gingivalis* RE1 by transmission electron microscopy. There was no significant difference in the thickness of the capsular layer between *P. gingivalis* 381 and *P. gingivalis* RE1. However, *P. gingivalis* RE1 lacked mature fimbria, which is a key virulence factor in the ability to attach (Fig. 3A). From this result, *gtfA* must be required for the development of mature fimbria. We examined the expression of FimA, a subunit of fimbriae, by Western blotting and RT-PCR and found no significant change in *fimA* expression in *P. gingivalis* RE1 (Fig. 3B and C), indicating that GtfA is not required for *fimA* expression. These results suggested that sugar transfer might be involved in fimbria development in *P. gingivalis*.
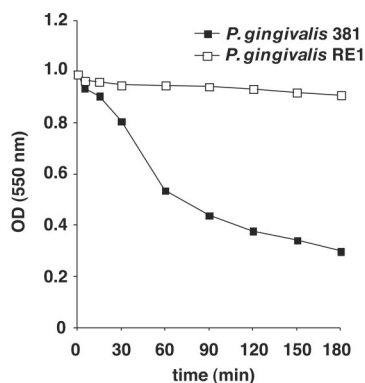
FIG. 4. Autoaggregation activities of wild-type and *gtfA*-deficient *P. gingivalis*. The OD of each *P. gingivalis* suspension was measured at the indicated times. Representative data from three independent experiments are shown. Open squares, *P. gingivalis* 381; solid squares, *P. gingivalis* RE1.
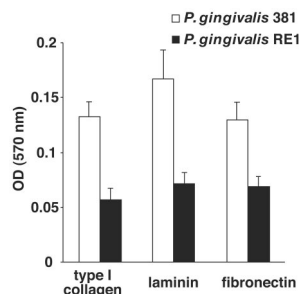

FIG. 5. Attachment to extracellular matrices. Ninety-six-well plates with immobilized type I collagen, fibronectin, or laminin were used. After incubation with *P. gingivalis* suspension, nonadherent cells were removed by washing, followed by crystal violet staining. The means of the OD at 570 nm and standard deviations are shown.

Autoaggregation activity of *gtfA*-deficient *P. gingivalis*. It has been reported that *P. gingivalis* shows autoaggregation and that this might be important for colonization of human oral surfaces (28, 29). Therefore, we examined whether the *gtfA* gene was involved in autoaggregation. The OD of cell suspensions was monitored to evaluate autoaggregation activity. In wild-type *P. gingivalis*, reduction of the OD was observed within several hours, whereas *P. gingivalis* RE1 showed only a slight reduction in OD (Fig. 4). This result indicated that *P. gingivalis* RE1 has low autoaggregation activity and that the *gtfA* gene is required for autoaggregation.

Role of *gtfA* in attachment to epithelial cells and extracellular matrices. It has been reported that *P. gingivalis* can adhere to cultured epithelial cells (6) and that this might be an important property in the colonization of *P. gingivalis*. Therefore, we also examined whether the *gtfA* gene is involved in the process of attachment to cultured epithelial cells. *P. gingivalis* suspensions were added to confluent HEp-2 epithelial cells and incubated for 90 min. Compared to *P. gingivalis* 381, *P. gingivalis* RE1 was defective in adherence to HEp-2 cells (Table 1). This result indicated that *gtfA* has an indispensable role in adherence to epithelial cells.

It has also been reported that *P. gingivalis* can attach to various proteins, including extracellular proteins such as collagen and salivary components (18). To investigate binding to extracellular matrices, we examined binding to type I collagen, laminin, and fibronectin. The ability of *P. gingivalis* RE1 to

attach to extracellular matrices was reduced to approximately 40 to 60% of that of the wild type (Fig. 5). These results indicated that *gtfA* is involved, at least in part, in attachment to extracellular matrices, including type I collagen, laminin, and fibronectin. Thus, GtfA plays a role in attachment to various proteins as well as to living epithelial cells.

DISCUSSION

In this report, we characterized the role of *gtfA*, a putative glycosyltransferase gene in *P. gingivalis*. Some glycosyltransferase genes have already been identified in *P. gingivalis*. The *narH* gene encodes β-*N*-acetylhexosaminidase, which is likely a lipoprotein (21). Duncan et al. identified an ORF for a putative glycosyltransferase from an expression screening to isolate ORFs related to attachment to epithelial cells (5). They indicated that overexpression of an ORF encoding a putative glycosyltransferase in *E. coli* could induce attachment to epithelial cells and that this result suggested that the glycosyltransferase might be involved in attachment to epithelial cells. However, the roles of these ORFs in *P. gingivalis* have remained unknown. Characterization of a loss-of-function mutant for these ORFs would be helpful in understanding their roles in *P. gingivalis*. A BLAST search of the *P. gingivalis* genomic database (data not shown) identified a number of ORFs for putative glycosyltransferases, and it might be of interest to determine the native role of each ORF.

P. gingivalis is a periodontopathogenic organism that has already been analyzed extensively, and the roles of virulence factors such as proteases and adhesion molecules have been characterized. In particular, *P. gingivalis* has the ability to interact with various surfaces, including other bacteria and epithelial cells, and also has the potential to be a virulence factor (19). Some reports have suggested that fimbriae have a central role in attachment to various surfaces; for example, a *fimA*-defective mutant showed loss of attachment ability as well as internalization into epithelial cells (23). Some proteases are also involved in the interaction between *P. gingivalis* and epithelial cells, and protease-defective mutants showed altered expression of *fimA*, suggesting that the expression of fimbriae might affect the ability to attach to epithelial cells (28, 29). In this study, we showed that *P. gingivalis* RE1 lacked mature fimbriae (Fig. 3A), even though expression of FimA protein or

TABLE 1. Attachment of wild-type and *gtfA*-deficient *P. gingivalis* to HEp-2 epithelial cells[a]

P. gingivalis strain	No. of cells/ml	Mean CFU (10^5) ± SD
381	10^6	1.26 ± 0.063
	10^7	2.12 ± 0.13
RE1	10^6	No viable colonies
	10^7	No viable colonies

[a] *P. gingivalis* suspensions were added to HEp-2 cells and allowed to adhere for 90 min. Adherent *P. gingivalis* cells were recovered, and viable cells were counted.

mRNA was not altered (Fig. 3B and 3C). This implies that a sugar transfer reaction might be involved in the production of fimbriae. The thickness of the capsular layer in *P. gingivalis* RE1 was similar to that of the wild type; however, the composition of the capsular layer remains unknown, and it is possible that the composition and function might differ.

We also showed that the *gtfA* gene is involved in autoaggregation; the interaction between *P. gingivalis* and epithelial cells, as well as binding to all the extracellular matrices used in this study. These phenotypes might be explained by the lack of fimbriae in *P. gingivalis* RE1. It is also possible that a surface matrix might have lost its potential to act as an adhesin. Both attachment and aggregation are important steps in biofilm formation (14). As *P. gingivalis* RE1 showed reduced autoaggregation and attachment to cell surfaces and several extracellular matrices, it is likely that the *gtfA* gene is involved in the development of *P. gingivalis* biofilms.

Our data showed that *gtfA* regulates autoaggregation and attachment to epithelial cells and extracellular matrices. The *gtfA* gene might regulate attachment ability in subgingival biofilms and act as a potent virulence factor.

ACKNOWLEDGMENTS

REFERENCES

1. **Amano, A., I. Nakagawa, K. Kataoka, I. Morisaki, and S. Hamada.** 1999. Distribution of *Porphyromonas gingivalis* strains with *fimA* genotypes in periodontitis patients. J. Clin. Microbiol. **37:**1426–1430.
2. **Amano, A., S. Shizukuishi, H. Horie, S. Kimura, I. Morisaki, and S. Hamada.** 1998. Binding of *Porphyromonas gingivalis* fimbriae to proline-rich glycoproteins in parotid saliva via a domain shared by major salivary components. Infect. Immun. **66:**2072–2077.
3. **Campbell, J. A., G. J. Davies, V. Bulone, and B. Henrissat.** 1997. A classification of nucleotide-diphospho-sugar glycosyltransferases based on amino acid sequence similarities. Biochem. J. **326:**929–939.
4. **Chen, W., R. J. Palmer, and H. K. Kuramitsu.** 2002. Role of polyphosphate kinase in biofilm formation by *Porphyromonas gingivalis*. Infect. Immun. **70:**4708–4715.
5. **Duncan, M. J., S. A. Emory, and E. C. Almira.** 1996. *Porphyromonas gingivalis* genes isolated by screening for epithelial cell attachment. Infect. Immun. **64:**3624–3631.
6. **Duncan, M. J., S. Nakao, Z. Skobe, and H. Xie.** 1993. Interactions of *Porphyromonas gingivalis* with epithelial cells. Infect. Immun. **61:**2260–2265.
7. **Fletcher, H. M., H. A. Schenkein, R. M. Morgan, K. A. Bailey, C. R. Berry, and F. L. Macrina.** 1995. Virulence of a *Porphyromonas gingivalis* W83 mutant defective in the *prtH* gene. Infect. Immun. **63:**1521–1528.
8. **Genevaux, P., P. Bauda, M. S. DuBow, and B. Oudega.** 1999. Identification of Tn10 insertions in the *rfaG*, *rfaP*, and *galU* genes involved in lipopolysaccharide core biosynthesis that affect *Escherichia coli* adhesion. Arch. Microbiol. **172:**1–8.
9. **Gerke, C., A. Kraft, R. Sussmuth, O. Schweitzer, and F. Gotz.** 1998. Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. J. Biol. Chem. **273:**18586–18593.
10. **Goulbourne, P. A., and R. P. Ellen.** 1991. Evidence that *Porphyromonas (Bacteroides) gingivalis* fimbriae function in adhesion to *Actinomyces viscosus*. J. Bacteriol. **173:**5266–5274.
11. **Grenier, D.** 1992. Demonstration of a bimodal coaggregation reaction between *Porphyromonas gingivalis* and *Treponema denticola*. Oral Microbiol. Immunol. **7:**280–284.
12. **Hirschfeld, M., J. J. Weis, V. Toshchakov, C. A. Salkowski, M. J. Cody, D. C. Ward, N. Qureshi, S. M. Michalek, and S. N. Vogel.** 2001. Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. Infect. Immun. **69:**1477–1482.
13. **Kinder, S. A., and S. C. Holt.** 1993. Localization of the *Fusobacterium nucleatum* T18 adhesin activity mediating coaggregation with *Porphyromonas gingivalis* T22. J. Bacteriol. **175:**840–850.
14. **Kolenbrander, P. E.** 2000. Oral microbial communities: biofilms, interactions, and genetic systems. Annu. Rev. Microbiol. **54:**413–437.
15. **Kumagai, Y., K. Konishi, T. Gomi, H. Yagishita, A. Yajima, and M. Yoshikawa.** 2000. Enzymatic properties of dipeptidyl aminopeptidase IV produced by the periodontal pathogen *Porphyromonas gingivalis* and its participation in virulence. Infect. Immun. **68:**716–724.
16. **Lamont, R. J., A. Chan, C. M. Belton, K. T. Izutsu, D. Vasel, and A. Weinberg.** 1995. *Porphyromonas gingivalis* invasion of gingival epithelial cells. Infect. Immun. **63:**3878–3885.
17. **Lamont, R. J., S. Gil, D. R. Demuth, D. Malamud, and B. Rosan.** 1994. Molecules of *Streptococcus gordonii* that bind to *Porphyromonas gingivalis*. Microbiology **140:**867–872.
18. **Lamont, R. J., and H. F. Jenkinson.** 1998. Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. Microbiol. Mol. Biol. Rev. **62:**1244–1263.
19. **Lamont, R. J., and H. F. Jenkinson.** 2000. Subgingival colonization by *Porphyromonas gingivalis*. Oral Microbiol. Immunol. **15:**341–349.
20. **Larsen, T.** 2002. Susceptibility of Porphyromonas gingivalis in biofilms to amoxicillin, doxycycline and metronidazole. Oral Microbiol. Immunol. **17:**267–271.
21. **Lovatt, A., and I. S. Roberts.** 1994. Cloning and expression in *Escherichia coli* of the *nahA* gene from *Porphyromonas gingivalis* indicates that beta-N-acetylhexosaminidase is an outer-membrane-associated lipoprotein. Microbiology **140:**3399–3406.
22. **Nakamura, T., A. Amano, I. Nakagawa, and S. Hamada.** 1999. Specific interactions between *Porphyromonas gingivalis* fimbriae and human extracellular matrix proteins. FEMS Microbiol. Lett. **175:**267–272.
23. **Njoroge, T., R. J. Genco, H. T. Sojar, N. Hamada, and C. A. Genco.** 1997. A role for fimbriae in *Porphyromonas gingivalis* invasion of oral epithelial cells. Infect. Immun. **65:**1980–1984.
24. **Noiri, Y., and S. Ebisu.** 2000. Identification of periodontal disease-associated bacteria in the "plaque-free zone." J. Periodontol. **71:**1319–1326.
25. **Noiri, Y., K. Ozaki, H. Nakae, T. Matsuo, and S. Ebisu.** 1997. An immunohistochemical study on the localization of *Porphyromonas gingivalis*, *Campylobacter rectus* and *Actinomyces viscosus* in human periodontal pockets. J. Periodontal Res. **32:**598–607.
26. **Ogawa, T., Y. Asai, M. Hashimoto, O. Takeuchi, T. Kurita, Y. Yoshikai, K. Miyake, and S. Akira.** 2002. Cell activation by *Porphyromonas gingivalis* lipid A molecule through Toll-like receptor 4- and myeloid differentiation factor 88-dependent signaling pathway. Int. Immunol. **14:**1325–1332.
27. **Stevenson, G., K. Andrianopoulos, M. Hobbs, and P. R. Reeves.** 1996. Organization of the *Escherichia coli* K-12 gene cluster responsible for production of the extracellular polysaccharide colanic acid. J. Bacteriol. **178:**4885–4893.
28. **Tokuda, M., M. Duncan, M. I. Cho, and H. K. Kuramitsu.** 1996. Role of *Porphyromonas gingivalis* protease activity in colonization of oral surfaces. Infect. Immun. **64:**4067–4073.
29. **Tokuda, M., T. Karunakaran, M. Duncan, N. Hamada, and H. Kuramitsu.** 1998. Role of Arg-gingipain A in virulence of *Porphyromonas gingivalis*. Infect. Immun. **66:**1159–1166.
30. **Yoshimura, F., K. Takahashi, Y. Nodasaka, and T. Suzuki.** 1984. Purification and characterization of a novel type of fimbriae from the oral anaerobe *Bacteroides gingivalis*. J. Bacteriol. **160:**949–957.