Journal of
PERIODONTAL RESEARCH

J Periodont Res 2015; 50: 1–8

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12255

Review Article

Porphyromonas gingivalis and related bacteria: from colonial pigmentation to the type IX secretion system and gliding motility

K. Nakayama
Division of Microbiology and Oral Infection, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Nakayama K. Porphyromonas gingivalis and related bacteria: from colonial pigmentation to the type IX secretion system and gliding motility. J Periodont Res 2015; 50: 1–8. © 2014 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

Porphyromonas gingivalis is a gram-negative, non-motile, anaerobic bacterium implicated as a major pathogen in periodontal disease. *P. gingivalis* grows as black-pigmented colonies on blood agar, and many bacteriologists have shown interest in this property. Studies of colonial pigmentation have revealed a number of important findings, including an association with the highly active extracellular and surface proteinases called gingipains that are found in *P. gingivalis*. The Por secretion system, a novel type IX secretion system (T9SS), has been implicated in gingipain secretion in studies using non-pigmented mutants. In addition, many potent virulence proteins, including the metallocarboxypeptidase CPG70, 35 kDa hemin-binding protein HBP35, peptidylarginine deiminase PAD and Lys-specific serine endopeptidase PepK, are secreted through the T9SS. These findings have not been limited to *P. gingivalis* but have been extended to other bacteria belonging to the phylum Bacteroidetes. Many Bacteroidetes species possess the T9SS, which is associated with gliding motility for some of these bacteria.

Koji Nakayama, DDS, PhD, Division of Microbiology and Oral Infection, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8588, Japan
Tel: +81 95 819 7648
Fax: +81 95 819 7650
e-mail: knak@nagasaki-u.ac.jp

Key words: gliding motility; *Porphyromonas gingivalis*; protease; protein secretion system; review; virulence factors

Accepted for publication November 19, 2014

Porphyromonas gingivalis is a gram-negative anaerobic bacterium considered a major pathogen in chronic periodontitis (1). Recently, it has been proposed that even in low abundance *P. gingivalis* is a keystone pathogen with community-wide effects that are critical for the development of dysbiosis in periodontal biofilm (2). In addition, epidemiological and experimental studies have shown that the bacterium may be associated with systemic conditions, such as cardiovascular diseases (3), preterm low birth weight (4), rheumatoid arthritis (5) and non-alcoholic fatty liver disease (6,7).

P. gingivalis requires protoheme for growth. In heme-deprived medium, *P. gingivalis* cells grow slowly and eventually stop growing after several passages in this medium. On blood agar, these bacterial cells form black-pigmented colonies (Fig. 1). The black pigment is derived from the protoheme in erythrocytes. The black pigment phenotype of *P. gingivalis* has been attributed to the accumulation of the μ-oxo bisheme complex of Fe(III) protoporphyrin IX, $[Fe(III)PPIX]_2O$ (8–10). This heme complex, also termed μ-oxo oligomers or dimeric heme, comprises two Fe(III) protoporphyrin IX moieties bridged by an oxygen atom (8). As the optimum pH for *P. gingivalis* growth is approximately 8 and this bacterium produces an alkaline terminal growth pH because of peptide and amino acid metabolism (11–14), the μ-oxo dimer $[Fe(III)PPIX]_2O$ is maintained at an alkaline pH. Interestingly,

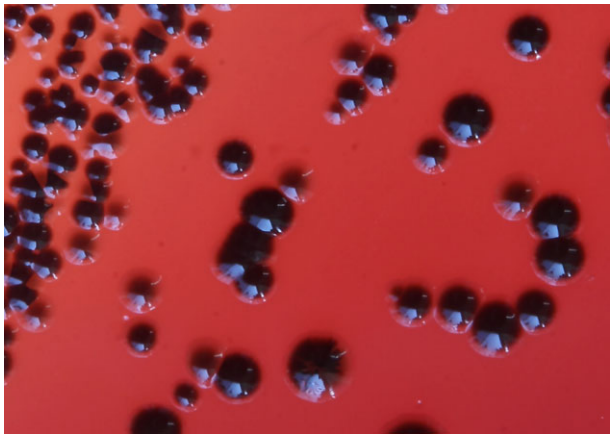

Fig. 1. The pigmentation of *Porphyromonas gingivalis* colonies on blood agar. *Porphyromonas gingivalis* cells were spread on to blood agar and anaerobically incubated for 7 d.

pigmented *Prevotella* species, such as *Prev. intermedia* and *Prev. nigrescens*, generate monomeric Fe(III)PPIX.OH from $[Fe(III)PPIX]_2O$ because the terminal growth pH of these bacteria on blood agar for 8 d is approximately 6 (14). In this review, we discuss novel findings, including the type IX secretion system (T9SS), obtained from genetic studies of colonial pigmentation.

Spontaneous pigment-less mutants

When *P. gingivalis* cells were grown under hemin excess in a chemostat at pH 7.5 for 2–3 wk (49–73 bacterial generations) and subsequently plated on to blood agar, colonies with atypical morphology were observed (15). One colony variant (W50/BE1) was beige in color, and another colony variant (W50/BR1) was brown. Both colonial variants exhibited decreased virulence (15), and W50/BE1 lacked gelatinase, collagenase and dipeptidyl aminopeptidase activities compared with the parent strain and exhibited reduced hydrophobicity, hemagglutination activity, fimbriation and extracellular vesicle production (16). *P. gingivalis* cells produce gingipains, including arginine-specific gingipains (Arg-gingipain A [RgpA] and Arg-gingipain B [RgpB]) and lysine-specific gingipains (Lys-gingipain [Kgp]), which are highly active extracellular and surface proteases that degrade numerous host proteins, including extracellular matrix proteins, cytokines, complement proteins, antibodies and proteinase inhibitors (17–19). Collinson *et al*. (20) showed that BE1 exhibited decreased Rgp activity compared with the wild type, and no Rgp enzyme with large glycan additions, which are associated with the outer membrane, was observed. The gene(s) responsible for the phenotypes of W50/BE1 and W50/BR1 has not been elucidated. However, these early findings suggest that colonial pigmentation is associated with the activity and localization of proteases in *P. gingivalis* cells. Moreover, Rgp was purified from the *P. gingivalis* strain HG66, which secreted soluble Rgp and lacked pigmentation (21,22).

The isolation of pigment-less mutants using transposon mutagenesis

Several studies have applied transposon mutagenesis to isolate pigment-less *P. gingivalis* mutants (23–26). Simpson *et al*. (26) reported a non-pigmented mutant with an insertion sequence element (IS*1126*) at the promoter locus of *kgp*. In addition, Chen *et al*. (25) isolated non-pigmented mutants with transposon Tn*4351* DNA within *kgp*. Previous studies have shown that the *kgp* mutant is less pigmented (27). These results demonstrated the involvement of *kgp* in pigmentation. However, non-*kgp* mutations causing non-pigmentation have also been identified (25,28). Chen *et al*. (25) reported a Tn*4351* insertion in a putative glucosyl (rhamnosyl) transferase-encoding gene in several non-pigmented mutants, and Abaibou *et al*. (28) demonstrated that the *vimA* gene, located downstream of *recA*, is responsible for pigmentation. Using Tn*4351* transposon mutagenesis, we isolated and characterized two non-pigmented mutants (*porR* and *porT*) (29,30).

Pigmentation-related genes

Pigmentation-related genes encode proteins with three types of functions: gingipain activity, gingipain transport and gingipain attachment (31). Rgp and Kgp proteinases are encoded by *rgpA*, *rgpB* and *kgp*. *rgpA* and *kgp* also encode hemagglutinins (adhesins) and the hemoglobin receptor at the 3′-terminal region of these genes. *kgp* single mutants and *rgpA rgpB kgp* triple mutants form less-pigmented and non-pigmented colonies, respectively, whereas *rgpA rgpB* double mutants form pigmented colonies (27,32). Smalley *et al*. (33) revealed that Rgp activity is crucial for converting oxyhemoglobin into methemoglobin, a form more susceptible to Kgp-mediated degradation, resulting in the release of iron(III) protoporphyrin IX and the production of μ-oxo heme dimers.

The *porR* mutant exhibited a pleiotropic phenotype: Rgp and Kgp proteinases were mainly present in the culture supernatant, mutant cells had no hemagglutinating activity and Rgp-mediated processing of fimbrillin was delayed (29). The *porR* mutant had altered phenol extractable polysaccharides. The monoclonal antibody (mAb) 1B5, which reacts with the sugar portions of *P. gingivalis* cell surface polysaccharides and membrane-type Rgp proteinases (17), did not react with cell lysates from the *porR* mutant, indicating that *porR* is involved in the biosynthesis of cell surface polysaccharides that might function as anchors for Rgp, Kgp, hemagglutinins and the hemoglobin receptor protein. *P. gingivalis* has two different lipopolysaccharide (LPS) molecules, O-LPS and A-LPS. O-LPS

possesses the conventional O-antigen, whereas A-LPS has a different O-antigen comprising an anionic polysaccharide repeat unit that reacts with mAb 1B5 (34,35). Recently, another mAb (TDC-5-2-1) that recognizes the O-antigen of O-LPS, which is present in almost all wild-type cells, was generated; however, the glycan epitope recognized by this mAb has not been identified (36,37). As the *porR* mutant reacts with mAb TDC-5-2-1, this mutant might possess O-LPS but lacks A-LPS. The *porR* gene encodes a putative transaminase (29). We recently proposed that the final product synthesized through the Wbp pathway, which involves WbpA (PGN_0613 [UgdA], PGN_1243), WbpB (PGN_0168), WbpE (PGN_1236 [PorR]) and WbpD (PGN_0002), is a sugar substrate required for the biosynthesis of A-LPS (38). The *P. gingivalis* strain HG66, typically used for gingipain purification, exhibits no pigmentation on blood agar. This strain has a nonsense mutation in the *wbpB* gene, which is responsible for the pigmentless phenotype of the strain (38).

The *porT* mutant was also isolated as a non-pigmented mutant using Tn*4351* transposon mutagenesis; however, the *porT* mutant was quite different from the *porR* mutant (30). The *porR* mutant exhibited gingipain activity in the culture supernatant, whereas the *porT* mutant demonstrated no gingipain activity either in the cell extract or in the culture supernatant. Subcellular fractionation and immunoblot analysis revealed that gingipain proproteins accumulate in the periplasmic space, indicating that the PorT protein is involved in gingipain transport across the outer membrane. The subcellular localization of the PorT protein is controversial. We treated the membrane fraction of *P. gingivalis* cells with 1% Triton X-100 to separate the outer and inner membrane fractions, and the PorT protein was detected in the inner membrane fraction (Triton X-100-soluble fraction). However, using fractionation with Sarkosyl treatment, Nguyen *et al.* (39) reported that the PorT protein is located in the outer membrane.

Genome sequence of *Porphyromonas gingivalis*

In 2003, researchers at TIGR and the Forsyth Institution determined the whole genome sequence of *P. gingivalis* W83 (40). The *P. gingivalis* W83 genome comprises 2.3 megabase pairs and encodes a range of pathways and virulence determinants associated with the novel biology of this oral pathogen. This genome size is consistent with previous measurements using pulsed field gel electrophoresis (41). We determined the whole genome sequence of a different strain, ATCC 33277, typically used as a type strain in studies of the pathogenicity and physiology of *P. gingivalis* (42). Via genomic comparison with strain W83, we identified 461 ATCC 33277-specific and 415 W83-specific CDSs, and extensive genomic rearrangements were observed between the two strains, including 175 regions in which genomic rearrangements occurred. Interestingly, the genomes of *P. gingivalis* strains did not encode proteins involved in known secretion systems, such as the type II and III secretion systems, suggesting that *P. gingivalis* possesses a novel secretion system.

Discovery of a new protein secretion system

Genes homologous to *porT* of *P. gingivalis* have been identified in many members of the large and diverse Bacteroidetes phylum, whereas there are no *porT* homologs in bacteria belonging to other phyla. In addition, a *porT* homolog is not present in a bacterium belonging to the genus *Bacteroides*, *B. thetaiotaomicron*. Most bacterial protein secretion systems comprise multiple proteins that form a complex in the cell envelope. Thus, a set of proteins, including PorT, required for a protein secretion system must exist in bacteria with the protein secretion system, but not in bacteria lacking the system. Therefore, we used Venn diagram analysis to identify genes involved in these protein secretion systems. We identified 55 genes, including *porT*, that are present in *P. gingivalis* and *Cytophaga hutchinsonii* but absent in *B. thetaiotaomicron* and constructed deletion mutants of the genes (43). *P. gingivalis* strains with deletion mutations in 46 of these genes were generated to determine involvement of these genes in a secretion system for gingipains. Among the 46 mutants, 10 mutations in *sov* (PGN_0832), which was previously implicated in gingipain secretion (44), *porK* (PGN_1676), *porL* (PGN_1675), *porM* (PGN_1674), *porP* (PGN_1677), *porQ* (PGN_0645), *porU* (PGN_0022), *porW* (PGN_1877), *porX* (PGN_1019) and *porY* (PGN_2001) resulted in decreased Rgp or Kgp activity in cells and culture supernatants. We named this novel secretion system the Por secretion system (PorSS), now referred to as the T9SS (43,45,46) (Fig. 2). PorK, PorN and PorP were detected in the outer membrane fraction, whereas PorL and PorM were detected in the inner membrane fraction. Blue native gel analysis revealed that PorK was associated with PorN, and PorL was associated with PorM (43,47). PGN_0023 (*pg27*/*lptO*/*porV*) is located downstream of *porU* (PGN_0022) on the *P. gingivalis* chromosome. PGN_0023, a mutant deficient in gingipain secretion (48,49), has been implicated in the *O*-deacylation of LPS (50). PGN_0022 (*porU*)-encoding protein is a C-terminal signal peptidase for proteins that possess conserved C-terminal domains (CTDs) and utilize the T9SS for translocation across the outer membrane (49). Veith *et al.* (51) first identified the CTDs of *P. gingivalis* proteins. Seers *et al.* (52) predicted a role for CTDs in export and attachment to the cell surface, and Shoji *et al.* (53) verified this role. PG27/LptO/PorV has been associated with PorU and several CTD proteins (54).

Two-component system for the T9SS of *Porphyromonas gingivalis*

porX (PGN_1019) and *porY* (PGN_2001) encode the response regulatory protein and histidine sensor kinase, respectively, of a two-component signal transduction system and have roles in regulating the expression of the genes in the transport system (43) (Fig. 2). These genes are located separately on the *P. gingivalis* chromosome, although the cognate

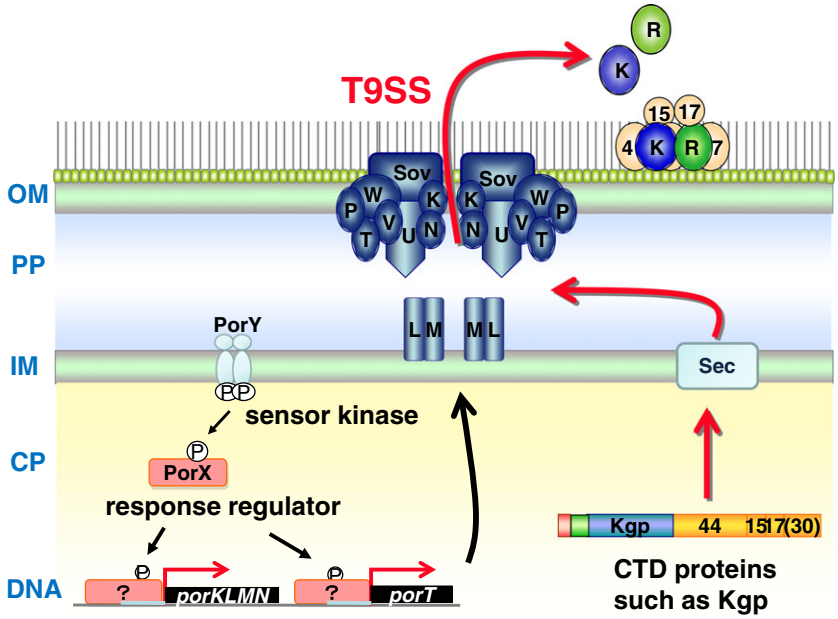

Fig. 2. Model of the T9SS of *Porphyromonas gingivalis*. T9SS comprises more than 10 proteins, including PorK, PorL, PorM, PorN, PorP, PorQ, PorT, PorU, PorV, PorW and Sov. Some of these proteins were expressed using the two-component system PorXY. PorX and PorY are a response regulator and a sensor kinase, respectively. CTD proteins, such as Kgp (K) and Rgp (R), are translocated across the IM via Sec machinery and subsequently secreted across the OM through the T9SS. CTD, C-terminal domains; CP, cytoplasm; IM, inner membrane; OM, outer membrane; PP, periplasm; T9SS, type IX secretion system.

partners of a two-component signal transduction system are typically located in tandem. Microarray and reverse transcription polymerase chain reaction analyses revealed that 20 genes were downregulated by < 60% in the *porX* deletion mutant compared with the wild-type parent strain. Among these genes were *porK*, *porL*, *porM*, *porN*, *porP*, *porT* and *sov*, which are involved in the T9SS. These genes were also downregulated in the *porY* mutant, suggesting that decreased expression of gingipains in the *porX* and *porY* single mutants reflects the downregulation of *porK*, *porL*, *porM*, *porN*, *porP*, *porT* and *sov*.

Extracellular and surface proteins secreted through the T9SS in *Porphyromonas gingivalis*

The T9SS was identified in a study examining the secretion of gingipains. PGN_1728 (*kgp*), PGN_1733 (*hagA*), PGN_1970 (*rgpA*) and PGN_1466 (*rgpB*) encode gingipain group proteins, with proproteins that possess CTDs at the C-terminus, and the secretion of these proteins is dependent on the T9SS. However, the T9SS is not specific for gingipain secretion. Seers *et al.* (52) predicted that CTD-containing proteins other than gingipains, including PG1326 (PGN_1115), PG2100 (PGN_0152, *tapA*), PG2102 (no PGN, *tapC*), PG1427 (PGN_0900, *prtT*), PG1374 (PGN_0852), PG0495 (PGN_1476), PG0232 (PGN_0335, *cpg70*), PG0611 (PGN_0654), PG0654 (PGN_0693), PG1798 (PGN_1767), PG0553 (PGN_1416, *pepK*), PG2216 (PGN_2080), PG0350 (PGN_1611), PG1795 (PGN_1770), PG0616 (PGN_0659, *hbp35*), PG1424 (PGN_0898, *pad*), PG0614 (PGN_0657), PG1030 (PGN_1321) and PG0290 (PGN_1674), are secreted through this secretion system. In addition, Veith *et al.* (55) reported that as well as the CTD proteins described above, the outer membrane vesicle contains the following CTD proteins: PG0026 (PGN_0022, *porU*), PG0182 (PGN_0291), PG0183 (no PGN), PG0411 (PGN_1556), PG0626 (no PGN), PG1548 (no PGN), PG1604 (PGN_0509), PG1969 (PGN_1770) and PG2172 (PGN_0123). We compared the proteomes of *P. gingivalis* strains *kgp rgpA rgpB* (T9SS-sufficient strain) and *kgp rgpA rgpB porK* (T9SS-deficient strain) using two-dimensional gel electrophoresis and peptide mass fingerprinting to identify other proteins secreted through the T9SS and identified the following 10 proteins: PGN_0152 (PG2100, *tapA*), PGN_0291 (PG0182), PGN_0335 (PG0232, *cpg70*), PGN_0654 (PG0611), PGN_0659 (PG0616, *hbp35*), PGN_0795 (PG0769), PGN_0898 (PG1424, *pad*), PGN_1416 (PG0553, *pepK*), PGN_1476 (PG0495) and PGN_1767 (PG1798) (56).

tapA (PGN_0152, PG2100) was associated with *tprA* (PGN_0876, PG1385). TprA is a tetratricopeptide repeat (TPR) protein that was upregulated in wild-type *P. gingivalis* (W83) cells placed in a mouse subcutaneous chamber, and the *tprA* mutant was clearly less virulent in the mouse subcutaneous abscess model (57). When the *tprA* mutant was placed in a mouse subcutaneous chamber, nine genes, including PG2102 (*tapA*), PG2101 (*tapB*) and PG2100 (*tapC*), were downregulated in the *tprA* mutant compared with the wild-type bacteria (58). These mutant genes were also downregulated in the culture medium. Yeast two-hybrid system analysis and *in vitro* protein binding assays with immunoprecipitation and surface plasmon resonance

detection revealed that the TprA protein, which has three TPR motifs (collectively known as a protein–protein interaction module), binds to the TapA and TapB proteins. The TapA protein is located on the outer membrane, whereas the TprA and TapB proteins are located in the periplasmic space. The *tapA* mutant is less virulent than the wild type in mouse subcutaneous infection experiments.

cpg70 (PGN_0335, PG0232) encodes a 69.8 kDa metallocarboxypeptidase (CPG70) that cleaves C-terminal Lys and Arg residues from peptides (59). Purified CPG70 is an N- and C-terminally truncated 91.5 kDa proprotein predicted from the *cpg70* gene. The *cpg70* mutant was less virulent than the wild type in a mouse subcutaneous infection experiment (59). The RgpA and Kgp proteinases and adhesins are C-terminally processed by CPG70 (60).

Abiko *et al.* (61) cloned *hbp35* (PGN_0659, PG0616), which encodes a *P. gingivalis* outer membrane protein that binds hemin and has a calculated molecular mass of 35,313 Da (62). Subcellular fractionation, sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot analysis using the anti-HBP35 antibody revealed that *hbp35* encodes three cytoplasmic proteins with molecular masses of 40, 29 and 27 kDa and a modified form of the 40 kDa protein on the cell surface (63). The 29 and 27 kDa proteins are N-terminal truncated forms of the 40 kDa protein, and the initiation codons for these proteins are located in the middle of the coding sequence of *hbp35*. HBP35 exhibits thioredoxin activity and is essential for hemin-depleted conditions. The CTD of HBP35 has been extensively characterized (53). The 22 C-terminal amino acid residues of the CTD of HBP35 are required for cell surface translocation and glycosylation. The CTD region functions as a recognition signal for the T9SS, and the glycosylation of CTD proteins occurs after removal of the CTD region, as CTD-containing peptides were not detected in samples of glycosylated HBP35 protein through peptide map fingerprinting analysis, and antibodies against CTD region peptides did not react with glycosylated HBP35 protein (53).

pad (PGN_0898, PG1424) encodes a prokaryotic peptidylarginine deiminase (PAD). McGraw *et al.* (64) purified and characterized the biochemical and enzymatic properties of the PAD enzyme from *P. gingivalis* and proposed that PAD, acting in concert with arginine-specific proteinases from *P. gingivalis*, promotes the growth of the pathogen in the periodontal pocket by enhancing the survivability of this bacterium and mediating the circumvention of host humoral defenses (64). Subsequently, research interests were focused on the relationship between *P. gingivalis* PAD and rheumatoid arthritis (5,65,66). Experimental evidence of a relationship between PAD and rheumatoid arthritis has recently been proposed. Using the chamber model, Maresz *et al.* (67) showed that infection with viable wild-type *P. gingivalis* exacerbated collagen-induced arthritis in a mouse model, manifested through earlier onset, accelerated progression and enhanced disease severity, including significantly increased bone and cartilage destruction. Additional studies showed that infection with wild-type *P. gingivalis* significantly increased levels of autoantibodies to collagen type II and citrullinated epitopes, as a PAD null mutant did not elicit similar host responses. Consistently, Gully *et al.* (68) reported that the development of experimental periodontitis was significantly reduced in PAD-deficient *P. gingivalis*, and the extent of collagen-induced arthritis was significantly reduced in animals exposed to previous induction of periodontal disease through oral inoculation with a PAD-deficient strain vs. the wild type.

PepK protein, encoded by *pepK* (PGN_1416, PG0553), is secreted via the T9SS and anchored on to the cell surface through binding to A-LPS (56,69). Enzymatic analysis using outer membrane fractions of wild-type, *pepK* and gingipain-deficient mutant strains suggests that PepK has Lys-specific serine endopeptidase activity, and the activation of this protein requires processing through Rgp (69).

T9SSs in other bacteria

The comparative analysis of 37 Bacteroidetes bacteria genomes revealed T9SS genes in bacteria belonging to the phylum Bacteroidetes (45). Mutant analysis has revealed functional T9SSs in three other bacterial species (*C. hutchinsonii*, *Flavobacterium johnsoniae*, *Tannerella forsythia*) in the phylum Bacteroidetes.

In *F. johnsoniae*, a gliding bacterium that digests insoluble chitin, a *chiA*-encoded chitinase (Fjoh_4555) is secreted via the T9SS (43,70). The *F. johnsoniae* genome encodes proteins with CTDs similar to the *P. gingivalis* CTD. However, the C-terminal region of ChiA is not similar to that of *P. gingivalis* CTD, although it is necessary for T9SS-mediated secretion (70).

Wang *et al.* (71) constructed an orthologous *porU* mutant in *C. hutchinsonii*, a widely distributed gram-negative cellulolytic bacterium, and this mutant showed defects in cellulose degradation and protein secretion. In addition, *C. hutchinsonii* CHU_0344, a dominant extracellular protein that possesses a C-terminal CTD, is secreted through the T9SS (71).

T. forsythia is one of the three bacteria implicated in the 'Red Complex' with *P. gingivalis* and *Treponema denticola*, which are important for chronic periodontitis (72). We constructed *porK*, *porT* and *sov* orthologous *T. forsythia* mutants and observed that these single mutants lack the surface layer (S-layer) and express less-glycosylated versions of the S-layer glycoproteins TfsA and TfsB (73). Compared with the proteins secreted from the *porK* and wild-type strains, the secretion of several proteins containing CTD-like sequences is *porK* gene-dependent. Tomek *et al.* (74) obtained similar results using *porK* and *porU* orthologous mutants, showing that the TfsA and TfsB glycoproteins in these mutants, which are N-terminally processed for Sec-mediated translocation across the cytoplasmic membrane, are

O-glycosylated, revealing that T9SS-mediated translocation across the outer membrane is not associated with *O*-glycan attachment. In wild-type bacteria, TfsA and TfsB are likely further glycosylated with rough-type LPS on the cell surface (74).

T9SS and gliding motility

While the periodontal pathogens *P. gingivalis* and *T. forsythia* are non-motile, the phylum Bacteroidetes includes many gliding bacteria, such as *F. johnsoniae* and *C. hutchinsonii* (75). *F. johnsoniae* cells attach to and move along surfaces at speeds of up to 5 μm/s in a process known as gliding motility (76). Electron microscopic analyses have failed to identify motility machines such as flagella and type IV pili on cells of *F. johnsoniae*, and analysis of the genome failed to identify genes encoding critical components of flagella and type IV pili, suggesting that *F. johnsoniae* gliding motility is achieved by another mechanism (77).

Bacteroidetes gliding motility is closely associated with the T9SS (43,45,46). *F. johnsoniae* genes *gldK*, *gldL*, *gldM*, *gldN*, *sprA*, *sprE* and *sprT*, which are essential for gliding motility, are homologous to *P. gingivalis* T9SS-related genes *porK*, *porL*, *porM*, *porN*, *sov*, *porW* and *porT*, respectively (43). This association may not be surprising because bacterial motility is typically associated with secretion systems. For example, flagellar motility and the type III secretion system have the same origin, and type IV pili, implicated in twitching motility, are associated with the type II secretion system.

Recently, we proposed a helical loop track model for the gliding motility of bacteria (78). In *F. johnsoniae*, the filamentous surface protein SprB is propelled along a left-handed helical loop on the bacterial cell surface (Fig. 3). When SprB adheres to a solid surface and can no longer move with respect to that surface, the cell is helically propelled in the opposite direction.

Coda

Many oral bacteria associated with periodontal diseases belong to the phylum Bacteroidetes. These bacteria are members of the genera *Porphyromonas*, *Prevotella*, *Tannerella* and *Capnocytophaga* and possess T9SS-related genes. Our unpublished data obtained from mutant studies suggest that *Prev. intermedia* has a functional T9SS. The proteins secreted through T9SSs include many virulence factors, such as gingipains in *P. gingivalis*. More than 10 proteins comprising T9SSs have been identified, but the precise interactions of these proteins remain unknown. Thus, the elucidation of T9SSs is only just commencing.

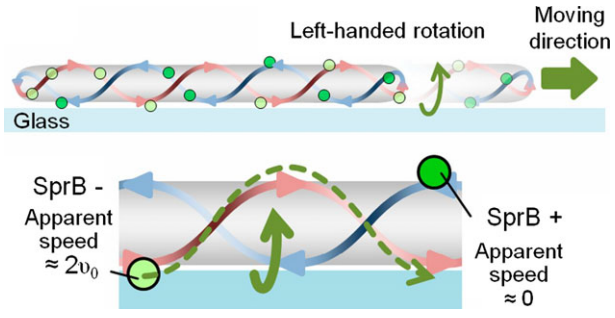

Fig. 3. Model of *Flavobacterium* gliding motility. A translocating cell on glass. SprB exhibits two different states: SprB moving toward the front of the cell, and SprB moving toward the rear of the cell. In a translocating cell, SprB moving toward the rear of the cell results in adhesion to the surface, generating left-handed rotation and right-directed translocation of the cell. SprB moves toward the front of the cell twice as fast with respect to the glass surface than SprB in a non-translocating cell. Reproduced from Nakane *et al.* (78).

Acknowledgements

Koji Nakayama would like to thank the members of the Division of Microbiology and Oral Infection, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, for their assistance.

References

1. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000* 1994; **5**:78–111.
2. Hajishengallis G, Lamont RJ. Breaking bad: manipulation of the host response by *Porphyromonas gingivalis*. *Eur J Immunol* 2014;**44**:328–338.
3. Pussinen PJ, Mattila K. Periodontal infections and atherosclerosis: mere associations? *Curr Opin Lipidol* 2004;**15**:583–588.
4. Madianos PN, Lieff S, Murtha AP *et al.* Maternal periodontitis and prematurity. Part II: maternal infection and fetal exposure. *Ann Periodontol* 2001;**6**: 175–182.
5. Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ. Periodontitis in RA – the citrullinated enolase connection. *Nat Rev Rheumatol* 2010;**6**:727–730.
6. Yoneda M, Naka S, Nakano K *et al.* Involvement of a periodontal pathogen, *Porphyromonas gingivalis* on the pathogenesis of non-alcoholic fatty liver disease. *BMC Gastroenterol* 2012;**12**:16.
7. Furusho H, Miyauchi M, Hyogo H *et al.* Dental infection of *Porphyromonas gingivalis* exacerbates high fat diet-induced steatohepatitis in mice. *J Gastroenterol* 2013;**48**:1259–1270.
8. Smalley JW, Silver J, Marsh PJ, Birss AJ. The periodontopathogen *Porphyromonas gingivalis* binds iron protoporphyrin IX in the μ-oxo dimeric form: an oxidative buffer and possible pathogenic mechanism. *Biochem J* 1998;**331**:681–685.
9. Smalley JW, Birss AJ, Withnall R, Silver J. Interactions of *Porphyromonas gingivalis* with oxyhaemoglobin and deoxyhaemoglobin. *Biochem J* 2002;**362**:239–245.
10. Smalley JW, Thomas MF, Birss AJ, Withnall R, Silver J. A combination of both arginine- and lysine-specific gingipain activity of *Porphyromonas gingivalis* is necessary for the generation of the μ-oxo bishaem-containing pigment from haemoglobin. *Biochem J* 2004;**379**: 833–840.
11. McDermid AS, McKee AS, Marsh PD. Effect of environmental pH on enzyme activity and growth of *Bacteroides gingivalis* W50. *Infect Immun* 1988;**56**:1096–1100.

12. Shah HN, Williams RAD. Utilization of glucose and amino acids by *Bacteroides intermedius* and *Bacteroides gingivalis*. *Curr Microbiol* 1987;**15**:241–246.
13. Shah HN, Williams RAD, Bowden GH, Hardie JM. Comparison of the properties of *Bacteroides melaninogenicus* from human dental plaque and other sites. *J Appl Bacteriol* 1976;**41**:473–492.
14. Smalley JW, Silver J, Birss AJ, Withnall R, Titler PJ. The haem pigment of the oral anaerobes *Prevotella nigrescens* and *Prevotella intermedia* is composed of iron (III) protoporphyrin IX in the monomeric form. *Microbiology* 2003;**149**: 1711–1718.
15. McKee AS, McDermid AS, Wait R, Baskerville A, Marsh PD. Isolation of colonial variants of *Bacteroides gingivalis* W50 with a reduced virulence. *J Med Microbiol* 1988;**27**:59–64.
16. Shah HN, Seddon SV, Gharbia SE. Studies on the virulence properties and metabolism of pleiotropic mutants of *Bacteroides gingivalis* W50. *Oral Microbiol Immunol* 1989;**4**:19–23.
17. Curtis MA, Kuramitsu HK, Lantz M *et al*. Molecular genetics and nomenclature of proteases of *Porphyromonas gingivalis*. *J Periodontal Res* 1999;**34**:464–472.
18. Kadowaki T, Nakayama K, Okamoto K *et al*. *Porphyromonas gingivalis* proteinases as virulence determinants in progression of periodontal diseases. *J Biochem* 2000;**128**:153–159.
19. Potempa J, Banbula A, Travis J. Role of bacterial proteinases in matrix destruction and modulation of host responses. *Periodontol 2000* 2000;**24**:153–192.
20. Collinson LM, Rangarajan M, Curtis MA. Altered expression and modification of proteases from an avirulent mutant of *Porphyromonas gingivalis* W50 (W50/BE1). *Microbiology* 1998;**144**:2487–2496.
21. Chen Z, Potempa J, Polanowski A, Wikstrom M, Travis J. Purification and characterization of a 50-kDa cysteine proteinase (gingipain) from *Porphyromonas gingivalis*. *J Biol Chem* 1992;**267**: 18896–18901.
22. Potempa J, Pike R, Travis J. The multiple forms of trypsin-like activity present in various strains of *Porphyromonas gingivalis* are due to the presence of either Arg-gingipain or Lys-gingipain. *Infect Immun* 1995;**63**:1176–1182.
23. Genco CA, Schifferle RE, Njoroge T, Forng RY, Cutler CW. Resistance of a Tn*4351*-generated polysaccharide mutant of *Porphyromonas gingivalis* to polymorphonuclear leukocyte killing. *Infect Immun* 1995;**63**:393–401.
24. Genco CA, Simpson W, Forng RY, Egal M, Odusnya BM. Characterization of a Tn*4351*-generated hemin uptake mutant of *Porphyromonas gingivalis*: evidence for the coordinate regulation of virulence factors by hemin. *Infect Immun* 1995;**63**:2459–2466.
25. Chen T, Dong H, Yong R, Duncan MJ. Pleiotropic pigmentation mutants of *Porphyromonas gingivalis*. *Microb Pathog* 2000;**28**:235–247.
26. Simpson W, Wang CY, Mikolajczyk-Pawlinska J *et al*. Transposition of the endogenous insertion sequence element IS*1126* modulates gingipain expression in *Porphyromonas gingivalis*. *Infect Immun* 1999;**67**:5012–5020.
27. Okamoto K, Nakayama K, Kadowaki T, Abe N, Ratnayake DB, Yamamoto K. Involvement of a lysine-specific cysteine proteinase in hemoglobin adsorption and heme accumulation by *Porphyromonas gingivalis*. *J Biol Chem* 1998;**273**: 21225–21231.
28. Abaibou H, Chen Z, Olango GJ, Liu Y, Edwards J, Fletcher HM. *vimA* gene downstream of *recA* is involved in virulence modulation in *Porphyromonas gingivalis* W83. *Infect Immun* 2001;**69**: 325–335.
29. Shoji M, Ratnayake DB, Shi Y *et al*. Construction and characterization of a nonpigmented mutant of *Porphyromonas gingivalis*: cell surface polysaccharide as an anchorage for gingipains. *Microbiology* 2002;**148**:1183–1191.
30. Sato K, Sakai E, Veith PD *et al*. Identification of a new membrane-associated protein that influences transport/maturation of gingipains and adhesins of *Porphyromonas gingivalis*. *J Biol Chem* 2005; **280**:8668–8677.
31. Sato K, Kido N, Murakami Y, Hoover CI, Nakayama K, Yoshimura F. Lipopolysaccharide biosynthesis-related genes are required for colony pigmentation of *Porphyromonas gingivalis*. *Microbiology* 2009;**155**:1282–1293.
32. Shi Y, Ratnayake DB, Okamoto K, Abe N, Yamamoto K, Nakayama K. Genetic analyses of proteolysis, hemoglogin binding, and hemagglutination of *Porphyromonas gingivalis*. Construction of mutants with a combination of *rgpA*, *rgpB*, *kgp*, and *hagA*. *J Biol Chem* 1999; **274**:17955–17960.
33. Smalley JW, Birss AJ, Szmigielski B, Potempa J. Sequential action of R- and K-specific gingipains of *Porphyromonas gingivalis* in the generation of the haem-containing pigment from oxyhaemoglobin. *Arch Biochem Biophys* 2007;**465**:44–49.
34. Paramonov N, Rangarajan M, Hashim A *et al*. Structural analysis of a novel anionic polysaccharide from *Porphyromonas gingivalis* strain W50 related to Arg-gingipain glycans. *Mol Microbiol* 2005;**58**:847–863.
35. Rangarajan M, Aduse-Opoku J, Paramonov N *et al*. Identification of a second lipopolysaccharide in *Porphyromonas gingivalis* W50. *J Bacteriol* 2008;**190**: 2920–2932.
36. Maruyama M, Hayakawa M, Zhang L, Shibata Y, Abiko Y. Monoclonal antibodies produced against lipopolysaccharide from *fimA* Type II *Porphyromonas gingivalis*. *Hybridoma (Larchmt)* 2009;**28**:431–434.
37. Shoji M, Yukitake H, Sato K *et al*. Identification of an O-antigen chain length regulator, WzzP, in *Porphyromonas gingivalis*. *Microbiologyopen* 2013;**2**:383–401.
38. Shoji M, Sato K, Yukitake H, Naito M, Nakayama K. Involvement of the Wbp pathway in the biosynthesis of *Porphyromonas gingivalis* lipopolysaccharide with anionic polysaccharide. *Sci Rep* 2014;**4**: 5056.
39. Nguyen K, Żylicz J, Szczesny P, Sroka A, Hunter N, Potempa J. Verification of a topology model of PorT as an integral outer membrane protein in *Porphyromonas gingivalis*. *Microbiology* 2009;**155**: 328–337.
40. Nelson KE, Fleischmann RD, DeBoy RT *et al*. Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. *J Bacteriol* 2003;**185**:5591–5601.
41. Nakayama K. Determination of the genome size of the oral anaerobic bacterium *Porphyromonas gingivalis* by pulsed field gel electrophoresis. *Dent Jpn* 1995; **32**:25–28.
42. Naito M, Hirakawa H, Yamashita A *et al*. Determination of the genome sequence of *Porphyromonas gingivalis* strain ATCC 33277 and genomic comparison with strain W83 revealed extensive genome rearrangements in *P. gingivalis*. *DNA Res* 2008;**15**:215–225.
43. Sato K, Naito M, Yukitake H *et al*. A protein secretion system linked to bacteroidete gliding motility and pathogenesis. *Proc Natl Acad Sci USA* 2010;**107**:276–281.
44. Saiki K, Konishi K. Identification of a *Porphyromonas gingivalis* novel protein sov required for the secretion of gingipains. *Microbiol Immunol* 2007;**51**: 483–491.
45. McBride MJ, Zhu Y. Gliding motility and Por secretion system genes are widespread among members of the phylum bacteroidetes. *J Bacteriol* 2013;**195**:270–278.
46. Chagnot C, Zorgani MA, Astruc T, Desvaux M. Proteinaceous determinants of surface colonization in bacteria: bacterial adhesion and biofilm formation from a protein secretion perspective. *Front Microbiol* 2013;**4**:303.
47. Glew MD, Veith PD, Chen D, Seers CA, Chen YY, Reynolds EC. Blue native-PAGE analysis of membrane protein complexes in *Porphyromonas gingivalis*. *J Proteomics* 2014;**110C**:72–92.

48. Ishiguro I, Saiki K, Konishi K. PG27 is a novel membrane protein essential for a *Porphyromonas gingivalis* protease secretion system. *FEMS Microbiol Lett* 2009;**292**:261–267.
49. Glew MD, Veith PD, Peng B *et al*. PG0026 is the C-terminal signal peptidase of a novel secretion system of *Porphyromonas gingivalis*. *J Biol Chem* 2012; **287**:24605–24617.
50. Chen YY, Peng B, Yang Q *et al*. The outer membrane protein LptO is essential for the O-deacylation of LPS and the co-ordinated secretion and attachment of A-LPS and CTD proteins in *Porphyromonas gingivalis*. *Mol Microbiol* 2011;**79**: 1380–1401.
51. Veith PD, Talbo GH, Slakeski N *et al*. Major outer membrane proteins and proteolytic processing of RgpA and Kgp of *Porphyromonas gingivalis* W50. *Biochem J* 2002;**363**:105–115.
52. Seers CA, Slakeski N, Veith PD *et al*. The RgpB C-terminal domain has a role in attachment of RgpB to the outer membrane and belongs to a novel C-terminal-domain family found in *Porphyromonas gingivalis*. *J Bacteriol* 2006;**188**: 6376–6386.
53. Shoji M, Sato K, Yukitake H *et al*. Por secretion system-dependent secretion and glycosylation of *Porphyromonas gingivalis* hemin-binding protein 35. *PLoS ONE* 2011;**6**:e21372.
54. Saiki K, Konishi K. *Porphyromonas gingivalis* C-terminal signal peptidase PG0026 and HagA interact with outer membrane protein PG27/LptO. *Mol Oral Microbiol* 2014;**29**:32–44.
55. Veith PD, Chen YY, Gorasia DG *et al*. Outer membrane vesicles obtained from *Porphyromonas gingivalis* contain exclusively outer membrane and periplasmic proteins and carry a cargo enriched with virulence factors. *J Proteome Res* 2014; **13**:2420–2432.
56. Sato K, Yukitake H, Narita Y, Shoji M, Naito M, Nakayama K. Identification of *Porphyromonas gingivalis* proteins secreted by the Por secretion system. *FEMS Microbiol Lett* 2013;**338**:68–76.
57. Yoshimura M, Ohara N, Kondo Y *et al*. Proteome analysis of *Porphyromonas gingivalis* cells placed in a subcutaneous chamber of mice. *Oral Microbiol Immunol* 2008;**23**:413–418.
58. Kondo Y, Ohara N, Sato K *et al*. Tetratricopeptide repeat protein-associated proteins contribute to the virulence of *Porphyromonas gingivalis*. *Infect Immun* 2010;**78**:2846–2856.
59. Chen YY, Cross KJ, Paolini RA, Fielding JE, Slakeski N, Reynolds EC. CPG70 is a novel basic metallocarboxypeptidase with C-terminal polycystic kidney disease domains from *Porphyromonas gingivalis*. *J Biol Chem* 2002;**277**:23433–23440.
60. Veith PD, Chen YY, Reynolds EC. *Porphyromonas gingivalis* RgpA and Kgp proteinases and adhesins are C terminally processed by the carboxypeptidase CPG70. *Infect Immun* 2004;**72**:3655–3657.
61. Abiko Y, Hayakawa M, Aoki H, Kikuchi T, Shimatake H, Takiguchi H. Cloning of a *Bacteroides gingivalis* outer membrane protein. *Arch Oral Biol* 1990;**35**:689–695.
62. Shibata Y, Hiratsuka K, Hayakawa M *et al*. A 35-kDa co-aggregation factor is a hemin binding protein in *Porphyromonas gingivalis*. *Biochem Biophys Res Commun* 2003;**300**:351–356.
63. Shoji M, Shibata Y, Shiroza T *et al*. Characterization of hemin-binding protein 35 (HBP35) in *Porphyromonas gingivalis*: its cellular distribution, thioredoxin activity and role in heme utilization. *BMC Microbiol* 2010;**10**:152.
64. McGraw WT, Potempa J, Farley D, Travis J. Purification, characterization, and sequence analysis of a potential virulence factor from *Porphyromonas gingivalis*, peptidylarginine deiminase. *Infect Immun* 1999;**67**:3248–3256.
65. Liao F, Li Z, Wang Y, Shi B, Gong Z, Cheng X. *Porphyromonas gingivalis* may play an important role in the pathogenesis of periodontitis-associated rheumatoid arthritis. *Med Hypotheses* 2009;**72**:732–735.
66. de Pablo P, Chapple IL, Buckley CD, Dietrich T. Periodontitis in systemic rheumatic diseases. *Nat Rev Rheumatol* 2009;**5**:218–224.
67. Maresz KJ, Hellvard A, Sroka A *et al*. *Porphyromonas gingivalis* facilitates the development and progression of destructive arthritis through its unique bacterial peptidylarginine deiminase (PAD). *PLoS Pathog* 2013;**9**:e1003627.
68. Gully N, Bright R, Marino V *et al*. *Porphyromonas gingivalis* peptidylarginine deiminase, a key contributor in the pathogenesis of experimental periodontal disease and experimental arthritis. *PLoS ONE* 2014;**9**:e100838.
69. Nonaka M, Shoji M, Kadowaki T *et al*. Analysis of a Lys-specific serine endopeptidase secreted via the type IX secretion system in *Porphyromonas gingivalis*. *FEMS Microbiol Lett* 2014;**354**:60–68.
70. Kharade SS, McBride MJ. *Flavobacterium johnsoniae* chitinase ChiA is required for chitin utilization and is secreted by the type IX secretion system. *J Bacteriol* 2014;**196**:961–970.
71. Wang Y, Wang Z, Cao J, Guan Z, Lu X. FLP-FRT-based method for unmarked deletions of *CHU_3237* (*porU*) and large genomic fragments of *Cytophaga hutchinsonii*. *Appl Environ Microbiol* 2014;**80**:6037–6045.
72. Holt SC, Ebersole JL. *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*: the "red complex", a prototype polybacterial pathogenic consortium in periodontitis. *Periodontol 2000* 2005;**38**:72–122.
73. Narita Y, Sato K, Yukitake H *et al*. Lack of a surface layer in *Tannerella forsythia* mutants deficient in the type IX secretion system. *Microbiology* 2014;**160**:2295–2303.
74. Tomek MB, Neumann L, Nimeth I *et al*. The S-layer proteins of *Tannerella forsythia* are secreted via a type IX secretion system that is decoupled from protein *O*-glycosylation. *Mol Oral Microbiol* 2014;**29**:307–320.
75. Jarrell KF, McBride MJ. The surprisingly diverse ways that prokaryotes move. *Nat Rev Microbiol* 2008;**6**: 466–476.
76. McBride MJ. Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. *Annu Rev Microbiol* 2001; **55**:49–75.
77. McBride MJ, Xie G, Martens EC *et al*. Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis. *Appl Environ Microbiol* 2009;**75**:6864–6875.
78. Nakane D, Sato K, Wada H, McBride MJ, Nakayama K. Helical flow of surface protein required for bacterial gliding motility. *Proc Natl Acad Sci USA* 2013; **110**:11145–11150.