Send Orders of Reprints at reprints@benthamscience.net

2594 *Current Pharmaceutical Design,* **2013,** *19,* **2594-2605**

Redox Proteins as Targets for Drugs Development Against Pathogens

Daniela L. Catalano-Dupuy, Arleth López-Rivero, Anabel Soldano and Eduardo A. Ceccarelli*

Molecular Biology Division, Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531. S2002LRK Rosario, Argentina

Abstract: Antimicrobial drug resistance in pathogens is an increasing human health problem. The rapid loss of effectiveness in antibiotics treatments and the accumulation of multi-resistant microbial strains are increasing worldwide threats. Moreover, several infectious diseases have been neglected for years and new antimicrobial treatments are lacking. In other cases, complexity of infectious organisms has exceeded the efforts to find new drugs to control them. Thus, strategies for the proper development of specific drugs are critically needed. Redox metabolism has already been proved to be a useful target for drug development. During the last years a significant number of electron carriers, enzymes, proteins and protein complexes have been studied and some of them were found to be essential for survival of several microbial pathogens. This review will focus on three major redox metabolic pathways which may provide promising strategies to fight against pathogens: the non-mevalonate pathway for isoprenoids biosynthesis, the iron metabolism and the iron-sulfur proteins. The common attractive link of all these processes is the plant-type ferredoxin-$NADP^+$ reductase, an enzyme that participates in numerous electron transfer reactions and has no homologous enzyme in humans. Research in these redox pathways will open new perspectives for the rational design of drugs against infectious diseases.

Keywords: Isoprenoids biosynthesis, heme oxygenase, ferredoxin-NADP(H) reductase, ferredoxin, iron-sulfur clusters, iron-sulfur proteins, drug targets, pathogenicity.

1. INTRODUCTION

The widespread and intensive use of antimicrobial agents for human treatment and veterinary purposes has increased the number of multi-resistant microbial strains [1,2]. Most of the first generation antibiotics will be unsuitable for the treatment of microbial infections in next years. Antibiotic resistance of any antimicrobial drug will inevitably appear between one to ten years from the time it begins its clinical use [3]. Moreover, the existence of multi and pan-drug resistant microbes [4] increases the human health risk particularly for those that are in hospitals or suffering immunodeficiencies. Antibiotic-resistant bacteria can be transferred from reservoirs to healthy people increasing the risk of infections, the use of antibiotics and adding extra costs of health and social care [2,5]. The current situation will put future patients in real danger and concerted international actions are urgently needed [6]. Moreover, several infectious diseases have been neglected for years [7] and no enough knowledge is available for development of specific antimicrobial drugs. In other cases, complexity of the organisms has exceeded the effort to find new drugs and appropriate tools to fight them are still lacking. These important health problems are widespread all over the world and in some cases worsened as a consequence of environmental conditions, poverty, unsafe water, poor sanitation, and proliferation of animal reservoirs and vectors. Consequently, new strategies and better antimicrobial drugs to overcome the above-mentioned threats need to be developed [6,8].

Redox metabolism has already been proved to be a useful target for drugs development. It involves multiple enzymes and processes. 14.9% of non-redundant entries of the Enzyme Data Bank March 2012 release, which contains 20,639,311 entries, are classified as oxidoreductases. To this group it should be added those enzymes in which there is not net electron exchange between substrates and products but a redox reaction occurs during the catalytic process, as in the case of some transferases, isomerases, synthases, hydratases, etc. Interestingly, some of these metabolic pathways and their enzymes are essential for several microbial pathogens which make them attractive targets for drugs development. The use of redox metabolism as target for antimicrobial drugs is also supported by the relevant differences that have been observed between redox pathways from humans and some infectious agents.

Moreover, many drugs are supplied as inactive forms that redox enzymes convert to compounds that act on the final targets [9,10]. On the contrary, some xenobiotics become inactive after oxidoreduction by the cell [11]. Consequently, avoiding these redox reactions may help to strengthen the effect of some drugs. On the other hand, a number of antimicrobial agents display their effects by inducing the generation of oxygen reactive species in conjunction with redox enzymes of the host. This strategy was proved to be useful against several microbial infections and cancer [12].

Understanding of microbial and host redox metabolisms could help in tailoring new drugs obtained by a rational design. In this article we review a number of findings related to different redox metabolic pathways and the usefulness as potentially attractive drug targets.

2. THE NON-MEVALONATE PATHWAY FOR ISOPRENOIDS BIOSYNTHESIS AS DRUG TARGET

2.1. Isoprenoids Biosynthesis

Isoprenoids are a broad group of natural compounds with carbon skeleton assembled from five-carbon building blocks derived from isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). Isoprenoids are broadly distributed among living organisms, both eukaryotes and prokaryotes. They are functionally important for many aspects of cell metabolism, for membrane structure and function. Many relevant biological isoprenoids roles have been described in photosynthesis, respiration, hormonal regulation of metabolism, regulation of growth and development, defense against pathogens, intracellular signal transduction, vesicular transport within the cell and as coenzymes [13]. Isoprenoids also serve as attractants for pollinators, seed dispersers [14], natural antibiotics and herbivore repellents as well as toxins in plants [15,16]. Several isoprenoids are known to influence membrane structure [16,17]. The specific precursor of all isoprenoids, mevalonate (MVA), was found to be synthesized by the condensa-

*Address correspondence to this author at the IBR, CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Ocampo y Esmeralda, 2000 Rosario, Argentina. Fax: +54 341 4390465.
Tel: +54 341-4237070. E-mail: ceccarelli@ibr-conicet.gov.ar

1873-4286/13 $58.00+.00

tion of three acetyl-CoA molecules via acetoacetyl-CoA and 3-hydroxy-3-methyl-glutaryl-CoA, yielding after phosphorylation and decarboxylation IPP [13]. However, it had been found an MVA-independent pathway for IPP formation in bacteria [18], green algae [19] and higher plants [20,21]. What is more, genome analysis of *Leptospira interrogans* allows us to suggest that a similar metabolic pathway is present in this pathogen [22,23]. However, some differences may exist. It has been shown that IPP in chloroplasts is synthesized from pyruvate and glyceraldehyde-3-phosphate (GA-3P) and not from MVA [18,19].

2.2. Biosynthesis of the Isoprenoids Precursor IPP via the DOXP/MEP Pathway

After decarboxylation of pyruvate, the acetaldehyde is added to the carbonyl group of GA-3P yielding 1-deoxy-D-xylulose 5-phosphate (DOXP) as the first intermediate [21,24]. The starting enzyme of this pathway is 1-deoxy-D-xylulose 5-phosphate synthase (Fig. **1**). The second enzymatic step, the reduction of DOXP to 2-C-methyl-D-erythritol 4-phosphate (MEP) is catalyzed by *dxr* gene product, namely DOXP-reductoisomerase in the presence of NADPH and Mn^{2+}. The further biosynthetic step consists in the conversion of MEP to 4-diphosphocytidyl-2-C-methyl-D-erythritol in a CTP-dependent reaction by the *ispD* gene product, a 4-(cytidine 5-diphospho)-2-C-methyl-D-erythritol synthase. The following two steps of the pathway were initially identified using bioinformatics methods. The *ispE* gene product, a 4-(cytidine 5-diphospho)-2-C-methyl-D-erythritol kinase, catalyzes the fourth step, the phosphorylation of CDP-methyl-D-erythritol (CDP-ME) at the 2-hydroxy group in an ATP-dependent reaction yielding 2-C-methyl-D-erythritol-2-phosphate. The *ispF* gene product catalyzes the next step in the pathway, the conversion of 2-C-methyl-D-erythritol-2-phosphate into 2-C-methyl-D-erythritol-2,4-cyclodiphosphate. This product is accumulated under stress conditions in several bacteria [25].

The last two steps leading to IPP and DMAPP with (E)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBPP) as intermediate are less well understood. The involved enzymes GcpE and LytB were characterized as oxygen sensitive iron-sulfur proteins most likely containing [4Fe-4S] clusters [26–29]. Both reactions depend on the transfer of two electrons. In *Escherichia coli* it was demonstrated that flavodoxin together with ferredoxin (flavodoxin)-$NADP^+$ reductase and NADPH can serve as electron donor for both GcpE and LytB [28–30] while in the cyanobacterium *Thermosynechococcus elongatus* ferredoxin was identified as electron

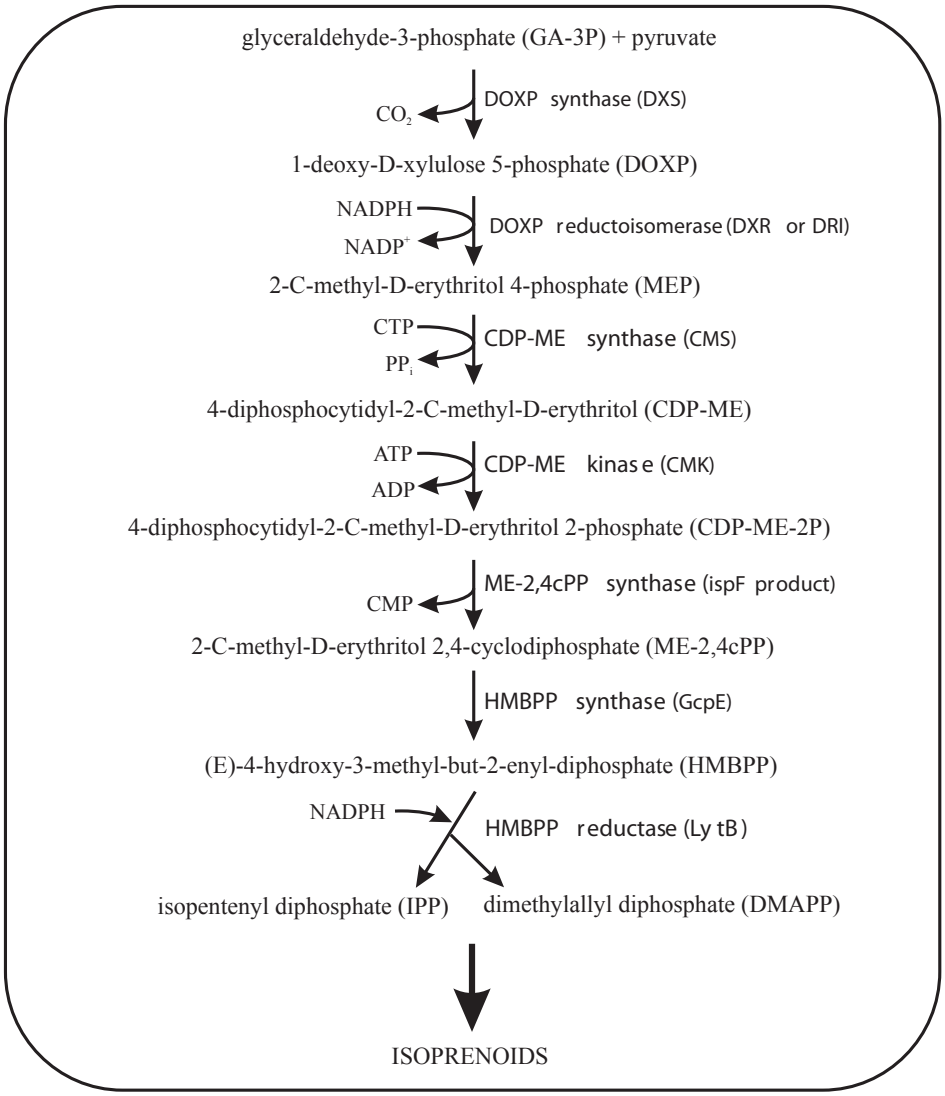

Fig. (1). Biosynthesis of the isoprenoid precursor IPP via the DOXP/MEP pathway.

donor for GcpE [31]. Although not yet proven, it has been hypothesized that the ferredoxin and ferredoxin-$NADP^+$ reductase (FNR) identified in *Plasmodium falciparum, Toxoplasma gondii* and other *Apicomplexa* may play a role in this process [32]. LytB leads to the simultaneous formation of IPP and DMAPP in a ratio approximately 5:1 from HMBPP. This ratio resembles the requirement of an excess of IPP for the downstream synthesis of isoprenoids [33].

The MEP pathway is found operative in most eubacteria [18], algae [20,21,24,34], cyanobacteria and diatoms [35], but is absent in animals, fungi, archaebacteria and certain bacteria like streptococci, staphylococci and lactobacilli [36]. Although higher plants possess both the MEP pathway as well as the MVA pathway for isoprenoids synthesis [21,37] distinct compartmentalization occurs [38–43]: the MVA pathway takes place in the cytosol and mitochondria (e.g. for sterols and ubiquinone) while the MEP pathway, for synthesis of both IPP and DMAPP, occurs in plastids (e.g. for isoprene, plastoquinone-9, carotenoids and phytol) [34,44].

2.3. The DOXP/MEP Pathway as an Antimicrobial Drug Target

Interestingly, it has been found that isoprenoids are synthesized exclusively via the DOXP/MEP pathway in various pathogenic bacteria like *E. coli*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Helicobacter pylori*, *Vibrio cholerae* and *Bacillus anthracis* and in the apicoplast of the apicomplexan protozoan *P. falciparum*. Since the amino acid sequences of the constituent enzymes of the DOXP/MEP pathway are highly conserved (except HMBPP synthase that contains a large plant-specific internal domain of unknown function) [45] and have no known human counterparts [43], these enzymes offer the potential for the development of novel antibacterial, antimalarial and herbicidal compounds, and perhaps without toxicity to humans.

The DOXP pathway of *P. falciparum* has attracted considerable interest as a target for new antimalarial drugs [46–48]. Fosmidomycin, a natural antibiotic, was found to inhibit the DOXP reductoisomerase enzyme [49]. The antimalarial effectiveness of fosmidomycin was evaluated in patients suffering from acute uncomplicated *P. falciparum* infections. The treatment with fosmidomycin was very well tolerated and efficient in initial parasite infection and fever clearance [50]. However, parasites re-appeared in several patients after termination of the treatment. Based on the proven clinical efficacy of fosmidomycin the characterization of additional *P. falciparum* derived enzymes of the DOXP pathway is highly desirable. These enzymes could finally serve as targets for other new antimalarial drugs, which may be used in combination with fosmidomycin. Such a combination able to shut down the DOXP pathway at two different steps is expected to result in a pronounced synergistic increase in efficacy [51].

Despite substantial progress, the quest for therapeutically significant inhibitors of DOXP-reductoisomerase has not yet led to the development of drugs that are clinically useful. Several inhibitors of DOXP-reductoisomerase have been identified, with fosmidomycin and its acetyl analogue being the most potent to date [52].

3. THE IRON METABOLISM AS DRUG TARGET

3.1. Iron as Nutrient

Iron is an essential nutrient for most organisms. The ability of this transition metal to easily accept and donate electrons allows it to participate in numerous redox reactions. Chemical versatility of iron makes it a valuable prosthetic group for incorporation into proteins as a biocatalytic center or as an electron carrier [53]. Therefore, iron is involved in a wide variety of important biological processes, such as oxygen storage and transport, respiration, photosynthesis, DNA synthesis, cell signaling and defense [54].

The majority of iron in mammals is as heme. This molecule is made up by a heterocyclic protoporphyrin IX ring and an iron atom in its ferrous reduction state. While heme is an important cofactor for many proteins, it also has the potential to cause toxicity at high concentrations. Accumulation of free heme in cells will result in cellular damage, oxidative stress and tissue injury because under aerobic conditions it is able to generate reactive oxygen species. In addition, heme is a small lipophilic molecule that can easily incorporate into membranes, disrupt lipid bilayers and destabilize the cytoskeleton [55,56]. Therefore, in mammals heme is mainly found bound to hemoproteins, in particular hemoglobin and myoglobin [57].

Pathogenic microorganisms, specially, need iron to colonize their hosts and cause disease. However, the level of free iron in mammals is very low as a strategy to avoid infections [58]. Although mammals had developed multiple methods of preventing microorganism from acquiring iron, pathogens have devised a variety of mechanisms to extract iron from the host iron-containing compounds [59].

3.2. Iron Acquisition in Pathogens

A common mechanism in bacteria is to produce and secrete compounds known as siderophores to chelate iron from the environment with high affinity. Another mechanism is to capture iron from iron-binding proteins such as transferrin, lactoferrin or ferritin, via outer membrane receptors. A third mechanism is to extract iron from host's heme or heme-containing proteins [56,60]. The latter two mechanisms are found only in pathogens.

In gram-negative bacteria have been identified three classes of heme acquisition systems [56,60]. The direct heme uptake system involves binding of heme or heme-containing protein to an outer membrane receptor and its transport via a TonB-dependent manner into the periplasm. Once in the periplasmic space, the heme forms a complex with a transport protein which shuttles it to an ATP-binding cassette transporter anchored to the inner membrane. Heme is then passed into the cytoplasm in an ATP-dependent process [56,60]. Pathogens that have this system include *Pseudomonas aeruginosa* [61], *V. cholerae* [62], *Yersinia enterocolitica* [63] and *Shigella dysenteriae* [64]. The second uptake system involves a secreted heme-binding protein called hemophore, an outer membrane receptor and an ATP-binding cassette transporter. Hemophores are able to take up free heme or extract heme from hemoproteins present in the external medium and bring it to the cell surface for uptake through a TonB-dependent specific outer membrane receptor [60]. The hemophore mediated heme uptake system has been identified in *P. aeruginosa* [61], *Serratia marcescens* [65] and *Haemophilus influenzae* [66]. A third heme acquisition system is the bipartite hemoglobin receptor HpuAB, identified in *Neisseria* spp. [67,68]. This system is composed of a TonB-dependent outer membrane receptor (HpuB) and an accessory outer membrane lipoprotein (HpuA). The HpuAB complex let the utilization of hemoglobin porphyrin as nutrient iron source [69].

Gram-positive heme uptake systems consist in a specific receptor that shuttles heme to an ATP-binding cassette transporter, which carries the heme across the cytoplasmic membrane and delivers the porphyrin ring into the cytoplasm [56,60]. One of the most studied heme acquisition system in gram-positive bacteria is the iron-regulated surface determinant system (Isd) of *Staphylococcus aureus* [70], which is also found in other gram-positive pathogens. The Isd system includes four proteins anchored to the cell wall (IsdABCH), a transpeptidase (SrtB), a membrane transport system (IsdDEF), and two cytoplasmic heme binding proteins (IsdG, IsdI) [60]. It has been shown that *S. aureus* is able to bind human hemoproteins, remove the heme molecule and degrade it leading to the formation of free iron that is used as a nutrient source [70]. Other gram-positive bacteria, such as *Corynebacterium diphtheriae* [71,72] and *Streptococcus pyogenes* [73,74], can also utilize heme and hemoglobin as iron source. Additionally, it has been reported one hemophore-mediated heme uptake system in *B. anthracis*. This gram-positive bacterium secretes two hemophores, IsdX1 and

IsdX2, which are able to extract heme directly from hemoglobin [75]. Heme is then imported into the cytoplasm through the Isd system.

It has been suggested that heme (in the form of hemoglobin) is likely to be the sole iron source for *L. interrogans* [76], mainly because the spirochete is hemolytic, displays chemotaxis towards hemoglobin [77] and possess multiple genes for putative heme acquisition and utilization [78]. Although little is known about the iron acquisition systems in *Leptospira*, it is proposed that the bacterium may secrete hemolysins to lyse red blood cells and make iron available as heme for further uptake [78].

In order to scavenging iron from heme or heme binding compounds, microbes must be able to access the iron atom contained within the porphyrin ring. The final step of this process requires enzymatic degradation of heme which usually involves its oxidative cleavage by a family of monooxygenases known as heme oxygenases [79] and an abundant source of reducing equivalents.

3.3. Heme Oxygenase Structure, Function and Mechanism

Heme oxygenase (HO) is the only enzyme which employs heme as both a substrate and a prosthetic group, although it is not considered an hemoprotein *per se* [80]. This enzyme, first characterized in eukaryotes, catalyzes the conversion of heme to biliverdin IX, carbon monoxide (CO) and free iron (Fe^{2+}) utilizing reducing equivalents. In mammals, biliverdin is reduced to bilirubin by a biliverdin reductase, finally conjugated to glucuronic acid and excreted [81].

Genes encoding heme oxygenases have been identified in a wide range of organisms including mammals [82], higher plants [83], algae [84,85], cyanobacteria [86], fungi [87,88], and some pathogenic microorganisms [76,89–92]. The first crystal structure of a heme oxygenase was obtained in 1999 for the human HO-1 in complex with heme [93]. Currently, several heme oxygenases structures are available [94–103]. Studies on structural aspects propose that the fold of heme oxygenases is a single compact domain consisting of mostly α-helices. The heme is sandwiched between two helices called proximal and distal, and is coordinated with a neutral imidazole group of histidine and an oxygen atom of water as the axial ligands [104].

Mammalian heme oxygenase exists as two isoforms, an inducible HO-1 and a constitutive HO-2. Both enzymes have a C-terminal hydrophobic domain that is involved in the binding to the microsomal membrane [105,106]. These proteins play important roles in different physiological processes. HO-1 is implicated in iron homeostasis and defense against oxidative and cellular stress, while HO-2 seems to be involved in neurotransmission by the generation of CO as a physiological messenger molecule [106]. In photosynthetic organisms heme oxygenases are soluble enzymes that participate in the biosynthesis of photoreceptive pigments. Cyanobacteria and algae utilize the biliverdin IXα produced by HO as a precursor of phycobilins used for light-harvesting during photosynthesis [86,107]. In higher plants, biliverdin is reduced to phytochromobilins, the chromophore of the phytochrome family of photoreceptors [108,109]. In pathogenic microorganisms the major role of heme oxygenases has been attributed to iron acquisition from host heme during infection [110], although some of them have been identified to protect against heme toxicity [60].

Heme degradation proceeds through a complex reaction that requires the input of seven electrons delivered by NADPH and three molecules of oxygen (Fig. **2**). The first step of the catalysis involves the heme binding to HO at a specific pocket to form the ferric heme-HO complex, followed by the reduction of the ferric-heme iron to the ferrous state using one electron. Immediately, molecular oxygen binds the complex to yield an oxy-ferrous form (Fe^{2+}-O_2) [111]. The subsequent one-electron reduction and protonation of the ferrous-O_2 heme gives a ferric hydroperoxide (Fe^{3+}-OOH). Lastly, the distal oxygen of the Fe^{3+}-OOH attacks the porphyrin ring on the α-meso-carbon to convert heme to ferric α-

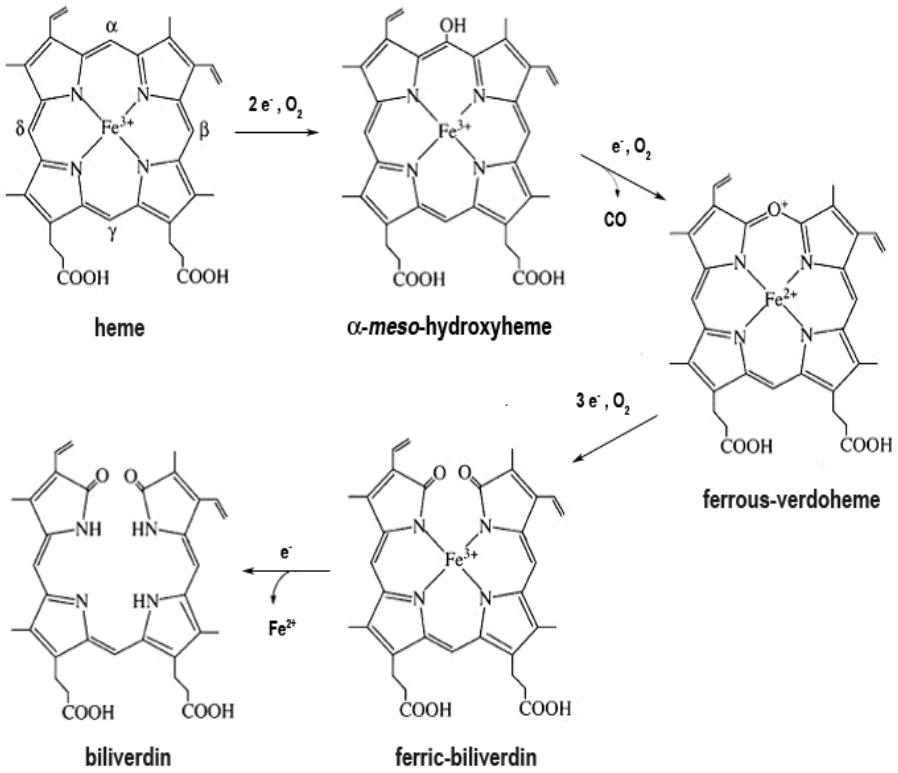

Fig. (2). Heme degradation pathway catalyzed by heme oxygenase. α, β, γ, and δ represent α-, β-, γ-, and δ-meso-carbons of heme, respectively.

meso-hydroxyheme [112]. The next step of heme breakdown involves the oxidation of the ferric α-meso-hydroxyheme to ferrous-verdoheme and CO by consuming one molecule of oxygen and one reducing equivalent [113]. Degradation of verdoheme to ferric-biliverdin requires both oxygen and three reducing equivalents [114]. Ferric-biliverdin is finally reduced to the ferrous state consuming one electron [115] followed by the rapid release of free iron (Fe^{2+}), and the slow dissociation of biliverdin from the protein, step that has been proposed to be the rate limiting [116]. In general, the IXα isomer of biliverdin is produced, although it has been reported that *P. aeruginosa* heme oxygenase displays selectivity for the δ-meso heme carbon and generates δ-biliverdin (70%) and β-biliverdin (30%) [91,117].

The mechanism of heme cleavage is broadly conserved between heme oxygenases from most organisms. However, the source of reducing equivalents is highly variable. In mammals the electrons required to complete the heme oxygenase reaction are derived from NADPH cytochrome P450 reductase [111,118], a flavoprotein that incorporates one FAD and one FMN as prosthetic groups. In contrast, plants, cyanobacteria and bacteria heme oxygenases seem to be ferredoxin-dependent. It has been suggested that the redox system NADPH/FNR/ferredoxin could be the electron donating partner of these enzymes [108,119,120]. In addition, it is proposed the requirement for a second (auxiliary) reductant, such as ascorbate or trolox, to carry out the full activity of bacterial HO [108]. Nevertheless, there is a report showing that catalytic activity of *P. aeruginosa* heme oxygenase is efficiently supported by FNR without the need of a ferredoxin [121].

3.4. Heme Oxygenase as an Antimicrobial Drug Target

Since iron is required for pathogen survival and infectivity, iron uptake and utilization became an attractive target for antimicrobial drug development [122]. Considering that many pathogenic microorganisms utilize heme oxygenase to acquire iron from the host's heme, it seems to be logical that the inhibition of this enzyme may prevent pathogen colonization of mammals. Therefore, HO may provide a potential target for new antimicrobials.

An important consideration of any drug target is that its function should be essential for microorganism survival. Notably, heme oxygenase has been shown to be required for growth in some microbial pathogens. A knockout of the heme oxygenase gene in *P. aeruginosa* (*pigA*) disables the bacterium to use heme iron and consequently shows severe growth defects [91]. Similar observations have been made in the pathogen *L. interrogans* [76]. Growth of a mutant in the *L. interrogans* heme oxygenase gene (*LB 186*) was impaired when there was only hemoglobin in the medium, suggesting that in the absence of this protein the spirochete cannot effectively utilize heme as an iron source. In addition, when the role of *L. interrogans* heme oxygenase in pathogenesis was investigated there has been shown that the enzyme contributes significantly to virulence in the hamster model of infection [123]. Collectively, these studies indicate that decreasing the amount of functional heme oxygenase is of crucial importance for the survival of these pathogens.

3.5. Heme Oxygenase Inhibitors

Many progresses have been made in finding new strategies for inhibiting heme oxygenases. It has been proposed that metal substituted porphyrins, such as Zn^{2+} protoporphyrin IX and Sn^{4+} protoporphyrin IX, are effective competitive inhibitors of mammalian heme oxygenase [124]. Those compounds bind to the HO active site via metal coordination to the proximal histidine and replace the natural substrate heme. However, because of their identical heterocyclic nucleus they are unselective and have been found to inhibit other heme dependent enzymes [125]. More recently, azole-based compounds have shown inhibition of the mammalian heme oxygenase in a non heme competitive manner [126]. These molecules derive from the chemical azalanstat and do not affect nitric oxide synthase or soluble guanylyl cyclase except at high millimolar concentrations, but are potent cytochromes P-450 inhibitors [127]. There is also evidence for the use of isocyanides as uncompetitive inhibitors of heme oxygenase. They completely stopped heme degradation by coordination to the verdoheme intermediate [125].

A significant consideration in the development of novel antibiotics targeting microbial heme oxygenase is its specificity for pathogen over mammalian enzyme. Computer-aided drug design was used to identify small-molecule inhibitors of the apo-form of *Neisseria meningitidis* HO and *P. aeruginosa* HO [124]. It has been hypothesized that finding inhibitors able to bind the heme free protein instead of compounds that coordinate heme iron would increase the selectivity toward bacterial heme oxygenase. By *in silico* screening of a large number of compounds several molecules that bind to HO at the heme binding pocket were identified. These compounds may preclude HO to be active with its natural substrate. Eight compounds were shown to bind to *Neisseria* and *P. aeruginosa* heme oxygenases *in vitro* with binding affinities in the micromolar range. Furthermore, many of them also inhibited HO activity *in vivo*. They were not toxic to cells and could decrease the production of biliverdin in a recombinant *E. coli* system. The compounds tested were also able to cross the cell membrane and specifically inhibit the growth of *P. aeruginosa* under iron limitation conditions where heme is the sole iron source. Further testing should be undertaken to ensure selectivity of these inhibitors toward bacterial versus mammalian enzymes and they may be optimized to improve their biological activity.

4. IRON-SULFUR CLUSTERS AND PATHOGENICITY

4.1. Iron-Sulfur Clusters in Nature

Iron-sulfur clusters are prosthetic groups involved in a variety of regulatory processes and redox reactions [128,129]. They are widely distributed in nature performing a myriad of functions. Chemical activity of iron and sulfur with variations in composition in a concerted association with the protein scaffold offer different redox potentials, oxidation states and physical accessibilities of clusters to the environment, making them one of the most used cofactors in nature for electron transfer reactions [128,130]. It is known that they participate in the normal physiological process of the cell but have been also related to several pathologies.

Although scientists have made efforts in understanding the metabolic insertion of iron-sulfur containing proteins and their biogenesis, still important aspects are not well established and more research is needed.

4.2. Iron-Sulfur Clusters Biogenesis

The understanding of the iron-sulfur clusters biosynthesis is an important topic for science and medicine. Research in this area involves the biogenesis of metal cofactors, the intracellular traffic of metal, the participation of other cofactors and proteins and the characterization of genes, their operons and products [129].

The general mechanism of clusters assembly is based on the presence of a protein scaffold which initially binds sulfur atoms from a cysteine desulfurase and iron atoms from a donor to synthesize a [2Fe-2S] or [4Fe-4S] cluster. Subsequently, the cluster is transferred to an apo-protein target with the assistance of some intermediaries and molecular chaperons [131] (Fig. **3**).

In prokaryotes, three distinct iron-sulfur cluster assembly mechanisms have been described: the iron-sulfur cluster (ISC), the nitrogen fixation (NIF) and sulfur mobilization (SUF) systems. NifU and NifS proteins, which belong to the NIF system, were identified as required for maturation of nitrogenase enzyme in *Azotobacter vinelandii* and other organisms, as well as in maturation of other iron-sulfur proteins. NIF proteins involved in the formation of iron-sulfur clusters can also be found in organisms that do not fix

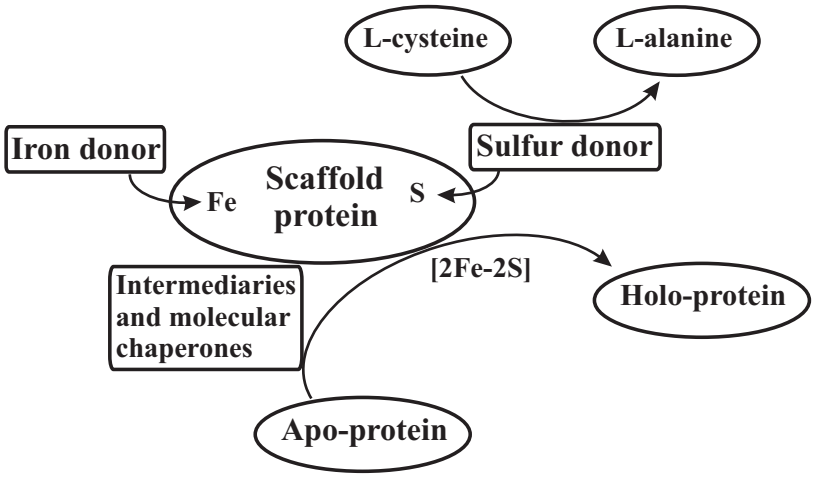

Fig. (3). Iron-sulfur cluster assembly mechanisms in bacteria. The scaffold protein, the central component of the system, receives iron from a donor not yet completely identified (see text). Isc protein donates sulfur from L-cysteine. Subsequently, scaffold protein assembles the [2Fe-2S] cluster, which is transferred to an apo-protein with the assistance of additional components.

nitrogen but no specific functions have been assigned to them. In *A. vinelandii* the operon *iscRUA-hscBA-fdx*, which encodes for a set of essential proteins for iron-sulfur clusters biosynthesis, was also identified [131]. This operon has been found in *E. coli* and in the genome of other organisms as well. Other genes playing important roles in iron-sulfur cluster formation were identified. The *sufAB-CDSE* operon encodes for a set of proteins whose expression is regulated by oxidative stress and/or iron starvation.

Phylogenetic distribution and metabolic insertion of these iron-sulfur clusters biogenesis systems are complex [132]. As mentioned above, ISC appears to be a housekeeping iron-sulfur cluster assembly system, while SUF is specifically adapted to synthesize iron-sulfur cluster in harsh environmental conditions such as oxidative stress and iron starvation. In cyanobacteria the SUF pathway is the major system for iron-sulfur cluster assembly compared to the ISC pathway. On the contrary, in *E. coli* the relative importance of SUF decreases in relation to ISC [133,134]. In eukaryotes, iron-sulfur cluster assembly exhibits a higher degree of complexity because of sub-cellular organization, in which different systems exist. It is remarkable that ISC is found in eubacteria and most eukarya whereas SUF is found in bacteria, archaea, plants and parasites [133, 134].

Despite of differences in function and localization of these iron-sulfur cluster assembly pathways, there are some similarities between them. IscS and SufS proteins are structurally similar to NifS. All three systems contain a cysteine desulfurase [135]. IscU is homologous to the N-terminal domain of NifU and contains three conserved cysteine residues that are essential for its function as a scaffold protein [128,133,135]. SufA and IscA bind iron and coordinate its delivery to iron-sulfur cluster in IscU [134]. The ISC machinery contains three additional components, two essential chaperons HscB and HscA, and a nonessential [2Fe-2S] ferredoxin [135]. Although the biochemical properties of the SUF-specific components are still under study, it is known that SufE interacts with SufS and stimulates its cysteine desulfurase activity [136]. The SufB, SufC, and SufD proteins are associated as a stable complex, and SufC has been shown to display ATPase activity [135,137].

Iron-sulfur cluster assembly needs to be tightly regulated. In *E. coli*, the *isc* operon is under the transcriptional control of the IscR repressor, which is encoded as part of the *iscRSUA* operon and auto-regulates its own expression as well as that of *iscSUA* [137–139]. IscR is a member of the Rrf2 transcriptional regulators family and possesses a winged helix-turn-helix DNA binding domain. IscR contains three cysteine residues and was shown to be an iron-sulfur protein with a $[2Fe\text{-}2S]^{+}$ cluster [138,139]. Likewise, the [2Fe-2S] cluster in IscR can interconvert from $[2Fe\text{-}2S]^{+}$ to $[2Fe\text{-}2S]^{2+}$ in response to oxidizing or reducing conditions. The regulatory activity of IscR iron-sulfur cluster may depend on the oxidation/reduction state of cluster as in the case of the SoxR superoxide-sensing transcription factor. However, the effect of a similar redox behavior in IscR DNA binding activity is unknown [133,139].

Iron binding to the iron-sulfur cluster is a process that has not yet been completely elucidated. Frataxin is a protein that in humans is localized in mitochondria and it seems to be involved in iron-sulfur clusters assembly. Frataxin homologues in prokaryotes and eukaryotes are postulated to deliver iron to the scaffold protein IscU [133,139–142]. However, the general mechanism by which frataxin allows incorporation of the metal into iron-sulfur proteins is not yet completely understood [142].

The other important element for clusters assembly is the sulfur. This is an essential element for life since it is involved in numerous metabolic processes [143]. In its reduced form plays a central role in biosynthesis of the amino acids cysteine and methionine [143].

Sulfur incorporation into iron-sulfur clusters is performed by cysteine desulfurases such as IscS y SufS, which acquire sulfur from free L-cysteine by a pyridoxal-5'-phosphate-dependent desulfuration [141–143]. Sulfur is transiently bound in the persulfide form to an active-site cysteine of desulfurase. Subsequently, it is transferred to the scaffold protein. In the SUF system there is an intermediary protein, SufE, which forms a complex with the SufS cysteine desulfurase for sulfur mobilization to the scaffold protein [136,142].

4.3. Iron-Sulfur Clusters and Pathogenesis

Currently, iron-sulfur clusters have been widely studied. Defects in their formation and/or activity are now recognized as cause of several human diseases [130]. Disruption of iron-sulfur cluster biogenesis is deleterious to cell leading to diseases such as Friederich's ataxia or sideroblastic anemia. Accumulation and mismanagement of iron in mitochondria have been related to diseases as type II diabetes [130,144]. Discovery of alterations in iron sulfur clusters biogenesis opens a new scene in the understanding, diagnosis and treatment of these pathologies [130].

Iron-sulfur proteins play an essential function in iron and sulfur regulation and as a result participate in the development and protection against infectious diseases [145,146]. As already mentioned, restriction of iron and sulfur availability is a strategy of vertebrates to avoid microbial infection. Conversely, iron-sulfur clusters

assembly allows the control of both compounds availability in bacteria. Consequently, these proteins have been related to virulence mechanisms of several pathogens, the efficiency with which these organisms gain access to their host, infect and colonize a new host [145,146]. Strategies used to ensure bacterial success in pathogenicity is an important field of research. The identification of factors involved in such strategies is crucial in diagnosis and drug development against pathogenic microbes and infectious diseases.

The capability of some microbes to access their hosts is largely influenced by some iron-sulfur proteins which act as sensors [128,145,147]. In *E. coli* cells, the *soxRS* regulon protects against superoxide and nitric oxide due to oxidation of the SoxR sensor, a [2Fe-2S]-containing transcriptional regulator that triggers the protection response [148]. Besides, ferredoxins are not major sources of iron storage but are critical in regulating the amount of free iron and in the detection of its availability in the host [145]. Altering the above mentioned processes will affect bacterial survival. Likewise, ferredoxins are the source of reducing power for several metabolic processes in bacteria, donating electrons initially provided by the FNR [149]. As some of these processes are unique and have been pointed as essential for bacterial survival, altering or knocking them will consequently affect the virulence mechanisms in some pathogenic organisms [133]. In this context, it has been observed that bacteria need to finely regulate homeostasis of the NADP(H) pool to enable proper deployment of their defensive response being glucose-6-phosphate dehydrogenase and FNR, among other enzymes, the ones that perform this task [148,150].

We have previously reported that despite being a bacterium *L. interrogans* contains a plastidic class FNR with high catalytic efficiency, at difference from the bacterial class FNRs [23]. The inclusion of a plastidic FNR in *Leptospira* metabolism and in its parasitic life cycle is not currently understood. Our studies also showed that in *L. interrogans* the FNR exchanges electrons with a bacterial-type [4Fe-4S] ferredoxin, process which has not been previously observed in nature. Ferredoxins usually work as electron hubs providing reduction equivalents for multiple metabolic pathways. It is expected that this FNR/ferredoxin couple participates in a variety of metabolic reactions in *Leptospira*. A very low potential [4Fe-4S] ferredoxin from *P. aeruginosa* with characteristics of a housekeeping protein, abolished bacterial growth when its gene was deleted, unless a plasmid copy of the gene was provided to mutant strain [151].

In *Mycobacterium*, some genes involved in sulfur metabolism have consistently been identified as up-regulated in response to oxidative stress, nutrient starvation, dormancy adaptation, and during macrophage infection. Mutants in sulfur metabolism genes are severely impaired in their ability to persist and cause disease. Moreover, the SUF assembly system is presumed to play a key role in the pathogenicity of this bacterium. Sulfur metabolic pathways and iron sulfur cluster assembly can be used as potential antimicrobial targets for this pathogen [143,152]. In other organisms such as *Bacillus subtilis*, genomic analysis of iron-sulfur cluster biosynthesis genes suggests that only SUF system is present. This was pointed as an essential system for the bacterium growth [141] and consequently constitutes an interesting drug target. In this context, genomic analysis of *Shigella flexneri*, a facultative intracellular pathogen of humans indicated that *sufABCDSE* operon is expressed during the infection stage, presumably to sense the environment and initiates an appropriate metabolic strategy [153]. Likewise, studies on formation of essential proteins for *H. pylori* viability indicate that proteins involved in iron-sulfur clusters assembly such as NifU and NifS are absolutely required for virulence and are responsible for the assembly of many (non-nitrogenase) iron-sulfur proteins [146].

5. FNR, A COMMON ATRACTIVE LINK

Plant-type FNRs are ubiquitous, hydrophilic and monomeric enzymes that contain noncovalently bound FAD as a prosthetic group. They catalyze the reversible electron transfer between NADP(H) and different low potential one-electron donors (e.g., ferredoxin, flavodoxin, heme oxygenase and iron). These flavoenzymes are present in plastids, bacteria, cyanobacteria and apicoplasts of intracellular parasites, and participate in processes as dissimilar as photosynthesis, steroid hydroxylation, nitrate reduction, anaerobic pyruvate assimilation and fatty acid desaturation. They have been also involved in xenobiotic detoxification, amino acid and deoxyribonucleotide synthesis, iron-sulfur cluster biogenesis and in the regulation of several metabolic pathways. In animals, yeast mitochondria and in a few bacteria, another flavoenzymes displaying FNR activity, the adrenodoxin reductases, have been characterized. These enzymes are evolutively unconnected to plant-type FNRs, do not share sequence similarities and belong to the disulphide oxidoreductase family of flavoproteins, whose prototype is glutathione reductase [154,155]. Plant-type and mitochondrial-type FNRs share some properties as NADP(H) binding and catalysis, but the reaction geometry with the nucleotide is different [155,156], encouraging the search for compounds that inhibit one enzyme without affecting the other.

Despite ample variations in amino acid sequences between plant-type FNRs, the chain topologies of all of them are highly conserved. The protein molecule is made up of two structural domains, the carboxyl terminal region includes most of the residues involved in NADP(H) binding, whereas the large cleft between the two domains accommodates the FAD group (see reviews on theses enzymes [157–159]).

FNR is involved in a direct or indirect way to all the metabolic processes that we have discussed in this work.

Non-mevalonate pathway for isoprenoids biosynthesis has been proposed as an attractive target for development of antibacterial compounds, especially for the fact that is present in clinically important pathogens and is not present in humans [23,32,160]. FNR was implicated in electron transfers occurring at the last two steps of this pathway [28–32] .

As mentioned before, iron is required for pathogen survival and infectivity and many pathogenic microorganisms utilize the heme oxygenase enzyme to acquire this essential nutrient from host's heme. In bacteria, electrons required for the heme oxygenase reaction are derived from ferredoxin. It has been suggested that the redox system NADPH/FNR/ferredoxin could be the electron donating partner of these enzymes [108,119,120]. Moreover, in the case of *P. aeruginosa* heme oxygenase the catalytic activity is efficiently supported by FNR without the need of a ferredoxin [121].

Iron-sulfur proteins play an essential function in iron and sulfur regulation and consequently in the development and protection against infectious diseases [146]. In particular, ferredoxins are critical in regulating the amount of free iron in eukaryotes and have ability to detect the availability of this compound in host environment [145]. In addition, ferredoxins are the source of reducing power for several metabolic processes in bacteria, donating electrons initially provided by FNRs [149]. Several of these processes have been considered as essential for bacterial survival and implicated in virulence mechanisms in some pathogenic organisms [145]. Different authors have suggested that the synthesis of iron-sulfur clusters in the apicoplast of some organisms is an essential event that could not be replaced by mitochondrial machinery and implies at least five ferredoxins. Based on this, it is likely that alteration of the redox system FNR/protein substrate could produce an important effect on more than one essential metabolic pathway [161]. These

effects could be induced either by inhibiting the reductase activity or affecting the interaction between the reductase and some of its substrates. Recent studies have proposed inhibition models by altering ferredoxins active sites (iron-sulfur clusters) assembly [162, 163].

The FNR insertion in these relevant metabolic pathways makes this enzyme a potential antimicrobial target to investigate in different pathogens. Moreover, as the enzyme participates as a redox hub (Fig. **4**), it may be expected that knocking down its activity will produce a profound effect on pathogen survival. Several inhibitors have been described for FNR enzymes and the potential to development of new ones has been analyzed [32].

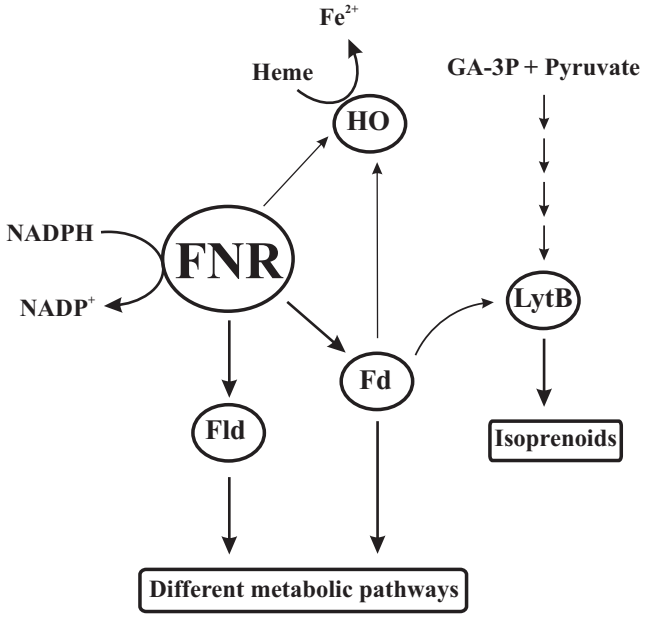

Fig. (4). Critical plant-type FNR catalyzed reactions. Arrows indicate catalytic processes already established (thick lines) or that have been suggested or detected in a small number of pathogens (thin lines).

In summary, it is evident that research in these redox pathways will open new perspectives contributing to the rational design of drugs for infectious disease control.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported by grants from CONICET and Agencia de Promoción Científica y Tecnológica (ANPCyT, Argentina)

ABBREVIATIONS

CO	=	carbon monoxide
DMAPP	=	dimethylallyl pyrophosphate
DOXP	=	1-deoxy-D-xylulose-5-phosphate
FNR	=	ferredoxin-NADP$^+$ reductase
GA-3P	=	glyceraldehyde-3-phosphate
HMBPP	=	(E)-4-hydroxy-3-methyl-but-2-enyl diphosphate
HO	=	heme oxygenase
IPP	=	Isopentenyl pyrophosphate
ISC	=	iron-sulfur cluster system
Isd	=	iron-regulated surface determinant system
MEP	=	2-C-methyl-D-erythritol 4-phosphate
MVA	=	mevalonate
NIF	=	nitrogen fixation system.
SUF	=	sulfur mobilization system

REFERENCES

[1] Hawkey PM, Jones AM. The changing epidemiology of resistance. J Antimicrob Chemother 2009; 64 Suppl 1: i3-10.

[2] Molbak K. Spread of resistant bacteria and resistance genes from animals to humans--the public health consequences. J Vet Med B Infect Dis Vet Public Health 2004; 51: 364-9.

[3] Walsh C. Where will new antibiotics come from? Nat Rev Microbiol 2003; 1: 65-70.

[4] Magiorakos AP, Srinivasan A, Carey RB, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18: 268-81.

[5] Gootz TD. The global problem of antibiotic resistance. Crit Rev Immunol 2010; 30: 79-93.

[6] Carlet J, Jarlier V, Harbarth S, *et al.* Ready for a world without antibiotics? The Pensieres Antibiotic Resistance Call to Action. Antimicrobial Resistance and Infection Control 2012; 1: 11.

[7] Hotez PJ, Bottazzi ME, Franco-Paredes C, Ault SK, Periago MR. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. PLoS Negl Trop Dis 2008; 2: e300.

[8] Bush K, Courvalin P, Dantas G, *et al.* Tackling antibiotic resistance. Nat Rev Microbiol 2011; 9: 894-6.

[9] Chen Y, Hu L. Design of anticancer prodrugs for reductive activation. Med Res Rev 2009; 29: 29-64.

[10] Graf N, Lippard SJ. Redox activation of metal-based prodrugs as a strategy for drug delivery. Adv Drug Deliv Rev 2012.

[11] Strolin Benedetti M. FAD-dependent enzymes involved in the metabolic oxidation of xenobiotics. Ann Pharm Fr 2011; 45-51.

[12] Albesa I, Becerra MC, Battan PC, Paez PL. Oxidative stress involved in the antibacterial action of different antibiotics. Biochem Biophys Res Commun 2004; 317: 605-9.

[13] Wanke M, Skorupinska-Tudek K, Swiezewska E. Isoprenoid biosynthesis via 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway. Acta Biochim Pol 2001; 48: 663-72.

[14] Langenheim JH. Higher plant terpenoids: a phytocentric overview of their ecological roles. J Chem Ecol 1994; 20: 1223-1280.

[15] Duke, Dayan, Romagni, Rimando Natural products as sources of herbicides: current status and future trends. Weed Research 2000; 40: 99-111.

[16] Sacchettini JC, Poulter CD Creating isoprenoid diversity. Science 1997; 277: 1788-9.

[17] Bach TJ, Boronat A, Campos N, Ferrer A, Vollack KU. Mevalonate biosynthesis in plants. Crit Rev Biochem Mol Biol 1999; 34: 107-22.

[18] Rohmer M, Knani M, Simonin P, Sutter B, Sahm H Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem J 1993; 295 (Pt 2): 517-24.

[19] Schwender J, Seemann M, Lichtenthaler HK, Rohmer M. Biosynthesis of isoprenoids (carotenoids, sterols, prenyl side-chains of chlorophylls and plastoquinone) via a novel pyruvate/glyceraldehyde 3-phosphate non-mevalonate pathway in the green alga *Scenedesmus obliquus*. Biochem J 1996; 316 (Pt 1): 73-80.

[20] Lichtenthaler HK, Schwender J, Disch A, Rohmer M. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. FEBS Lett 1997; 400: 271-4.

[21] Schwender J, Zeidler J, Groner R, *et al.* Incorporation of 1-deoxy-D-xylulose into isoprene and phytol by higher plants and algae. FEBS Lett 1997; 414: 129-34.

[22] Catalano-Dupuy DL, Ceccarelli EA. Searching for the role of the plastidic-type Ferredoxin-NADP(H) reductase in *Leptospira interrogans*. In: Frago S, Gomez-Moreno C, Medina M, editors. Flavins and Flavoproteins.Prensas Universitarias de Zaragoza. 2008; pp. 249-54.

[23] Catalano-Dupuy DL, Musumeci MA, Lopez-Rivero A, Ceccarelli EA. A highly stable plastidic-type ferredoxin-NADP(H) reductase

in the pathogenic bacterium *Leptospira interrogans*. PLoS One 2011; 6: e26736.

[24] Arigoni D, Sagner S, Latzel C, *et al*. Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. Proc Natl Acad Sci USA 1997; 94: 10600-5.

[25] Ostrovsky D, Diomina G, Lysak E, *et al*. Effect of oxidative stress on the biosynthesis of 2-C-methyl-D-erythritol-2,4-cyclopyrophosphate and isoprenoids by several bacterial strains. Arch Microbiol 1998; 171: 69-72.

[26] Altincicek B, Duin EC, Reichenberg A, *et al*. LytB protein catalyzes the terminal step of the 2-C-methyl-D-erythritol-4-phosphate pathway of isoprenoid biosynthesis. FEBS Lett 2002; 532: 437-40.

[27] Kollas AK, Duin EC, Eberl M, *et al*. Functional characterization of GcpE, an essential enzyme of the non-mevalonate pathway of isoprenoid biosynthesis. FEBS Lett 2002; 532: 432-6.

[28] Wolff M, Seemann M, Tse Sum BB, *et al*. Isoprenoid biosynthesis via the methylerythritol phosphate pathway: the (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase (LytB/IspH) from *Escherichia coli* is a [4Fe-4S] protein. FEBS Lett 2003; 541: 115-20.

[29] Grawert T, Groll M, Rohdich F, Bacher A, Eisenreich W. Biochemistry of the non-mevalonate isoprenoid pathway. Cell Mol Life Sci 2011; 68: 3797-814.

[30] Seemann M, Bui BT, Wolff M, *et al*. Isoprenoid biosynthesis through the methylerythritol phosphate pathway: the (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE) is a [4Fe-4S] protein. Angew Chem Int Ed Engl 2002; 41: 4337-9.

[31] Okada K, Hase T. Cyanobacterial non-mevalonate pathway: (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase interacts with ferredoxin in *Thermosynechococcus elongatus* BP-1. J Biol Chem 2005; 280: 20672-79.

[32] Seeber F, Aliverti A, Zanetti G. The plant-type ferredoxin-$NADP^+$ reductase/ferredoxin redox system as a possible drug target against apicomplexan human parasites. Curr Pharm Des 2005; 11: 3159-72.

[33] Wiesner J, Jomaa H. Isoprenoid biosynthesis of the apicoplast as drug target. Curr Drug Targets 2007; 8: 3-13.

[34] Disch A, Hemmerlin A, Bach TJ, Rohmer M. Mevalonate-derived isopentenyl diphosphate is the biosynthetic precursor of ubiquinone prenyl side chain in tobacco BY-2 cells. Biochem J 1998; 331 (Pt 2): 615-21.

[35] Cvejic JH, Rohmer M. CO2 as main carbon source for isoprenoid biosynthesis via the mevalonate-independent methylerythritol 4-phosphate route in the marine diatoms *Phaeodactylum tricornutum* and *Nitzschia ovalis*. Phytochemistry 2000; 53: 21-8.

[36] Eberl M, Hintz M, Reichenberg A, *et al*. Microbial isoprenoid biosynthesis and human gammadelta T cell activation. FEBS Lett 2003; 544: 4-10.

[37] Zeidler JG, Lichtenthaler HK, May HU, Lichtenthaler FW. Is isoprene emitted by plants synthesized via the novel isopentenyl pyrophosphate pathway ? Z Naturforsch 1997; 52: 15-23.

[38] Lichtenthaler HK, Rohmer M, Schwender J. Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. Physiologia Plantarum 1997; 101: 643-52.

[39] Eisenreich W, Schwarz M, Cartayrade A, *et al*. The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. Chem Biol 1998; 5: R221-33.

[40] Rohmer M. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Nat Prod Rep 1999; 16: 565-74.

[41] Eisenreich W, Rohdich F, Bacher A. Deoxyxylulose phosphate pathway to terpenoids. Trends Plant Sci 2001; 6: 78-84.

[42] Rodriguez-Concepcion M, Boronat A. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. Plant Physiol 2002; 130: 1079-89.

[43] Lange BM, Rujan T, Martin W, Croteau R. Isoprenoid biosynthesis: the evolution of two ancient and distinct pathways across genomes. Proc Natl Acad Sci USA 2000; 97: 13172-7.

[44] Hoeffler JF, Hemmerlin A, Grosdemange-Billiard C, Bach TJ, Rohmer M. Isoprenoid biosynthesis in higher plants and in *Escherichia coli*: on the branching in the methylerythritol phosphate pathway and the independent biosynthesis of isopentenyl diphosphate and dimethylallyl diphosphate. Biochem J 2002; 366: 573-83.

[45] Querol J, Campos N, Imperial S, Boronat A, Rodriguez-Concepcion M. Functional analysis of the *Arabidopsis thaliana* GCPE protein involved in plastid isoprenoid biosynthesis. FEBS Lett 2002; 514: 343-6.

[46] Wiesner J, Borrmann S, Jomaa H Fosmidomycin for the treatment of malaria. Parasitol Res 2003; 90 Suppl 2: S71-6.

[47] Rohmer M, Grosdemange-Billiard C, Seemann M, Tritsch D. Isoprenoid biosynthesis as a novel target for antibacterial and antiparasitic drugs. Curr Opin Investig Drugs 2004; 5: 154-62.

[48] Wiesner J, Seeber F. The plastid-derived organelle of protozoan human parasites as a target of established and emerging drugs. Expert Opin Ther Targets 2005; 9: 23-44.

[49] Kuzuyama T, Shimizu T, Takahashi S, Seto H. Fosmidomycin, a specific inhibitor of 1-deoxy-d-xylulose 5-phosphate reducto-isomerase in the nonmevalonate pathway for terpenoid biosynthesis. Tetrahedron Letters 1998; 39: 7913-6.

[50] Missinou MA, Borrmann S, Schindler A, *et al*. Fosmidomycin for malaria. Lancet 2002; 360: 1941-2.

[51] Rohrich RC, Englert N, Troschke K, *et al*. Reconstitution of an apicoplast-localised electron transfer pathway involved in the isoprenoid biosynthesis of *Plasmodium falciparum*. FEBS Lett 2005; 579: 6433-8.

[52] Singh N, Cheve G, Avery MA, McCurdy CR. Targeting the methyl erythritol phosphate (MEP) pathway for novel antimalarial, antibacterial and herbicidal drug discovery: inhibition of 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) enzyme. Curr Pharm Des 2007; 13: 1161-77.

[53] Andrews SC, Robinson AK, Rodriguez-Quinones F. Bacterial iron homeostasis. FEMS Microbiol Rev 2003; 27: 215-37.

[54] Lieu PT, Heiskala M, Peterson PA, Yang Y. The roles of iron in health and disease. Mol Aspects Med 2001; 22: 1-87.

[55] Ryter SW, Tyrrell RM. The heme synthesis and degradation pathways: role in oxidant sensitivity: Heme oxygenase has both pro- and antioxidant properties. Free Radical Biology and Medicine 2000; 28: 289-309.

[56] Tong Y, Guo M. Bacterial heme-transport proteins and their heme-coordination modes. Arch Biochem Biophys 2009; 481: 1-15.

[57] Bothwell TH. Overview and mechanisms of iron regulation. Nutr Rev 1995; 53: 237-45.

[58] Bullen JJ, Rogers HJ, Spalding PB, Ward CG. Natural resistance, iron and infection: a challenge for clinical medicine. J Med Microbiol 2006; 55: 251-8.

[59] Wandersman C, Stojiljkovic I. Bacterial heme sources: the role of heme, hemoprotein receptors and hemophores. Curr Opin Microbiol 2000; 3: 215-20.

[60] Anzaldi LL, Skaar EP. Overcoming the heme paradox: heme toxicity and tolerance in bacterial pathogens. Infect Immun 2010; 78: 4977-89.

[61] Ochsner UA, Johnson Z, Vasil ML. Genetics and regulation of two distinct haem-uptake systems, phu and has, in *Pseudomonas aeruginosa*. Microbiology 2000; 146 (Pt 1): 185-98.

[62] Henderson DP, Payne SM. *Vibrio cholerae* iron transport systems: roles of heme and siderophore iron transport in virulence and identification of a gene associated with multiple iron transport systems. Infect Immun 1994; 62: 5120-5.

[63] Stojiljkovic I, Hantke K. Hemin uptake system of *Yersinia enterocolitica:* similarities with other TonB-dependent systems in gram-negative bacteria. EMBO J 1992; 11: 4359-67.

[64] Mills M, Payne SM. Genetics and regulation of heme iron transport in *Shigella dysenteriae* and detection of an analogous system in *Escherichia coli* O157:H7. J Bacteriol 1995; 177: 3004-9.

[65] Ghigo JM, Letoffe S, Wandersman C. A new type of hemophore-dependent heme acquisition system of *Serratia marcescens* reconstituted in *Escherichia coli*. J Bacteriol 1997; 179: 3572-9.

[66] Maciver I, Latimer JL, Liem HH, *et al*. Identification of an outer membrane protein involved in utilization of hemoglobin-haptoglobin complexes by nontypeable *Haemophilus influenzae*. Infect Immun 1996; 64: 3703-12.

[67] Lewis LA, Gray E, Wang YP, Roe BA, Dyer DW. Molecular characterization of hpuAB, the haemoglobin-haptoglobin-utilization operon of *Neisseria meningitidis*. Mol Microbiol 1997; 23: 737-49.

[68] Schryvers AB, Stojiljkovic I. Iron acquisition systems in the pathogenic *Neisseria*. Mol Microbiol 1999; 32: 1117-1123.

[69] Rohde KH, Gillaspy AF, Hatfield MD, Lewis LA, Dyer DW. Interactions of haemoglobin with the *Neisseria meningitidis* receptor HpuAB: the role of TonB and an intact proton motive force. Mol Microbiol 2002; 43: 335-54.

[70] Skaar EP, Humayun M, Bae T, DeBord KL, Schneewind O. Iron-source preference of *Staphylococcus aureus* infections. Science 2004; 305: 1626-8.

[71] Schmitt MP. Utilization of host iron sources by *Corynebacterium diphtheriae*: identification of a gene whose product is homologous to eukaryotic heme oxygenases and is required for acquisition of iron from heme and hemoglobin. J Bacteriol 1997; 179: 838-45.

[72] Drazek ES, Hammack CA, Schmitt MP. *Corynebacterium diphtheriae* genes required for acquisition of iron from haemin and haemoglobin are homologous to ABC haemin transporters. Mol Microbiol 2000; 36: 68-84.

[73] Francis RT, Jr., Booth JW, Becker RR Uptake of iron from hemoglobin and the haptoglobin-hemoglobin complex by hemolytic bacteria. Int J Biochem 1985; 17: 767-73.

[74] Bates CS, Montanez GE, Woods CR, Vincent RM, Eichenbaum Z. Identification and characterization of a *Streptococcus pyogenes* operon involved in binding of hemoproteins and acquisition of iron. Infect Immun 2003; 71: 1042-55.

[75] Fabian M, Solomaha E, Olson JS, Maresso AW. Heme transfer to the bacterial cell envelope occurs via a secreted hemophore in the Gram-positive pathogen *Bacillus anthracis*. J Biol Chem 2009; 284: 32138-46.

[76] Murray GL, Ellis KM, Lo M, Adler B. *Leptospira interrogans* requires a functional heme oxygenase to scavenge iron from hemoglobin. Microbes Infect 2008; 10: 791-7.

[77] Yuri K, Takamoto Y, Okada M, *et al*. Chemotaxis of *leptospires* to hemoglobin in relation to virulence. Infect Immun 1993; 61: 2270-2272.

[78] Louvel H, Bommezzadri S, Zidane N, *et al*. Comparative and functional genomic analyses of iron transport and regulation in *Leptospira* spp. J Bacteriol 2006; 188: 7893-904.

[79] Li C, Stocker R. Heme oxygenase and iron: from bacteria to humans. Redox Rep 2009; 14: 95-101.

[80] Yoshida T, Kikuchi G. Features of the reaction of heme degradation catalyzed by the reconstituted microsomal heme oxygenase system. J Biol Chem 1978; 253: 4230-6.

[81] Ortiz de Montellano PR, Wilks A. Heme oxygenase structure and mechanism. Adv Inorg Chem 2001; 51: 359-407.

[82] Tenhunen R, Marver HS, Schmid R. Microsomal heme oxygenase. Characterization of the enzyme. J Biol Chem 1969; 244: 6388-94.

[83] Davis SJ, Bhoo SH, Durski AM, Walker JM, Vierstra RD. The heme-oxygenase family required for phytochrome chromophore biosynthesis is necessary for proper photomorphogenesis in higher plants. Plant Physiol 2001; 126: 656-69.

[84] Beale SI, Cornejo J. Enzymatic heme oxygenase activity in soluble extracts of the unicellular red alga, *Cyanidium caldarium*. Arch Biochem Biophys 1984; 235: 371-84.

[85] Richaud C, Zabulon G. The heme oxygenase gene (pbsA) in the red alga *Rhodella violacea* is discontinuous and transcriptionally activated during iron limitation. Proc Natl Acad Sci USA 1997; 94: 11736-41.

[86] Cornejo J, Willows RD, Beale SI. Phytobilin biosynthesis: cloning and expression of a gene encoding soluble ferredoxin-dependent heme oxygenase from *Synechocystis* sp. PCC 6803. Plant J 1998; 15: 99-107.

[87] Pendrak ML, Chao MP, Yan SS, Roberts DD. Heme oxygenase in *Candida albicans* is regulated by hemoglobin and is necessary for metabolism of exogenous heme and hemoglobin to alpha-biliverdin. J Biol Chem 2004; 279: 3426-33.

[88] Kim D, Yukl ET, Moenne-Loccoz P, Montellano PR. Fungal heme oxygenases: Functional expression and characterization of Hmx1 from S*accharomyces cerevisiae* and CaHmx1 from *Candida albicans*. Biochemistry 2006; 45: 14772-80.

[89] Wilks A, Schmitt MP. Expression and characterization of a heme oxygenase (Hmu O) from *Corynebacterium diphtheriae*. Iron acquisition requires oxidative cleavage of the heme macrocycle. J Biol Chem 1998; 273: 837-41.

[90] Zhu W, Hunt DJ, Richardson AR, Stojiljkovic I. Use of heme compounds as iron sources by pathogenic neisseriae requires the product of the hemO gene. J Bacteriol 2000; 182: 439-47.

[91] Ratliff M, Zhu W, Deshmukh R, Wilks A, Stojiljkovic I. Homologues of neisserial heme oxygenase in gram-negative bacteria: degradation of heme by the product of the pigA gene of *Pseudomonas aeruginosa*. J Bacteriol 2001; 183: 6394-403.

[92] Okada K The novel heme oxygenase-like protein from *Plasmodium falciparum* converts heme to bilirubin IX alpha in the apicoplast. FEBS Lett 2009; 583: 313-9.

[93] Schuller DJ, Wilks A, Ortiz De Montellano PR, Poulos TL. Crystal structure of human heme oxygenase-1. Nat Struct Biol 1999; 6: 860-7.

[94] Sugishima M, Omata Y, Kakuta Y, *et al*. Crystal structure of rat heme oxygenase-1 in complex with heme. FEBS Lett 2000; 471: 61-6.

[95] Schuller DJ, Zhu W, Stojiljkovic I, Wilks A, Poulos TL. Crystal structure of heme oxygenase from the gram-negative pathogen *Neisseria meningitidis* and a comparison with mammalian heme oxygenase-1. Biochemistry 2001; 40: 11552-8.

[96] Hirotsu S, Chu GC, Unno M, *et al*. The crystal structures of the ferric and ferrous forms of the heme complex of HmuO, a heme oxygenase of *Corynebacterium diphtheriae*. J Biol Chem 2004; 279: 11937-47.

[97] Friedman J, Lad L, Li H, Wilks A, Poulos TL. Structural basis for novel delta-regioselective heme oxygenation in the opportunistic pathogen *Pseudomonas aeruginosa*. Biochemistry 2004; 43: 5239-45.

[98] Sugishima M, Migita CT, Zhang X, Yoshida T, Fukuyama K. Crystal structure of heme oxygenase-1 from cyanobacterium *Synechocystis* sp. PCC 6803 in complex with heme. Eur J Biochem 2004; 271: 4517-25.

[99] Sugishima M, Hagiwara Y, Zhang X, *et al*. Crystal structure of dimeric heme oxygenase-2 from *Synechocystis* sp. PCC 6803 in complex with heme. Biochemistry 2005; 44: 4257-66.

[100] Zhang R, Zhang J, Guo G, *et al*. Crystal structure of *Campylobacter jejuni* ChuZ: a split-barrel family heme oxygenase with a novel heme-binding mode. Biochem Biophys Res Commun 2011; 415: 82-7.

[101] Hu Y, Jiang F, Guo Y, *et al*. Crystal structure of HugZ, a novel heme oxygenase from *Helicobacter pylori*. J Biol Chem 2011; 286: 1537-44.

[102] Rahman MN, Vlahakis JZ, Vukomanovic D, *et al*. X-ray crystal structure of human heme oxygenase-1 with (2R,4S)-2-[2-(4-chlorophenyl)ethyl]-2-[(1H-imidazol-1-yl)methyl]-4[((5-trifluorom ethylpyridin-2-yl)thio)methyl]-1,3-dioxolane: a novel, inducible binding mode. J Med Chem 2009; 52: 4946-50.

[103] Rahman MN, Vlahakis JZ, Vukomanovic D, *et al*. A novel, "double-clamp" binding mode for human heme oxygenase-1 inhibition. PLoS One 2012; 7: e29514.

[104] Unno M, Matsui T, Ikeda-Saito M. Structure and catalytic mechanism of heme oxygenase. Nat Prod Rep 2007; 24: 553-70.

[105] Maines MD, Trakshel GM, Kutty RK. Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible. J Biol Chem 1986; 261: 411-9.

[106] Maines MD. The heme oxygenase system: a regulator of second messenger gases. Annu Rev Pharmacol Toxicol 1997; 37: 517-54.

[107] Migita CT, Zhang X, Yoshida T. Expression and characterization of cyanobacterium heme oxygenase, a key enzyme in the phycobilin synthesis. Properties of the heme complex of recombinant active enzyme. Eur J Biochem 2003; 270: 687-98.

[108] Muramoto T, Tsurui N, Terry MJ, Yokota A, Kohchi T. Expression and biochemical properties of a ferredoxin-dependent heme oxygenase required for phytochrome chromophore synthesis. Plant Physiol 2002; 130: 1958-66.

[109] Davis SJ, Kurepa J, Vierstra RD. The *Arabidopsis thaliana* HY1 locus, required for phytochrome-chromophore biosynthesis, encodes a protein related to heme oxygenases. Proc Natl Acad Sci USA 1999; 96: 6541-6.

[110] Frankenberg-Dinkel N. Bacterial heme oxygenases. Antioxid Redox Signal 2004; 6: 825-34.

[111] Yoshida T, Noguchi M, Kikuchi G. Oxygenated form of heme . heme oxygenase complex and requirement for second electron to initiate heme degradation from the oxygenated complex. J Biol Chem 1980; 255: 4418-20.

[112] Wilks A, Torpey J, Ortiz De Montellano PR. Heme oxygenase (HO-1). Evidence for electrophilic oxygen addition to the porphyrin ring in the formation of alpha-meso-hydroxyheme. J Biol Chem 1994; 269: 29553-6.

[113] Matsui T, Unno M, Ikeda-Saito M. Heme oxygenase reveals its strategy for catalyzing three successive oxygenation reactions. Acc Chem Res 2010; 43: 240-7.

[114] Kikuchi G, Yoshida T, Noguchi M. Heme oxygenase and heme degradation. Biochem Biophys Res Commun 2005; 338: 558-67.

[115] Yoshida T, Kikuchi G. Reaction of the microsomal heme oxygenase with cobaltic protoporphyrin IX, and extremely poor substrate. J Biol Chem 1978; 253: 8479-82.

[116] Liu Y, Ortiz De Montellano PR. Reaction intermediates and single turnover rate constants for the oxidation of heme by human heme oxygenase-1. J Biol Chem 2000; 275: 5297-307.

[117] Caignan GA, Deshmukh R, Wilks A, *et al*. Oxidation of heme to beta- and delta-biliverdin by *Pseudomonas aeruginosa* heme oxygenase as a consequence of an unusual seating of the heme. J Am Chem Soc 2002; 124: 14879-92.

[118] Schacter BA, Nelson EB, Marver HS, Masters BS. Immunochemical evidence for an association of heme oxygenase with the microsomal electron transport system. J Biol Chem 1972; 247: 3601-7.

[119] Gohya T, Zhang X, Yoshida T, Migita CT. Spectroscopic characterization of a higher plant heme oxygenase isoform-1 from *Glycine max* (soybean)--coordination structure of the heme complex and catabolism of heme. FEBS J 2006; 273: 5384-99.

[120] Wegele R, Tasler R, Zeng Y, Rivera M, Frankenberg-Dinkel N. The heme oxygenase(s)-phytochrome system of *Pseudomonas aeruginosa*. J Biol Chem 2004; 279: 45791-802.

[121] Wang A, Zeng Y, Han H, *et al*. Biochemical and structural characterization of *Pseudomonas aeruginosa* Bfd and FPR: ferredoxin $NADP^+$ reductase and not ferredoxin is the redox partner of heme oxygenase under iron-starvation conditions. Biochemistry 2007; 46: 12198-211.

[122] Ballouche M, Cornelis P, Baysse C. Iron metabolism: a promising target for antibacterial strategies. Recent Pat Antiinfect Drug Discov 2009; 4: 190-205.

[123] Murray GL, Srikram A, Henry R, *et al*. *Leptospira interrogans* requires heme oxygenase for disease pathogenesis. Microbes Infect 2009; 11: 311-4.

[124] Furci LM, Lopes P, Eakanunkul S, *et al*. Inhibition of the bacterial heme oxygenases from *Pseudomonas aeruginosa* and *Neisseria meningitidis*: novel antimicrobial targets. J Med Chem 2007; 50: 3804-13.

[125] Evans JP, Kandel S, Ortiz De Montellano PR. Isocyanides inhibit human heme oxygenases at the verdoheme stage. Biochemistry 2009; 48: 8920-8.

[126] Rahman MN, Vlahakis JZ, Roman G, *et al*. Structural characterization of human heme oxygenase-1 in complex with azole-based inhibitors. J Inorg Biochem 2010; 104: 324-30.

[127] Kinobe RT, Vlahakis JZ, Vreman HJ, *et al*. Selectivity of imidazole-dioxolane compounds for *in vitro* inhibition of microsomal haem oxygenase isoforms. Br J Pharmacol 2006; 147: 307-15.

[128] Beinert H, Holm RH, Munck E. Iron-sulfur clusters: nature's modular, multipurpose structures. Science 1997; 277: 653-9.

[129] Sheftel A, Stehling O, Lill R. Iron-sulfur proteins in health and disease. Trends Endocrinol Metab 2010.

[130] Rouault TA, Tong WH. Iron-sulfur cluster biogenesis and human disease. Trends Genet 2008; 24: 398-407.

[131] Fontecave M, Ollagnier-de-Choudens S. Iron-sulfur cluster biosynthesis in bacteria: Mechanisms of cluster assembly and transfer. Arch Biochem Biophys 2008; 474: 226-37.

[132] Vinella D, Brochier-Armanet C, Loiseau L, Talla E, Barras F. Iron-sulfur (Fe/S) protein biogenesis: phylogenomic and genetic studies of A-type carriers. PLoS Genet 2009; 5: e1000497.

[133] Ayala-Castro C, Saini A, Outten FW. Fe-S cluster assembly pathways in bacteria. Microbiol Mol Biol Rev 2008; 72: 110-25.

[134] Johnson DC, Dos Santos PC, Dean DR. NifU and NifS are required for the maturation of nitrogenase and cannot replace the function of isc-gene products in *Azotobacter vinelandii*. Biochem Soc Trans 2005; 33: 90-93.

[135] Johnson DC, Dean DR, Smith AD, Johnson MK. Structure, function, and formation of biological iron-sulfur clusters. Annu Rev Biochem 2005; 74: 247-81.

[136] Outten FW, Wood MJ, Munoz FM, Storz G. The SufE protein and the SufBCD complex enhance SufS cysteine desulfurase activity as part of a sulfur transfer pathway for Fe-S cluster assembly in *Escherichia coli*. J Biol Chem 2003; 278: 45713-9.

[137] Nachin L, Loiseau L, Expert D, Barras F. SufC: an unorthodox cytoplasmic ABC/ATPase required for [Fe-S] biogenesis under oxidative stress. EMBO J 2003; 22: 427-37.

[138] Giel JL, Rodionov D, Liu M, Blattner FR, Kiley PJ. IscR-dependent gene expression links iron-sulphur cluster assembly to the control of O2-regulated genes in *Escherichia coli*. Mol Microbiol 2006; 60: 1058-75.

[139] Schwartz CJ, Giel JL, Patschkowski T, *et al*. IscR, an Fe-S cluster-containing transcription factor, represses expression of *Escherichia coli* genes encoding Fe-S cluster assembly proteins. Proc Natl Acad Sci USA 2001; 98: 14895-900.

[140] Barras F, Loiseau L, Py B. How *Escherichia coli* and *Saccharomyces cerevisiae* build Fe/S proteins. Adv Microb Physiol 2005; 50: 41-101.

[141] Albrecht AG, Netz DJ, Miethke M, *et al*. SufU is an essential iron-sulfur cluster scaffold protein in *Bacillus subtilis*. J Bacteriol 2010.

[142] Lill R. Function and biogenesis of iron-sulphur proteins. Nature 2009; 460: 831-8.

[143] Bhave DP, Muse WB, III, Carroll KS. Drug targets in mycobacterial sulfur metabolism. Infect Disord Drug Targets 2007; 7: 140-58.

[144] Zuris JA, Harir Y, Conlan AR, *et al*. Facile transfer of [2Fe-2S] clusters from the diabetes drug target mitoNEET to an apo-acceptor protein. Proc Natl Acad Sci USA 2011; 108: 13047-52.

[145] Mietzner TA, Morse SA. The role of iron-binding proteins in the survival of pathogenic bacteria. Annu Rev Nutr 1994; 14: 471-93.

[146] Olson JW, Agar JN, Johnson MK, Maier RJ. Characterization of the NifU and NifS Fe-S cluster formation proteins essential for viability in *Helicobacter pylori*. Biochemistry 2000; 39: 16213-9.

[147] Moura JJ, Moura I, Kent TA, *et al*. Interconversions of [3Fe-3S] and [4Fe-4S] clusters. Mossbauer and electron paramagnetic resonance studies of *Desulfovibrio gigas* ferredoxin II. J Biol Chem 1982; 257: 6259-67.

[148] Krapp AR, Humbert MV, Carrillo N. The soxRS response of *Escherichia coli* can be induced in the absence of oxidative stress and oxygen by modulation of NADPH content. Microbiology 2011; 157: 957-65.

[149] Carrillo N, Vallejos RH. Ferredoxin-$NADP^+$ oxidoreductase. In *The Light Reactions*. In: Barber J, editors. *Topics in Photosynthesis*. Amsterdam-New York-Oxford: Elsevier.1987; pp. 527-60.

[150] Giro M, Carrillo N, Krapp AR. Glucose-6-phosphate dehydrogenase and ferredoxin-NADP(H) reductase contribute to damage repair during the soxRS response of *Escherichia coli*. Microbiology 2006; 152: 1119-28.

[151] Elsen S, Efthymiou G, Peteinatos P, *et al*. A bacteria-specific 2[4Fe-4S] ferredoxin is essential in *Pseudomonas aeruginosa*. BMC Microbiol 2010; 10: 271.

[152] Huet G, Daffe M, Saves I. Identification of the *Mycobacterium tuberculosis* SUF machinery as the exclusive mycobacterial system of [Fe-S] cluster assembly: evidence for its implication in the pathogen's survival. J Bacteriol 2005; 187: 6137-46.

[153] Runyen-Janecky L, Daugherty A, Lloyd B, *et al*. Role and regulation of iron-sulfur cluster biosynthesis genes in *Shigella flexneri* virulence. Infect Immun 2008; 76: 1083-92.

[154] Hanukoglu I, Gutfinger T. cDNA sequence of adrenodoxin reductase. Identification of NADP-binding sites in oxidoreductases. Eur J Biochem 1989; 180: 479-84.

[155] Ziegler GA, Schulz GE. Crystal structures of adrenodoxin reductase in complex with $NADP^+$ and NADPH suggesting a mechanism for the electron transfer of an enzyme family. Biochemistry 2000; 39: 10986-95.

[156] Deng Z, Aliverti A, Zanetti G, *et al*. A productive $NADP^+$ binding mode of ferredoxin-$NADP^+$ reductase revealed by protein engineering and crystallographic studies. Nat Struct Biol 1999; 6: 847-53.

[157] Carrillo N, Ceccarelli EA. Open questions in ferredoxin-$NADP^+$ reductase catalytic mechanism. Eur J Biochem 2003; 270: 1900-15.

[158] Aliverti A, Pandini V, Pennati A, de Rosa M, Zanetti G Structural and functional diversity of ferredoxin-NADP(+) reductases. Arch Biochem Biophys 2008; 474: 283-91.

[159] Medina M, Gomez-Moreno C. Interaction of Ferredoxin-$NADP^+$ Reductase with Its Substrates: Optimal Interaction for Efficient Electron Transfer. Photosynth Res 2004; 79: 113-31.

[160] Seeber F. Biosynthetic pathways of plastid-derived organelles as potential drug targets against parasitic apicomplexa. Curr Drug Targets Immune Endocr Metabol Disord 2003; 3: 99-109.

[161] Seeber F. Malaria and anti-malarials - a focused view. In: P.Selzer, editors. Antiparasitic and Antibacterial Drug Discovery in Infectious Diseases - From Molecular Targets to Drug Candidates.Wiley-VCH, Weinheim. 2009; pp. 277-298.

[162] Wang K, Wang W, No JH, *et al*. Inhibition of the Fe(4)S(4)-cluster-containing protein IspH (LytB): electron paramagnetic resonance, metallacycles, and mechanisms. J Am Chem Soc 2010; 132: 6719-27.

[163] Wang W, Li J, Wang K, Smirnova TI, Oldfield E. Pyridine inhibitor binding to the 4Fe-4S protein *A. aeolicus* IspH (LytB): a HYSCORE Investigation. J Am Chem Soc 2011; 133: 6525-8.

Received: September 13, 2012 **Accepted: October 30, 2012**