Published in final edited form as:

Future Microbiol. 2012 April ; 7(4): 497–512. doi:10.2217/fmb.12.17.

Oxidative stress resistance in *Porphyromonas gingivalis*

Leroy G Henry¹, Rachelle ME McKenzie^{1,2}, Antonette Robles¹, and Hansel M Fletcher^{1,*}

¹Division of Microbiology & Molecular Genetics, School of Medicine, Loma Linda, CA 92350, USA

²Center for Dental Research, School of Dentistry, Loma Linda University, Loma Linda, CA 92350, USA

Abstract

Porphyromonas gingivalis, a black-pigmented, Gram-negative anaerobe, is an important etiologic agent of periodontal disease. The harsh inflammatory condition of the periodontal pocket implies that this organism has properties that will facilitate its ability to respond and adapt to oxidative stress. Because the stress response in the pathogen is a major determinant of its virulence, a comprehensive understanding of its oxidative stress resistance strategy is vital. We discuss multiple mechanisms and systems that clearly work in synergy to defend and protect *P. gingivalis* against oxidative damage caused by reactive oxygen species. The involvement of multiple hypothetical proteins and/or proteins of unknown function in this process may imply other unique mechanisms and potential therapeutic targets.

Keywords

7,8-dihydro-8-oxoguanine; antioxidant protein; gingipain; oxidative stress; *Porphyromonas gingivalis*; reactive oxygen species; ROS

Oxidative stress can be functionally defined as an excess of pro-oxidants, such as reactive oxygen species (ROS), in the cell. ROS, such as the superoxide radical (O₂^{•-}), hydroxyl radical (HO[•]) and hydrogen peroxide (H₂O₂), pose a significant threat to cellular integrity in terms of their range of damage to proteins, lipids, RNA and DNA [1]. Virtually all organisms have evolved complex defense and repair mechanisms to protect themselves from the damaging effects of ROS. Although these protective mechanisms are especially important for strict anaerobes, little is known about ROS in these organisms.

Porphyromonas gingivalis is a Gram-negative, black-pigmented anaerobic rod that is an important causative agent of periodontal disease. This organism normally grows without oxygen (or in the presence of minimal concentrations of oxygen) and is found in anaerobic periodontal pockets. In order to reach the periodontal pocket, *P. gingivalis* is transferred through different sites in the oral cavity (saliva, tongue and buccal mucosa) where it is inevitably exposed to oxygen. Additionally, it has been shown that this organism has the

© 2012 Future Medicine Ltd.

*Author for correspondence: Tel.: +1 909 558 8497, Fax: +1 909 558 4035, hfletcher@llu.edu.

Financial & competing interests disclosure

This work was supported by Loma Linda University, Schools of Medicine and Dentistry and by Public Health Service grants DE13664 and DE019730 from NIDCR (to HM Fletcher). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

For reprint orders, please contact: reprints@futuremedicine.com

ability to invade and survive within eukaryotic cells where it is exposed to intracellular ROS [2]. Thus, in order to survive an oxidatively stressed environment, *P. gingivalis* has had to develop efficient oxidative stress protection mechanisms.

The robustness of this bacterium is not only due to the strong oxidative stress mechanisms that protect its DNA, proteins and lipid layer from oxidative damage, but also because of its excellent DNA repair process that accomplishes an efficient and precise removal of deleterious lesions. Oxidative damage produced by intracellular ROS results in base modifications, single- and double-strand breaks and the formation of apurinic/aprimidinic lesions, many of which are toxic and/or mutagenic [3]. The most notable is the highly mutagenic guanine residue 7,8-dihydro-8-oxoguanine, which is by far the most common DNA lesion formed as a result of oxidative stress [4]. When the genome of *P. gingivalis* was surveyed [5,101], essential components that are involved in base excision repair (BER) [6] and nucleotide excision repair [7] were observed, and investigation of these components revealed that the mechanism *P. gingivalis* uses to repair DNA damage in comparison to *Escherichia coli* [8] and other well-studied organisms [3,9] is undoubtedly unique. There is still a gap in our understanding as to how all of these components interact to bring about the resiliency of *P. gingivalis* to oxidative stress.

This review is intended to highlight oxidative stress as it relates to *P. gingivalis* and the myriad of methods this organism has employed to promote its continued survival in the periodontal pocket. We believe that with increasing evolution of genetic tools to study this organism, tremendous progress has been made towards understanding the genetic and physiological responses to oxidative stress, and eventually, the data generated will provide us with the means to completely eradicate this major periodontal pathogen.

Sources of oxidative stress

Exposure of *P. gingivalis* to air can give rise to the metabolic conversion of atmospheric oxygen to ROS inside bacterial cells. ROS are also produced by macrophages and neutrophils during the immune inflammatory response that is mediated by a process called the 'oxidative burst'. In this process, there is an increase in the consumption of oxygen and a rapid increase in the amount of ROS in response to external stimuli. Generally, ROS are usually produced by several one-electron reductions of molecular oxygen. One-, two- and three-electron reductions of molecular oxygen yield O_2^- , H_2O_2 and $HO\cdot$, respectively. In a slightly acidic or neutral pH, superoxides can dismutate spontaneously or by the action of superoxide dismutase, to form H_2O_2 and oxygen. Additionally, O_2^- can readily combine with nitric oxide to form another free radical, peroxynitrite ($ONOO\cdot$) [10]. On the other hand, H_2O_2 can react with free iron or copper (Fenton reaction) or with O_2^- (Haber-Weiss reaction), leading to the formation of $HO\cdot$. The ability of H_2O_2 to cross cell membranes easily makes it one of the most potent ROS, as its damaging effects can occur at sites that are distant from its formation.

Consequences of oxidative stress

ROS can have deleterious effects on many cellular components such as proteins, lipids, RNA and DNA [10]. In an oxidatively stressed environment, ROS can damage lipids. Free radicals can directly attack polyunsaturated lipids and initiate a chain reaction of lipid peroxidation [11]. Lipid peroxidation results in altered membrane function and damage to membrane-bound proteins due to loss of membrane fluidity. The chain reaction of lipid peroxidation can degrade lipids to more toxic byproducts, such as aldehydes. Because aldehydes are more reactive, they can travel further and exert effects on macromolecules, such as proteins. Among the many different aldehydes that can cause lipid peroxidation, 4-hydroxynonenal is the most extensively studied [11].

Proteins can also be oxidized during oxidative stress. Damage to proteins can occur by oxidation of sulfhydryl groups, reduction of disulfides, protein–protein cross-linking, peptide fragmentation and oxidative adduction of amino acid residues close to metal-binding sites via catalyzed oxidation. These modifications to proteins cause obvious deleterious effects on the bacterial cell and result in death or severe impairment of function.

DNA damage caused by ROS induces mutagenesis. Thus, mutation prevention or avoidance is of utmost priority. Additionally, there is a wide spectrum of oxidative DNA lesions generated as a result of oxidative stress. Lesions in DNA can cause deletions, mutations and other lethal genetic effects. Characterization of this DNA damage has indicated that both the sugar and the base moieties are susceptible to oxidation, causing base degradation, single-strand breakage and cross-linking to proteins. Degradation of the base produces numerous products including hydroxymethyl urea, urea, thymine glycol, thymine and adenine ring-opened and -saturated products and, most notably, the highly mutagenic guanine residue 7,8-dihydro-8-oxoguanine, which is by far the most common DNA lesion formed as a result of oxidative stress [12]. It is formed by oxidation, yielding a guanine with an extra oxygen at the C8 position.

7,8-dihydro-8-oxoguanine is formed in DNA by multiple pathways, including intracellular metabolism, oxidative stress, ionizing radiation or tobacco smoke. It is mutagenic due to its miscoding nature, in that it mispairs with adenine, producing primarily GC–TA transversion mutations [4]. Inevitably, all of these lesions must be repaired if the integrity of the DNA is to be maintained. Thus, it is crucial that *P. gingivalis* utilizes an arsenal of mechanisms to either prevent or fix oxidative damage resulting from ROS in order to survive in a hostile environment.

Regulators of oxidative stress

In aerobes and facultative anaerobic bacteria, the expression of antioxidant-related genes is usually regulated by transcriptional modulators that sense oxidative stress-generating agents. The SoxR/SoxS and the OxyR systems are examples of these regulators that respond to superoxide-generating compounds and H₂O₂, respectively. OxyR is a redox-sensitive protein in the LysR family of DNA-binding transcriptional modulators.

When exposed to oxidative stresses, *E. coli* induces one or both of two regulatory systems, the OxyR and SoxR/SoxS regulons. Expression of OxyR-regulated genes (OxyR regulons) is induced by the transcription factor OxyR and activated by peroxides such as H₂O₂. An OxyR homolog has also been identified in the aerotolerant anaerobe *Bacteroides fragilis* [13]. In *B. fragilis*, several OxyR-regulated genes are induced not only after H₂O₂ addition, as occurs in *E. coli*, but also after exposure to air. However, *oxyR* is necessary only for resistance to H₂O₂; its inactivation does not affect the aerotolerance of *B. fragilis*, perhaps because of compensatory mechanisms that are not OxyR-dependent.

An *oxyR* homolog has been identified in the *P. gingivalis* genome sequence [5]. *P. gingivalis* possesses the *sod* gene encoding Fe/Mn-containing SOD, which contributes to the relatively high aerotolerance of this organism. However, other genes for oxidative stress defense and regulation of their expression in this organism have been poorly characterized. Inspection of the *P. gingivalis* genome yielded several genes putatively involved in oxidative stress defense, including genes homologous to *bcp*, *tpx* and *oxyR*. It also revealed that there were no genes homologous to *soxR* or *soxS* in *P. gingivalis* genomic DNA [5].

In one study, oxidative stress responses of *P. gingivalis* regulated by the OxyR transcriptional regulator were investigated and it was found that *sod* gene expression in this bacterium was under OxyR control [14]. This was in contrast to the observation that SoxR/

SoxS, not OxyR, regulates *sodA* in *E. coli*. Additionally, the role of *oxyR* in *P. gingivalis* was evaluated. Diaz *et al.* concluded that putative OxyR-controlled genes, identified by microarray analysis, were not inducible after H₂O₂ treatment [15]. However, their expression during anaerobic growth required the presence of a functional OxyR [15]. It is clear that *P. gingivalis oxyR* is important not only for resistance to H₂O₂, but also for the aerotolerance of the microorganism.

Another transcriptional response to oxidative stress employs the use of the RprY response regulator. Response regulators can activate and/or repress transcription of their target genes. When the *P. gingivalis* genome was surveyed, the open reading frame of PG1089 was found to encode a 28-kDa protein with 61% identity and 74% similarity to a previously identified response regulator, RprY, from *B. fragilis*. In *P. gingivalis*, several strategies were used to identify promoter targets of RprY with the goal of building a comprehensive picture of biological function that would aid the identification of a partner sensor histidine kinase, and provide information on the environmental conditions that activate RprY [16]. The data revealed two potential and possibly related roles; first, the regulation of transporters including the primary sodium pump, Na⁺-translocating NADH ubiquinone oxidoreductase; and second, direct and indirect interaction with functions that may be associated with oxidative stress.

In *P. gingivalis*, ROS are converted to peroxide by superoxide dismutase. The organism does not possess catalase, therefore peroxide is detoxified to water by alkyl hydroperoxide [AhpC-F]) [16]. *Trx* (PG0616), however, was identified in *P. gingivalis*, which is immediately upstream from *ahpCF* (PG0618 and PG0619). The open reading frame annotated as PG0617 has been identified as the promoter of *ahpCF* [14]. It was demonstrated that RprY could bind to this promoter sequence, establishing a direct link between RprY and the oxidative stress response. Interestingly, *ahpCF* expression was activated by OxyR [14], raising the possibility of more complex modulation of this activity.

In *P. gingivalis*, extracytoplasmic function (ECF)- σ factors, known to be regulated at the transcriptional level, have the ability to regulate virulence factors. In a recent study, a PCR-based linear transformation strategy was used to inactivate five putative ECF- σ factors in *P. gingivalis* [17]. When the virulence-related characteristics of these proteins were analyzed, the results suggested that ECF- σ factors may be involved in the post-transcriptional regulation of gingipains and may play a role in H₂O₂-induced oxidative stress resistance in *P. gingivalis*. These studies showed that ECF- σ factors could likely modulate the virulence potential of *P. gingivalis*, leading to oxidative stress resistance. There is also emerging evidence of a unique regulatory mechanism(s) for gingipain biogenesis in *P. gingivalis* (PG2096, designated *regT*) that plays a role in regulating the gingipains at elevated temperatures [18]. RegT was shown to be involved in resistance to oxidative stress in *P. gingivalis*; however, the exact mechanism is still unclear.

Mechanisms of oxidative stress resistance

Overcoming oxidative stress is a key element in the survival of *P. gingivalis* in the inflammatory environment of the periodontal pocket. *P. gingivalis* has developed several mechanisms to combat oxidative stress. These include mechanisms that can, first, modulate/decrease the host immune response (e.g., polymorphonuclear [PMN] leukocytes and macrophages), which are major sources of ROS. Second, the bacteria have extracytoplasmic components that protect the cell from oxidative stress produced by the host. These include a hemin layer or manganese transporters, which contribute to homeostasis. Third, *P. gingivalis* can protect itself from ROS that enter the cell with antioxidant enzymes, which attempt to

neutralize the ROS before it damages cellular components. Fourth, DNA damage as a result of oxidative stress can then be repaired with various enzymes.

Modulating host response

P. gingivalis is able to combat oxidative stress by modulating the host immune response. Recent studies show that *P. gingivalis* can change host cellular ROS production in order to increase its survival [2]. Extracellular ATP (eATP) released by infected cells can act as warning signals, which stimulate ROS production by eATP binding to the P2X7 host plasma membrane receptor. *P. gingivalis* secretes Ndk, which can consume eATP, resulting in diminished ROS production. A recent study evaluated cellular ROS and mitochondrial ROS production by gingival epithelial cells infected with either wild-type or Ndk-mutant *P. gingivalis* and treated with eATP [2]. There was a significant decrease in ROS production over time from gingival epithelial cells infected with wild-type *P. gingivalis* versus the Ndk mutant, which was unable to suppress eATP–P2X7-induced ROS production.

P. gingivalis can also modulate the host immune response via its capsule. The capsule represents one of many *P. gingivalis* virulence factors. In fact, it has been shown that *P. gingivalis* strains with capsules are more virulent than strains without capsules [19]. Although past studies have suggested that the capsule in other pathogens plays a role in evading the host immune system, the exact role of the capsule in *P. gingivalis* has not been fully determined. There are currently six serotypes, which consist of serotypes K1–K6 [20]. In general, capsules play a role in evading the host immune system by preventing the host immune factors from reaching the microbe. Recent studies now suggest that the capsule may also play a role in covering the surface antigens of the microbe and thus decreasing the host response to those antigens [19]. Recent findings by Brunner *et al.* demonstrated that when human gingival fibroblasts were challenged with the W83 wild-type versus an *epsC* mutant, cytokine (IL-1 β , IL-6 and IL-8) expression levels were much higher in the host cells infected with the *epsC* mutant [20]. Cytokines IL-1 β , IL-6 and IL-8 are all mediators of the inflammatory response. *epsC* is an epimerase-coding gene involved in capsule synthesis. These experiments suggest that the capsule reduces the host proinflammatory immune response. Similarly, Singh *et al.* showed that macrophages challenged with nonencapsulated *P. gingivalis* had higher upregulation of certain host genes encoding cytokines and chemokines versus the encapsulated microbe [19]. The nonencapsulated strain also had increased phagocytosis or was killed quickly by macrophages and dendritic cells. The authors speculate that the presence of a capsule in *P. gingivalis* may also protect against oxidative stress, as seen in *Cryptococcus neoformans* [21]. These studies demonstrate that with a capsule, not only is the host response decreased, but phagocytosis is reduced and there is an increase in survival of the bacteria.

Finally, *P. gingivalis* can also modulate the immune response via its gingipains. Gingipains, the major proteases produced by *P. gingivalis*, are endopeptidases, which are both extracellular and cell-associated [7,22]. These proteases consist of arginine-specific proteases, RgpA and RgpB, as well as the lysine-specific protease, Kgp. Rgp is encoded by two genes, *rgpA* and *rgpB*, while Kgp is encoded by *kgp*. RgpA and Kgp both contain a catalytic domain and an adhesin/hem-agglutinin domain in comparison with RgpB, which does not contain an adhesin/hemagglutinin domain. Gingipains can cleave various host proteins [23,24] including cytokines such as IL-8 and IL-6, both proinflammatory cytokines, which potentially affects the production of ROS. One study carried out by Mikolajczyk-Pawlinska *et al.* showed the degradation of IL-8 by vesicle-associated gingipains [25], while another study by Banbula and colleagues showed the rapid cleavage of IL-6 by the gingipains [26]. In a related study, it was demonstrated that Kgp and Arg gingipains inhibited the production of ROS from activated PMN leukocytes [27]. Using guinea pig

PMN leukocytes stimulated by serum-activated zymosan incubated with Lys or Arg gingipains, results showed that there was a marked decrease in PMN leukocyte function as indicated by the luminol-dependent chemiluminescence response.

Hemin layer

The hemin layer is a unique mechanism of oxidative stress resistance. *P. gingivalis* has an absolute growth requirement for hemin. Nutritionally, hemin provides the bacteria with iron and protoporphyrin IX, which *P. gingivalis* cannot synthesize [28]. Hemin also regulates virulence-associated activities. Of particular interest, cell surface hemin gives the bacteria its characteristic black pigmentation and is responsible for protecting the cell against ROS by scavenging them [7]. Since lysed erythrocytes are found in abundance in the gingival crevicular fluid in the periodontal pocket, hemoglobin derived from red blood cells is a primary source of amino acids/peptides for cellular growth, as well as hemin. Although *P. gingivalis* has the ability to utilize other compounds such as haptoglobin, hemopexin and albumin as sources of hemin, Shizukuishi *et al.* found that *P. gingivalis* uses hemoglobin more efficiently versus other iron-containing compounds [29]. Hemin acquisition from erythrocytes involves several important steps: binding/hemagglutination of red blood cells, hemolysis, hemoglobin binding and hemoglobin degradation. Gingipains have been shown to play a crucial role in this process.

The exact cellular elements responsible for binding hemin at the cell surface have not been determined [30]. However, it has been shown that HbR, part of the C-terminal domain of Kgp and RgpA, binds hemin as well as hemoglobin [31]. In addition, there are studies to suggest that lipopolysaccharide biosynthesis-related genes may play a role in cell surface hemin binding [32].

Binding of iron protoporphyrin IX in the μ -oxodimeric form on the surface of the cell protects it from oxidative stress. This hemin-containing pigment is made up of two iron (III) protoporphyrin IX molecules covalently linked via an oxygen atom. Thus, by interacting with dioxygen and toxic oxygen derivatives, it is able to protect the cell by acting as an oxidative buffer [33]. In addition, the cell surface μ -oxobishaem protects against H_2O_2 , possibly by inactivating/degrading H_2O_2 . In fact, cells grown in the presence of μ -oxobishaem were protected against H_2O_2 versus cells not grown with μ -oxobishaem. The μ -oxodimer on the bacterial cell surface can also serve to block ROS. The HbR domain of RgpA and Kgp play an important role in forming this μ -oxodimer [31].

Other studies have further confirmed a role for the cell surface hemin layer as a protective mechanism against oxidative stress [4,34] [McKenzie RME, Johnson NA, Aruni AW, Dou Y, Masinde G, Fletcher HM. Differential response of *Porphyromonas gingivalis* to varying levels and duration of hydrogen peroxide-induced oxidative stress (2012), Submitted]. In addition to altered antibiotic sensitivities and abnormal protein expression in *P. gingivalis* FLL92 (a *vimA*-defective mutant) [35], there is aberrant gingipain activation/maturation and distribution, and thus the cells have no protective hemin layer and are nonpigmented [36,37]. *P. gingivalis* FLL92, when compared with wild-type *P. gingivalis* W83 exposed to H_2O_2 , showed increased sensitivity [McKenzie RME, Johnson NA, Aruni AW, Dou Y, Masinde G, Fletcher HM. Differential response of *Porphyromonas gingivalis* to varying levels and duration of hydrogen peroxide-induced oxidative stress (2012), Submitted] and was found to have higher levels of 8-oxoguanine in its chromosomal DNA [38]. In addition, cell extracts from *P. gingivalis* FLL92 had increased repair activity when compared with *P. gingivalis* W83.

Manganese homeostasis

Manganese homeostasis is a major mechanism of oxidative stress resistance in other bacteria such as *Neisseria gonorrhoeae* and *Lactobacillus planatarum* [39]. In *N. gonorrhoeae*, accumulation of manganese by the bacterium has intrinsic superoxide dismutase activity and can scavenge/quench ROS [39]. The mechanism by which Mn(II) scavenges ROS is similar to a process that occurs in *L. planatarum* [39]. *N. gonorrhoeae* contains a Mn(II) uptake system that is crucial for protection against oxidative stress. In fact, when a mutant in *mntC*, a gene involved in manganese uptake, was exposed to oxidative stress, the mutant was highly sensitive [40].

Studies indicate that *P. gingivalis* may have a similar protective mechanism in place. *P. gingivalis* has two gene products (FeoB1 and FeoB2) with sequence similarity to the FeoB of *E. coli*, which is involved in ferrous iron transport and located in the cytoplasmic membrane [41]. Previous studies have shown that *P. gingivalis* FeoB1 is a ferrous iron transporter, while FeoB2 plays a major role in manganese transport [42]. A study carried out by He *et al.* determined that FeoB2 is involved in manganese transport and also has a protective role against oxidative stress [43]. The *feoB2* mutant had reduced rates of manganese uptake versus the wild-type. In addition, the mutants had reduced rates of survival when exposed to H₂O₂ and to atmospheric oxygen versus the wild-type strain. The growth of the wild-type strain under anaerobic conditions was unaffected when manganese was absent from the media. By contrast, the same strain had no growth under aerobic conditions (6% oxygen) without manganese in the media. The FeoB2 mutants grew in anaerobic conditions without manganese, but did not grow in the presence of oxygen with or without manganese added to the growth media. Taken together, this indicates the significance of manganese homeostasis as a mechanism of resistance against oxidative stress. In another study, it was also observed using DNA microarray analysis that *P. gingivalis* exposed to 6% oxygen had increased expression of *feoB2* [44].

Antioxidant proteins

The most documented protective mechanisms in *P. gingivalis* are the antioxidant proteins. The role of these proteins in the protection of numerous bacteria from oxidative stress is well documented in the scientific literature [45]. In *P. gingivalis*, several of these antioxidant proteins including AhpC, SOD and Bcp have been identified and their specific roles in oxidative stress resistance studied. Interestingly, catalase, a major antioxidant enzyme in bacteria, is absent in *P. gingivalis*.

SOD

SOD plays a significant role in protection against oxidative stress and is a major factor in the defense mechanism in bacteria, specifically by catalyzing the disproportion of superoxide arising from the univalent reduction of oxygen [46]. A survey of SOD in obligately anaerobic bacteria has revealed that its activity appears to be correlated with aerotolerance. The SOD protein from *P. gingivalis* has been purified [47], expressed as a recombinant protein in *E. coli* and its crystal structure has been determined. This SOD was predicted to be an Fe-SOD based on its amino acid sequence, but experimental data revealed that it is a single, cambialistic protein that can bind either iron or manganese. Although SOD was initially shown to protect *P. gingivalis* from killing by PMN leukocytes, the expression of the protein was induced upon exposure to air. As confirmation of its importance in aerotolerance, a *sod*-deficient mutant demonstrated rapid loss of viability when compared with the parent after exposure to air. Additionally, *sod* appears to be positively regulated by the peroxide-sensing transcription activator OxyR [14,48], which is known to be an important regulator in aerotolerance in other bacteria. These and other studies [49] indicate

that the presence of SOD is important for the survival of *P. gingivalis* under aerobic conditions.

AhpC–F

In facultative and aerobic bacteria, the role in protecting cells against exogenous or endogenously generated toxic peroxides is exerted, in part, by the cytoplasmic, peroxide-scavenging enzyme Ahp [50]. AhpC–F belong to the peroxiredoxin oxidoreductase family and consist of two components: a small 22,000-Da peroxiredoxin protein (AhpC) with peroxidase activity and a larger 57,000-Da dedicated ‘AhpC reductase’ flavoprotein (AhpF) [45]. These two proteins act together and have a wide substrate range that includes H₂O₂, organic peroxides and OONO[•]. AhpF uses NADH or NADPH as an electron donor to AhpC, reducing physiological lipid peroxides such as linoleic acid hydroperoxide and thymine hydroperoxide and nonphysiological alkyl hydroperoxides to their respective nontoxic alcohol forms and water [51]. Apart from catalyzing the reduction of oxygen, alkyl hydroperoxidases show extremely high peroxide reductase activity for H₂O₂. Homologs of both *ahpC* and *ahpF* have been identified in *P. gingivalis* W83 [5,101], and both genes appear to be transcriptionally coupled [52]. Diaz *et al.* first demonstrated that AhpC–F contributed to the moderate tolerance of *P. gingivalis* to ROS by metabolizing H₂O₂ [52]. AhpC also appears to protect the organism from oxygen-induced stress [53]. In other studies, a *P. gingivalis* W83 *ahpC* mutant showed more sensitivity to organic peroxide-mediated oxidative stress than the wild-type, but its virulence in a murine model was unaffected. In addition, we have demonstrated that the *ahpC* homolog from *P. gingivalis* could complement the defect in an *E. coli* *ahpC* mutant [50]. Similar to SOD, AhpC appears to be positively regulated by OxyR in *P. gingivalis* [14]. Our studies indicate that AhpC protects against organic peroxides but does not affect the virulence of *P. gingivalis* W83 [50]. Taken together, AhpC appears to play an important role in aerotolerance and resistance to peroxides in *P. gingivalis*.

Bcp

Bcp is a member of the TSA/AhpC peroxiredoxin family and demonstrates Trx-dependent Tpx activity with a preference for the linoleic acid hydroperoxide substrate over H₂O₂ or t-butyl hydroperoxide [54]. AhpC and Bcp appear to have overlapping functions and activities [54] and may be part of a redundant mechanism for the reduction of peroxides. In *E. coli*, Bcp expression was induced threefold when cultures were shifted from anaerobic to aerobic growth conditions. A *bcp*-null mutant grew more slowly in aerobic conditions than the wild-type and showed increased sensitivity to the oxidants H₂O₂, t-butyl hydroperoxide and linoleic acid hydroperoxide. These peroxide hypersensitivities were complemented by expression of the *bcp* gene in the null mutant [54]. Induced expression of Bcp was also demonstrated by 2D protein gel electrophoresis and confirmed by reverse transcriptase PCR in the anaerobe *Desulfovibrio vulgaris* Hildenborough after exposure to oxygen [55]. The only reference to possible regulation of the *bcp* gene was demonstrated in *Staphylococcus aureus*, where PerR was found to control the expression of several oxidative stress resistance genes, including *bcp* [56]. In addition to its role in oxidative stress resistance, Bcp may also be involved in efficient host colonization, as shown in *Helicobacter pylori* [54].

In *P. gingivalis*, Bcp can play a role in oxidative stress resistance [34]. The *bcp* gene is part of a unique transcriptional unit with the *recA*, *vimA*, *vimE* and *vimF* genes in *P. gingivalis* [5,57–59]. Taken together, this suggests a coordinate relationship between gingipain activity, oxidative stress resistance and virulence by the gene cluster at the *bcp*–*recA*–*vimA*–*vimE*–*vimF* locus. The cloned *P. gingivalis* *bcp* gene increased resistance to H₂O₂ in a *bcp*-defective *E. coli* mutant, supporting its conserved function in both species. Its role in

virulence is questioned. While *P. gingivalis* FLL302, the *bcp*-defective mutant, showed decreased aerotolerance and increased sensitivity to H₂O₂, its virulence potential in a mouse model was unaltered when compared with the wild-type [34]. It is interesting that the *recA* and *bcp* genes are part of the same transcriptional unit along with the *vim* genes that are involved in modulating proteolytic activity [5]. This kind of association may be significant since a response to oxidative stress will involve binding of oxygen and its toxic derivatives to iron accumulated on the surface of the cell via the gingipains [60].

Tpx

Thiol peroxidases are part of a large family of enzymes (peroxiredoxins) found in both prokaryotes and eukaryotes [45]. They work together with Trx, Trx reductases and NADPH, and use a wide variety of peroxides as substrates (H₂O₂ and organic/lipid peroxides). Tpx proteins may be periplasmic or cytoplasmic. In several bacteria, they appear to play a significant role in resistance to peroxides, superoxides and high oxygen concentrations. A Tpx homolog has been identified in *P. gingivalis*. The expression of *tpx* was increased when *P. gingivalis* was subjected to microaerophilic conditions [44] or atmospheric oxygen [53] and appears to be partially OxyR -dependent [53]. The Tpx protein has also been shown to be actively produced by *P. gingivalis* cells during infection in a mouse model, where it induced a typical Th1 immune response in the host [61]. Additionally, Tpx may be regulated by the stationary phase protein, UstA since its expression is increased in the UstA mutant [62].

Rbr

Aerobic organisms typically deal with hyperoxic stress using two principal enzymes, SOD and catalase, which catalyze the disproportionation of superoxide and H₂O₂, respectively. Air-sensitive and strict anaerobes have the ability to use non-heme iron proteins, such as rubrerythrins, as an efficient, alternate antioxidant defense system against the toxic effects of oxygen and products of its incomplete reduction [63]. Rbr appears to be involved in protection against oxidative stress in several anaerobic bacteria and archaeobacteria, most likely functioning as the terminal component of an NAD(P) H peroxidase [64]. These proteins are involved in the reduction rather than disproportionation of superoxide or H₂O₂. Rbr contains an oxo-bridged di-iron site and a rubredoxin-like [Fe(SCys)₄] site within separate domains of a single subunit. The di-iron site is similar to those in a class of enzymes that activate dioxygen. To date, no *in vivo* substrate for Rbr has been conclusively identified, but the fully reduced (all-ferrous) form of *D. vulgaris* Rbr reduces H₂O₂ much more rapidly than dioxygen *in vitro* [65]. Purified rubrerythrins can serve as the terminal component of an NADH peroxidase by catalyzing two-electron reduction of H₂O₂. Little or no SOD or catalase activities have been reported for three different purified rubrerythrins using standard assays. *D. vulgaris* Rbr, when overexpressed in an *E. coli* *sodA sodB* strain, failed to restore an aerobic growth phenotype, indicating that this Rbr does not protect against intracellular superoxide [65]. On the other hand, the *Clostridium perfringens* Rbr, when overexpressed in the same *E. coli* *sodA sodB* strain, was reported to restore a limited degree of aerobic growth. Based on such evidence and on the detection of an *rbr* homolog in the genome sequence, Rbr was suggested as a possible peroxide stress-protective protein in *P. gingivalis*. It has been proposed that Rbr protects *P. gingivalis* against aerobic stress by functioning as a cytoplasmic peroxidase that reduces H₂O₂ to water [66]. Sztukowska *et al.* demonstrated that transcription of the *rbr* gene homolog in *P. gingivalis* was increased after exposure to H₂O₂ and dioxygen and, as expected, *rbr*-deficient mutants were more sensitive to both oxidants [67]. Additionally, Rbr also provided protection against reactive nitrogen species, thereby ensuring the survival of *P. gingivalis* in the infected host [68].

DNA-binding proteins

The DNA binding protein (Dps) family of proteins is a diverse group of bacterial stress-inducible polypeptides that bind DNA and likely confer resistance to peroxide damage during periods of oxidative stress and long-term nutrient limitation. Studies have demonstrated that a diverse group of Dps homologs are found in various prokaryotes, including *Synechococcus* spp., *Bacillus subtilis*, *Listeria innocua*, *Streptococcus mutans* and *B. fragilis*, and are related to the ferritin–bacterioferritin–rubrerythrin superfamily. Structurally, Dps forms a ferritin-like spherical oligomeric structure. In addition, the Dps monomer displays essentially the same protein fold (four-helix bundle) as the ferritin monomer. The Dps homologs are widely conserved among the prokaryotes and may suggest an important role for this protein in oxidative stress. The expression of Dps has been shown to be regulated by σ^{38} , σ^{70} and OxyR. In *E. coli*, as the most abundant protein in the stationary phase [69], Dps plays an important role in the protection of cells from peroxide stress by exhibiting nonspecific DNA- and iron-binding activities. Additionally, this protein has several other protective functions including protection from UV and γ -irradiation, iron and copper toxicity, thermal stress and acid and base shock [69] and protection of the chromosome from iron-induced free radical damage [70]. Dps proteins are also involved in iron homeostasis as they have ferritin-like activity, and most importantly, have the capacity to attenuate the production of ROS. This latter function allows bacterial pathogens that lack catalase (e.g., *P. gingivalis*) to survive in an aerobic environment and resist peroxide stress [71]. In *P. gingivalis*, a Dps homolog was shown to play an important role in the protection of cells from peroxide stress. Even though there was a significant OxyR-dependent induction of the Dps protein after exposure to atmospheric oxygen, a Dps-deficient mutant showed no loss of viability after 6 h exposure to atmospheric oxygen. Instead, the mutant was shown to be highly susceptible to H_2O_2 , with reduced viability after exposure [72]. Similar to *B. fragilis*, the *P. gingivalis* *dps* homolog was regulated in an OxyR-dependent manner and was upregulated under peroxide challenge, implicating its protection of the organism from peroxide stress.

Other protective proteins

Other less-known and less-characterized proteins have been shown to be involved in protection of *P. gingivalis* from oxidative stress. An *uspA* gene homolog has been identified in the genome of *P. gingivalis*. UspA is a member of the conserved universal stress proteins involved in the stress responses of prokaryotes and, to a lesser extent, eukaryotes. In *P. gingivalis*, the *uspA* gene has been shown to be involved in resistance to H_2O_2 , as a *uspA* mutant demonstrated a drastically decreased survival rate in comparison with the wild-type when treated with H_2O_2 [73]. While the mechanism of resistance against H_2O_2 and other environmental stressors have not been defined for UspA, its function may be indirect. The expression of *uspA* was increased during biofilm formation in *P. gingivalis*, and bacteria in biofilms are more resistant to various chemical agents, antibiotics or host immune factors than planktonic cells. Immediately upstream of *uspA* is a novel gene for the protein UstA, which is predicted to be localized in the cytoplasm in *P. gingivalis* and has been shown to be involved in protection from oxidative stress [62]. Expression of the *ustA* gene has been demonstrated to be increased not only in the stationary growth phase, but is also markedly increased after exposure to atmospheric oxygen [62]. Additionally, its role in oxidative stress resistance has been suggested because of the apparent compensatory overexpression of redox proteins such as SOD, Tpx and Trx in an *ustA* mutant. Homologs of the *ustA* genes have only been identified in the closely related *B. fragilis* and *Bacteroides thetaiotaomicron* [62,74], the mechanism of conferred resistance to oxidative stress has not been elucidated.

DNA repair mechanisms

Prevotella melaninogenica was the first anaerobe that was shown to be highly sensitive to oxidative DNA damage when exposed to O₂ or H₂O₂ [75]. Additionally, the elevated 8-oxoguanine levels detected correlated with its decreased survival [38]. In our studies, we also identified the presence of 8-oxoguanine in the chromosomal DNA of *P. gingivalis* W83 and the nonpigmented isogenic mutant FLL92 after exposure to H₂O₂ [38].

Previously, we deduced that *P. gingivalis* RecA protein showed strong similarities to RecA proteins from other Gram-negative anaerobes [76]. Additionally, two conserved ATP-binding motifs were observed in the deduced *P. gingivalis* RecA protein, which agrees with the evolutionary conservation of this motif across bacterial species. We were able to complement the *recA* mutation in *E. coli* by using the *recA* homolog from *P. gingivalis*, which suggested functional conservation of the RecA protein from *P. gingivalis*. Insertional inactivation of the *recA* gene with the *ermF-ermAM* cassette [77] significantly reduced the ability for DNA repair in both *P. gingivalis* and *E. coli* [34,77]. This confirmed that the *recA* gene in *P. gingivalis* W83 plays an important role in DNA repair [78,79].

The mechanism of mismatch repair has been studied most thoroughly in *E. coli* [80]. The research groups of Modrich, Kolodner and others have reconstituted the repair process from purified proteins [81]. The proteins that initiate the repair process are MutS, MutL and MutH. Most mismatches are due to replication errors. However, mismatches can also be produced by other mechanisms, such as deamination of 5-methyl cytosine to produce thymidine improperly paired to guanine or oxidative stress. Regardless of the mechanism by which mismatches are produced, mismatches can always be repaired by the mismatch repair pathway. In cases where the appropriate DNA *N*-glycosylase is available, mismatches can also be repaired by the BER pathway.

E. coli DNA is normally methylated at GATC sequences, but the newly synthesized strand is not immediately methylated. The fact that the old strand, but not the new, is methylated near the replication fork allows *E. coli* cells to distinguish the old (presumably correct) strand from the newly synthesized (presumably incorrect) strand. Briefly, MutS recognizes and binds distorted double-helix mismatches. Subsequent binding of MutL stabilizes the complex. The MutS–MutL complex activates MutH, which locates a nearby methyl group and nicks the newly synthesized strand opposite the methyl group. Excision is accomplished by cooperation between the UvrD (hHelicase II) protein, which unwinds from the nick in the direction of the mismatch, and a single-strand-specific exo-nuclease of appropriate polarity (one of several in *E. coli*), followed by resynthesis (polymerase III) and ligation (DNA ligase). A survey of the *P. gingivalis* genome reveals three sequence homologs for the *mutS* gene and one sequence homolog for the *mutL* gene, but none for the *mutH* gene [5,101]. Even though a sequence homolog for *mutH* was not found, there is still a possibility that a functional homolog of the *E. coli* MutH may be present in *P. gingivalis*. It is still yet to be determined whether *P. gingivalis* has the ability to repair DNA damage by the mismatch repair pathway.

In a previous report, the ability of *P. gingivalis* to repair DNA that contained the deleterious 8-oxoguanine product was investigated [38]. Briefly, bacterial extracts from the *P. gingivalis* isogenic strains grown in the presence or absence of H₂O₂ were used in glycosylase assays with a 5'-end-labeled [γ -³²P]-ATP 8-oxodG:C-containing oligonucleotide (24-mer) [38]. If 8-oxoguanine is removed by a BER mechanism, a cleavage product corresponding to a 12-mer would be observed because the 8-oxodG:C was placed in the middle of the 24-mer. The Fpg enzyme generated the expected 12-mer fragment in oligonucleotides containing 8-oxoguanine. However, in *P. gingivalis* strains W83 and FLL92, a cleavage product of

approximately 17 bases was observed, which indicates that the repair mechanism of 8-oxoguanine is different in *P. gingivalis* when compared with *E. coli*. In addition, when compared with other anaerobic periodontal pathogens, the removal of 8-oxoguanine was unique to *P. gingivalis* and suggests a mechanism that needs to be defined.

The *P. gingivalis* genome contains genes that encode for the UvrA, UvrB and UvrC proteins [5,101]. UvrB is the central component of bacterial nucleotide excision repair. It is directly involved in distinguishing damaged from undamaged DNA and guides the DNA from recognition to repair synthesis [82,83]. To further evaluate if nucleotide excision repair played a role in this repair activity given the size of the cleavage product [38], the *uvrB* gene in *P. gingivalis* was inactivated [4]. In contrast to the wild-type *P. gingivalis* W83, the *uvrB*-deficient mutant FLL144 was significantly more sensitive to UV irradiation. However, the enzymatic removal of 8-oxoguanine was unaffected by the inactivation of the *uvrB* gene. Collectively, these results suggest that the *uvrB* gene in *P. gingivalis* may not be involved in the removal of 8-oxoguanine and that another as-yet unidentified mechanism may be employed in its repair.

The pathway most commonly employed to remove incorrect bases (e.g., uracil) or damaged bases (e.g., 3-methyladenine) is BER. Specificity of the various pathways is conferred by the different types of DNA *N*-glycosylases. Studies with *E. coli* have shown that three different repair activities of MutM/Fpg [84], MutY [6] and MutT cooperate to prevent mutations from being formed at 8-oxoguanine lesions. Fpg removes 8-oxoguanine as well as formamidopyrimidines from DNA.

MutM and MutY are glycosylases that act on oxidized guanine residues in DNA. In the absence of both MutY and MutM, an increase in the frequency of CG to AT mutations is observed, whereas a much lower frequency is detected in cells lacking either MutM or MutY. Genetic experiments have shown that the overproduction of *mutM* suppresses the mutability of the *mutY* mutant, indicating that *mutM* acts before *mutY*. In addition, the *mutT* gene encodes a nucleoside triphosphate that preferentially hydrolyzes 8-oxodGTP and 8-oxoGTP, thereby preventing incorporation of the oxidized base into DNA and RNA.

Typical removal of 8-oxoguanine takes place in a fairly sequential or coordinated manner in both eukaryotic and prokaryotic systems. Error-free repair of 8-oxoguanine-containing DNA is carried out by BER, initiated mainly by the enzyme MutM/Fpg. Since adenine is the nucleotide most frequently misincorporated opposite 8-oxoguanine, cells have evolved a mechanism that removes adenine from DNA in a process initiated by the enzyme MutY. Since cytosine is usually inserted opposite 8-oxoguanine, this will enable a second attempt of error-free BER, initiated by MutM/Fpg.

Uracil DNA glycosylase is another important enzyme involved in BER. This generally removes bases that cause minor structural changes in DNA [85]. These structural changes include deamination of cytosine to uracil, alkylation caused by normal cellular metabolites such as *S*-adenosylmethionine, (e.g., 3-methyladenine), oxidative damage caused by ROS from oxidative metabolism and errors in DNA replication. Removal of the damaged base is the only catalytic function of monofunctional DNA glycosylases, such as the uracil DNA glycosylase (UNG), the mismatch-specific thymine/uracil DNA glycosylase and the methylpurine DNA glycosylases. Whereas UNG and thymine/uracil DNA glycosylase have narrow substrate specificities, methylpurine DNA glycosylase removes a large array of damaged bases that have a weakened glycosylic bond as their only common feature.

Surveys of the *P. gingivalis* genome has revealed the presence of MutY, which was shown to play an important role in oxidative stress resistance [6]. The results implied that a system involving MutY is a primary mechanism for repair of 8-oxoG:A in addition to other

redundant mechanism(s). Surveys of the *P. gingivalis* genome also revealed MutT and UNG sequence homologs, and studies in our laboratory have determined the function of UNG in the removal of uracil mismatches in duplex DNA [38]. However, function of the MutT DNA glycosylases are still to be investigated.

Conclusion

Systems that defend and protect *P. gingivalis* against oxidative damage are varied and complex. These include antioxidant enzymes [45], Dps [69], the hemin layer [7] and enzymatic removal of deleterious products (e.g., 8-oxoguanine) caused by ROS [4,6,38]. Additionally, several response regulators have been implicated in oxidative stress resistance [17,18,86]. Taken together, there is no doubt that *P. gingivalis* is a robust organism that has developed numerous strategies to survive in the harsh environment of the periodontal pocket. Its successful strategies leading to persistence play an important role in the initiation and progression of adult periodontal diseases.

Invariably, the hemin layer acts as the primary line of defense against oxidative stress by scavenging ROS. Studies have shown that the hemin layer acts as a protective mechanism/barrier against oxidative stress [87]. However, if this layer is compromised, major protective mechanisms located internally exist to prevent the deleterious effects of oxidative stress on major macromolecules of the cell that are essential for its survival [10].

Based on our observations and literature review, we can postulate that *P. gingivalis* has not only evolved mechanisms to combat cellular damage, but there is also evidence that indicates that *P. gingivalis* may have specific proteins that preferentially bind to DNA as well [4]. The evidence suggests that these proteins may have properties that confer protection to DNA from oxidative stress, hence enhancing their oxidative stress-resistance capabilities. Clearly, all the essential components involved in oxidative stress resistance in *P. gingivalis* work in synergy to bring about its continued survival in the periodontal pocket (Figure 1).

Future perspective

Recently, DNA microarray analysis has also played an integral role in further elucidating the complexities of oxidative stress resistance in *P. gingivalis* [McKenzie RME, Johnson NA, Aruni AW, Dou Y, Masinde G, Fletcher HM. Differential response of *Porphyromonas gingivalis* to varying levels and duration of hydrogen peroxide-induced oxidative stress (2012), Submitted]. When *P. gingivalis* was initially exposed to a subinhibitory concentration of H₂O₂, an adaptive response to higher concentrations could be induced. Transcriptome analysis demonstrated that oxidative stress can modulate several functional classes of genes depending on the severity and duration of the exposure. A shorter exposure to H₂O₂ revealed increased expression of genes involved in DNA damage and repair, while after a longer exposure showed genes involved in protein fate, protein folding and stabilization were upregulated. The majority of these induced genes were classified as hypothetical or of unknown function. Collectively, these data indicate the adaptive ability of *P. gingivalis* to oxidative stress and further underscores the complex nature of its resistance strategy under those conditions.

There is still a significant gap in our understanding of all the mechanisms involved in DNA damage and repair in *P. gingivalis* and we have yet to elucidate what roles the metabolic activity of the cell or newly discovered hypothetical proteins play in oxidative stress resistance. As information accumulates pertaining to the innate resiliency of this organism, the identification of novel therapeutics to combat this process has become increasingly feasible. Already, the use of chemotherapeutics such as chlorohexidine has been shown to

decrease plaque by up to 61% and reduces gingivitis by up to 80%; however, this chemical causes staining, alters taste perception and is impaired in terms of efficacy in the presence of toothpaste [88]. Alternatively, essential oil mouth rinses have been shown to effectively kill bacteria, but were not as efficient in doing so as chlorohexidine. Natural remedies such as the increased uptake of ascorbic acid or the addition of vitamin A to toothpaste could be useful in treating periodontal disease, since fewer deep pockets and reduced gingival bleeding were found following its use [88]. Another author suggested the use of isolated adhesins as vaccines to inhibit *P. gingivalis* colonization, although antibody-based control of infections at mucus membranes is difficult to accomplish [89]. As an alternative, blocking agents targeted to active domains of adhesins or proteases could be developed to control both adhesion and tissue destruction [89]. Additionally, it has also been implied by Kirkwood *et al.* that alveolar bone loss may be delayed or prevented by the use of host-modulating agents [90]. New families of endogenous chemical mediators that possess potent anti-inflammatory properties – the resolvins – have been investigated in the inflammatory disease model and have provided valuable insight into alternative methods of treating inflammatory diseases [91]. Other therapies that offer a more targeted approach to plaque-related diseases, such as selective light-activated killing, functional inhibition of specific virulence factors and microbial replacement therapy (reviewed in [88]) have been employed whereas more natural therapies (e.g., plant-based products) have been steadily gaining acceptance in mainstream healthcare. Ultimately, it is hoped that treatment will be as simple as applying a cream that contains a potent cocktail of inhibitors directed against inflammatory agents (e.g., gingipains or proteases) [92]. With emerging technology, we expect to eventually elucidate possible unique mechanism(s) for oxidative stress resistance in *P. gingivalis*. The design of specific small molecules to inhibit the active site of these novel proteins should become a significant therapeutic target. It is our belief that by 2020, suitable therapeutic agents, based on this information, will be developed to combat this periodontal pathogen.

Acknowledgments

We apologize to all our colleagues whose important work could not be directly cited.

References

Papers of special note have been highlighted as:

■ of interest

■ of considerable interest

1. Strand KR, Sun C, Li T, Jenney FE Jr, Schut GJ, Adams MW. Oxidative stress protection and the repair response to hydrogen peroxide in the hyperthermophilic archaeon *Pyrococcus furiosus* and in related species. *Arch Microbiol.* 2010; 192(6):447–459. [PubMed: 20379702]
- 2■■. Spooner R, Yilmaz O. The role of reactive-oxygen-species in microbial persistence and inflammation. *Int J Mol Sci.* 2011; 12(1):334–352. Highlights how opportunistic pathogens maintain chronic infections and persist in highly inflammatory environments. [PubMed: 21339989]
3. Salmon TB, Evert BA, Song B, Doetsch PW. Biological consequences of oxidative stress-induced DNA damage in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 2004; 32(12):3712–3723. [PubMed: 15254273]
4. Henry LG, Sandberg L, Zhang K, Fletcher HM. DNA repair of 8-oxo-7,8-dihydroguanine lesions in *Porphyromonas gingivalis*. *J Bacteriol.* 2008; 190(24):7985–7993. [PubMed: 18849425]
- 5■■. Nelson KE, Fleischmann RD, DeBoy RT, et al. Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. *J Bacteriol.* 2003; 185(18):5591–

5601. Published genome of *Porphyromonas gingivalis* W83 allowed for homology searches for genes known to be involved in oxidative stress defense in other bacterial species. [PubMed: 12949112]
6. Robles AG, Reid K, Roy F, Fletcher HM. *Porphyromonas gingivalis* *mutY* is involved in the repair of oxidative stress-induced DNA mispairing. *Mol Oral Microbiol.* 2011; 26(3):175–186. [PubMed: 21545695]
 7. Sheets SM, Robles-Price AG, McKenzie RM, Casiano CA, Fletcher HM. Gingipain-dependent interactions with the host are important for survival of *Porphyromonas gingivalis*. *Front Biosci.* 2008; 13:3215–3238. Reviews the importance of the gingipains and highlights the significance of oxidative stress in the survival in *P. gingivalis*. [PubMed: 18508429]
 8. Vasilenko NL, Nevinskii GA. Enzymes of direct, excision, and corrected systems of repair of higher and lower organisms and their biological roles. *Mol Biol (Mosk).* 2003; 37(6):944–960. [PubMed: 14714489]
 9. Slade D, Radman M. Oxidative stress resistance in *Deinococcus radiodurans*. *Microbiol Mol Biol Rev.* 2011; 75(1):133–191. [PubMed: 21372322]
 10. Dickinson BC, Chang CJ. Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat Chem Biol.* 2011; 7(8):504–511. [PubMed: 21769097]
 11. Niki E. Lipid peroxidation: physiological levels and dual biological effects. *Free Radic Biol Med.* 2009; 47(5):469–484. [PubMed: 19500666]
 12. Kasai H, Chung MH, Jones DS, et al. 8-hydroxyguanine, a DNA adduct formed by oxygen radicals: its implication on oxygen radical-involved mutagenesis/carcinogenesis. *J Toxicol Sci.* 1991; 16(Suppl 1):95–105. [PubMed: 1656061]
 13. Herren CD, Rocha ER, Smith CJ. Genetic analysis of an important oxidative stress locus in the anaerobe *Bacteroides fragilis*. *Gene.* 2003; 316:167–175. [PubMed: 14563563]
 14. Ohara N, Kikuchi Y, Shoji M, Naito M, Nakayama K. Superoxide dismutase-encoding gene of the obligate anaerobe *Porphyromonas gingivalis* is regulated by the redox-sensing transcription activator OxyR. *Microbiology.* 2006; 152(Pt 4):955–966. [PubMed: 16549660]
 15. Diaz PI, Slakeski N, Reynolds EC, Morona R, Rogers AH, Kolenbrander PE. Role of *oxyR* in the oral anaerobe *Porphyromonas gingivalis*. *J Bacteriol.* 2006; 188(7):2454–2462. [PubMed: 16547032]
 16. Duran-Pinedo AE, Nishikawa K, Duncan MJ. The RprY response regulator of *Porphyromonas gingivalis*. *Mol Microbiol.* 2007; 64(4):1061–1074. [PubMed: 17501928]
 17. Dou Y, Osbourne D, McKenzie R, Fletcher HM. Involvement of extracytoplasmic function sigma factors in virulence regulation in *Porphyromonas gingivalis* W83. *FEMS Microbiol Lett.* 2010; 312(1):24–32. [PubMed: 20807237]
 18. Vanterpool E, Aruni AW, Roy F, Fletcher HM. *regT* can modulate gingipain activity and response to oxidative stress in *Porphyromonas gingivalis*. *Microbiology.* 2010; 156(Pt 10):3065–3072. [PubMed: 20595264]
 19. Singh A, Wyant T, Anaya-Bergman C, et al. The capsule of *Porphyromonas gingivalis* leads to a reduction in the host inflammatory response, evasion of phagocytosis, and increase in virulence. *Infect Immun.* 2011; 79(11):4533–4542. [PubMed: 21911459]
 20. Brunner J, Scheres N, El Idrissi NB, et al. The capsule of *Porphyromonas gingivalis* reduces the immune response of human gingival fibroblasts. *BMC Microbiol.* 2010; 10:5. [PubMed: 20064245]
 21. Zaragoza O, Rodrigues ML, De Jesus M, Frases S, Dadachova E, Casadevall A. The capsule of the fungal pathogen *Cryptococcus neoformans*. *Adv Appl Microbiol.* 2009; 68:133–216. [PubMed: 19426855]
 22. Roy F, Vanterpool E, Fletcher HM. HtrA in *Porphyromonas gingivalis* can regulate growth and gingipain activity under stressful environmental conditions. *Microbiology.* 2006; 152(Pt 11):3391–3398. [PubMed: 17074908]
 23. Sheets SM, Potempa J, Travis J, Casiano CA, Fletcher HM. Gingipains from *Porphyromonas gingivalis* W83 induce cell adhesion molecule cleavage and apoptosis in endothelial cells. *Infect Immun.* 2005; 73(3):1543–1552. [PubMed: 15731052]

24. Sheets SM, Potempa J, Travis J, Fletcher HM, Casiano CA. Gingipains from *Porphyromonas gingivalis* W83 synergistically disrupt endothelial cell adhesion and can induce caspase-independent apoptosis. *Infect Immun*. 2006; 74(10):5667–5678. [PubMed: 16988242]
25. Mikolajczyk-Pawlinska J, Travis J, Potempa J. Modulation of interleukin-8 activity by gingipains from *Porphyromonas gingivalis*: implications for pathogenicity of periodontal disease. *FEBS Lett*. 1998; 440(3):282–286. [PubMed: 9872387]
26. Banbula A, Bugno M, Kuster A, Heinrich PC, Travis J, Potempa J. Rapid and efficient inactivation of IL-6 gingipains, lysine- and arginine-specific proteinases from *Porphyromonas gingivalis*. *Biochem Biophys Res Commun*. 1999; 261(3):598–602. [PubMed: 10441472]
27. Abe N, Kadowaki T, Okamoto K, Nakayama K, Ohishi M, Yamamoto K. Biochemical and functional properties of lysine-specific cysteine proteinase (Lys-gingipain) as a virulence factor of *Porphyromonas gingivalis* in periodontal disease. *J Biochem*. 1998; 123(2):305–312. [PubMed: 9538207]
28. Lewis JP. Metal uptake in host-pathogen interactions: role of iron in *Porphyromonas gingivalis* interactions with host organisms. *Periodontol* 2000. 2010; 52(1):94–116. [PubMed: 20017798]
29. Shizukuishi S, Tazaki K, Inoshita E, Kataoka K, Hanioka T, Amano A. Effect of concentration of compounds containing iron on the growth of *Porphyromonas gingivalis*. *FEMS Microbiol Lett*. 1995; 131(3):313–317. [PubMed: 7557343]
30. Smalley JW, Byrne DP, Birss AJ, et al. HmuY haemophore and gingipain proteases constitute a unique syntrophic system of haem acquisition by *Porphyromonas gingivalis*. *PLoS ONE*. 2011; 6(2):E17182. [PubMed: 21390208]
31. Smalley JW, Birss AJ, Szmigielski B, Potempa J. The HA2 haemagglutinin domain of the lysine-specific gingipain (Kgp) of *Porphyromonas gingivalis* promotes micro-oxo bishaem formation from monomeric iron(III) protoporphyrin IX. *Microbiology*. 2006; 152(Pt 6):1839–1845. [PubMed: 16735746]
32. 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(Pt 4):1282–1293. [PubMed: 19332829]
33. 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 micro-oxo bishaem-containing pigment from haemoglobin. *Biochem J*. 2004; 379(Pt 3):833–840. [PubMed: 14741050]
34. Johnson NA, McKenzie RM, Fletcher HM. The *bcp* gene in the *bcp-recA-vimA-vimE-vimF* operon is important in oxidative stress resistance in *Porphyromonas gingivalis* W83. *Mol Oral Microbiol*. 2011; 26(1):62–77. [PubMed: 21214873]
35. Osbourne DO, Aruni W, Roy F, et al. Role of *vimA* in cell surface biogenesis in *Porphyromonas gingivalis*. *Microbiology*. 2010; 156(Pt 7):2180–2193. [PubMed: 20378652]
36. Vanterpool E, Roy F, Zhan W, Sheets SM, Sangberg L, Fletcher HM. VimA is part of the maturation pathway for the major gingipains of *Porphyromonas gingivalis* W83. *Microbiology*. 2006; 152(Pt 11):3383–3389. [PubMed: 17074907]
37. Olango GJ, Roy F, Sheets SM, Young MK, Fletcher HM. Gingipain RgpB is excreted as a proenzyme in the *vimA*-defective mutant *Porphyromonas gingivalis* FLL92. *Infect Immun*. 2003; 71(7):3740–3747. [PubMed: 12819055]
38. Johnson NA, McKenzie R, McLean L, Sowers LC, Fletcher HM. 8-oxo-7,8-dihydroguanine is removed by a nucleotide excision repair-like mechanism in *Porphyromonas gingivalis* W83. *J Bacteriol*. 2004; 186(22):7697–7703. First paper to identify a unique mechanism for the removal of 8-oxoguanine adducts from DNA in *P. gingivalis*. [PubMed: 15516584]
39. Seib KL, Tseng HJ, McEwan AG, Apicella MA, Jennings MP. Defenses against oxidative stress in *Neisseria gonorrhoeae* and *Neisseria meningitidis*: distinctive systems for different lifestyles. *J Infect Dis*. 2004; 190(1):136–147. [PubMed: 15195253]
40. Tseng HJ, Srikhanta Y, McEwan AG, Jennings MP. Accumulation of manganese in *Neisseria gonorrhoeae* correlates with resistance to oxidative killing by superoxide anion and is independent of superoxide dismutase activity. *Mol Microbiol*. 2001; 40(5):1175–1186. [PubMed: 11401721]

41. Anaya-Bergman C, He J, Jones K, Miyazaki H, Yeudall A, Lewis JP. *Porphyromonas gingivalis* ferrous iron transporter FeoB1 influences sensitivity to oxidative stress. *Infect Immun*. 2010; 78(2):688–696. [PubMed: 19917713]
42. Dashper SG, Butler CA, Lissel JP, et al. A novel *Porphyromonas gingivalis* FeoB plays a role in manganese accumulation. *J Biol Chem*. 2005; 280(30):28095–28102. [PubMed: 15901729]
43. He J, Miyazaki H, Anaya C, Yu F, Yeudall WA, Lewis JP. Role of *Porphyromonas gingivalis* FeoB2 in metal uptake and oxidative stress protection. *Infect Immun*. 2006; 74(7):4214–4223. [PubMed: 16790796]
44. Lewis JP, Iyer D, Anaya-Bergman C. Adaptation of *Porphyromonas gingivalis* to microaerophilic conditions involves increased consumption of formate and reduced utilization of lactate. *Microbiology*. 2009; 155(Pt 11):3758–3774. [PubMed: 19684063]
45. Dubbs JM, Mongkolsuk S. Peroxiredoxins in bacterial antioxidant defense. *Subcell. Biochem*. 2007; 44:143–193.
46. Imlay JA. Cellular defenses against superoxide and hydrogen peroxide. *Annu Rev Biochem*. 2008; 77:755–776. Comprehensive review of mechanisms employed by bacteria in defense against reactive oxygen species. [PubMed: 18173371]
47. Amano A, Nakagawa I, Okahashi N, Hamada N. Variations of *Porphyromonas gingivalis* fimbriae in relation to microbial pathogenesis. *J Periodontal Res*. 2004; 39(2):136–142. [PubMed: 15009522]
48. Wu J, Lin X, Xie H. OxyR is involved in coordinate regulation of expression of *fimA* and *sod* genes in *Porphyromonas gingivalis*. *FEMS Microbiol Lett*. 2008; 282(2):188–195. [PubMed: 18355277]
49. Diaz PI, Rogers AH. The effect of oxygen on the growth and physiology of *Porphyromonas gingivalis*. *Oral Microbiol Immunol*. 2004; 19(2):88–94. [PubMed: 14871347]
50. Johnson NA, Liu Y, Fletcher HM. Alkyl hydroperoxide peroxidase subunit C (AhpC) protects against organic peroxides but does not affect the virulence of *Porphyromonas gingivalis* W83. *Oral Microbiol Immunol*. 2004; 19(4):233–239. [PubMed: 15209993]
51. Poole LB. Bacterial defenses against oxidants: mechanistic features of cysteine-based peroxidases and their flavoprotein reductases. *Arch Biochem Biophys*. 2005; 433(1):240–254. [PubMed: 15581580]
52. Diaz PI, Zilm PS, Wasinger V, Corthals GL, Rogers AH. Studies on NADH oxidase and alkyl hydroperoxide reductase produced by *Porphyromonas gingivalis*. *Oral Microbiol Immunol*. 2004; 19(3):137–143. [PubMed: 15107063]
53. Meuric V, Gracieux P, Tamanai-Shacoori Z, Perez-Chaparro J, Bonnaure-Mallet M. Expression patterns of genes induced by oxidative stress in *Porphyromonas gingivalis*. *Oral Microbiol Immunol*. 2008; 23(4):308–314. First paper to use DNA transcriptome profiling to identify genes modulated in response to exposure of *P. gingivalis* to atmospheric oxygen. [PubMed: 18582330]
54. Wang G, Olczak AA, Walton JP, Maier RJ. Contribution of the *Helicobacter pylori* thiol peroxidase bacterioferritin comigratory protein to oxidative stress resistance and host colonization. *Infect Immun*. 2005; 73(1):378–384. [PubMed: 15618175]
55. Fournier M, Aubert C, Dermoun Z, Durand MC, Moinier D, Dolla A. Response of the anaerobe *Desulfovibrio vulgaris* Hildenborough to oxidative conditions: proteome and transcript analysis. *Biochimie*. 2006; 88(1):85–94. [PubMed: 16040186]
56. Horsburgh MJ, Clements MO, Crossley H, Ingham E, Foster SJ. PerR controls oxidative stress resistance and iron storage proteins and is required for virulence in *Staphylococcus aureus*. *Infect Immun*. 2001; 69(6):3744–3754. [PubMed: 11349039]
57. Vanterpool E, Roy F, Fletcher HM. Inactivation of *vimF*, a putative glycosyltransferase gene downstream of *vimE*, alters glycosylation and activation of the gingipains in *Porphyromonas gingivalis* W83. *Infect Immun*. 2005; 73(7):3971–3982. [PubMed: 15972484]
58. Vanterpool E, Roy F, Fletcher HM. The *vimE* gene downstream of *vimA* is independently expressed and is involved in modulating proteolytic activity in *Porphyromonas gingivalis* W83. *Infect Immun*. 2004; 72(10):5555–5564. [PubMed: 15385452]

59. Vanterpool E, Roy F, Sandberg L, Fletcher HM. Altered gingipain maturation in *vimA*- and *vimE*-defective isogenic mutants of *Porphyromonas gingivalis*. *Infect Immun*. 2005; 73(3):1357–1366. [PubMed: 15731033]
60. 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(1): 325–335. [PubMed: 11119521]
61. Goncalves RB, Leshem O, Bernards K, Webb JR, Stashenko PP, Campos-Neto A. T-cell expression cloning of *Porphyromonas gingivalis* genes coding for T helper-biased immune responses during infection. *Infect Immun*. 2006; 74(7):3958–3966. [PubMed: 16790769]
62. Kikuchi Y, Ohara N, Sato K, et al. Novel stationary-phase-upregulated protein of *Porphyromonas gingivalis* influences production of superoxide dismutase, thiol peroxidase and thioredoxin. *Microbiology*. 2005; 151(Pt 3):841–853. [PubMed: 15758230]
63. Briukhanov AL. Non-heme iron proteins as an alternative system of antioxidant defense in the cells of strictly anaerobic microorganisms: a review. *Prikl Biokhim Mikrobiol*. 2008; 44(4):373–386. [PubMed: 18924402]
64. Weinberg MV, Jenney FE Jr, Cui X, Adams MW. Rubrerythrin from the hyperthermophilic archaeon *Pyrococcus furiosus* is a rubredoxin-dependent, iron-containing peroxidase. *J Bacteriol*. 2004; 186(23):7888–7895. [PubMed: 15547260]
65. Lumpio HL, Shenvi NV, Summers AO, Voordouw G, Kurtz DM Jr. Rubrerythrin and rubredoxin oxidoreductase in *Desulfovibrio vulgaris*: a novel oxidative stress protection system. *J Bacteriol*. 2001; 183(1):101–108. [PubMed: 11114906]
66. Sroka A, Sztukowska M, Potempa J, Travis J, Genco CA. Degradation of host heme proteins by lysine- and arginine-specific cysteine proteinases (gingipains) of *Porphyromonas gingivalis*. *J Bacteriol*. 2001; 183(19):5609–5616. [PubMed: 11544223]
67. Sztukowska M, Bugno M, Potempa J, Travis J, Kurtz DM Jr. Role of rubrerythrin in the oxidative stress response of *Porphyromonas gingivalis*. *Mol Microbiol*. 2002; 44(2):479–488. [PubMed: 11972784]
68. Mydel P, Takahashi Y, Yumoto H, et al. Roles of the host oxidative immune response and bacterial antioxidant rubrerythrin during *Porphyromonas gingivalis* infection. *PLoS Pathog*. 2006; 2(7):E76. [PubMed: 16895445]
69. Nair S, Finkel SE. Dps protects cells against multiple stresses during stationary phase. *J Bacteriol*. 2004; 186(13):4192–4198. [PubMed: 15205421]
70. Andrews SC, Robinson AK, Rodriguez-Quinones F. Bacterial iron homeostasis. *FEMS Microbiol Rev*. 2003; 27(2–3):215–237. [PubMed: 12829269]
71. Chiancone E, Ceci P, Ilari A, Ribacchi F, Stefanini S. Iron and proteins for iron storage and detoxification. *Biometals*. 2004; 17(3):197–202. [PubMed: 15222465]
72. Ueshima J, Shoji M, Ratnayake DB, et al. Purification, gene cloning, gene expression, and mutants of Dps from the obligate anaerobe *Porphyromonas gingivalis*. *Infect Immun*. 2003; 71(3):1170–1178. [PubMed: 12595429]
73. Chen W, Honma K, Sharma A, Kuramitsu HK. A universal stress protein of *Porphyromonas gingivalis* is involved in stress responses and biofilm formation. *FEMS Microbiol Lett*. 2006; 264(1):15–21. [PubMed: 17020544]
74. Xu J, Bjursell MK, Himrod J, et al. A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. *Science*. 2003; 299(5615):2074–2076. [PubMed: 12663928]
75. Takeuchi T, Nakaya Y, Kato N, Watanabe K, Morimoto K. Induction of oxidative DNA damage in anaerobes. *FEBS Lett*. 1999; 450(3):178–180. [PubMed: 10359070]
76. Abaibou H, Ma Q, Olango GJ, Potempa J, Travis J, Fletcher HM. Unaltered expression of the major protease genes in a non-virulent *recA*-defective mutant of *Porphyromonas gingivalis* W83. *Oral Microbiol Immunol*. 2000; 15(1):40–47. [PubMed: 11155163]
77. Fletcher HM, Morgan RM, Macrina FL. Nucleotide sequence of the *Porphyromonas gingivalis* W83 *recA* homolog and construction of a *recA*-deficient mutant. *Infect Immun*. 1997; 65(11): 4592–4597. [PubMed: 9353038]
78. Liu Y, Fletcher HM. Environmental regulation of *recA* gene expression in *Porphyromonas gingivalis*. *Oral Microbiol Immunol*. 2001; 16(3):136–143. [PubMed: 11358535]

79. Liu Y, Fletcher HM. The *recA* gene in *Porphyromonas gingivalis* is expressed during infection of the murine host. *Oral Microbiol Immunol*. 2001; 16(4):218–223. [PubMed: 11442846]
80. Fukui K. DNA mismatch repair in eukaryotes and bacteria. *J Nucleic Acids*. 2010; 2010:260512. [PubMed: 20725617]
81. Kunkel TA, Erie DA. DNA mismatch repair. *Annu Rev Biochem*. 2005; 74:681–710. [PubMed: 15952900]
82. Skorvaga M, Theis K, Mandavilli BS, Kisker C, Van Houten B. The beta-hairpin motif of UvrB is essential for DNA binding, damage processing, and UvrC-mediated incisions. *J Biol Chem*. 2002; 277(2):1553–1559. [PubMed: 11687584]
83. Truglio JJ, Croteau DL, Van HB, Kisker C. Prokaryotic nucleotide excision repair: the UvrABC system. *Chem Rev*. 2006; 106(2):233–252. [PubMed: 16464004]
84. Fowler RG, White SJ, Koyama C, Moore SC, Dunn RL, Schaaper RM. Interactions among the *Escherichia coli* *mutT*, *mutM*, and *mutY* damage prevention pathways. *DNA Repair (Amst)*. 2003; 2(2):159–173. [PubMed: 12531387]
85. Krokan HE, Nilsen H, Skorpen F, Otterlei M, Slupphaug G. Base excision repair of DNA in mammalian cells. *FEBS Lett*. 2000; 476(1–2):73–77. [PubMed: 10878254]
86. Pomposiello PJ, Demple B. Global adjustment of microbial physiology during free radical stress. *Adv Microb Physiol*. 2002; 46:319–341. [PubMed: 12073656]
87. Simpson W, Olczak T, Genco CA. Lysine-specific gingipain K and heme/hemoglobin receptor HmuR are involved in heme utilization in *Porphyromonas gingivalis*. *Acta Biochim Pol*. 2004; 51(1):253–262. [PubMed: 15094847]
88. Allaker RP, Douglas CW. Novel antimicrobial therapies for dental plaque-related diseases. *Int J Antimicrob Agents*. 2009; 33(1):8–13. [PubMed: 18804350]
89. Lamont RJ, Jenkinson HF. Subgingival colonization by *Porphyromonas gingivalis*. *Oral Microbiol Immunol*. 2000; 15(6):341–349. [PubMed: 11154429]
90. Kirkwood KL, Cirelli JA, Rogers JE, Giannobile WV. Novel host response therapeutic approaches to treat periodontal diseases. *Periodontol 2000*. 2007; 43:294–315. [PubMed: 17214846]
91. Van Dyke TE. Control of inflammation and periodontitis. *Periodontol 2000*. 2007; 45:158–166. [PubMed: 17850455]
92. Fitzpatrick RE, Wijeyewickrema LC, Pike RN. The gingipains: scissors and glue of the periodontal pathogen, *Porphyromonas gingivalis*. *Future Microbiol*. 2009; 4(4):471–487. [PubMed: 19416015]
93. Smalley JW, Birss AJ, Silver J. The periodontal pathogen *Porphyromonas gingivalis* harnesses the chemistry of the mu-oxo bishaem of iron protoporphyrin IX to protect against hydrogen peroxide. *FEMS Microbiol Lett*. 2000; 183(1):159–164. [PubMed: 10650220]
94. Konkel ME, Tilly K. Temperature-regulated expression of bacterial virulence genes. *Microbes Infect*. 2000; 2(2):157–166. [PubMed: 10742688]
95. Percival RS, Marsh PD, Devine DA, et al. Effect of temperature on growth, hemagglutination, and protease activity of *Porphyromonas gingivalis*. *Infect Immun*. 1999; 67(4):1917–1921. [PubMed: 10085036]
96. Park Y, Lamont RJ. Contact-dependent protein secretion in *Porphyromonas gingivalis*. *Infect Immun*. 1998; 66(10):4777–4782. [PubMed: 9746578]

Website

101. Oral Genomic and Metagenomic Resource. www.oralgen.lanl.gov

Executive summary

Background

- Oxidative stress poses a significant threat to the proteins, lipids, RNA and DNA of cells.
- *Porphyromonas gingivalis* has developed a strategy to survive oxidative stress.

Sources of oxidative stress

- The main sources of oxidative stress to the bacterium include exposure to air or the oxidative burst generated by immune cells involved in the host inflammatory response.
- Reactive oxygen species include the superoxide radical, hydrogen peroxide and the hydroxyl radical.

Consequences of oxidative stress

- Reactive oxygen species have a deleterious effect on several bacterial cellular components.
- Lipid peroxidation occurs when free radicals attack polyunsaturated lipids.
- Damage to proteins includes oxidation of sulfhydryl groups, reduction of disulfides, protein–protein cross-linking, peptide fragmentation and oxidative adduction of amino acid residues.
- Oxidative stress generates lesions in the sugar or base moieties of DNA.

Regulators of oxidative stress

- The OxyR homolog in *P. gingivalis* appears to be important in aerotolerance.
- RprY, ECF- σ factors and RegT are other regulators that are involved in oxidative stress resistance in *P. gingivalis*.

Mechanisms of oxidative stress resistance

- Modulating host response:
 - *P. gingivalis* can modulate the host response by secreting Ndk.
 - The capsule of *P. gingivalis* may play a role in evasion of the host immune system.
 - Gingipains can modulate immune response.
- Hemin layer:
 - The unique hemin layer on the surface of *P. gingivalis* acts as an oxidative buffer against dioxygen, hydrogen peroxide and other toxic oxygen derivatives.
- Manganese homeostasis:
 - Manganese homeostasis may play an important role in the survival of *P. gingivalis* under aerobic conditions.
- Antioxidant proteins:
 - SOD, AhpC–F, Bcp, Tpx, Rbr and Dps play roles in oxidative stress resistance in *P. gingivalis*.

- UspA and UstA have been shown to protect against hydrogen peroxide and atmospheric oxygen, respectively.

DNA repair mechanisms

- The *P. gingivalis* RecA protein plays a role in DNA repair.
- A unique mechanism for the removal of 8-oxoguanine from damaged DNA has been discovered in *P. gingivalis*.
- Removal of 8-oxoguanine does not involve the *uvrB* gene in this organism.
- The MutY homolog of *P. gingivalis* plays an important role in the repair of DNA lesions.

Conclusion

- The systems involved in protecting *P. gingivalis* against oxidative stress are varied and complex and may be controlled by several response regulators.
- The survival of *P. gingivalis* in the periodontal pocket indicates the development of successful strategies and mechanism(s) to ensure its persistence.

Future perspective

- With emerging technology, we expect to fully elucidate the possibly unique mechanism(s) for oxidative stress resistance in *P. gingivalis*.
- In the near future, suitable therapeutic agents will be developed to combat this periodontal pathogen.

Figure 1. Summary of oxidative stress defense mechanisms in *Porphyromonas gingivalis*
Protection against oxidative damage may utilize multiple mechanisms in *Porphyromonas gingivalis*. The hemin layer can form μ -oxodimers in the presence of ROS and can give rise to the catalytic degradation of hydrogen peroxide [93]. Antioxidant enzymes (e.g., Rbr, AhpC and Bcp) and Dps may become upregulated to combat the increase in oxidative stress. Because the gingipains are downregulated at elevated temperatures, which is typical of the inflammatory microenvironment of the periodontal pocket [94,95] or when *P. gingivalis* contacts host cells [96], DNA damage repair and/or protein stabilization/repair may become more important for survival. As the bacterial hemin layer is further compromised by the assault from ROS, *P. gingivalis* is now more vulnerable to oxidant-induced DNA damage, especially guanine oxidation. A mechanism for the repair of this lesion, which may likely be novel, is still unknown. The involvement of multiple hypothetical proteins and/or proteins of unknown function in this process may imply other unique mechanisms and potential therapeutic targets.

8-oxoG: 7,8-dihydro-8-oxoguanine; BER: Base excision repair; eATP: Extracellular ATP; NER: Nucleotide excision repair; ROS: Reactive oxygen species.