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REVIEW

VimA mediates multiple functions that control virulence in *Porphyromonas gingivalis*

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SUMMARY

***Porphyromonas gingivalis*, a black-pigmented, gram-negative anaerobe, is an important etiological agent of periodontal disease. Its ability to survive in the periodontal pocket and orchestrate the microbial/host activities that can lead to disease suggest that *P. gingivalis* possesses a complex regulatory network involving transcriptional and post-transcriptional mechanisms. The *vimA* (virulence modulating) gene is part of the 6.15-kb *bcp-recA-vimA-vimE-vimF-aroG* locus and plays a role in oxidative stress resistance. In addition to the glycosylation and anchorage of several surface proteins including the gingipains, VimA can also modulate sialylation, acetyl coenzyme A transfer, lipid A and its associated proteins and may be involved in protein sorting and transport. In this review, we examine the multifunctional role of VimA and discuss its possible involvement in a major regulatory network important for survival and virulence regulation in *P. gingivalis.* It is postulated that the multifunction of VimA is modulated via a post-translational mechanism involving acetylation.**

INTRODUCTION

The human oral cavity has always been a challenging environment for various bacterial taxa to inhabit. Although home to more than 600 species, only a subset of microbes, which now includes previously unrecognized and uncultivated species, are associated with disease (Dewhirst *et al.*, 2010; Gross *et al.*, 2010; Griffen *et al.*, 2012). The 'red complex', consisting of *P. gingivalis*, *Tannerella forsythia* and *Treponema denticola*, is well established to be associated with periodontal disease (Holt & Ebersole, 2000; Kumar *et al.*, 2003; Dewhirst *et al.*, 2010). *P. gingivalis*, now designated as a 'keystone' species, even when present in low numbers, is able to manipulate the host immune system, thus eliciting a major effect on the composition of the oral microbial community which significantly contributes and may be ultimately responsible for the pathology of periodontitis (Hajishengallis, 2011; Hajishengallis *et al.*, 2011; Darveau *et al.*, 2012; Hajishengallis & Lamont, 2012).

P. gingivalis is known to possess several outer membrane structures, major outer membrane proteins and secreted proteins that have contributed to cell adherence, survival and virulence (Lamont & Jenkinson, 1998). To facilitate its 'keystone' species function, this organism requires a complex regulatory network. Integrated as part of this network are regulatory circuits using transcriptional and post-transcriptional mechanisms that are guided by the external environment including microbial and host-induced cues. A variety of transcriptional regulatory mechanisms such as the two component system (Hasegawa *et al.*, 2003; Nishikawa & Duncan, 2010), extracytoplasmic function sigma factor (Dou *et al.*, 2010), and transposase-mediated regulation (Lewis *et al.*, 2009),

have been reported in *P. gingivalis*. However, a key element in modulating the pathogenic potential of *P. gingivalis* is the post-translational modification of several of the major surface components. For example, the major proteases, called gingipains, consist of arginine-specific (Arg-gingipain; Rgp) and lysine-specific (Lys-gingipain; Kgp) proteases that are both extracellular and cell-membrane-associated. The maturation pathway of the gingipains is linked to carbohydrate biosynthesis and is regulated by several proteins including the PorR, PorT, Sov, Rfa, VimA, VimE and VimF (Vanterpool *et al.*, 2004, 2005a,b, 2006; Sato *et al.*, 2009, 2010; Saiki & Konishi, 2010; Shoji *et al.*, 2011).

VimA is a 39-kDa protein that is encoded for by the *vimA* gene. This gene is part of the 6.15-kb *bcp-recA-vimA-vimE-vimF-aroG* locus (Fig. 1). A role for the *vimA* gene in oxidative stress resistance has been demonstrated in *P. gingivalis*, but the VimA protein is believed to be multifunctional (Fig. 2). In addition to the glycosylation and anchorage of several surface proteins including the gingipains, VimA can also modulate sialylation (Aruni *et al.*, 2011), acetyl coenzyme A (acetyl-CoA) transfer, lipid A and its associated proteins, and may be involved in protein sorting and transport (Aruni *et al.*, 2012). In this review, we examine the multifunctional role of VimA and discuss its possible involvement in a major regulatory network important for survival and virulence regulation in *P. gingivalis.* It is postulated that the multifunction of VimA is modulated via a post-translational mechanism involving acetylation.

VIMA IS INVOLVED IN OXIDATIVE STRESS RESISTANCE

There are multiple complex systems that defend and protect *P. gingivalis* against oxidative damage generated in the inflammatory environment of the periodontal pocket (reviewed in Henry *et al.*, 2012). Components of these systems, which include antioxidant enzymes (Mydel *et al.*, 2006), DNA binding proteins (Meuric *et al.*, 2008), the hemin layer (McKenzie *et al.*, 2012) and enzymatic removal of reactive oxygen species-induced deleterious products, are coordinately regulated (Henry *et al.*, 2008). Multiple transcriptional modulators (including OxyR, RprY and extracytoplasmic function sigma factors) that sense oxidative-stress-generating agents and induce the appropriate response in *P. gingivalis* have been described (Henry

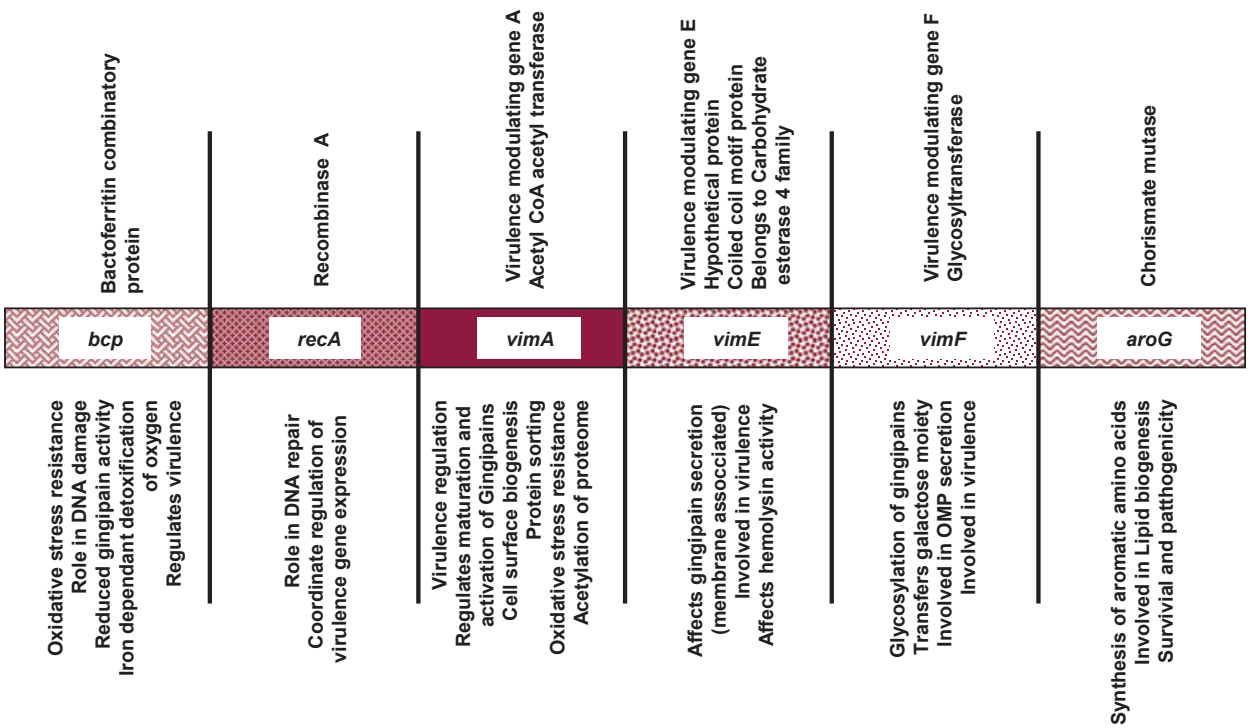

Figure 1 Genome architecture of *bcp- aroG* locus in *Porphyromonas gingivalis* showing the annotation of the genes (top panel) and their putative functions (bottom panel).

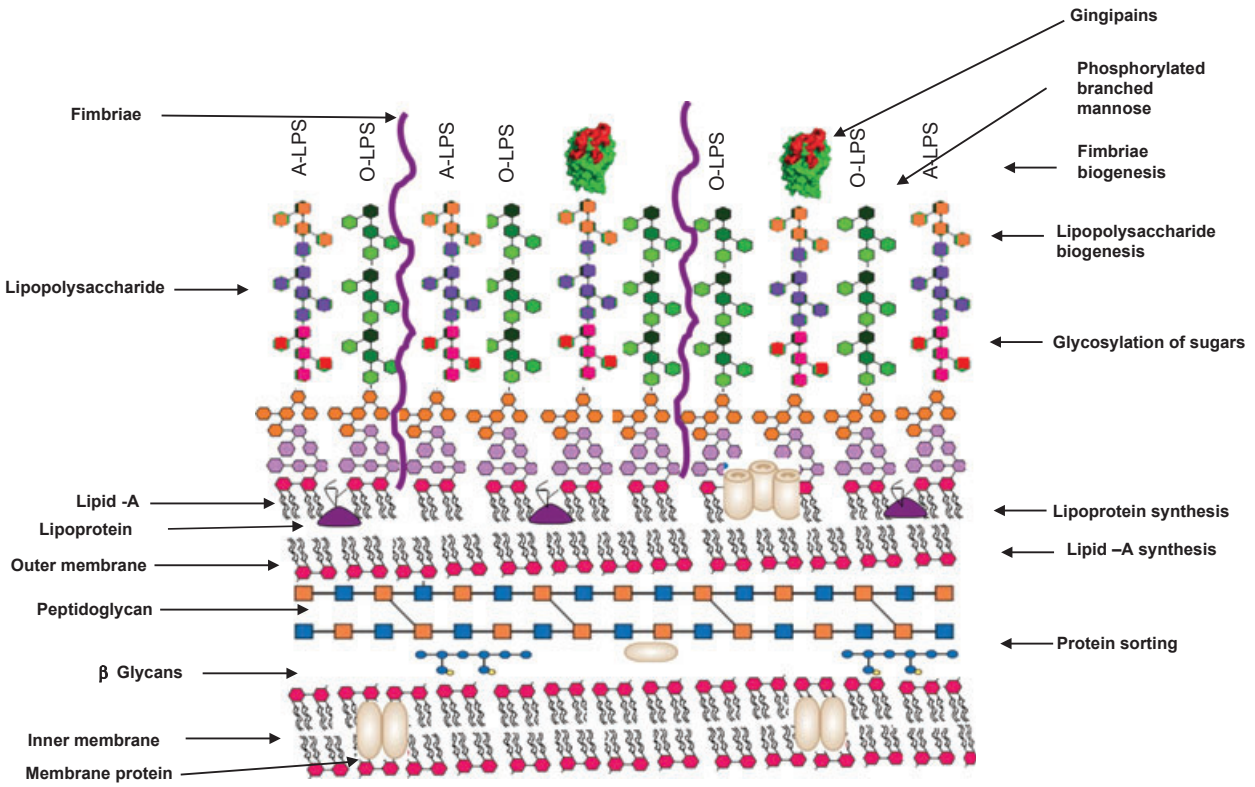

Figure 2 Functions of VimA in surface biogenesis. Multifunction of VimA in various cell surface modifications. Right panel shows the VimA functions and the left panel shows various surface structures.

et al., 2008; Lewis *et al.*, 2009; Dou *et al.*, 2010; McKenzie *et al.*, 2012). Collectively, these data suggest that *P. gingivalis* may have a redundant mechanism(s) to defend against oxidative stress.

Inactivation of the *vimA* gene in *P. gingivalis* generated a non-polar isogenic mutant (Abaibou *et al.*, 2001) that showed increased sensitivity to hydrogen peroxide (Vanterpool *et al.*, 2006). This mutant, designated *P. gingivalis* FLL92, also displayed a non-black-pigmented phenotype and had reduced gingipain activity (Vanterpool *et al.*, 2005b). The involvement of the gingipains in heme acquisition and binding (Okamoto *et al.*, 1998) and the ability of the heme layer to act as an 'oxidative sink' to neutralize reactive oxygen intermediates (Smalley *et al.*, 2000, 2004) would be consistent with the sensitivity of *P. gingivalis* FLL92 to hydrogen peroxide-induced oxidative stress.

This model system has uncovered other mechanisms that may be involved in oxidative stress resistance in *P. gingivalis*. In the chromosomal DNA of *P. gingivalis* FLL92 there was an elevated level of 8-oxo-7,8-dihydroguanine (8-oxoG) after exposure to hydrogen peroxide (Johnson *et al.*, 2004). The ability to repair these lesions used a novel non-base excision repair mechanism that was upregulated in *P. gingivalis* FLL92 compared with the wild-type strain (Henry *et al.*, 2008). A gene expression profile using DNA microarray analysis revealed that about 5.7 and 3.45% of the *P. gingivalis* genome displayed altered expression in response to hydrogen peroxide exposure at 10 and 15 min, respectively in FLL92 compared with the wild-type W83 strain. The *P. gingivalis* FLL92 isogenic mutant in response to hydrogen peroxide-induced oxidative stress showed upregulation of several genes including some with unknown function and others known to be involved in oxidative stress resistance in other pathogenic bacteria. In particular, after 15 min of exposure to hydrogen peroxide the *P. gingivalis vimA* mutant had high upregulation (between 9.6 and 11.9-fold) of transposase-encoding genes (PG0051, PG0194, PG0812, PG813, PG0944 and PG2169). Increase in transposase activity in response to oxidative stress has previously been

reported in *P. gingivalis* (Diaz *et al.*, 2006). However, we find them to be upregulated only during prolonged exposure (15 min) to oxidative stress and not modulated after 10 min exposure. While the significance of this is unclear, this could be an inherent strategy of *P. gingivalis* to induce genomic rearrangement that may lead to survival under unstable hostile environmental conditions (Diaz *et al.*, 2006).

In silico analysis of the metabolome of the *vimA*-defective mutant during oxidative stress indicated an increase in pyruvate synthesis and glycine catabolism that can result in the production of more endogenous CO_2. The use of alternative energy substrates such as fumarate and formate was noted (unpublished results). Hence during oxidative stress, *P. gingivalis* may resort to a metabolic state where the oxidative reactions are reduced and there is a shift to reduction reactions that bring about increase in cellular CO_2.

The interaction of VimA with other proteins may also facilitate oxidative stress resistance in *P. gingivalis*. Pull-down experiments using the recombinant VimA protein showed the ability of this protein to interact with the sialidase protein (Vanterpool *et al.*, 2006). Furthermore, in a *vimA* mutant, sialidase activity was reduced (Vanterpool *et al.*, 2006). An isogenic mutant defective in the sialidase gene showed increased sensitivity to hydrogen peroxide (Aruni *et al.*, 2011). Release of free monomeric sialic acid when it is cleaved from the sugar chain can detoxify hydrogen peroxide (Iijima *et al.*, 2004). This reaction reduces the hydrogen peroxide and sialic acid (*N*-acetylneuraminic acid) into H_2O and non-toxic carboxylic acid (Iijima *et al.*, 2004). The ability of the *P. gingivalis* sialidase to cleave multiple substrates that could result in the release of sialic acid would be consistent with the increased sensitivity of the sialidase-defective isogenic mutants to hydrogen peroxide compared with the wild-type strain. The VimA-dependent regulation of the sialidase activity in *P. gingivalis* is unclear and may include both transcriptional and post-translational mechanisms. The sialidase gene was not expressed in the *vimA*-defective mutant (Aruni *et al.*, 2011).

VIMA IS INVOLVED IN CELL SURFACE BIOGENESIS

The cell surface components play an important role in establishing the organism in the host and are involved in adhesion, invasion and colonization. In *P. gingivalis,* surface components like capsule, fimbria, outer membrane proteins, peptidoglycan and lipopolysaccharide (LPS), contribute to virulence (Lamont & Jenkinson, 1998). Autoaggregation is an important phenomenon in virulence and correlates with the absence of capsule and the presence of fimbriae (Davey & Duncan, 2006). Although non-virulent, it was noted that the *P. gingivalis vimA* mutant FLL92 showed enhanced autoaggregation (Osbourne *et al.*, 2010). Major differences in the capsule and fimbriae were noted between the wild-type and this *vimA*-defective mutant. The wild-type showed a well-defined capsule in contrast to a less defined, irregular and fuzzy capsule in FLL92. Also, the FLL92 strain showed distinct fine structures resembling fimbriae that were not present in the wild-type strain (Osbourne *et al.*, 2010). This could be the result of changes in the phenotypic expression of fimbrial protein. This was confirmed using Western blot analysis with anti-FimA antibody on the outer membrane and total protein fractions of W83 and FLL92. The immunoreactive band corresponding to the FimA (between 41 and 43 kDa) was missing in the wild-type W83 strain but was present in the FLL92 outer membrane fraction (Osbourne *et al.*, 2010). Immunogold electron microcopy also identified appendage-like structures in FLL92 that were reactive to the FimA antibody. To confirm that *vimA* plays a role only at the post-translational level of fimbrial expression, a reverse transcription PCR was performed; the *fimA* gene was similarly expressed in both the wild-type and the *vimA*-defective isogenic mutant FLL92 (Osbourne *et al.*, 2010).

To clarify the effect of the *vimA* mutation on glycosylation of outer-membrane proteins, a lectin assay was performed and the results showed that outer membrane proteins with galactose (β1,3)*N*-acetylgalactosamine, *N*-acetyl-α-D-galactosamine, galactose (β1,4)*N*-acetylglucosamine, *N*-acetyl-D-galactosamine and sialic acid (*N*-acetyl neuraminic acid) were affected by the *vimA* mutation (Osbourne *et al.*, 2010). Ultrastructural studies on the peptidoglycan sacculi of the *P. gingivalis vimA* mutant showed a distinct difference with uneven surface variations in comparison to that of W83, suggesting a role of *vimA* in peptidoglycan synthesis (unpublished results). The peptidoglycan sacculus is a rigid exoskeleton structure, which is involved in maintaining cytoplasmic

pressure. One likely conclusion from these studies could infer a variation in the structure of the sacculi caused by faulty peptide cross-linkages in the *vimA* mutant. The cross-linked peptide bridges found in the cell wall typically include alanine (Barnard & Holt, 1985). Alanine tRNA synthetase is involved in alanine transport and formation of peptide cross-linkages. Using the *P. gingivalis* recombinant VimA protein produced in *Escherichia coli*, an interaction with alanine tRNA synthetase was demonstrated (Vanterpool *et al.*, 2006). This was confirmed using the VimA chimera where it was also shown to interact with isoleucyl tRNA synthetase in addition to 20 other proteins (Aruni *et al.*, 2012). The direct effect of VimA on the functional role of these proteins is unclear. Of the 21 VimA interacting proteins, a majority were cytoplasmic and membrane-bound, seven were found to be involved in cell surface biogenesis, and all had an N-terminal cleavage signal with C-terminal cell wall sorting motif (Aruni *et al.*, 2012). Among these proteins, five were related to LPS synthesis (Aruni *et al.*, 2012). Further, we have also shown that a defect in *vimA* causes alteration in lipid A biogenesis and membrane lipoproteins (Aruni *et al.*, 2012). Analysis of the polysaccharide component of LPS after removal of lipid-A showed shorter lengths in the *vimA*-defective mutant (Vanterpool *et al.*, 2006). Hence, collectively, we could conclude that *vimA* is involved in cell surface biogenesis.

VIMA MODULATES GINGIPAIN ACTIVITY

The proteolytic cleavage of multiple substrates by the *P. gingivalis* cysteine proteases called gingipains is considered to be important for its survival and to play a significant role in virulence (Eley & Cox, 2003; Vanterpool *et al.*, 2005b). More specifically, these proteases are involved in several processes known to be important for bacterial growth and can compromise cellular integrity and host cell functions by several mechanisms triggered for example, by inactivation of cytokines, platelet aggregation and apoptosis (Lamont & Jenkinson, 1998; Kadowaki *et al.*, 2000; Sheets *et al.*, 2008). Activation of gingipains is associated with several *vim* genes that are involved in post-translational modification of the gingipains (Abaibou *et al.*, 2001; Vanterpool *et al.*, 2004, 2005a,b, 2006; Osbourne *et al.*, 2010; Aruni *et al.*, 2011, 2012). Glycosylation, which is involved in the addition of carbohydrate moieties to the gingipains, is one of the important post-translational modifications in gingipain biogenesis (Curtis *et al.*, 1999, 2001; Gallagher *et al.*, 2003; Vanterpool *et al.*, 2005b).

Among the *vim* genes, *vimA* is a key player in modulating the phenotypic expression of the gingipains. Inactivation of the *vimA* gene resulted in isogenic mutants that showed decreased gingipain activity during the exponential growth phase (Vanterpool *et al.*, 2005b). These activities, however, increased to approximately 60% during stationary phase in the wild-type strain. Throughout all the growth phases, Rgp and Kgp activities were mostly soluble, in contrast to those of the wild-type strain. Expression of the gingipain genes was unchanged in the *vimA*-defective mutants compared with the parent strains (Vanterpool *et al.*, 2005b). The gingipain proenzyme species were observed in these mutants providing some of the first evidence for post-translational regulation of protease activity in *P. gingivalis* (Olango *et al.*, 2003; Vanterpool *et al.*, 2005b). Variation in the glycosylation profile of the gingipains was noted including no immunoreactivity to monoclonal antibody 1B5 (mAb1B5) known to recognize the phosphorylated branched mannan (Vanterpool *et al.*, 2006; Rangarajan *et al.*, 2008). In addition, methanolysis and gas chromatography/mass spectrometry analysis used to evaluate the monosaccharide composition of the inactive RgpB proenzyme from the *P. gingivalis vimA*-defective mutant (FLL92) showed that the *N*-Ac-glucosamine and *N*-Ac-galactosamine moieties were not detectable in comparison to the active forms of the gingipain (unpublished results).

The *vim* genes play a coordinated role in the glycosylation of the gingipains (Sheets *et al.*, 2008). Inactivation of the *vimE* and *vimF* genes that are downstream of *vimA* and located on the same operon resulted in isogenic mutants that showed no gingipain activity (Vanterpool *et al.*, 2006). Expression of the gingipain genes was also unchanged in these isogenic mutants compared with the parent strain (Vanterpool *et al.*, 2004, 2005a,b, 2006). The gingipain proenzyme species were also observed in these mutants (Olango *et al.*, 2003; Vanterpool *et al.*, 2005b). However, in contrast to the *vimA*-defective mutant, which only had the RgpB gingipain cell associated form, the *vimE*-defective and *vimF*-defective mutants had both cell-associated and extracellular inactive forms of the gingipains (Olango *et al.*, 2003;

Vanterpool *et al.*, 2005b, 2006). Throughout all the growth phases, no activation of the gingipains was observed. Again, variation in the glycosylation profile of the gingipains including the missing phosphorylated branched mannan was noted (Vanterpool *et al.*, 2006; Rangarajan *et al.*, 2008). Collectively, these observations suggest that the *vimE* and *vimF* genes that encode for a putative carbohydrate esterase and glycosyltransferase, respectively (Vanterpool *et al.*, 2005a,b), are essential for the post-translational modification required for gingipain activation.

There are multiple VimA-dependent mechanisms that can modulate gingipain activity in *P. gingivalis* (Vanterpool *et al.*, 2005b, 2006; Aruni *et al.*, 2012). VimA was shown to interact with proteins such as the HtrA, RegT and sialidases that in other bacteria have been shown to be involved in post-translational regulation of proteases (Vanterpool *et al.*, 2006). Inactivation of the genes encoding these proteins in *P. gingivalis* resulted in reduced gingipain activity (Roy *et al.*, 2006; Vanterpool *et al.*, 2010; Aruni *et al.*, 2012). In the sialidase-defective mutant, for example, because the level of expression of the gingipain genes was unaltered, it is likely that the sialidase gene is involved in the post-translational regulation of the gingipains. The breakdown of sialic acid residues and sialoconjugates by sialidases contributes to a wide range of important biological functions and conformational stabilization of glycoproteins (Angata & Varki, 2002). Analysis of the monosaccharide composition of the gingipains indicates the presence of at least nine different sugars, including high levels of sialic acid (Rangarajan *et al.*, 2005; Sakai *et al.*, 2007). The level of sialylation and its role in gingipain maturation/activation are unclear and are under further investigation.

VIMA IS LIKELY INVOLVED IN PROTEIN SORTING

Covalent attachment of extracellular proteins to the cell wall peptidoglycan is a fundamental feature of cell surface biogenesis in gram-positive bacteria. These cell wall anchored surface proteins are known to carry LPXTG, a sortase recognition motif (Schneewind *et al.*, 1995) and use transpeptidase enzymes called sortases to covalently connect their substrates to a target molecule (reviewed in Marraffini *et al.*, 2006). A similar mechanism involving the use of these sortases, as observed in gram-positive bacteria, is less clear in gram-negative bacteria. Although protein secretion mediated through various secretory systems, such as contact-dependent secretion and Type I–Type VI secretory systems in gram-negative bacteria are highly conserved (Thanassi & Hultgren, 2000), the sorting of proteins from the cytoplasm by means of a consensus protein sorting signal is now starting to emerge (Mazmanian *et al.*, 2001). Preferentially observed in bacteria from sediments, soils and biofilms, proteins with several key characteristic sorting signals were contained in a short C-terminal (CTERM) homology domain (Haft *et al.*, 2006). This domain, designated PEP-CTERM, includes a near-invariant motif Pro-Glu-Pro (PEP) that is considered a possible recognition or processing site, followed by a predicted transmembrane helix and a cluster rich in basic amino acids. These target proteins are usually destined to transit cellular membranes during their biosynthesis and undergo additional posttranslational modifications such as glycosylation (Haft *et al.*, 2012).

P. gingivalis may have multiple protein sorting/secretion systems. In contrast with the *P. gingivalis* wild-type strain, studies in our laboratory indicate that several proteins were aberrantly expressed or missing from the outer membrane and extracellular fractions of the *vimA*-defective isogenic mutant (Osbourne *et al.*, 2012). Several of the missing extracellular proteins in *P. gingivalis* FLL92 carried an N-terminal signal peptide, a common C-terminal motif with a common consensus Gly-Gly–CTERM pattern and a polar tail consisting of aromatic amino acid residues. Both the C-terminal motif with its common consensus Gly-Gly–CTERM pattern and polar tail are known to have protein sorting characteristics in other organisms (Ton-That *et al.*, 2004; Haft & Varghese, 2011). Other protein pull-down assays using the His-tagged chimeric VimA showed several proteins that contained conserved LXXTG or LPXTG motifs(Aruni *et al.*, 2012). These predicted putative sorting motifs were present in all the membrane or extracellular proteins that interacted with VimA. Hence, the unique C-terminal domain (CTD) with a glycine-rich region and a general sorting signal motif-like region (LxxxG) could be a general mechanism of VimA-mediated protein sorting.

Interestingly, studies from other groups demonstrated that in *P. gingivalis*, a group of surface proteins, including RgpB, with a common CTD was

shown to be exported by a novel secretion system (the Por secretion system; PorSS) to the surface, where they are covalently attached (Sato *et al.*, 2010; Shoji *et al.*, 2011). It was proposed that the CTD acts as a site of recognition by a *P. gingivalis* processing enzyme(s), possibly a novel sortase-like enzyme that cleaves the CTD sequence and attaches the C-terminal carbonyl to a sugar amine of a novel anionic LPS, which can be modulated by its level of acylation (Pallen *et al.*, 2001; Dramsi *et al.*, 2008; Haurat *et al.*, 2011). The identity of a specific sortase-like enzyme or recognition motif was not identified. Interrogation of these proteins including RgpB that are transported through the PorSS, showed the same Gly-Gly–CTERM pattern and the conserved YDMGRX and LXXG motif that we previously identified (Fig. 3 and Fig. S1). Hence, it is likely that VimA may play a role in this transport/secretion system. This is further supported by the demonstration that mutations in the *vimA* gene and the other genes that are part of the PorSS had a similar phenotype (Nguyen *et al.*, 2007; Chen *et al.*, 2011). A recently proposed mechanism for protein sorting into the outer membrane vesicles of *P. gingivalis* showed a critical role for LPS and its level of acylation in this process (Haurat *et al.*, 2011). Only a few of the most abundant proteins identified from the outer membrane vesicles were similar to those that interacted with the VimA chimera and had a general sorting signal motif-like region. It is unclear if they carry any specific sorting signals or use a similar putative VimA-dependent mechanism. In gram-positive bacteria, multiple sortase systems have been described (Dramsi *et al.*, 2008). However, in gram-negative bacteria there is a gap in such information, and this gap requires further exploration. Sortase homologs have only recently been identified in gram-negative bacteria (Pallen *et al.*, 2001), while a pattern of sorting through PEP- CTERM/exosortase was identified in some gram-negative bacteria (Haft *et al.*, 2006). *In silico* analysis of VimA also showed a DUF482/CH1444 domain that is a part of the PEP-CTERM system (Fig. 4 and Fig. S2; Haft *et al.*, 2006; Osbourne *et al.*, 2010). It is likely that in *P. gingivalis* there may be multiple systems, some of which may have novel characteristics.

VIMA IS INVOLVED IN PROTEIN ACETYLATION

Post-translational modification of proteins serves as a means for cells to react quickly to changes in the environment. Acetylation and deacetylation in pathogenic bacteria like *Salmonella enterica* (Starai & Escalante-Semerena, 2004) and *Bacillus* spp. (Gardner *et al.*, 2006) have previously been found to play an important role in such regulations. Among the various post-translational modifications, acetylation modifications were identified as a major post-translational modification equal to phosphorylation (Smith & Workman, 2009). It is important to note that involvement of protein acetylation has already been reported in a stress-induced transcription network in *E. coli* (Lima *et al.*, 2011), and a novel feedback inhibition regulating energy production in *E. coli* was reported for protein acetylation/deacetylation involving the transfer of CoA (Starai *et al.*, 2005). Hence, acetylome modulation could be considered part of a universal switch to regulate important functions of prokaryotes.

There is growing evidence to suggest that VimA is an acetyl-CoA transferase and that it may be involved in protein acetylation. Domain architecture shows a conserved acetyltransferase domain and an *N*-acetyl transferase superfamily domain (Fig. 4 and Fig. S2). The conserved nature of the *vimA* gene was noted among various bacteria (Aruni *et al.*, 2012). Orthologs of *vimA* were found in many anaerobic bacteria such as *Clostridium botulinium*, *Rhodobacter sphaeroides* and *Parabacteroides distasonis*. Multiple sequence alignment and phylogenetic analysis of the protein sequence also show its molecular relatedness to exopolysaccharide synthesis family proteins among other bacteria and homologous to the acetyl-CoA transferases of other oral bacteria such as *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Rhodobacter shpaeroides* (Aruni *et al.*, 2012). A

Figure 3 Common C- terminal domain (CTD) architecture of VimA interacting proteins and CTD proteins showing three consensus conserved LxxG, YDMNGRx, G-G-Cterm motifs.

PEP - PEP C term domain
Acetyltransf - Acetyl transferase domain
NAT-SF - N-acetyl transferase superfamily

Figure 4 Domain architecture of VimA showing conserved acetyl transferase domain, *N*-acetyl transferase superfamily domain between positions 138 and 257, and a PEP-Cterm domain between amino acid positions 60 and 138.

comparison with other *P. gingivalis* acetyl-CoA transferases showed VimA to be closely related to the lipid biosynthesis transferase (PG2222) and transpeptidase (PG0794). It showed molecular relatedness with CoA transferase (PG1013) and acetyl transferase (PG1358). Protein modeling showed the conserved $\alpha/\beta/\alpha$ domain structure of acetyl-CoA *N*-acetyl transferase superfamily (Osbourne *et al.*, 2010; Aruni *et al.*, 2012).

The VimA acetyltransferase function was demonstrated with its ability to transfer acetyl-CoA to isoleucine (Aruni *et al.*, 2012). The alteration in this transfer resulted in a reduction in branched-chain amino acids of approximately 40% in the *P. gingivalis vimA*-defective mutant. In addition, VimA was also shown to regulate the levels of acetyl-CoA in *P. gingivalis* (Aruni *et al.*, 2012). In *P. gingivalis*, several outer membrane proteins are lipid modified (Yoshimura *et al.*, 2009). One of the common lipid modifications seen on the outer membrane proteins of prokaryotes is a process involving the addition of an acyl group (Eichler & Adams, 2005). In a ^{3}H–labelled palmitic acid assay, the extracellular fraction of the *P. gingivalis* wild-type strain showed more acylated proteins than the *P. gingivalis vimA* mutant FLL92 (Osbourne *et al.*, 2010). Collectively, these observations have attributed a role for VimA in the acetylation process in *P. gingivalis*. Furthermore, variation in the acetylation profile is observed in the *vimA*-defective mutant compared with the wild-type (unpublished results).

A picture is now emerging that could suggest a role for acetylation/deacetylation in gingipain biogenesis and protein secretion/localization in *P. gingivalis.* The outer membrane protein LptO (PG0027) involved in *O*-deacetylation of LPS has been demonstrated to coordinate secretion/attachment of A-LPS and CTD proteins in *P. gingivalis* (Chen *et al.*, 2011). In our preliminary studies using monoclonal antibodies in Western blot analysis against *N*-acetylated lysine, more acetylated proteins were observed in the extracellular and membrane fractions of the wild-type compared with the *vimA*-defective mutant (unpublished results). In other studies we have shown a common putative sorting motif in the VimA interacting proteins (Aruni *et al.*, 2012), other membrane proteins that are missing in the *vimA*-defective mutant (Osbourne *et al.*, 2012) and other CTD proteins (Seers *et al.*, 2006; Nguyen *et al.*, 2007; Slakeski *et al.*, 2011). Together, these observations may suggest a common secretion system and raise questions on a mechanism for VimA-dependent acetylation and LptO-dependent deacetylation in the process. This requires further study.

VIMA IS INVOLVED IN VIRULENCE REGULATION

Collectively, the *P. gingivalis* VimA protein has been shown to affect gingipain maturation, sialidase activity, autoaggregation, hemolysis, hemagglutination, LPS synthesis, capsular synthesis and fimbrial expression, and plays a role in the glycosylation and anchorage of several surface proteins (Abaibou *et al.*, 2001; Olango *et al.*, 2003; Vanterpool *et al.*, 2006; Osbourne *et al.*, 2010, 2012; Aruni *et al.*, 2011, 2012). These phenotypic traits can be correlated with the virulence of *P. gingivalis* (Kilian, 1998). Hence, their decreased expression in the *vimA*-defective mutant is manifested in its increased sensitivity to oxidative stress resistance and reduced ability to invade or induce apoptosis of host epithelial and endothelial cells (Olango *et al.*, 2003). This is also consistent with the reduced virulence of the *vimA*-defective mutant of *P. gingivalis* compared with the parent strain in a mouse model (Abaibou *et al.*, 2001).

The multiple phenotypic properties of the *vimA*-defective mutant can result from a cascade of events suggesting that the *vimA* gene product is part of a complex regulatory network using both direct and indirect transcriptional and post-transcriptional mecha-

nisms. For example, carbohydrate biosynthesis is linked to the maturation pathway of the gingipains. A VimA-dependent alteration in the addition of the appropriate carbohydrate moieties to the gingipains results in a mutant with decreased gingipain activity, hemolysis and hemagglutination and increased sensitivity to oxidative stress (Vanterpool *et al*., 2005b, 2006). Similarly, a defect in LPS biosynthesis in *P. gingivalis* can influence the attachment of the gingipains to the cell surface, and autoaggregation (Osbourne *et al*., 2010; Yamaguchi *et al*., 2010). Several genes, including *vimA*, have shown the importance of the O side chain polysaccharide (O-LPS) and anionic polysaccharide (A-LPS) in these processes (Shoji *et al*., 2002; Vanterpool *et al*., 2004, 2005a, 2010; Sato *et al*., 2009; Yamaguchi *et al*., 2010). Analysis of cell surface LPS isolated from the parent W83 strain and isogenic mutants grown under the same conditions showed that the LPS of the *vimA*-defective mutant was truncated compared with that of the wild type (Vanterpool *et al*., 2006). Removal of the lipid A from the LPS resulted in a polysaccharide profile of the *vimA*-defective mutant in which the LPS was shorter than that of the parent strain, also suggesting that in the absence of VimA, polysaccharide modification could result in the loss of surface-associated gingipain proteinases and strong autoaggregation. VimA was found to mediate acetyl-CoA transfer to isoleucine that can result in a reduction in branched-chain amino acids and lipid A content in *P. gingivalis* (Aruni *et al*., 2012). The metabolic pathway of isoleucine degradation is known to provide the substrate (acetyl-CoA) that is important in lipid biosynthesis (Mahmud *et al*., 2005). The lower level of acetyl-CoA observed in the mutants could help to explain the VimA-dependent effect on lipid A biosynthesis that is possible via fatty acid chain elongation (Bugg & Brandish, 1994; Tatar *et al*., 2007). It is noteworthy that some of the proteins (PG1346, PG1347 and PG1348) that are predicted to play a role in lipid biosynthesis interacted with the purified VimA (Aruni *et al*., 2012). Together, the alterations in this pathway(s) could lead to the incorrect localization or targeting of proteins, resulting in reduced gingipain activity, hemolysis and hemagglutination. Because the gingipains are involved in hemin acquisition and uptake (Sheets *et al*., 2008), increased sensitivity to oxidative stress is expected as observed in the *vimA*-deficient mutant.

The inability to express the sialidase gene in the *vimA*-defective mutant could highlight the transcriptional effect of VimA on gene expression in *P. gingivalis*. A unifying theme that could therefore satisfy both transcriptional and post-translational VimA-dependent mechanisms may include acetylation. There is a gap in our understanding about the impact of protein acetylation on bacterial gene expression or physiology. In *E. coli* there is evidence that acetylation of the RNA polymerase subunit can play a crucial role in the transcriptional regulation of a periplasmic stress-responsive promoter when those cells were grown in an environment that induces protein acetylation (Lima *et al*., 2011). A similar VimA-dependent mechanism in *P. gingivalis* is unclear and is the subject of further investigation. Outer-membrane proteins including the gingipains that are glycosylated with acetylated carbohydrates moieties were affected by the *vimA* mutation. These observations could implicate VimA-dependent acetylation in gingipain biogenesis and could explain the truncated A-LPS species. Finally, there is evidence of a role for acetylation in protein sorting and transport in gram-negative bacteria (Craig *et al*., 2011). The missing or aberrantly expressed proteins in the *vimA*-defective mutants and their interaction with the purified VimA protein raise questions on a role for acetylation in protein sorting and transport in *P. gingivalis*. This awaits further confirmation in the laboratory.

FUTURE PERSPECTIVES

The VimA protein is conserved in many bacterial species and is multifunctional in *P. gingivalis* (Fig. 5). It is functionally characterized as an acetyl-CoA transferase in *P. gingivalis* and is involved in the acetylation process via acetyl-CoA, one of the keystone molecules of central metabolism. Acetylation is now emerging as a significant player with a broad impact on bacterial physiology/pathogenesis and could be as important as other post-translational modifications such as phosphorylation. Collectively, our observations have supported a role for the VimA protein in virulence modulation in *P. gingivalis* via its impact on the organism's acetylome. In addition to enhancing the ability of bacteria to subvert the innate immune system (Moynihan & Clarke, 2011), acetylation is also known to be important in bacterial antimicrobial resistance (Strahilevitz *et al*., 2009). It is noteworthy that

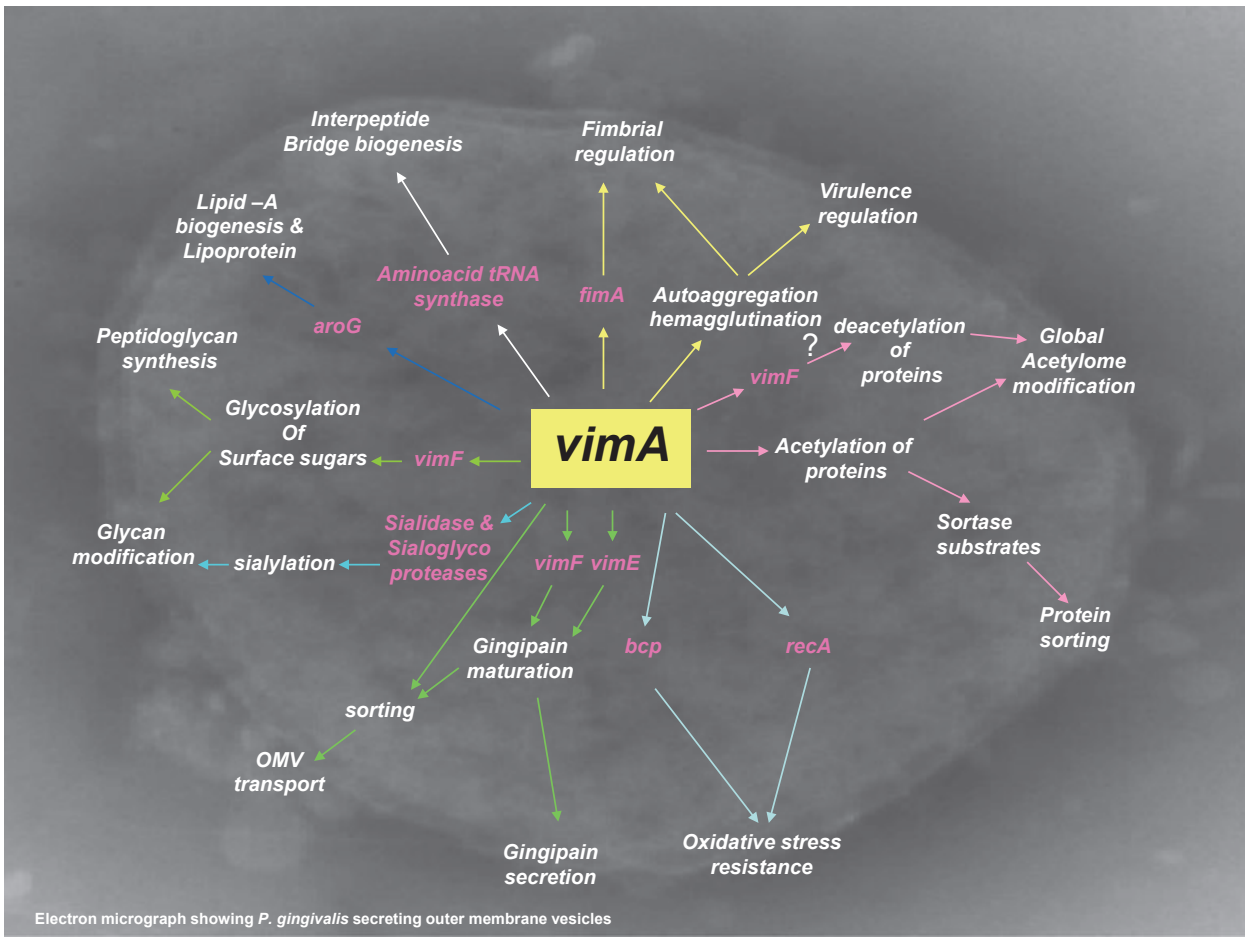

Figure 5 Operon function of *vimA* showing modulation of other related genes; *vimA* regulates *fimA* and fimbrial expression (Osbourne *et al*., 2010). Amino acid tRNA synthase, alanine tRNA synthase and isoleucine tRNA synthase are involved in transport of amino acids and formation of interpeptide bridges of peptidoglycans (Osbourne *et al*., 2010; Aruni *et al*., 2012). *vimA* interacts with *vimF* in glycosylation of surface sugars and peptidoglycan synthesis. In association with *vimA, vimE* and *vimF* are involved in gingipain maturation and secretion (Vanterpool *et al*., 2005a,b). *vimA* modulates glycan modification and sialidase activity (Aruni *et al*., 2012). *vimA* is involved in acetylation of proteins, lipid biogenesis and mediates protein sorting (Aruni *et al*., 2012; Osbourne *et al*., 2012). *vimA* is involved in autoaggregation and hemagglutination and thereby in virulence modulation (Vanterpool *et al*., 2004, 2006; Osbourne *et al*., 2010).

the *vimA*-defective mutant has reduced virulence and is more sensitive to globomycin and vancomycin (Osbourne *et al*., 2010). Taken together, VimA function should be an important target for therapeutic intervention. A comprehensive understanding of the VimA-dependent post-translational regulatory mechanism(s) awaits further investigation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Common C- terminal domain architecture of VimA interacting proteins and reported C-terminal domain (CTD) proteins. Multiple sequence alignment of CTD proteins and the VimA-interacting proteins showing three consensus conserved LxxG, YDMNGRx, G-G-Cterm motifs.

Figure S2. Multiple sequence alignment of VimA with related proteins from other organisms.