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The Interplay Between Iron, Haem and Manganese in *Porphyromonas gingivalis*

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Abstract

Background: Transition metals including iron and manganese are necessary for life because of their ability to donate and accept electrons. Approximately one third of all proteins require essential transition metal ions to perform catalytic, structural and regulatory functions. These essential metal ions react differently to the presence of oxygen radicals with iron directly involved in the formation of toxic reactive oxygen species, whilst manganese can protect against oxidative stress.

Highlight: Anaerobic bacterial species have been poorly studied with regard to transition metal homeostasis and behave differently in many respects when compared with aerobic or aerotolerant species. To optimize catabolism whilst protecting themselves from unwanted reactions bacterial cells must maintain intracellular metal levels in a very narrow range that varies, dependent on the environment. To maintain metal ion homoeostasis, bacteria have evolved complex regulatory mechanisms of metal uptake, secretion and storage. In this review we examine how iron, haem and manganese availability dictate the lifestyle and virulence of the anaerobic Gram-negative, periodontal pathogen *Porphyromonas gingivalis*.

Conclusion: *P. gingivalis* has novel haem, iron and manganese transporters and metalloregulatory proteins that enable it to switch rapidly between an energy efficient iron-dependent virulent phase and a protective manganese-dependent survival phase.

Key words: *P. gingivalis*; metal ion homeostasis; virulence; survival

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1. Introduction

Chronic periodontitis is the most common of the destructive periodontal diseases amongst adults and its prevalence and severity increase with age. The global age-standardised prevalence of severe periodontitis between 1990 and 2010 was 11%, however the exact percentage varies between and within countries [1-3]. In the USA, 38% of the adult population 30 years and older and 65% of adults 65 years and older have either severe or moderate periodontitis [4, 5]. Epidemiological surveys have shown that clinical indicators of chronic periodontal disease are associated with a greater risk of certain cancers such as squamous cell carcinoma of the head, neck, and oesophagus [6], cancer of the tongue [7] and pancreatic cancer [8-10]. There is also a relationship between chronic periodontitis and systemic diseases and disorders such as cardiovascular disease [11], preterm and underweight birth [12], systemic inflammation in solid-organ transplant recipients [13], diabetes and rheumatoid arthritis [11, 14-16].

The bacterial aetiology of chronic periodontitis is acknowledged to be polymicrobial in nature. Whilst the concepts of the roles of particular oral bacterial species in disease have changed over the past two decades, there is consensus that the anaerobic, proteolytic, amino acid fermenting species *Porphyromonas gingivalis* plays a significant role in either initiation or progression of disease [17-20]. Based on animal model data *P. gingivalis* has recently been proposed to be a "keystone pathogen" that manipulates the host response to favour the proliferation of a pathogenic polymicrobial biofilm (dysbiosis) and development of disease [19]. We have previously demonstrated in a longitudinal human study that the imminent progression of chronic periodontitis could be predicted by increases in the relative levels of *P. gingivalis* and/or *Treponema denticola* in subgingival plaque [21], which is consistent with other clinical studies demonstrating that *P. gingivalis* levels in subgingival plaque are

predictive of human disease progression [22-24]. *P. gingivalis* is also capable of causing periodontitis in animal models of disease [25, 26].

2. Divalent metal cations

All living cells acquire transition metal ions to meet their basic cellular needs, with iron, manganese, copper, zinc, nickel and cobalt being of greatest physiological relevance [27, 28]. It has been estimated that about one third of all proteins require essential transition metal ions to perform catalytic, structural and regulatory functions [29, 30]. Metals such as iron, copper, chromium, manganese and cobalt are capable of redox cycling in which a single electron may be accepted or donated by the metal. These actions catalyse reactions that play critical roles in the function of many organisms but may also produce reactive radicals and reactive oxygen species [31-34].

Iron (Fe^{2+}/Fe^{3+}) is an obligate requirement for the vast majority of bacteria as it is a versatile prosthetic component incorporated into many proteins as a biocatalyst or electron carrier [35, 36]. It has a role as a prosthetic group in many biological enzymatic systems including cytochromes, RNA polymerase and various amino acid hydrolases [37, 38].

Manganese is also essential with greater than 20 identified functions in enzymes and proteins involved in metabolism, signal transduction and as a stimulus for virulence gene regulation [36, 39-44]. It is a key cofactor of many metalloenzymes including oxidases and dehydrogenases, DNA and RNA polymerases, kinases, sugar transferases and decarboxylases [45].

$Mn^{3+} + e^- \rightleftharpoons M\,n^{2+}$ has a standard reducing potential of +1.51 V, higher than $Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$ whose standard reducing potential is +0.77 V, thus Mn^{2+} has a lower potential to donate an electron compared with Fe^{2+} and thereby a lower potential to reduce other molecules [41]. Although manganese has similar characteristics to iron being a transition

metal capable of cycling readily *in vivo* between the +2 and +3 oxidation states [41], instead of catalyzing oxidative damage like iron, manganese can protect cells against oxidative damage via enzyme-dependent and protein-independent mechanisms [46, 47]. Manganese appears to play a significant role in oxidative defence systems in most pathogenic bacteria [46].

3. Oxidative stress

Stepwise reduction of molecular oxygen (O_2) by high-energy exposure or electron-transfer reactions leads to production of highly reactive oxygen species (ROS). The conversion of atmospheric oxygen to ROS occurs inside actively respiring aerobic or facultative bacterial cells [48]. However, few ROS are generated intracellularly by anaerobic bacteria due to the absence of molecular oxygen in their environment. Commensal and pathogenic bacteria can also be exposed to the oxidative burst of macrophages and neutrophils of the host inflammatory immune response [49]. Transition metal ions can play a major role in the exacerbation or relief of oxidative stress. Most biological molecules cannot be damaged at a significant rate by direct reactions with molecular oxygen, superoxide anion (O_2^-) [50, 51] or hydrogen peroxide (H_2O_2) [52]. However, they can be oxidized by the highly reactive hydroxyl radical (HO•). This species is formed when a single electron is transferred to hydrogen peroxide.

$$e^- + H_2O_2 \rightarrow HO^\bullet + OH^-$$

In vivo the most facile donor of single electrons to H_2O_2 is the transition metal, ferrous iron (Fe^{2+}) via the Fenton reaction [53].

$$Fe^{2+} + H_2O_2 \rightarrow HO^\bullet + OH^- + Fe^{3+}$$

This reaction is driven to the right by the subsequent formation of poorly soluble $Fe(OH)_3$. The hydroxyl radicals formed are extremely damaging for cellular components such

as nucleic acids (both DNA and RNA), proteins and lipids [35]. The presence of such metals in an uncomplexed form in biological systems can significantly increase the level of oxidative stress.

Living organisms use defence systems to maintain the concentration of O_2-derived radicals at acceptable levels or repair any damage caused by the toxic ROS [36]. These oxidative stress protection systems also utilise transition metal ions, in particular manganese ions, as co-factors for metalloenzymes that defend against reactive oxygen species.

It is not surprising that some bacteria have evolved metabolic and survival strategies that minimize oxidative damage by acquiring Mn^{2+} instead of Fe^{2+}/Fe^{3+}. *Borrelia burgdorferi*, the etiological agent of Lyme disease and *Lactobacillus plantarum*, a probiotic bacterium were found to be free from Fe^{2+} requirements for their growth [42, 44]. Manganese was demonstrated to be the most essential divalent cation for these bacteria instead of iron [42, 44]. The absence of iron in these bacteria may be an advantage, as they are able to overcome the iron limitation found in most hosts and there is no requirement to minimize oxygen free radicals generated from the Fenton reaction [44]. However Mn^{2+} has a lower potential to donate an electron compared to Fe^{2+}, thus limiting its effectiveness as a metabolic enzyme cofactor.

4. *Porphyromonas gingivalis*

P. gingivalis is a Gram-negative, sessile, obligate anaerobe that has an absolute requirement for iron and its growth and virulence are dependent on the availability of iron complexes such as haem [54-57] or ferrous iron [58]. In addition *P. gingivalis* cannot synthesize protoporphyrin IX [59], a porphyrin derivative that combines with ferrous iron to form haem, a cofactor for several enzymes, which can be bound transiently [60], or remain bound to the protein permanently [61].

P. gingivalis relies on the anaerobic fermentation of amino acids for energy production, which requires a number of iron-containing proteins that are involved in redox reactions (Fig. 1). The *P. gingivalis* W83 genome contains at least 20 genes encoding predicted non-haem, iron-sulphur proteins with similarity to ferredoxins and other iron-containing enzymes. These include the fumarate reductase iron-sulfur subunit FrdB (PG1614), an iron-containing alcohol dehydrogenase 4hbD (PG0689), the indolepyruvate ferredoxin oxidoreductase IorA and IorB (PG0675, PG0674), the pyruvate ferredoxin/flavodoxin oxidoreductase family protein (PG0548), an iron-dependent fumarate hydratase FumB (PG1417) and a range of putative, uncharacterised ferredoxins (PG0472, PG1172, PG1421, PG1813). Some of these enzymes have been demonstrated biochemically to be involved in amino acid fermentation in *P. gingivalis* [62, 63]. The ferredoxins each contain two or more 4Fe-4S clusters.

Several *P. gingivalis* proteins have been predicted to form complexes as part of the respiratory chain of this organism for the production of ATP [64]. These include the sodium-dependent NADH: ubiquinone oxidoreductase (Na^+-Nqr) complex composed of NqrA-F (PG2182-2177) which is the main ion pump and primary entry site for electrons into the respiratory chain [64, 65]. The Nqr complex mediates electron transfer from NADH to quinone, and uses iron as a redox cofactor in the 2Fe-2S centre of NqrF [65].

Although there is little known about the function of the Rnf complex (RnfABCDGE; PG0303-0308) in *P. gingivalis*, characterisation in other anaerobic bacteria has shown it mediates electron transfer from ferredoxin to NAD^+ [66] and utilises six 4Fe-4S clusters and two 4Fe-4S clusters as cofactors in RnfB and RnfC, respectively [65]. Due to the large amount of iron required by this complex, genes encoding Rnf proteins are down-regulated when *P. gingivalis* is grown in iron-limited conditions [67].

Fumarate reductase, FrdBAC (PG1614-1616), is a trimeric enzyme complex belonging to the succinate:quinone oxidoreductase (SQOR) family that couples the reduction of

fumarate to succinate to the oxidation of quinol to quinone during anaerobic respiration [64]. This facilitates the formation of a proton/sodium gradient across the inner membrane coupled to ATP generation [64]. FrdB contains a 2Fe-2S, a 4Fe-4S and a 3Fe-4S cluster, whilst FrdC contains two haem molecules [62] and appears to be the main user of haem as a redox cofactor in the anaerobic respiratory chain of *P. gingivalis* [64]. Inhibition of fumarate reductase activity by oxantel pamoate stopped the growth of the bacterium and strongly inhibited biofilm formation, demonstrating the essential role of this enzyme in *P. gingivalis* metabolism [68, 69].

5. Metal acquisition systems of *Porphyromonas gingivalis*

P. gingivalis like most anaerobic bacteria does not produce siderophores to scavenge environmental iron or compete with transferrin or lactoferrin for ferric iron binding [70]. *P. gingivalis* utilises human transferrin as a source of iron and peptides via proteolytic cleavage by the cell surface Arg- and Lys-specific cysteine proteinases, RgpA/B and Kgp, collectively known as gingipains [71, 72]. In the absence of gingipains *P. gingivalis* cannot remove the iron from transferrin [71]. The resulting degradation products of transferrin can catalyse the formation of a highly reactive hydroxyl radical ($OH^{\bullet}$), due to the fragments containing iron or due to the release of iron [72].

P. gingivalis has been reported to have a high-affinity receptor which binds lactoferrin before complete cleavage by the gingipains [73]. Lactoferrin does have an inhibitory effect on bacterial growth due to its ability to sequester iron [74], and it also has an antimicrobial domain at the N-terminus, which when isolated has potent bactericidal activity [75]. Bovine lactoferrin inhibits *P. gingivalis* planktonic growth and biofilm formation [76] which may in part be due to its sustained inhibition of the gingipains which are required for biofilm formation [77].

Haem is preferentially obtained by *P. gingivalis* from haemoglobin and is acquired through the activity of the gingipains [78-80] and other haem-binding proteins that some researchers have proposed act as haemophores [81]. Haemophores are specialized bacterial proteins that are secreted from the cell and released into the environment which then acquire haem and facilitate uptake through a specific cell surface transporter [82]. Although the gingipains have been shown to cleave haemoglobin and the HA2 domain of the gingipains Kgp and RgpA and the haemagglutinin HagA binds haemoglobin or haem via an iron-independent mechanism that recognises the porphyrin ring [81, 83, 84], these proteins are covalently attached at the cell surface but are released by *P. gingivalis* on outer membrane vesicles (*vide infra*) [85].

In fact, *P. gingivalis* produces a range of haem-binding lipoproteins anchored to the outer membrane (Fig. 2). The best studied example is HmuY, which uses two His residues to bind haem or haemin in a 1:1 molar ratio [86, 87] and is part of a haem acquisition mechanism with HmuR, a TonB-linked outer-membrane receptor involved in haem transport through the outer membrane [84, 88]. The proposed mechanism of action of the HmuY/HmuR acquisition system is that HmuY scavenges haem liberated by the cleavage of host haem-carrier proteins by the gingipains [87, 89]. Binding of haem leads to tetramerisation of HmuY, protecting the haem from host scavengers [87]. HmuR then induces disruption of the HmuY tetramer via its His axial ligands to enable haem transfer [87]. Haem is then passed through the outer membrane HmuR to the periplasm where it is transported from the periplasm to cytoplasm, presumably by the other *hmu* operon proteins HmuSTUV (Fig 2.) [90]. Expression of the entire *hmu* locus is upregulated under haemin-limited growth [62].

More recently, the novel haem binding protein HusA has been identified in *P. gingivalis* and was found to have more than 1,000-fold greater affinity for haem than HmuY.

With this high haem binding affinity and a fast haem association rate HusA could compete directly with host haemoproteins such as serum albumin [91]. HusA has a preference for dimeric haem and may serve as the predominant bishaem chelating protein under low haem growth conditions [91]. Like HmuY, HusA is bound to the cell surface and once dimeric haem is bound, is proposed to deliver haem to HusB, an integral outer membrane protein for transport to the periplasm (Fig. 2) [91].

Several other haemin binding outer membrane proteins in *P. gingivalis* have been described including OMP26, OMP32, HBP35, HtrE (Tlr) and IhtB, many of which are expressed under low haemin growth conditions (Fig. 2) [62, 92-96]. The lipoprotein IhtB is an outer membrane haemin-binding ferrochelatase [93] homologous to a precorrin-2 cobalt chelatase [59]. The close proximity of the *ihtB* gene to a gene encoding a predicted TonB-linked outer membrane protein (IhtA) led to the proposal that IhtB removes iron from haem prior to IhtA-mediated iron transport through the outer membrane [93].

HtrE (Tlr) is a TonB-linked outer membrane transporter that is essential for growth at low concentrations of haemin [95]. The gene encoding HtrE is located adjacent to an operon encoding a putative ATP binding cassette transport system with sequence similarity to haem transport systems of other bacteria, thus together, these genes may encode a haem transport system [95]. The PG1019-1020 locus of *P. gingivalis* encodes a predicted outer membrane lipoprotein and an outer membrane TonB-linked receptor respectively that are greatly increased in abundance during haem-limitation [62] and iron-limitation [67], also suggesting a role in haem/iron transporter (Fig. 2).

Strikingly, many of the outer membrane components of these putative iron-complex transport systems are composed of a haem-binding lipoprotein coupled with a TonB-linked transmembrane transporter (Fig. 2). A proteomics-based study of the outer membrane vesicles (OMVs) produced by *P. gingivalis* indicated that the lipoproteins HmuY and IhtB

were preferentially packaged onto the vesicle surface whilst their cognate TonB-linked receptor proteins HmuR and IhtA remained on the cell surface [85]. The increased abundance of these haem binding lipoproteins and the gingipains on OMVs suggests that OMVs may extend the functionality of these proteins and that *P. gingivalis* OMVs may be important for haem acquisition by acting as haemophores [85]. For example, the release of OMVs containing the gingipains from the biofilm on the tooth root into the gingival tissue has been suggested to play a role in vascular disruption and immune dysregulation [85, 97, 98]. Through the concerted action of the gingipains and haem-binding proteins in the tissue the OMVs may become loaded with haem. The resulting inflammation and gingival exudate could then return the loaded vesicles back to the biofilm allowing haem transfer to the biofilm cells.

P. gingivalis like many other Gram-negative bacteria transports ferrous ion across the cytoplasmic membrane using the transmembrane FeoB protein, FB1 [58, 99]. FeoB proteins are 700-800 amino acids in length and have a cytoplasmic G protein domain directly tethered to a polytopic membrane domain [100]. GTP binding to the G protein domain initiates the transport of Fe^{2+} across the membrane, which is completed by the hydrolysis of GTP to GDP. The GTPase activity of FeoB is activated by K^{+} which leads to a 20-fold acceleration in its hydrolysis rate, bringing it close to the active transport rate of hydrolysis of the ATP-binding cassette transporters [101]. FB1 is the only ferrous ion transporter in *P. gingivalis* as inactivation of this transporter abolished ferrous ion transport and the iron content of the mutant was half that of the wild-type (Fig. 2) [58]. The FB1 mutant was avirulent in a mouse model of disease indicating the importance of this transporter to the *in vivo* survival of *P. gingivalis* [58].

The major manganese transporter in *P. gingivalis* has been identified as a FeoB transport protein homologue called FB2 that had likely arisen by gene duplication [58]. FB2

was found to contribute to the survival of *P. gingivalis* in human umbilical vein endothelial cells (HUVECs) [102]. Although the full mechanism has not been elucidated, it is the acquisition of manganese that is required for intracellular survival of *P. gingivalis* in host cells [102].

Little is known about the translocation of manganese and other divalent cations across the outer membrane and into the periplasm; it was generally thought that they would diffuse through porins in the outer membrane [103, 104]. However, it was recently demonstrated in *Bradyrhizobium japonicum* that Mn^{2+} does not diffuse through the outer membrane but is transported through a selective outer membrane channel that is expressed specifically under manganese limitation [105]. The gene encoding this outer membrane channel was in the same operon as the gene encoding the inner membrane Mn^{2+} transporter in this organism, MntH, ensuring co-ordinated expression of the whole transport system [105]. Such an outer membrane channel has not been identified in *P. gingivalis*, nor is there an outer membrane protein predicted to be encoded in the same operon as the Mn^{2+} transporter FB2.

Whilst examining the *P.gingivalis* W50 global pattern of protein and transcript abundances in response to haem-limitation in continuous culture, 160 genes and 70 proteins were found differentially regulated by haem availability, with broad agreement between the transcriptomic and proteomic data (Fig. 1) [62]. Haem-limitation caused upregulation of a number of gene products in *P. gingivalis* that are linked to metabolism, oxidative stress response, virulence and invasion of host cells [62]. A change in abundance of the iron and haem containing enzymes of the aspartate and glutamate catabolic pathways was observed during haem-limitation which was reflected in organic acid end products. This included down-regulation of the fumarate reductase which is essential for energy production [62, 64].

There was a notable increase in expression of two haem transport systems encoded by the *hmu* and *htr* operons, as well as a large increase in the abundance of alkyl hydroperoxide

reductase subunits (AhpC, PG0618; AhpF, PG0619), a peroxide scavenging enzyme shown to play an important role in peroxide resistance in *P. gingivalis*. Haem-limitation also reduced *P. gingivalis* biofilm development with a 56% decrease in biomass and a 78% decrease in biofilm depth [77].

Vascular disruption and bleeding are characteristics of periodontitis, providing a protein/peptide and iron/haem rich environment for bacterial growth during disease progression. However, inflamed gingival tissues contain considerable numbers of polymorphonuclear leucocytes (PMNs) that produce O_2^- and H_2O_2 as part of their bactericidal armoury. As a consequence of this the bacterium must have a defence system against oxidative stress. Under conditions of haem excess the bacterium forms an oxidative shield by accumulating haem from haemoglobin on the cell surface as μ-oxo bishaem which binds reactive oxygen intermediates, hence maintaining a locally reduced environment [106]. This haem layer protects the bacterium from direct contact with reactive oxidants generated by neutrophils in periodontal lesions [106]. This is also a novel way to store reactive iron outside the cell where it can't cause damage to intracellular components.

P. gingivalis has developed various intracellular oxidative stress defence systems, including superoxide dismutase (SOD) which can utilise either iron or manganese as co-factor, [107], the DNA-binding protein from starved cells (Dps) [108, 109], alkyl hydroperoxide peroxidase subunit C (AhpC) [110] and rubrerythrin (Rbr) [111]. Superoxide dismutase (SOD) is the only known *P. gingivalis* oxidative defence system which requires manganese as a cofactor, however, the intracellular accumulation of manganese itself has been shown to have anti-oxidative properties, protecting *P. gingivalis* from atmospheric oxygen and hydrogen peroxide [102].

6. The polymicrobial biofilm nature of health and disease

P. gingivalis is a normal component of the human oral microbiota and is a late colonizer of polymicrobial oral biofilms, relying on complex interactions with a range of other oral bacteria including *Streptococcus gordonii*, *Fusobacterium nucleatum, Tannerella forsythia* and *T. denticola* [112-114]. Therefore although much has been learnt by studying *P. gingivalis* in isolation, its interactions with other bacterial species in the biofilm will have a considerable influence on its role as an opportunistic pathogen in inducing dysbiosis and disease. For example results from a polymicrobial biofilm analysis showed a decrease in abundance of HtrE (Tlr), IhtB, HmuY and fumarate reductase which could possibly be due to the cross feeding of succinate from *T. denticola* to *P. gingivalis* thereby reducing the need for haem (Fig. 1), or due to reduced growth rates in the biofilm [115]. This is in contrast to findings in a monospecies biofilm, which would have a similar growth rate to a polymicrobial biofilm, where HmuY was more abundant than in planktonic cells [116]. Commensurate with this polymicrobial approach Mashburn *et al*. [117] have shown that *Pseudomonas aeruginosa* relies on *Staphylococcus aureus* as an iron source *in vivo*. It has also been shown that the presence of *T. denticola* reduces energy consuming processes of *P. gingivalis* such as fatty acid synthesis, which would reduce the need for cellular iron [114]. The expression of 134 *P. gingivalis* genes was modulated by the presence of *T. denticola* and the two species showed a range of symbioses and syntrophy that resulted in higher biomass when grown in coculture [114, 118].

When in association with *S. gordonii* 10 of the 33 genes that altered in expression in *P. gingivalis* were classified as encoding proteins involved in metabolic pathways whilst a further 4 encoded transport and binding proteins, including HmuY that was down-regulated [119]. These results suggested that the initial adaptation of *P. gingivalis* to a polymicrobial biofilm with *S. gordonii* involved a shift in metabolic and physiologic status, and that the

cells were stressed, as both superoxide dismutase and excinuclease were also upregulated [119].

F. nucleatum is capable of generating a CO_2-enriched environment [120] that enables it to support the growth of *P. gingivalis* in aerated and CO_2-depleted environments in which *P. gingivalis* would not survive on its own [120]. When *P. gingivalis* was grown in a three species community with *F. nucleatum* and *S. gordonii*, proteomic differences were again noted that implied extensive interactions between the three organisms and suggested a favourable environment, which resulted in increased *P. gingivalis* protein expression and decreased stress [113].

P. gingivalis cells use LuxS-dependent signalling to communicate with each other in the biofilm [121, 122] and to mediate interspecies communication in mixed-species biofilms [123, 124]. Thus *P. gingivalis* interacts with other members of the polymicrobial biofilm that will modify its iron complex acquisition and use.

In the healthy oral cavity *P. gingivalis* is exposed to low iron/haem environments that are also likely to have a higher oxygen exposure. In response to this dynamic environment, *P. gingivalis* must regulate gene expression to survive.

7. Metalloregulatory Proteins

To protect against the toxic effect of the Fenton reaction, cells must utilize, store and maintain iron concentrations with careful management of cellular free iron sequestered in high affinity protein-bound forms [125]. Intracellular concentrations of metal ions in living cells are maintained and co-ordinated through a system known as metal ion homeostasis that involves metal ion influx across the cell membrane depending on the intracellular metal ion concentration, availability and demand. Excess metal uptake may lead to toxic effects and cell death. In order to maintain and balance intracellular metal ion concentration, metal

homeostasis needs to be regulated at the level of transcription [30]. Proteins that are responsible for regulation are known as metalloregulatory or 'metal sensor' proteins, in which metal ions bind directly to the protein which in turn can then either repress, derepress or activate gene transcription depending on its mode of action [126]. To date, ten major families of metalloregulatory proteins in prokaryotic organisms have been identified and characterized as the ArsR (or ArsR/SmtB), MerR, DtxR, Fur, NikR, CopY, TetR, MarR, LysR and CsoR/RcnR families (Table 1.0) [27, 30]. These metalloregulatory proteins have been divided into two groups in relation to their functions: protein families that control gene expression linked to metal efflux / sequestration (ArsR, MerR, CopY, CsoR, TetR) and protein families that control the expression of genes for metal ion uptake (DtxR, Fur, NikR, MarR, LysR) (Table 1.0). Of these ten structural superfamilies of metalloregulatory proteins, only two are known to contain members that sense manganese and are thus required for manganese homeostasis (Table 1.0). These are MurR from the Fur superfamily and MntR from the DtxR superfamily. *P. gingivalis* encodes one homologue from each of the Fur and DtxR superfamilies of metalloregulators.

In Gram-negative bacteria, gene regulation in response to intracellular iron availability is usually mediated by the ferric uptake regulatory (Fur) protein [127]. Fur is a small, approximately 17 kDa, global transcriptional regulator that in the presence of iron regulates the expression of genes involved in iron acquisition, transport, storage, oxidative stress and virulence [128]. The Fur protein of the facultative generalist bacterium, *Escherichia coli* (EC-Fur) is the best characterised representative of this family of metalloregulatory repressor proteins. Fur acts as a transcriptional repressor due to its Fe^{2+}-dependent DNA binding activity [129]. Fur binds free intracellular Fe^{2+} as its co-repressor, acquiring a conformation able to bind specific DNA sequences known as Fur boxes which overlap gene promoters, thus preventing transcription of these genes. When iron is scarce, Fur no longer binds Fe^{2+} or

DNA, thus the RNA polymerase can access the promoters and the genes are expressed [127]. Structural and functional studies of EC-Fur revealed that Fur exists as a dimer, with each monomer containing two metal-binding sites [130]. The N-terminal domains are involved in DNA binding, whilst the C-termini are involved in dimerisation [130]. Although the EC-Fur crystal structure has not been solved, the crystal structure derived from the *Pseudomonas aeruginosa* Fur (PA-Fur; Fig. 3) protein has provided a model for the EC-Fur structure. These structural studies predict that both the EC-Fur and PA-Fur share similar domain structures, they both exist as dimers and contain one Zn^{2+} and one Fe^{2+} binding site per monomer [130, 131]. Later structural and biochemical studies of Fur orthologues HpFur and BsFur from *Helicobacter pylori* and *Bacillus subtilis* respectively showed three functional metal binding sites in each protein [132, 133]. Disruption of Site 3 in HpFur significantly reduced DNA binding affinity [132].

P. gingivalis W83 has one Fur orthologue (PG0465) encoded in its genome, but the molecular mechanisms of iron-dependent regulation appear to be novel in *P. gingivalis* as the deletion of the Fur protein had no effect on the expression of iron-regulated genes or manganese-regulated genes [67, 134]. Instead, this Fur orthologue, called Har for haem associated regulator, was demonstrated to regulate haem-responsive biofilm formation [134]. Har dimerises in the presence of Zn^{2+} and binds one haemin molecule per monomer with high affinity via the haem regulatory motif Cys97-Pro98 [134]. The binding affinity of Har for haemin (K_d of 0.23 μM) [134] was comparable to the affinity for haemin for the *Anabaena* FurA (0.35 μM) [135] and *E. coli* Fur (<1 μM) [136]. When Har was inactivated, there was no significant change in metal content of *P. gingivalis*, suggesting that *P. gingivalis* does not use its only Fur orthologue to regulate metal homeostasis [134]. Instead Har conferred the ability to respond to environmental haem and develop biofilms, both of which are key attributes for the *in vivo* survival and pathogenicity of *P. gingivalis*.

P. gingivalis is an iron-dependent Gram-negative bacterium that has a distinct iron-responsive regulon [67] but does not utilize a member of the Fur superfamily to regulate iron homeostasis, instead linking the transport of haem and ferrous iron from exogenous sources with quorum sensing via LuxS. James *et al*. [137] have shown that LuxS was required for a 1.5-fold increase in transcript levels of the ferrous ion transport system but negative regulators of this system have not yet been identified.

The DtxR family of transcriptional regulators was characterized after being discovered as the first iron metalloregulator in *Corynebacterium diphtheriae* (CdDtxR) [138]. CdDtxR is a 226 amino acid polypeptide, which functions as a homodimer [139, 140]. Each CdDtxR monomer consists of 3 domains. Domain 1 (residues 1-73) is the DNA binding domain, which contains the helix-turn-helix (HTH) motif. Domain 2 (residues 74-140) is the dimerisation domain and has two iron binding sites. The ancillary site has a higher iron-binding affinity than the primary site and binds iron prior to the primary site [140]. Domain 3 (residues 145-226) provides two amino acids to the ancillary iron-binding site and has structural similarity to an SH3 domain, an important domain in signal transduction in eukaryotes [141]. Between Domain 2 and 3 is a flexible poly-proline rich linker sequence. SH3 domains recognize poly-proline-rich sequences and are involved in protein-protein interactions [142]. The C-terminal domain of DtxR shares function and structural similarity with its SH3 counterpart but without sequence similarity [142]. The SH3 tail of DtxR plays two roles, providing amino acids Glu^{170} and Gln^{173} for iron-binding as part of the ancillary iron binding site of the dimeric DtxR holorepressor and binding the poly-proline linker found between domain 2 and domain 3 in a deep crevice of the monomeric DtxR aporepressor, thus acting as a regulatory switch that modulates the activation of repressor activity [143-146]. Domain 3 of DtxR is now known as a FeoA domain, due to a common-fold in bacterial FeoA

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4 proteins and eukaryotic SH3 domains as revealed by crystal structures [99], suggesting a
5
6 similar role in mediating protein-protein interactions [147].
7
8 A homologue of CdDtxR, IdeR (MtIdeR) [148] and orthologues of DtxR, MntR
9
10
11 (BsMntR) [149] and TroR (TpTroR) [43] were first discovered in *Mycobacterium*
12
13 *tuberculosis, Bacillus subtilis* and *Treponema pallidum* respectively. Although MtIdeR
14
15 responds to iron, BsMntR and TpTroR were found to respond to manganese [43, 149]. Other
16
17
18 experimentally characterized DtxR-related manganese-responsive homologues identified are
19
20 SirR (*Staphylococcus epidermis*; SeSirR) [150], ScaR (*Streptococcus gordonii*, SgScaR)
21
22 [151], EfaR (*Enterococcus faecalis*; EfEfaR) [152] and SloR (*Streptococcus mutans*;
23
24
25 SmSloR) [153]. Work by Guedon *et al* showed that specificity for Mn^{2+} originates from the
26
27 primary metal binding site [154].
28
29 The DtxR homologue of *P. gingivalis* W83 (PG1044; PgMntR) is encoded in the same
30
31
32 operon as the FB2 manganese transporter and based on its predicted amino acid sequence,
33
34 has aspects of both an iron-binding and a manganese-binding primary metal binding site [58].
35
36 Recombinant PgMntR was used to probe the specificity of metal binding and its impact on
37
38
39 PgMntR structure and DNA binding (unpublished). PgMntR dimerised in the absence of a
40
41 structural divalent transition metal cation and unusually bound three Mn(II) or two Fe(II) per
42
43 monomer. *In vitro*, Mn^{2+} increased the DNA binding affinity of PgMntR to the promoter
44
45
46 region of the gene encoding the FB2 manganese transporter whilst Fe^{2+} destabilised the
47
48 protein-DNA complex which would result in the derepression of the transcription of Mn^{2+}
49
50 transport genes (unpublished). This may suggest a novel regulatory mechanism of the
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52
53 interplay between iron and manganese in bacterial pathogenesis.
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55 Although not a metalloregulatory protein, OxyR activity is significantly upregulated
56
57 when *P. gingivalis* is grown in a haem-limited environment, indicating that *P. gingivalis*
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59
60 coordinately regulates expression of oxidative-stress-related genes by a haemin
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concentration-dependent pathway [155]. The OxyR regulatory protein of *P. gingivalis* functions differently compared to the OxyR of facultative anaerobes and aerobic microorganisms where this regulator co-ordinates the response of these microorganisms to H_2O_2 [156]. Instead, OxyR does not act as a sensor of H_2O_2 in *P. gingivalis* but constitutively activates transcription of oxidative-stress-related genes under anaerobic growth. Common OxyR-regulated genes such as *dps* and *ahpFC* were not positively regulated in *P. gingivalis* in response to H_2O_2 [156], instead expression of *sod*, *dps*, and *ahpC* were upregulated when OxyR activity was increased in low-haemin growth conditions [155]. Phenotypic characterisation of an *oxyR* mutant showed that OxyR plays a role in both the resistance to H_2O_2 and the aerotolerance of *P. gingivalis*.

8. Walking the tightrope: The nexus between haem, iron, manganese and oxygen

There is interplay between iron and manganese homeostasis in *P. gingivalis* as in a FeoB mutant, which had half the cellular iron of wild-type, there was a concomitant three-fold increase in cellular manganese [58]. This increase in cellular manganese content in the *P. gingivalis* mutant was attributed to manganous ions binding to vacant sites of ferrous ion binding proteins thus lowering the free manganous ion concentration within the cell. Given the link between increased OxyR expression under haem-limitation resulting in increased oxidative stress protection, this increase in Mn^{2+} could also be *P. gingivalis* using the antioxidative properties of Mn^{2+} itself [102, 125] or replacing iron in key enzymes susceptible to oxidative attack. *E. coli* cells shift from an iron to manganese central metabolism during oxidative stress and mononuclear enzymes such as ribulose-5-phosphate epimerase switch to using Mn^{2+} as a cofactor [157]. The shift to the use of manganese in *P. gingivalis* highlights the interdependence of these two ions and their critical role in virulence and survival. The close linkage between iron and manganese accumulation in *P. gingivalis* is

also reflected in the cambialistic nature of its superoxide dismutase (SOD, PG1545), which can utilise either manganese or iron to give maximum specific activity for the disproportionation of superoxide radicals into hydrogen peroxide and molecular oxygen [158-161]. This flexibility in superoxide dismutase metal ion specificity may have evolved to aid *P. gingivalis* exploit habitats where iron is not freely available and may have resulted in a more coordinated balance between iron and manganese cellular content.

The combination of a cambialistic SOD that is able to utilize Mn^{2+} or Fe^{2+} as well as the ability to use Mn^{2+} for oxidative stress protection, an OxyR-dependent peroxidase activity catalysed by Dps and a surface layer of μ-oxo-bishaem endows *P. gingivalis* with a high degree of aerotolerance to survive in the oral cavity.

The tight interplay between iron and manganese in *P. gingivalis* has also extended to metal transport with the discovery of a ferrous ion transport system, Feo that has evolved to transport manganese; the first report of a FeoB orthologue used to transport a metal other than iron [58]. Thus *P. gingivalis* has two FeoB transporters, FB1 which transports ferrous iron and FB2 which transports manganese. Both FeoB transporters are required for *P. gingivalis* to colonise and cause disease in the oral cavity. When the FB1 transporter is inactivated *P. gingivalis* is avirulent in a murine abscess model of disease whereas when the FB2 transporter is inactivated *P. gingivalis* is not able to survive intracellularly [58, 102].

The interplay between Mn^{2+} and Fe^{2+} in *P. gingivalis* is also apparent in the PgMntR metalloregulatory protein which has a primary metal binding site capable of binding Mn(II) or Fe(II) [58](unpublished).

9. Conclusion

The clear interplay between iron, manganese, haem and oxidative stress protection may enable the anaerobic *P. gingivalis* to maintain a high level of intracellular ferrous iron to maximise growth and virulence using energy efficient iron-dependent metabolism, but to rapidly replace this potentially deadly metal with manganese for survival during oxidative stress by switching to a more protective, but much more restrictive, manganese-based physiology.

Ethical Approval

Ethical approval was not required.

Conflict of Interest

There are no potential conflicts of interest to be disclosed.

References

[1] Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global Burden of Severe Periodontitis in 1990-2010: A Systematic Review and Meta-regression. J Dent Res. 2014;93:1045-53.

[2] Dye BA. Global periodontal disease epidemiology. Periodontol 2000. 2012;58:10-25.

[3] Petersen PE, Baehni PC. Periodontal health and global public health. Periodontol 2000. 2012;60:7-14.

[4] Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ. Prevalence of Periodontitis in Adults in the United States: 2009 and 2010. J Dent Res. 2012;91:914-20.

[5] Papapanou PN. The Prevalence of Periodontitis in the US: Forget What You Were Told. J Dent Res. 2012;91:907-8.

[6] Guha N, Boffetta P, Wunsch Filho V, Eluf Neto J, Shangina O, Zaridze D, Curado MP, Koifman S, Matos E, Menezes A, Szeszenia-Dabrowska N, Fernandez L, Mates D, Daudt AW, Lissowska J, Dikshit R, Brennan P. Oral health and risk of squamous cell carcinoma of the head and neck and esophagus: results of two multicentric case-control studies. Am J Epidemiol. 2007;166:1159-73.

[7] Tezal M, Sullivan MA, Reid ME, Marshall JR, Hyland A, Loree T, Lillis C, Hauck L, Wactawski-Wende J, Scannapieco FA. Chronic periodontitis and the risk of tongue cancer. Arch Otolaryngol Head Neck Surg. 2007;133:450-4.

[8] Michaud DS, Joshipura K, Giovannucci E, Fuchs CS. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. J Natl Cancer Inst. 2007;99:171-5.

[9] Stolzenberg-Solomon RZ, Dodd KW, Blaser MJ, Virtamo J, Taylor PR, Albanes D. Tooth loss, pancreatic cancer, and *Helicobacter pylori*. Am J Clin Nutr. 2003;78:176-81.

[10] Hujoel PP, Drangsholt M, Spiekerman C, Weiss NS. An exploration of the periodontitis-cancer association. Ann Epidemiol. 2003;13:312-6.

[11] Genco RJ, Van Dyke TE. Prevention: Reducing the risk of CVD in patients with periodontitis. Nat Rev Cardiol. 2010;7:479-80.

[12] Spahr A, Klein E, Khuseyinova N, Boeckh C, Muche R, Kunze M, Rothenbacher D, Pezeshki G, Hoffmeister A, Koenig W. Periodontal infection and coronary heart disease. Arch Intern Med. 2006;166:554-9.

[13] Ioannidou E, Kao D, Chang N, Burleson J, Dongari-Bagtzoglou A. Elevated serum interleukin-6 (IL-6) in solid-organ transplant recipients is positively associated with tissue destruction and IL-6 gene expression in the periodontium. J Periodontol. 2006;77:1871-8.

[14] Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ. Periodontitis in RA-the citrullinated enolase connection. Nat Rev Rheumatol. 2010;6:727-30.

[15] Lalla E, Papapanou PN. Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. Nat Rev Endocrinol. 2011;7:738-48.

[16] Gully N, Bright R, Marino V, Marchant C, Cantley M, Haynes D, Butler C, Dashper S, Reynolds E, Bartold M. *Porphyromonas gingivalis* Peptidylarginine Deiminase, a Key Contributor in the Pathogenesis of Experimental Periodontal Disease and Experimental Arthritis. PLoS ONE. 2014;9:e100838.

[17] Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25:134-44.

[18] Darveau R. Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol. 2010;8:481-90.

[19] Hajishengallis G, Liang S, Payne Mark A, Hashim A, Jotwani R, Eskan Mehmet A, McIntosh Megan L, Alsam A, Kirkwood Keith L, Lambris John D, Darveau Richard P, Curtis Michael A. Low-Abundance Biofilm Species Orchestrates Inflammatory Periodontal

Disease through the Commensal Microbiota and Complement. Cell Host Microbe. 2011;10:497-506.

[20] Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012;10:717-25.

[21] Byrne SJ, Dashper SG, Darby IB, Adams GG, Hoffmann B, Reynolds EC. Progression of chronic periodontitis can be predicted by the levels of *Porphyromonas gingivalis* and *Treponema denticola* in subgingival plaque. Oral Microbiol Immunol. 2009;24:469-77.

[22] Brown LF, Beck JD, Rozier RG. Incidence of Attachment Loss in Community-Dwelling Older Adults. J Periodontol. 1994;65:316-23.

[23] Haffajee AD, Socransky SS, Smith C, Dibart S. Relation of baseline microbial parameters to future periodontal attachment loss. J Clin Periodontol. 1991;18:744-50.

[24] Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. Prevalence of *Porphyromonas gingivalis* and Periodontal Health Status. J Clin Microbiol. 1998;36:3239-42.

[25] Orth RH, O’Brien‐Simpson N, Dashper S, Reynolds E. Synergistic virulence of *Porphyromonas gingivalis* and *Treponema denticola* in a murine periodontitis model. Mol Oral Microbiol. 2011;26:229-40.

[26] Pathirana RD, O'Brien-Simpson NM, Brammar GC, Slakeski N, Reynolds EC. Kgp and RgpB, but not RgpA, are important for *Porphyromonas gingivalis* virulence in the murine periodontitis model. Infect Immun. 2007;75:1436-42.

[27] Ma Z, Jacobsen FE, Giedroc DP. Coordination Chemistry of Bacterial Metal Transport and Sensing. Chem Rev. 2009;109:4644-81.

[28] Van Ho A, Ward DM, Kaplan J. Transition Metal Transport In Yeast. Annu Rev Microbiol. 2002;56:237-61.

[29] Rosenzweig AC. Metallochaperones: Bind and Deliver. Chem Biol. 2002;9:673-7.

[30] Pennella MA, Giedroc DP. Structural determinants of metal selectivity in prokaryotic metal-responsive transcriptional regulators. Biometals. 2005;18:413-28.

[31] Kovacic P. Unifying electrostatic mechanism for metal cations in receptors and cell signaling. J Recept Signal Transduct. 2008;28:153-61.

[32] Olson T, Williams J, Allen J. Influence of protein interactions on oxidation/reduction midpoint potentials of cofactors in natural and *de novo* metalloproteins. BBA Bioenergetics. 2013;1827:914-22.

[33] Pratviel G. Oxidative DNA Damage Mediated by Transition Metal Ions and Their Complexes. In: Sigel A, Sigel H, Sigel RKO, editors. Interplay between Metal Ions and Nucleic Acids: Springer Netherlands; 2012. p. 201-16.

[34] Theil EC, Raymond KN. Transition-metal storage, transport and biomineralization. In: Bertini I, Gray H, Lippard S, Valentine J, editors. Bioinorg Chem. Mill Valley, California: University Science Books; 1994. p. 1-37.

[35] Andrews SC, Robinson AK, Rodríguez-Quiñones F. Bacterial Iron Homeostasis. FEMS Microbiol Rev. 2003;27:215-37.

[36] Jakubovics NS, Jenkinson HF. Out of the iron age: new insights into the critical role of manganese homeostasis in bacteria. Microbiology. 2001;147:1709-18.

[37] Wooldridge K, Williams P. Iron uptake mechanisms of pathogenic bacteria. FEMS Microbiol Rev. 1993;12:325-48.

[38] Braun V, Killmann H. Bacterial Solutions to the Iron-Supply Problem. Trends Biochem Sci. 1999;24:104-9.

[39] Papp-Wallace KM, Maguire ME. Manganese transport and the role of manganese in virulence. Annu Rev Microbiol. 2006;60:187-209.

[40] Zaharik ML, Finlay BB. Mn^{2+} and Bacterial Pathogenesis. Front Biosci. 2004;9:1035-42.

[41] Kehres DG, Maguire ME. Emerging Themes in Manganese Transport, Biochemistry and Pathogenesis in Bacteria. FEMS Microbiol Rev. 2003;27:263-90.

[42] Posey JE, Gherardini FC. Lack of a role for iron in the Lyme disease pathogen. Science. 2000;288:1651-3.

[43] Posey JE, Hardham JM, Norris SJ, Gherardini FC. Characterization of a manganese-dependent regulatory protein, TroR, from *Treponema pallidum*. Proc Natl Acad Sci U S A. 1999;96:10887-92.

[44] Archibald F. Manganese - Its Acquisition by and Function in the Lactic-Acid Bacteria. CRC Crit Rev Microbiol. 1986;13:63-109.

[45] Culotta VC, Yang M, Hall MD. Manganese Transport and Trafficking: Lessons Learned from *Saccharomyces cerevisiae*. Eukaryot Cell. 2005;4:1159-65.

[46] Aguirre JD, Culotta VC. Battles with Iron: Manganese in Oxidative Stress Protection. J Biol Chem. 2012;287:13541-8.

[47] Tseng H-J, 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;40:1175-86.

[48] Cadet J, Douki T, Gasparutto D, Ravanat J-L. Oxidative damage to DNA: formation, measurement and biochemical features. Mutat Res-Fundam Mol Mech Mutag. 2003;531:5-23.

[49] Freitas M, Lima JLFC, Fernandes E. Optical probes for detection and quantification of neutrophils' oxidative burst. A review. Anal Chim Acta. 2009;649:8-23.

[50] Pacher P, Beckman JS, Liaudet L. Nitric Oxide and Peroxynitrite in Health and Disease. Physiol Rev. 2007;87:315-424.

[51] Sawyer DT, Valentine JS. How super is superoxide? Acc Chem Res. 1981;14:393-400.

[52] Cheeseman KH, Slater TF. An Introduction to Free Radical Biochemistry. Br Med Bull. 1993;49:481-93.

[53] Stohs S, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med. 1995;18:321-36.

[54] Genco CA. Regulation of Hemin and Iron Transport in *Porphyromonas gingivalis*. Adv Dent Res. 1995;9:41-7.

[55] Marsh PD. The effect of growth rate and haemin on the virulence and proteolytic activity of *Porphyromonas gingivalis* W50. Microbiology. 1994;140:861.

[56] McKee AS, McDermid AS, Baskerville A, Dowsett AB, Ellwood DC, Marsh PD. Effect of hemin on the physiology and virulence of *Bacteroides gingivalis* W50. Infect Immun. 1986;52:349-55.

[57] Olczak T, Simpson W, Liu X, Genco CA. Iron and heme utilization in *Porphyromonas gingivalis*. FEMS Microbiol Rev. 2005;29:119-44.

[58] Dashper SG, Butler CA, Lissel JP, Paolini RA, Hoffmann B, Veith PD, O'Brien-Simpson NM, Snelgrove SL, Tsiros JT, Reynolds EC. A Novel *Porphyromonas gingivalis* FeoB Plays a Role in Manganese Accumulation. J Biol Chem. 2005;280:28095-102.

[59] Roper JM, Raux E, Brindley AA, Schubert HL, Gharbia SE, Shah HN, Warren MJ. The Enigma of Cobalamin (Vitamin B12) Biosynthesis in *Porphyromonas gingivalis* : Identification and Characterization of a Functional Corrin Pathway. J Biol Chem. 2000;275:40316-23.

[60] Hou S, Reynolds MF, Horrigan FT, Heinemann SH, Hoshi T. Reversible Binding of Heme to Proteins in Cellular Signal Transduction. Acc Chem Res. 2006;39:918-24.

[61] Bowman SEJ, Bren KL. The Chemistry and Biochemistry of Heme *c*: Functional Bases for Covalent Attachment. Nat Prod Rep. 2008;25:1118-30.

[62] Dashper SG, Ang C-S, Veith PD, Mitchell HL, Lo AWH, Seers CA, Walsh KA, Slakeski N, Chen D, Lissel JP, Butler CA, O'Brien-Simpson NM, Barr IG, Reynolds EC. Response of *Porphyromonas gingivalis* to Heme Limitation in Continuous Culture. J Bacteriol. 2009;191:1044-55.

[63] Takahashi N, Sato T, Yamada T. Metabolic Pathways for Cytotoxic End Product Formation from Glutamate- and Aspartate-Containing Peptides by *Porphyromonas gingivalis*. J Bacteriol. 2000;182:4704-10.

[64] Meuric V, Rouillon A, Chandad F, Bonnaure-Mallet M. Putative respiratory chain of *Porphyromonas gingivalis*. Future Microbiol. 2010;5:717-34.

[65] Reyes-Prieto A, Barquera B, Juárez O. Origin and Evolution of the Sodium -Pumping NADH: Ubiquinone Oxidoreductase. PLoS ONE. 2014;9:e96696.

[66] Biegel E, Schmidt S, González J, Müller V. Biochemistry, evolution and physiological function of the Rnf complex, a novel ion-motive electron transport complex in prokaryotes. Cell Mol Life Sci. 2011;68:613-34.

[67] Anaya-Bergman C, Rosato A, Lewis JP. Iron- and Hemin-Dependent Gene Expression of *Porphyromonas gingivalis*. Mol Oral Microbiol. 2014;doi: 10.1111/omi.12066.

[68] Dashper S, Ang C-S, Liu SW, Paolini R, Veith P, Reynolds E. Inhibition of *Porphyromonas gingivalis* biofilm by oxantel. Antimicrob Agents Chemother. 2010;54:1311-4.

[69] Dashper S, O'Brien-Simpson N, Liu SW, Paolini R, Mitchell H, Walsh K, D'Cruze T, Hoffmann B, Catmull D, Zhu Y, Reynolds E. Oxantel disrupts polymicrobial biofilm development of periodontal pathogens. Antimicrob Agents Chemother. 2014;58:378-85.

[70] Bramanti TE, Holt SC. Roles of porphyrins and host iron transport proteins in regulation of growth of *Porphyromonas gingivalis* W50. J Bacteriol. 1991;173:7330-9.

[71] Brochu V, Grenier D, Nakayama K, Mayrand D. Acquisition of iron from human transferrin by *Porphyromonas gingivalis*: a role for Arg- and Lys-gingipain activities. Oral Microbiol Immunol. 2001;16:79-87.

[72] Goulet V, Britigan B, Nakayama K, Grenier D. Cleavage of Human Transferrin by *Porphyromonas gingivalis* Gingipains Promotes Growth and Formation of Hydroxyl Radicals. Infect Immun. 2004;72:4351-6.

[73] de Lillo A, Teanpaisan R, Fierro JF, Douglas CWI. Binding and degradation of lactoferrin by *Porphyromonas gingivalis*, *Prevotella intermedia* and *Prevotella nigrescens*. FEMS Immunol Med Microbiol. 1996;14:135-43.

[74] Oram JD, Reiter B. Inhibition of bacteria by lactoferrin and other iron-chelating agents. BBA General Subjects. 1968;170:351-65.

[75] Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M. Identification of the bactericidal domain of lactoferrin. BBA Protein Structure and Molecular Enzymology. 1992;1121:130-6.

[76] Wakabayashi H, Yamauchi K, Kobayashi T, Yaeshima T, Iwatsuki K, Yoshie H. Inhibitory Effects of Lactoferrin on Growth and Biofilm Formation of *Porphyromonas gingivalis* and *Prevotella intermedia*. Antimicrob Agents Chemother. 2009;53:3308-16.

[77] Dashper SG, Pan Y, Veith PD, Chen Y-Y, Toh ECY, Liu SW, Cross KJ, Reynolds EC. Lactoferrin Inhibits *Porphyromonas gingivalis* Proteinases and Has Sustained Biofilm Inhibitory Activity. Antimicrob Agents Chemother. 2012;56:1548-56.

[78] Dashper SG, Cross KJ, Slakeski N, Lissel P, Aulakh P, Moore C, Reynolds EC. Hemoglobin hydrolysis and heme acquisition by *Porphyromonas gingivalis*. Oral Microbiol Immunol. 2004;19:50-6.

[79] Shi Y, Ratnayake DB, Okamoto K, Abe N, Yamamoto K, Nakayama K. Genetic Analyses of Proteolysis, Hemoglobin Binding, and Hemagglutination of *Porphyromonas*

gingivalis : Construction of Mutants with a Combination of *rgpA*, *rgpB*, *kgp* and *hagA*. J Biol Chem. 1999;274:17955-60.

[80] 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;131:313-7.

[81] Paramaesvaran M, Nguyen K-A, Caldon E, McDonald JA, Najdi S, Gonzaga G, Langley DB, DeCarlo A, Crossley MJ, Hunter N, Collyer CA. Porphyrin-Mediated Cell Surface Heme Capture from Hemoglobin by *Porphyromonas gingivalis*. J Bacteriol. 2003;185:2528-37.

[82] Krewulak KD, Vogel HJ. Structural biology of bacterial iron uptake. BBA Biomebranes. 2008;1778:1781-804.

[83] DeCarlo AA, Paramaesvaran M, Yun PLW, Collyer C, Hunter N. Porphyrin-Mediated Binding to Hemoglobin by the HA2 Domain of Cysteine Proteinases (Gingipains) and Hemagglutinins from the Periodontal Pathogen *Porphyromonas gingivalis*. J Bacteriol. 1999;181:3784-91.

[84] Olczak T, Dixon DW, Genco CA. Binding Specificity of the *Porphyromonas gingivalis* Heme and Hemoglobin Receptor HmuR, Gingipain K, and Gingipain R1 for Heme, Porphyrins, and Metalloporphyrins. J Bacteriol. 2001;183:5599-608.

[85] Veith PD, Chen Y-Y, Gorasia DG, Chen D, Glew MD, O'Brien-Simpson NM, Cecil JD, Holden JA, Reynolds EC. *Porphyromonas gingivalis* Outer Membrane Vesicles Exclusively Contain Outer Membrane and Periplasmic Proteins and Carry a Cargo Enriched with Virulence Factors. J Proteome Res. 2014;13:2420-32.

[86] Olczak T, Siudeja K, Olczak M. Purification and initial characterization of a novel *Porphyromonas gingivalis* HmuY protein expressed in *Escherichia coli* and insect cells. Protein Expr Purif. 2006;49:299-306.

[87] Wójtowicz H, Guevara T, Tallant C, Olczak M, Sroka A, Potempa J, Solà M, Olczak T, Gomis-Rüth FX. Unique Structure and Stability of HmuY, a Novel Heme-Binding Protein of *Porphyromonas gingivalis*. PLoS Path. 2009;5:e1000419.

[88] Simpson W, Olczak T, Genco CA. Characterization and Expression of HmuR, a TonB-Dependent Hemoglobin Receptor of *Porphyromonas gingivalis*. J Bacteriol. 2000;182:5737-48.

[89] Smalley JW, Byrne DP, Birss AJ, Wojtowicz H, Sroka A, Potempa J, Olczak T. HmuY Haemophore and Gingipain Proteases Constitute a Unique Syntrophic System of Haem Acquisition by *Porphyromonas gingivalis*. PLoS ONE. 2011;6:e17182.

[90] Lewis JP, Plata K, Yu F, Rosato A, Anaya C. Transcriptional organization, regulation and role of the *Porphyromonas gingivalis* W83 *hmu* haemin-uptake locus. Microbiology. 2006;152:3367-82.

[91] Gao J-L, Nguyen K-A, Hunter N. Characterization of a hemophore-like protein from *Porphyromonas gingivalis*. J Biol Chem. 2010;285:40028-38.

[92] Bramanti TE, Holt SC. Effect of porphyrins and host iron transport proteins on outer membrane protein expression in *Porphyromonas* (*Bacteroides*) *gingivalis*: identification of a novel 26 kDa hemin-repressible surface protein. Microb Pathog. 1992;13:61-73.

[93] Dashper SG, Hendtlass A, Slakeski N, Jackson C, Cross KJ, Brownfield L, Hamilton R, Barr I, Reynolds EC. Characterization of a Novel Outer Membrane Hemin-Binding Protein of *Porphyromonas gingivalis*. J Bacteriol. 2000;182:6456-62.

[94] Shoji M, Shibata Y, Shiroza T, Yukitake H, Peng B, Chen YY, Sato K, Naito M, Abiko Y, Reynolds EC, Nakayama K. Characterization of hemin-binding protein 35 (HBP35) in *Porphyromonas gingivalis*: its cellular distribution, thioredoxin activity and role in heme utilization. BMC Microbiol. 2010;10:152.

[95] Slakeski N, Dashper SG, Cook P, Poon C, Moore C, Reynolds EC. A *Porphyromonas gingivalis* genetic locus encoding a heme transport system. Oral Microbiol Immunol. 2000;15:388-92.

[96] Smalley JW, Birss AJ, McKee AS, Marsh PD. Haemin-binding proteins of *Porphyromonas gingivalis* W50 grown in a chemostat under haemin-limitation. J Gen Microbiol. 1993;139:2145-50.

[97] O'Brien-Simpson NM, Pathirana RD, Walker GD, Reynolds EC. *Porphyromonas gingivalis* RgpA-Kgp proteinase-adhesin complexes penetrate gingival tissue and induce proinflammatory cytokines or apoptosis in a concentration-dependent manner. Infect Immun. 2009;77:1246-61.

[98] O'Brien-Simpson NM, Veith PD, Dashper SG, Reynolds EC. *Porphyromonas gingivalis* gingipains: the molecular teeth of a microbial vampire. Curr Protein Pept Sci. 2003;4:409-26.

[99] Cartron ML, Maddocks S, Gillingham P, Craven CJ, Andrews SC. Feo – Transport of Ferrous Iron into Bacteria. Biometals. 2006;19:143-57.

[100] Marlovits TC, Haase W, Herrmann C, Aller SG, Unger VM. The membrane protein FeoB contains an intramolecular G protein essential for Fe (II) uptake in bacteria. Proc Natl Acad Sci U S A. 2002;99:16243-8.

[101] Ash MR, Guilfoyle A, Clarke RJ, Guss M, Maher MJ, Jormakka M. Potassium-activated GTPase Reaction in the G Protein-coupled Ferrous Iron Transporter B. J Biol Chem. 2010;285:14594-602.

[102] 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;74:4214-23.

[103] Duy D, Soll J, Philippar K. Solute channels of the outer membrane: from bacteria to chloroplasts. Biol Chem. 2007;388:879-89.

[104] Nikaido H. Molecular Basis of Bacterial Outer Membrane Permeability Revisited. Microbiol Mol Biol Rev. 2003;67:593-656.

[105] Hohle TH, Franck WL, Stacey G, O'Brian MR. Bacterial outer membrane channel for divalent metal ion acquisition. Proc Natl Acad Sci U S A. 2011;108:15390-5.

[106] Smalley JW, Silver J, Marsh PJ, Birss AJ. The periodontopathogen *Porphyromonas gingivalis* binds iron protoporphyrin IX in the mu-oxo dimeric form: an oxidative buffer and possible pathogenic mechanism. Biochem J. 1998;331 681-5.

[107] Amano A, Ishimoto T, Tamagawa H, Shizukuishi S. Role of superoxide dismutase in resistance of *Porphyromonas gingivalis* to killing by polymorphonuclear leukocytes. Infect Immun. 1992;60:712-4.

[108] Ueshima J, Shoji M, Ratnayake DB, Abe K, Yoshida S, Yamamoto K, Nakayama K. Purification, gene cloning, gene expression, and mutants of Dps from the obligate anaerobe *Porphyromonas gingivalis*. Infect Immun. 2003;71:1170-8.

[109] Gao J-L, Lu Y, Browne G, Yap BC-M, Trewhella J, Hunter N, Nguyen K-A. The role of heme binding by DNA-protective protein from starved cells (Dps) in the tolerance of *Porphyromonas gingivalis* to heme toxicity. J Biol Chem. 2012;287:42243-58.

[110] Johnson N, Liu Y, Fletcher H. Alkyl hydroperoxide peroxidase subunit C (*ahpC*) protects against organic peroxides but does not affect the virulence of *Porphyromonas gingivalis* W83. Oral Microbiol Immunol. 2004;19:233-9.

[111] Mydel P, Takahashi Y, Yumoto H, Sztukowska M, Kubica M, Gibson III FC, Kurtz Jr DM, Travis J, Collins LV, Nguyen K-A. Roles of the host oxidative immune response and bacterial antioxidant rubrerythrin during *Porphyromonas gingivalis* infection. PLoS Path. 2006;2:e76.

[112] Kolenbrander PE, Palmer RJ, Jr., Periasamy S, Jakubovics NS. Oral multispecies biofilm development and the key role of cell-cell distance. Nat Rev Microbiol. 2010;8:471-80.

[113] Kuboniwa M, Hendrickson EL, Xia Q, Wang T, Xie H, Hackett M, Lamont RJ. Proteomics of *Porphyromonas gingivalis* within a model oral microbial community. BMC Microbiol. 2009;9:98.

[114] Tan KH, Seers CA, Dashper SG, Mitchell HL, Pyke JS, Meuric V, Slakeski N, Cleal SM, Chambers JL, McConville MJ, Reynolds EC. *Porphyromonas gingivalis* and *Treponema denticola* Exhibit Metabolic Symbioses. PLoS Path. 2014;10:e1003955.

[115] Zainal-Abidin Z, Veith PD, Dashper SG, Zhu Y, Catmull DV, Chen YY, Heryanto DC, Chen D, Pyke JS, Tan K, Mitchell HL, Reynolds EC. Differential proteomic analysis of a polymicrobial biofilm. J Proteome Res. 2012;11:4449-64.

[116] Ang CS, Veith PD, Dashper SG, Reynolds EC. Application of 16O/18O reverse proteolytic labeling to determine the effect of biofilm culture on the cell envelope proteome of *Porphyromonas gingivalis* W50. Proteomics. 2008;8:1645-60.

[117] Mashburn LM, Jett AM, Akins DR, Whiteley M. *Staphylococcus aureus* serves as an iron source for *Pseudomonas aeruginosa* during *in vivo* coculture. J Bacteriol. 2005;187:554-66.

[118] Zhu Y, Dashper SG, Chen Y-Y, Crawford S, Slakeski N, Reynolds EC. *Porphyromonas gingivalis* and *Treponema denticola* Synergistic Polymicrobial Biofilm Development. PLoS ONE. 2013;8:e71727.

[119] Simionato MR, Tucker CM, Kuboniwa M, Lamont G, Demuth DR, Tribble GD, Lamont RJ. *Porphyromonas gingivalis* Genes Involved in Community Development with *Streptococcus gordonii*. Infect Immun. 2006;74:6419-28.

[120] Diaz PI, Zilm PS, Rogers AH. *Fusobacterium nucleatum* supports the growth of *Porphyromonas gingivalis* in oxygenated and carbon-dioxide-depleted environments. Microbiology. 2002;148:467-72.

[121] Burgess NA, Kirke DF, Williams P, Winzer K, Hardie KR, Meyers NL, Aduse-Opoku J, Curtis MA, Camara M. LuxS-dependent quorum sensing in *Porphyromonas gingivalis* modulates protease and haemagglutinin activities but is not essential for virulence. Microbiology. 2002;148:763-72.

[122] Chung WO, Park Y, Lamont RJ, McNab R, Barbieri B, Demuth DR. Signaling system in *Porphyromonas gingivalis* based on a LuxS protein. J Bacteriol. 2001;183:3903-9.

[123] Frias J, Olle E, Alsina M. Periodontal pathogens produce quorum sensing signal molecules. Infect Immun. 2001;69:3431-4.

[124] AANNT JournalGuo L, He X, Shi W. Intercellular communications in multispecies oral microbial communities. Front Microbiol. 2014;5.

[125] Lewis JP. Metal uptake in host–pathogen interactions: role of iron in *Porphyromonas gingivalis* interactions with host organisms. Periodontol 2000. 2010;52:94-116.

[126] Silver S, Phung LT. Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol. 1996;50:753-89.

[127] Escolar L, Perez-Martin J, de Lorenzo V. Opening the Iron Box: Transcriptional Metalloregulation by the Fur Protein. J Bacteriol. 1999;181:6223-9.

[128] Hantke K. Iron and metal regulation in bacteria. Curr Opin Microbiol. 2001;4:172-7.

[129] Bagg A, Neilands J. Ferric uptake regulation protein acts as a repressor, employing iron (II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. Biochemistry (Mosc). 1987;26:5471-7.

[130] Coy M, Neilands JB. Structural dynamics and functional domains of the Fur protein. Biochemistry (Mosc). 1991;30:8201-10.

[131] Pohl E, Haller JC, Mijovilovich A, Meyer-Klaucke W, Garman E, Vasil ML. Architecture of a protein central to iron homeostasis: crystal structure and spectroscopic analysis of the ferric uptake regulator. Mol Microbiol. 2003;47:903-15.

[132] Dian C, Vitale S, Leonard GA, Bahlawane C, Fauquant C, Leduc D, Muller C, de Reuse H, Michaud-Soret I, Terradot L. The structure of the *Helicobacter pylori* ferric uptake regulator Fur reveals three functional metal binding sites. Mol Microbiol. 2011;79:1260-75.

[133] Ma Z, Faulkner MJ, Helmann JD. Origins of specificity and cross - talk in metal ion sensing by *Bacillus subtilis* Fur. Mol Microbiol. 2012;86:1144-55.

[134] Butler CA, Dashper SG, Zhang Z, Seers CA, Mitchell HL, Catmull DV, Glew MD, Heath JE, Tan Y, Khan HSG, E.C. R. The *Porphyromonas gingivalis* Ferric Uptake Regulator Orthologue Binds Hemin and Regulates Hemin-Responsive Biofilm Development. PloS One. 2014;DOI: 10.1371/journal.pone.0111168.

[135] Pellicer S, González A, Peleato ML, Martinez JI, Fillat MF, Bes MT. Site-directed mutagenesis and spectral studies suggest a putative role of FurA from *Anabaena* sp. PCC 7120 as a heme sensor protein. FEBS J. 2012;279:2231-46.

[136] Smith A, Hooper NI, Shipulina N, Morgan WT. Heme binding by a bacterial repressor protein, the gene product of the ferric uptake regulation (*fur*) gene of *Escherichia coli*. J Protein Chem. 1996;15:575-83.

[137] James CE, Hasegawa Y, Park Y, Yeung V, Tribble GD, Kuboniwa M, Demuth DR, Lamont RJ. LuxS Involvement in the Regulation of Genes Coding for Hemin and Iron Acquisition Systems in *Porphyromonas gingivalis*. Infect Immun. 2006;74:3834-44.

[138] Boyd J, Oza MN, Murphy JR. Molecular cloning and DNA sequence analysis of a diphtheria tox iron-dependent regulatory element (*dtxR*) from *Corynebacterium diphtheriae*. Proc Natl Acad Sci U S A. 1990;87:5968-72.

[139] Qiu X, Verlinde CL, Zhang S, Schmitt MP, Holmes RK, Hol WG. Three-dimensional structure of the diphtheria toxin repressor in complex with divalent cation co-repressors. Structure. 1995;3:87-100.

[140] Spiering MM, Ringe D, Murphy JR, Marletta MA. Metal stoichiometry and functional studies of the diphtheria toxin repressor. Proc Natl Acad Sci U S A. 2003;100:3808-13.

[141] Qiu X, Pohl E, Holmes RK, Hol WG. High-resolution structure of the diphtheria toxin repressor complexed with cobalt and manganese reveals an SH3-like third domain and suggests a possible role of phosphate as co-corepressor. Biochemistry (Mosc). 1996;35:12292-302.

[142] D'Aquino JA, Ringe D. Determinants of the SRC homology domain 3-like fold. J Bacteriol. 2003;185:4081-6.

[143] Feese MD, Ingason BP, Goranson-Siekierke J, Holmes RK, Hol WG. Crystal structure of the iron-dependent regulator from *Mycobacterium tuberculosis* at 2.0-A resolution reveals the Src homology domain 3-like fold and metal binding function of the third domain. J Biol Chem. 2001;276:5959-66.

[144] Love JF, VanderSpek JC, Murphy JR. The SRC homology 3-like domain of the diphtheria toxin repressor (DtxR) modulates repressor activation through interaction with the ancillary metal ion-binding site. J Bacteriol. 2003;185:2251-8.

[145] Wang G, Wylie GP, Twigg PD, Caspar DL, Murphy JR, Logan TM. Solution structure and peptide binding studies of the C-terminal src homology 3-like domain of the diphtheria toxin repressor protein. Proc Natl Acad Sci U S A. 1999;96:6119-24.

[146] Wylie GP, Rangachari V, Bienkiewicz EA, Marin V, Bhattacharya N, Love JF, Murphy JR, Logan TM. Prolylpeptide binding by the prokaryotic SH3-like domain of the diphtheria toxin repressor: a regulatory switch. Biochemistry (Mosc). 2005;44:40-51.

[147] Su Y-C, Chin K-H, Hung H-C, Shen G-H, Wang AHJ, Chou S-H. Structure of *Stenotrophomonas maltophilia* FeoA complexed with zinc: a unique prokaryotic SH3-domain protein that possibly acts as a bacterial ferrous iron-transport activating factor. Acta Crystallogr F-Struct Biol Cryst Commun. 2010;66:636-42.

[148] Rodriguez GM, Voskuil MI, Gold B, Schoolnik GK, Smith I. *IdeR*, an essential gene in *Mycobacterium tuberculosis*: role of IdeR in iron-dependent gene expression, iron metabolism, and oxidative stress response. Infect Immun. 2002;70:3371-81.

[149] Que Q, Helmann JD. Manganese homeostasis in *Bacillus subtilis* is regulated by MntR, a bifunctional regulator related to the diphtheria toxin repressor family of proteins. Mol Microbiol. 2000;35:1454-68.

[150] Hill PJ, Cockayne A, Landers P, Morrissey JA, Sims CM, Williams P. SirR, a Novel Iron-Dependent Repressor in *Staphylococcus epidermidis*. Infect Immun. 1998;66:4123-9.

[151] Jakubovics NS, Smith AW, Jenkinson HF. Expression of the virulence - related Sca (Mn^{2+}) permease in *Streptococcus gordonii* is regulated by a diphtheria toxin metallorepressor - like protein ScaR. Mol Microbiol. 2000;38:140-53.

[152] Low YL, Jakubovics NS, Flatman JC, Jenkinson HF, Smith AW. Manganese-dependent regulation of the endocarditis-associated virulence factor EfaA of *Enterococcus faecalis*. J Med Microbiol. 2003;52:113-9.

[153] Rolerson E, Swick A, Newlon L, Palmer C, Pan Y, Keeshan B, Spatafora G. The SloR/Dlg metalloregulator modulates *Streptococcus mutans* virulence gene expression. J Bacteriol. 2006;188:5033-44.

[154] Guedon E, Helmann JD. Origins of metal ion selectivity in the DtxR/MntR family of metalloregulators. Mol Microbiol. 2003;48:495-506.

[155] Xie H, Zheng C. OxyR Activation in *Porphyromonas gingivalis* in Response to a Hemin-Limited Environment. Infect Immun. 2012;80:3471-80.

[156] Diaz PI, Slakeski N, Reynolds EC, Morona R, Rogers AH, Kolenbrander PE. Role of *oxyR* in the oral anaerobe *Porphyromonas gingivalis*. J Bacteriol. 2006;188:2454-62.

[157] Sobota JM, Imlay JA. Iron enzyme ribulose-5-phosphate 3-epimerase in *Escherichia coli* is rapidly damaged by hydrogen peroxide but can be protected by manganese. Proc Natl Acad Sci U S A. 2011;108:5402-7.

[158] Amano A, Shizukuishi S, Tamagawa H, Iwakura K, Tsunasawa S, Tsunemitsu A. Characterization of superoxide dismutases purified from either anaerobically maintained or aerated *Bacteroides gingivalis*. J Bacteriol. 1990;172:1457-63.

[159] Lynch MC, Kuramitsu HK. Role of superoxide dismutase activity in the physiology of *Porphyromonas gingivalis*. Infect Immun. 1999;67:3367-75.

[160] Nakayama K. The superoxide dismutase-encoding gene of the obligately anaerobic bacterium *Bacteroides gingivalis*. Gene. 1990;96:149-50.

[161] Sugio S, Hiraoka BY, Yamakura F. Crystal structure of cambialistic superoxide dismutase from *Porphyromonas gingivalis*. Eur J Biochem. 2000;267:3487-95.

[162] Novichkov PS, Kazakov AE, Ravcheev DA, Leyn SA, Kovaleva GY, Sutormin RA, Kazanov MD, Riehl W, Arkin AP, Dubchak I, Rodionov DA. RegPrecise 3.0--a resource for genome-scale exploration of transcriptional regulation in bacteria. BMC Genomics. 2013;14:745.

[163] Busenlehner LS, Pennella MA, Giedroc DP. The SmtB/ArsR family of metalloregulatory transcriptional repressors: structural insights into prokaryotic metal resistance. FEMS Microbiol Rev. 2003;27:131-43.

[164] Brown NL, Stoyanov JV, Kidd SP, Hobman JL. The MerR family of transcriptional regulators. FEMS Microbiol Rev. 2003;27:145-63.

[165] Cuthbertson L, Nodwell JR. The TetR Family of Regulators. Microbiol Mol Biol Rev. 2013;77:440-75.

Table 1.0. Superfamilies of bacterial metalloregulatory proteins.

Super-family[a]	Mode of action	Metallo-regulator	Metal Effector
ArsR[b]	Derepression	ArsR	As(III), Sb(III)
		AztR	Zn(II), Cd(II), Pb(II)
		BxmR	Cu(I), Ag(I), Zn(II), Cd(II)
		CadC	Cd(II), Pb(II), Zn(II)
		CmtR	Cd(II), Pb(II)
		CzrA	Zn(II), Co(II)
		SmtB	Zn(II), Co(II), Cd(II)
MerR[c]	Activation	CadR	Cd(II)
		CueR	Cu(I), Ag(I), Au(I)
		HmrR	Cu(I)
		MerR	Hg(II)
		PbrR	Pb(II)
		ZntR	Zn(II), Cd(II), Pb(II)
CsoR	Derepression	CsoR	Cu(I)
		RcnR	Ni(II), Co(II)
CopY	Derepression	CopR	Cu(II)
TetR[d]	Derepression	SczA	Zn(II)
		ComR	Cu(II)
Fur	Corepression	Fur	Fe(II)
		Har[e]	Fe(II) of Haem
		Irr	Haem
		Mur	Mn(II)
		Nur	Ni(II)
		Zur	Zn(II)
DtxR	Corepression	DtxR	Fe(II)
		IdeR	Fe(II)
		MntR	Mn(II)
NikR	Corepression	NikR	Ni(II)
MarR	Corepression	AdcR	Zn(II)
LysR	Corepression	ModE	Mo(II)

Metals up regulate metal efflux / sequestration systems (ArsR through ComR)

Metals down regulate metal uptake systems (Fur through ModE)

[a] [162] (http://regprecise.lbl.gov/RegPrecise/collections_tffam.jsp)
[b] [163]
[c] [164]
[d] [165]
[e] [134]

Figure Captions

Figure 1. Major *P. gingivalis* metabolic pathways, highlighting the dependence on iron. Enzymes are shown that were identified in a proteomic analysis of *P. gingivalis* W50 grown in haem limitation and were found to be increased (▲), decreased (▼) or unchanged (▬) in abundance relative to growth in haem excess. Enzymes identified in the transcriptomic analysis are underlined. Enzymes that have iron as a cofactor are shaded. Figure modified from [62].

Figure 2. Characterised and proposed iron (complex) and manganese acquisition systems of *P. gingivalis*. Sources of haem and iron such as haemoglobin and transferrin are proteolytically cleaved by the surface associated gingipains Kgp and RgpA. The released haem is actively transported across the outer membrane (OM) via TonB-linked outer membrane proteins either with or without an associated lipoprotein; this transport is energized by TonB/ExbBD complexes. Once in the periplasm, haem is transported through the cell wall (CW) and across the inner membrane (IM) via ABC transporters where A is a periplasmic binding protein, B is an inner membrane permease and C is an ATPase. ABC transporters have been predicted as part of the Htr and Hmu transport systems. An ABC transporter system was also predicted for the transport of Fe^{2+} through the inner membrane following the removal of Fe^{2+} from haem by the ferrochelatase IhtB and transport through IhtA into the periplasm. Fe^{2+} and Mn^{2+} are also predicted to enter the periplasm via specific outer membrane channels prior to active transport across the inner membrane by FB1 and FB2, respectively.

Figure 3. Ribbon diagram of the crystal structure of the *P. aeruginosa* ferric uptake regulator (PA-Fur) dimer [131]. Each monomer consists of an N-terminal DNA-binding domain, a C-terminal dimerisation domain and two metal binding sites represented by spheres. One monomer is boxed.

Figure 1
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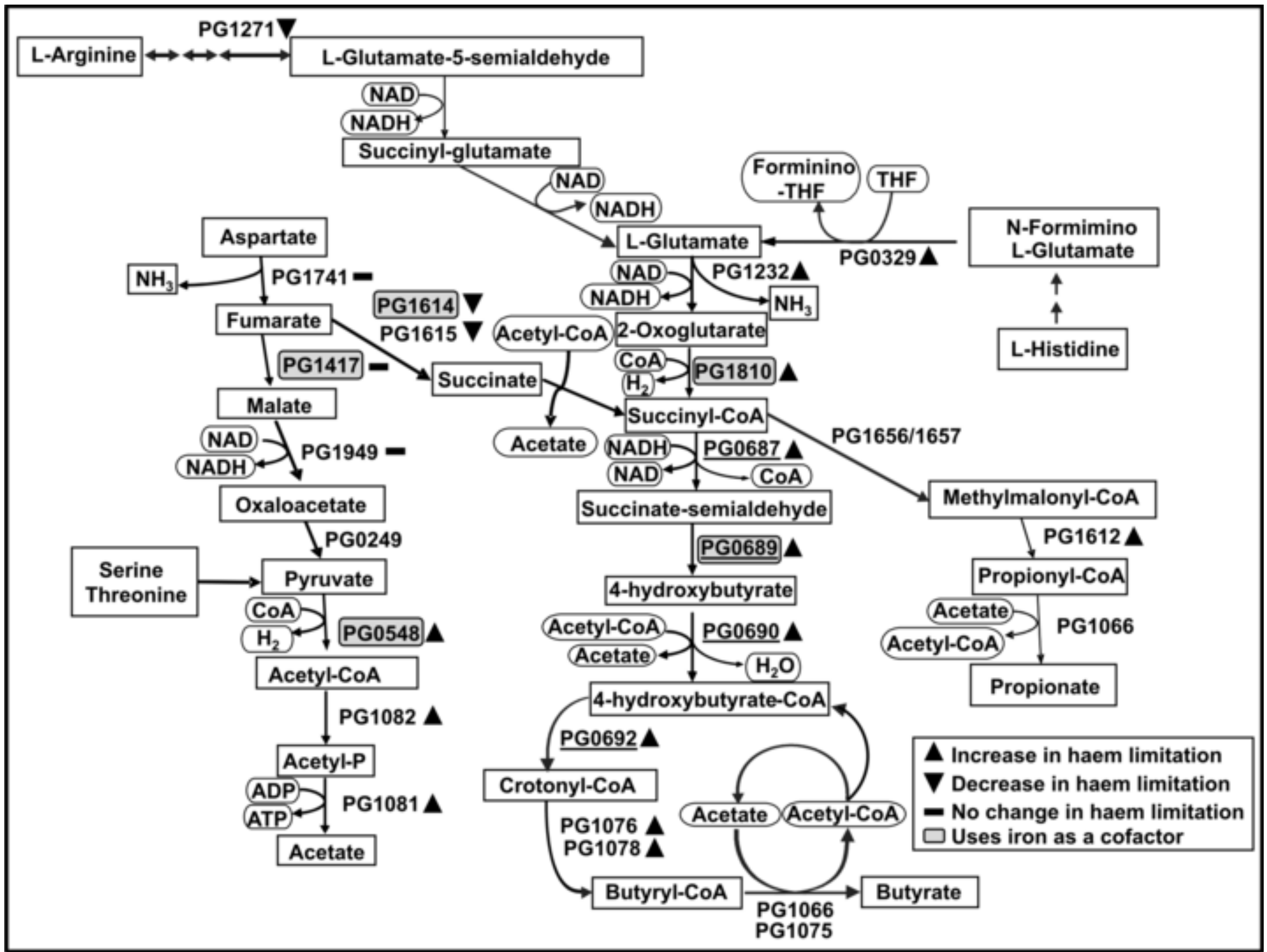

Figure 2
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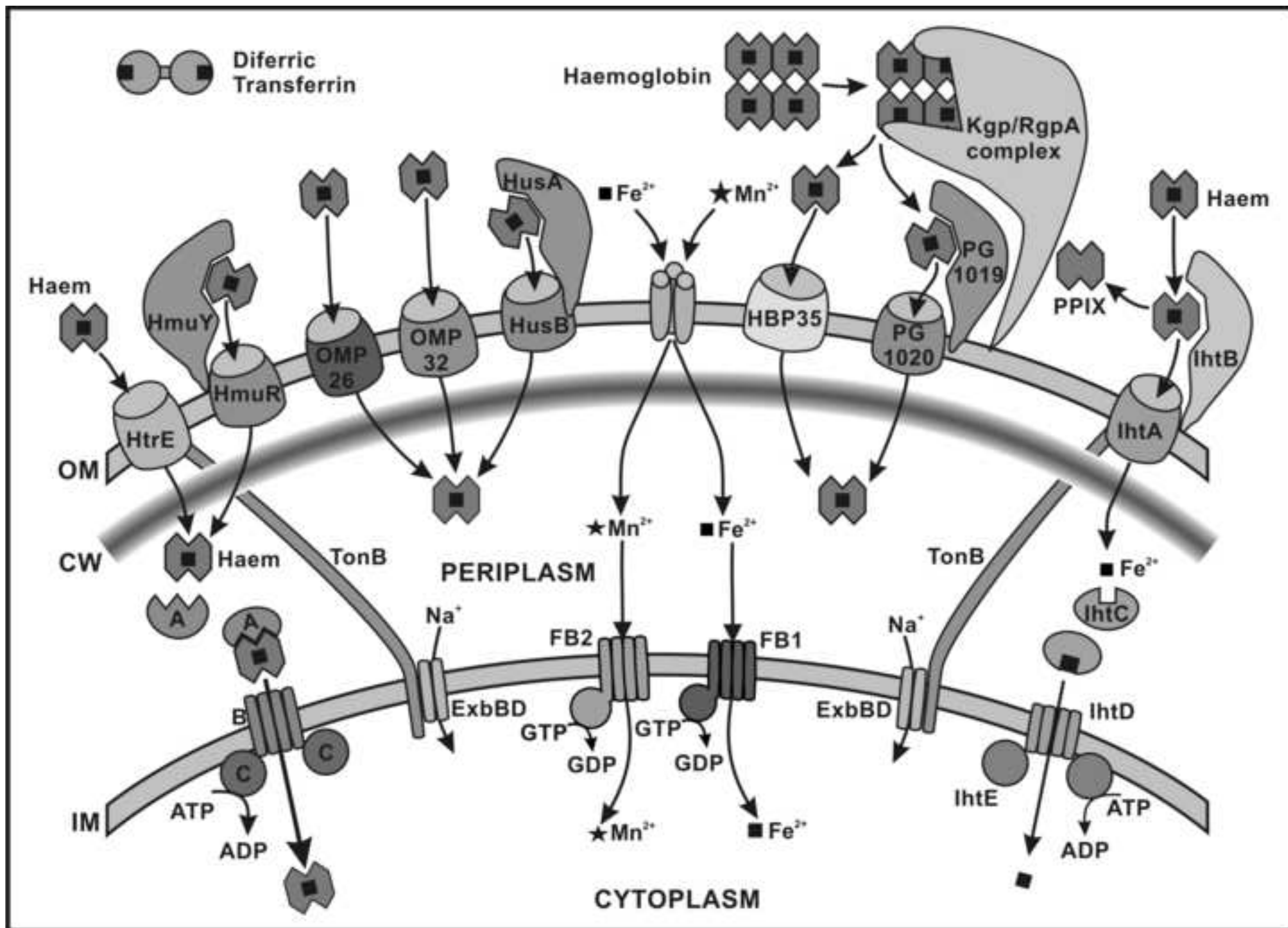

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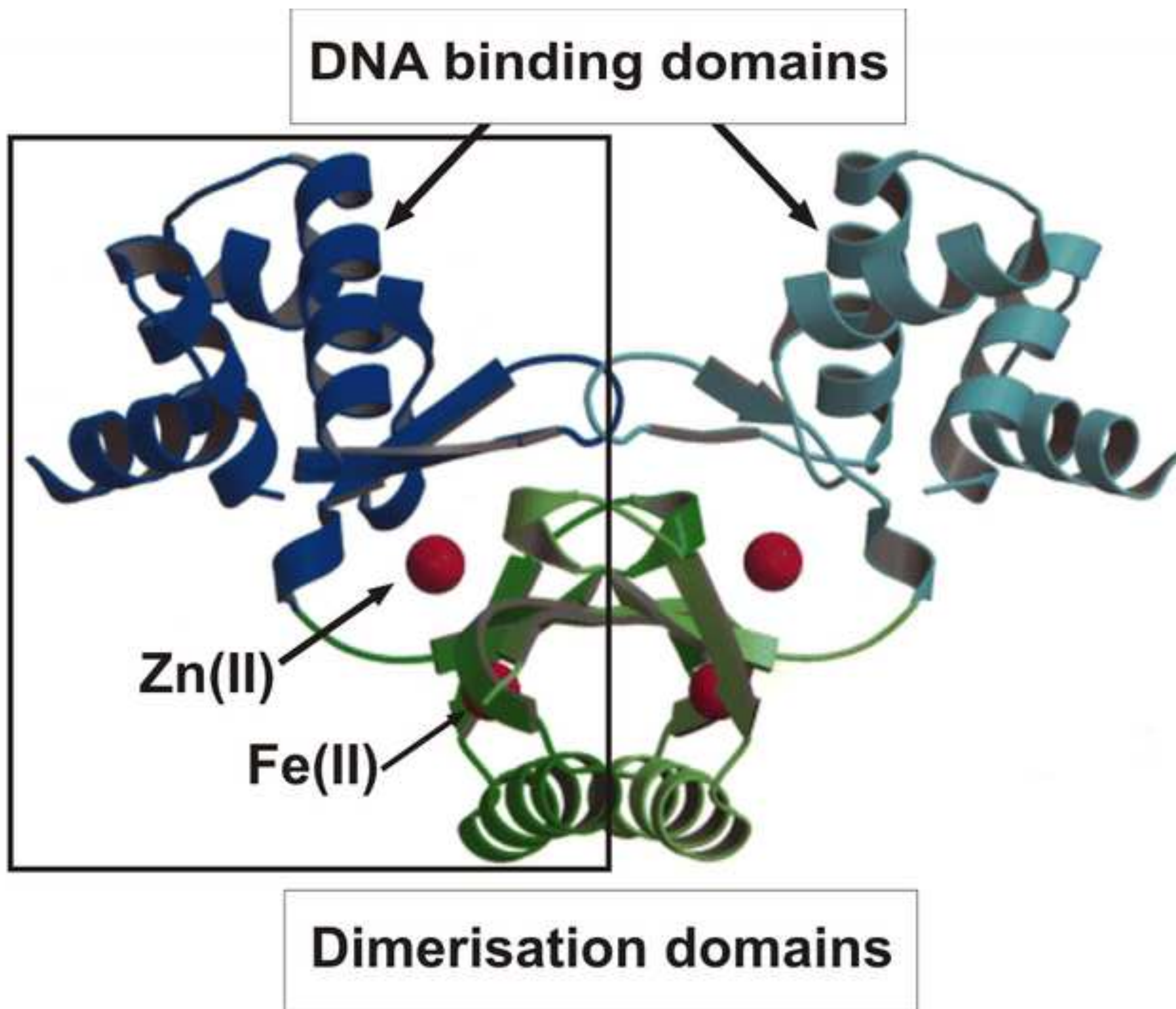

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