Coordination Chemistry Reviews 452 (2022) 214286

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Regulation of bacterial haem biosynthesis

Jordi Zamarreño Beas [1], Marco A.M. Videira [1], Lígia M. Saraiva *

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

ARTICLE INFO

Article history:
Received 23 April 2021
Received in revised form 28 September 2021
Accepted 19 October 2021
Available online 8 November 2021

Keywords:
Haem biosynthesis
Regulation
Protein–protein interactions
Sirohaem

ABSTRACT

Haem *b* and sirohaem are two iron-chelated modified tetrapyrroles that serve as prosthetic groups in proteins with crucial roles in a variety of biological functions, such as gas transport, respiration, and nitrite and sulphite reduction. These tetrapyrroles are synthesised from 5-aminolaevulinic acid and share a common pathway until the formation of uroporphyrinogen III, from where the synthesis diverges. In bacteria, sirohaem is produced from uroporphyrinogen III through the activities of one, two or three separate proteins, while haem *b* is synthesised through three distinct pathways. The biosynthesis of haem *b* and sirohaem comprises intermediates and end-products that are unstable or potentially hazardous to the cell. Therefore, the cellular metabolic fluxes of tetrapyrroles need to be tightly controlled by substrate channelling and/or other regulatory processes. This review summarises the recent advances on the regulation and protein–protein interactions controlling the formation of sirohaem and haem *b* in bacteria.

Contents

* Corresponding author.
E-mail address: lst@itqb.unl.pt (L.M. Saraiva).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ccr.2021.214286
0010-8545/

1. Introduction

Tetrapyrroles are molecules present in all domains of life and include chlorophylls, coenzyme F430, bilins, cobalamin (vitamin B12), haem *b* and sirohaem [1]. These compounds derive all from the universal common tetrapyrrole precursor, uroporphyrinogen III (uro'gen III) that is synthesised from 5-aminolaevulinic acid (ALA). The iron-containing tetrapyrroles haem *b* and sirohaem are ubiquitous tetrapyrroles derivatives involved in a plethora of cellular functions [2]. Haem iron is a source or sink of electrons during electron transfer or redox chemistry due to the ability of iron to alternate between two oxidation states, Fe^{2+} and Fe^{3+}. Several types of haem molecules exist (haem *a*, *b*, *c*, *d*, *o* and derivatives), but haem *b* that is the precursor of all other types of haems is the most common one [3].

A myriad of haem proteins play key functions in cells, such as, cytochromes that promote electron transfer in the respiratory chains, haemoglobin that is responsible for the transport/storage of oxygen, and catalases/peroxidases with major roles in oxidative stress detoxification. In addition, several transcription factors contain a haem cofactor that allows sensing different extracellular stimuli and, consequently, promote the regulation of various genes [4,5].

Sirohaem is an iron-containing isobacteriochlorin that is typically linked to the reduction of sulphur and nitrogen oxides [1,6]. Sirohaem is the prosthetic group of sulphite and nitrite reductases, which catalyse the six-electron reduction of either sulphite or nitrite to yield sulphide and ammonia, respectively. Sirohaem is also an intermediate of anaerobic haem *b* synthesis, as detailed below.

Both sirohaem and haem *b* (hereinafter referred to only as haem) are synthesized endogenously by the vast majority of prokaryotes through biosynthetic pathways that have been extensively studied, and are described in several reviews [6–15]. Despite its important functional role, high intracellular levels of tetrapyrroles derivatives are toxic to cells [16,17]. Therefore, the biosynthesis of these cofactors needs to be regulated to meet cellular requirements while preventing harmful accumulation [18,19].

In this review, we focus on the regulation of the genes involved in the biosynthesis of sirohaem and haem. Therefore, in the following sections we present a brief overview of the steps and enzymes of the several pathways. Next, we describe the regulatory mechanisms that may occur at the transcriptional level, via transcriptional regulators that modulate gene expression, post-transcriptionally through mRNA degradation, and post-translationally via protein–protein interactions and substrate-protein inhibition. Moreover, we present the currently known network of protein–protein interactions in which these enzymes are involved.

2. Towards uroporphyrinogen III

2.1. Synthesis of ALA

The first common precursor of uro'gen III synthesis is 5-aminolaevulinic acid (ALA) that may be formed by the C_4 or the C_5 pathway (Fig. 1).

In the C_4 or "Shemin" pathway, the pyridoxal-5'-phosphate-dependent enzyme AlaS catalyses the condensation of succinyl-CoA and glycine to ALA, releasing CO_2 and coenzyme A as side-products. This pathway is mainly found in metazoans, fungi and alphaproteobacteria [20,21].

The C_5 pathway converts glutamyl-tRNA to ALA in a two-step pathway carried out by the enzymes glutamyl-tRNA reductase (GtrR) and glutamate-1-semialdehyde-2,1-aminomutase (GsaM).

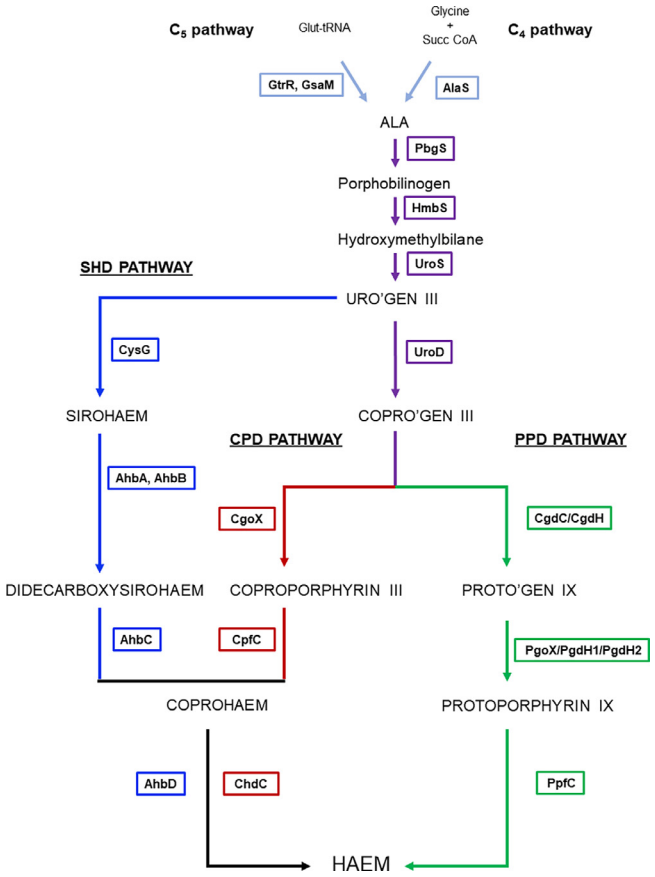

Fig. 1. Schematic representation of bacterial metabolic pathways leading to the formation of haem. Haem biosynthesis starts with synthesis of ALA through either C_4 (AlaS) or C_5 (GtrR, GsaM) pathways (light blue), followed by formation of uro'gen III (purple) by the enzymes PbgS, HmbS, and UroS. Uro'gen III is the common precursor from which three branches are originated, namely Sirohaem-Dependent (SHD) (dark blue), Coproporphyrin-Dependent (CPD) (red) and Protoporphyrin-Dependent (PPD) (green) pathway. In SHD pathway, sirohaem is an intermediate formed by CysG, and haem is synthesised by the subsequent activity of a cascade of the enzymes AhbA, AhbB, AhbC, AhbD. In both CPD and PPD pathways, UroD converts uro'gen III to copro'gen III. In the CPD pathway, copro'gen III is converted to coproporphyrin III by CgoX, and the last two enzymes that lead to haem are CpfC and ChdC. In the PPD Pathway, copro'gen III is converted to proto'gen IX by CgdC or CgdH enzyme, and the subsequent reactions forming haem are done by PgoX/PgdH1/PgdH2 and PpfC. Abbreviations: uro'gen III, uroporphyrinogen III; copro'gen III, coproporphyrinogen III; and proto'gen IX, protoporphyrinogen IX.

GtrR catalyses the reduction of glutamyl-tRNA to glutamate-1-semialdehyde (GSA) in a NADPH-dependent manner, and subsequently, the PLP-dependent GsaM enzyme transforms GSA into the final product ALA [22–26]. GtrR and GsaM occur often in bacteria, archaea and plants.

2.2. Regulation of ALA synthesis

In general, expression of the genes encoding haem and sirohaem biosynthesis enzymes are regulated mainly by intracellular haem and oxygen levels, as it is the case of *gtrR* (Table 1).

In *Escherichia coli* and *Salmonella typhimurium*, transcription of *gtrR* is induced in ALA and haem-starving cells and under low oxygen conditions. The latter involves two global oxygen responsive regulators, namely the fumarate and nitrate reductase regulator FNR and the two-component ArcAB, formed by the transcriptional regulator ArcA and the sensor kinase ArcB [27–30]. A detailed description of these global regulatory systems is reviewed in [31–33].

Table 1
Regulators linked to bacterial haem and sirohaem biosynthesis.

Pathway	Gene/Protein	Organism	Regulator	Signal	References
ALA synthesis	*gtrR*	*P. aeruginosa*	Dnr; IHF; NarXL; Anr	O_2	[44–48]
		E. coli	$ArcAB^{nt}$; Fnr^{nt}		[27–30]
		C. glutamicum	HrrSA	Haem/Haemoglobin	[53]
		C. diphteriae	HrrSA; ChrSA		[50,51]
		S. aureus; B. subtilis	HemX		[40,41,43]
		E. coli; S. enterica	ClpAP; Lon		[34–37]
		C. glutamicum; C. diphteriae	DtxR	Iron	[50,52,53,170]
		B. subtilis	PerR	H_2O_2	[38,39]
Uro'gen III synthesis	*hmbS*	*B. subtilis*	PerR	H_2O_2	
		P. aeruginosa	AlgZR	nt	[64,65]
	pbgS	*B. subtilis*	PerR	H_2O_2	[38,39]
	uroS	*P. aeruginosa*	AlgZR	nt	[64,65]
Sirohaem synthesis	*cysG*	*E. coli; S. typhimurium*	FNR	O_2	[95–100]
		E. coli	IHF; FIS		
			NarP, NarL	NO_3, NO_2	
			RyhB	Iron	
	uroM	*P. denitrificans*	nt	Uro'gen III, SAH	[103]
			NNR; NirI	O_2, NO	[101,102]
		K. aerogenes	CysB	S	[78]
		S. aureus	NreABC	O_2	[104,105]
		B. subtilis	TnrA	N_2	[106,107]
			ResDE	O_2	[106]
	shfC	*D. nodosus*	Fur	Iron	[108]
SHD	*ahbAB*	*M. barkeri*	nt	Haem	[114]
PPD/CPD	*uroD*	*P. aeruginosa*	Dnr; Anr	O_2	[49,140–142]
		L. monocytogenes	SpxA1		[165]
		C. glutamicum	DtxR	Iron	[53,168,170]
			HrrSA	Haem/Haemoglobin	[53]
PPD	*cgdC*	*P. aeruginosa*	Dnr: Anr	O_2	[49,140–142]
		E. coli	OxyR	H_2O_2	[147–149]
	cgdH	*P. aeruginosa*	Dnr; Anr	O_2	[49,140–142]
			AlgZR	nt	[64,65,143]
		A. eutrophus	Fnr	O_2	[146]
	ppfC	*E. coli*	OxyR	H_2O_2	[147–149]
CPD	*cgoX*	*C. glutamicum*	DtxR	Iron	[53,168,170]
			HrrSA	Haem/Haemoglobin	[53]
	cpfC	*L. monocytogenes*	SpxA1	O_2	[165]

PPD-Protoporphyrin-Dependent pathway; CPD-Coproporphyrin-Dependent pathway; SHD-Sirohaem-Dependent pathway; SAH-S-adenosyl-L-homocysteine. nt: not tested.

In *S. typhimurium*, GtrR is also post-transcriptionally regulated by proteases ClpAP (two-chain ATP-dependent) and Lon (single-chain ATP-dependent). These proteases promote degradation of GtrR when there is an increase of the intracellular haem concentration [34–37].

Bacillus subtilis is so far the only organism in which regulation of *gtrR* by reactive oxygen species has been identified through repression by the general oxidative stress regulator PerR, which is lifted upon exposure to H_2O_2 [38,39].

Also in *B. subtilis*, the membrane protein HemX, whose gene is clustered in the operon that encodes the enzymes involved in uro'gen III biosynthesis, modulates the steady state levels of GtrR. The regulatory role of HemX was deduced from the observation that GtrR protein levels were higher in the Δ*hemX* mutant than in the wild type strain (∼15-fold higher).

Furthermore, the co-expression of *B. subtilis gtrR* and *hemX* genes in *E. coli* decreased the amount of GtrR protein produced, indicating a direct negative effect of HemX on GtrR expression. Since pulse chase experiments showed that HemX does not modify the protein stability of GtrR, the regulation appears to result from a control at the level of GtrR protein synthesis rate [40–42].

Similar results were observed in *Staphylococcus*, since *S. aureus hemX* mutant cells contained higher amounts of GtrR protein and porphyrins, namely uro'gen III, coproporphyrin III, coprohaem and haem, as shown by liquid chromatography-multiple reaction monitoring-tandem mass spectrometry [43]. Moreover, the haem biosynthesis deficient *S. aureus* Δ*cdhC* strain contained more GtrR protein than Δ*hemX*. This phenotype was further shown to be dependent on the activity of HemX, as the amount of GtrR was lower in the double mutant Δ*hemX* Δ*cdhC* than in Δ*cdhC*, but similar to Δ*hemX*. Still, the mechanism through which GtrR protein levels are modulated by HemX and haem concentrations remains to be elucidated [43].

In *Pseudomonas aeruginosa*, the induction of *gtrR* under oxygen limiting conditions in nitrite-grown cells involves Dnr, the denitrification regulator/nitric oxide reductase regulator, Anr for anaerobic regulation of arginine deiminase and nitrate reduction, IHF the integration host factor that is a sequence-specific, histone-like, multifunctional DNA-binding and DNA-bending protein, and NarXL a two-component nitrate regulatory system [44–49].

An apparently more complex regulation seems to occur in *Corynebacterium diphtheriae* through the two-component system HrrSA (formed by the haem sensor HrrS and the response regulator activator HrrA) and ChrSA (*Corynebacterium* haem-responsive sensor and activator) [50,51]. In this organism, *gtrR* is repressed by both systems through a possibly linked process. HrrA is proposed to be the predominant repressor, independently of its phosphorylation state and the presence of haem. On the contrary, *gtrR* repression by ChrA only occurs under haemoglobin replete conditions (concentrations between 140 and 200 μg/mL). Moreover, the iron regulator DtxR represses the HrrSA system, increasing the complexity of regulation of *gtrR* as well as of the other HrrSA/ChrSA gene targets [50,52,53].

Interestingly, in *Caulobacter crescentus*, a different type of regulation seems to exist. The enzyme AlaS, that catalyses the formation of ALA from glycine and succinyl-CoA, is directly inhibited by haem as shown by spectroscopic analyses done with the purified protein [54].

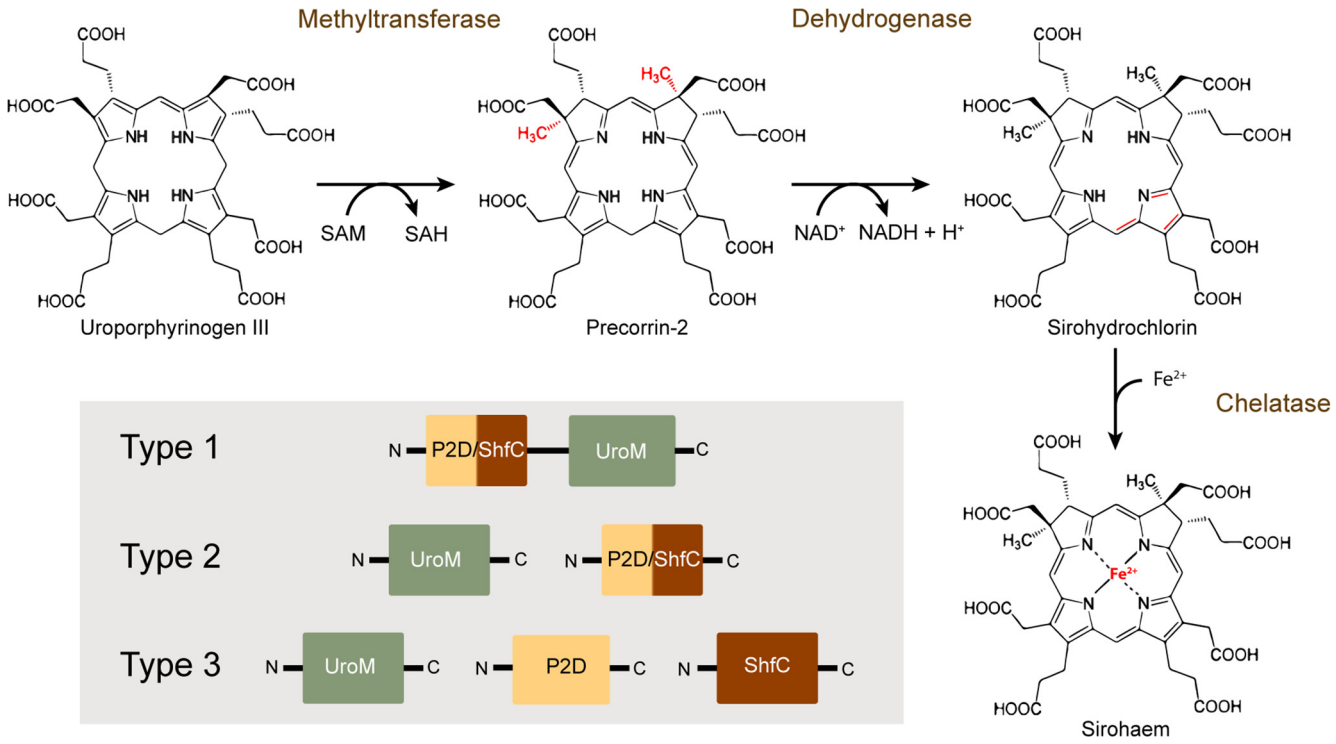

Fig. 2. Schematic representation of sirohaem synthesis. Sirohaem is formed from uroporphyrinogen III in three consecutive steps [73]. The three steps can be held by Type 1, Type 2 or Type 3 routes comprising one, two or three separate proteins, respectively. Chemical groups of products that undergo modification are highlighted in red. Abbreviations: SAM, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocysteine; NAD^+, nicotinamide adenine dinucleotide; and NADH, reduced nicotinamide adenine dinucleotide.

2.2.1. Synthesis of uroporphyrinogen III

Conversion of ALA to uro'gen III occurs in three steps. In the first, a porphobilinogen synthase (PbgS) promotes the asymmetric condensation of two ALA molecules generating porphobilinogen. In general, PbgS is a homooctamer metalloenzyme that contains Zn^{2+} (mammals, yeast, bacteria) or Mg^{2+} (plants, bacteria) [55–57]. One exception is the *Rhodobacter capsulatus* PbgS, whose activity is independent of any of these metals ions [58]. In the second step, hydroxymethylbilane synthase (HmbS) combines four porphobilinogen molecules to give hydroxymethylbilane. Usually, the enzyme is a monomer and requires a dipyrromethane cofactor for its activity [59,60]. In the final step, uroporphyrinogen III synthase (UroS) converts hydroxymethylbilane into uro'gen III. The enzyme catalyses the cyclisation of hydroxymethylbilane, and promotes rearrangement of the molecule to avoid formation of uroporphyrinogen I [8,61–63].

Uro'gen III is the last common intermediate of the downstream formation of sirohaem and haem. Three alternative pathways, namely the protoporphyrin-dependent pathway, coproporphyrin-dependent pathway, and sirohaem-dependent pathway, which are described below, do the synthesis of haem.

2.3. Regulation of the uroporphyrinogen III synthesis

The information currently available on regulation of the genes involved in uro'gen III synthesis is indicated in Table 1.

In *P. aeruginosa*, *hmbS* and *uroS* genes are co-transcribed with *algZR,* a two-component system that regulates the expression of several iron-related genes [64,65].

In *B. subtilis*, oxidative stress and low haem levels induce the expression of *hmbS* and *pbgS,* in a regulation that involves PerR [39].

Recently, a post-transcriptional regulation mechanism was described for *Vibrio cholerae* PbgS whose activity was shown to decrease upon binding of haem, indicating that the intracellular haem levels directly modulate the PbgS activity [66].

In cyanobacteria, iron modulates the expression of *hmbS* and *pbgS*. For example, in *Anabaena* sp. PCC7120 the holo form of FurA, a homologue of the ferric uptake regulator (Fur) [67], represses the transcription of *pbgS* and *hmbS* through direct binding to their promoter region, as shown by quantitative real-time PCR and electrophoretic mobility shift assays (EMSA) [68].

3. Synthesis of sirohaem from uroporphyrinogen III

Sirohaem is synthesised from uro'gen III in three steps (Fig. 2). First, uro'gen III is methylated at positions C2 and C7, giving rise to precorrin-2 [69], an intermediate that also occurs in the biosynthesis of cobalamin, haem d_1 and cofactor F_{430} [6,70,71]. Next, precorrin-2 is oxidised to sirohydrochlorin, and then iron is inserted into the macrocycle to produce sirohaem [6,72].

Depending on the organism, the three reactions of sirohaem biosynthesis are performed by a single multifunctional protein, two different proteins, or three separate proteins, and these routes were labelled as Type 1, Type 2 and Type 3, respectively (Fig. 2) [73]. In some bacteria, the presence in the same genome of enzymes associated with the three pathways suggests that more than one sirohaem-producing route can be used, but how and when it occurs still needs to be investigated. Examples of the different routes are found in *Fimbriimonas ginsengisoli* and *Photobacterium gaetbulicola* (Type 1 and Type 2), *Magnetococcus marinus* (Type 2 and Type 3), and Desulfuromonadales *Pelobacter carbinolicus* and *Geobacter lovleyi* (Type 1 and Type 3) [73].

The enzyme active in Type 1 route is the sirohaem synthase CysG, with *E. coli* and *S. enterica* being the best characterized. CysG

is composed of $CysG^A$, formed by domains A and B connected by a flexible loop and harbouring uro'gen III methyltransferase activity, and $CysG^B$ that has dehydrogenase/chelatase activities [74–76]. $CysG^B$ possesses a Rossmann fold, characteristic of a NAD^+ substrate binding site required for the dehydrogenase activity, and a helical domain that endows the ferrochelatase activity [76]. The mechanism that allows the two activities in the same active site of the $CysG^B$ domain is not yet fully understood. A recent structural study of *E. coli* CysG identified the binding conformation for both substrates and for the metallated sirohydrochlorin, and proposed residues P133 and D104 to be important for the bifunctional activity. Moreover, site-directed mutagenesis of residue D104 in $CysG^B$ generated a protein lacking the two activities [77]. Interestingly, in some bacteria, two functional copies of the *cysG* gene are present in the genome, such as in *Klebsiella aerogenes, Enterobacter aerogenes* and *Yersinia pestis* [73,78].

The Type 2 route includes an uro'gen methyltransferase (UroM), that converts uro'gen III to precorrin-2, and the P2D-ShfC fusion protein that converts precorrin-2 in sirohaem.

UroM is a S-adenosyl-L-methionine-dependent enzyme that catalyses the sequential insertion of two methyl groups in rings A and B of uro'gen III [71,79]. It shares high amino acid sequence similarity to the C-terminal part of CysG. In Firmicutes and Actinobacteria, such as *Propionibacterium*, *Corynebacterium* and *Mycobacterium* species, UroM and UroS constitute a single protein [73,80,81].

P2D-ShfC has domains for precorrin-2 dehydrogenase (P2D) and sirohydrochlorin ferrochelatase (ShfC). P2D domain has a Rossman fold for binding of the NAD^+ substrate, and converts precorrin-2 to sirohydrochlorin [82]. P2D-ShfC enzymes share sequence similarity with $CysG^B$ proteins, namely of *E. coli* and *S. cerevisiae* [83,84]. Although initially considered to occur only in eukaryotic yeasts, a recent bioinformatics analysis revealed that P2D-ShfC homologues are also present in several bacterial genomes [73].

ShfC is a chelatase that inserts iron into the sirohydrochlorin macrocycle, and is evolutionarily related to protoporphyrin ferrochelatases [73,85–88]. In *S. aureus*, His22 and His87 of ShfC predicted, by homology modelling, to be located at the active site were shown to control the ferrochelatase activity [73]. In plants, ShfC contains a [2Fe–2S] cluster that is not required for sirohydrochlorin ferrochelatase activity [89,90]. In the sulphate-reducer *Desulfovibrio vulgaris*, the enzyme $CbiK^C$ exhibits both sirohydrochlorin ferro- and cobalto-chelatase activities [91].

The Type 3 route that synthesises sirohaem via the three individual enzymes, UroM, P2D and ShfC, is found mainly in Firmicutes phylum [73]. Individual P2D enzymes are widespread in bacteria other than Firmicutes, e.g., in *Desulfovibrio vulgaris* [80].

Bacillus megaterium and *S. aureus* are two bacteria using Type 3 route. *In vitro* enzymatic assays and complementation experiments proved the function of each enzyme [73,82,92,93]. Complementation experiments in a sirohaem deficient *E. coli* strain demonstrated that *S. aureus* ShfC inserts iron into sirohydrochlorin, and the absence of nitrite consumption of a *S. aureus* Δ*shfc* mutant confirmed that ShfC is required for nitrite reductase activity [73]. Although it acts as a monofunctional enzyme, the *B. megaterium* P2D structure reveals a P2D-ShfC-like overall architecture. Additionally, residues N98 and Ser124 were found to be important for the dehydrogenase activity [92].

Interestingly, in plants the three consecutive steps of sirohaem synthesis are carried out only by two enzymes, UroM and a smaller ShfC-like protein, as no precorrin-2 dehydrogenase has, so far, been described [89].

A phylogenetic analysis of the bacterial sirohaem routes showed that Type 1 is the most used pathway and it occurs in Gammaproteobacteria and Streptomycetales. Type 2 predominates in Fibriobacteres and Vibrionales, and Type 3 in Firmicutes of the Baciliales order. Thus, the occurrence of several sirohaem pathways, that changes at the genus or species level and within taxa, may result of evolutionary multiple fusion/fission events.

3.1. Regulation of sirohaem synthesis

The genes encoding enzymes for sirohaem synthesis can appear clustered in a single operon, as it frequently occurs for genes of the Type 3 pathway, or are widespread in the genome (Fig. 3). In several cases, these genes are in operons that contain other genes involved in the metabolism of nitrate or nitrite and are regulated by nitrate, nitrite and oxygen [94].

In *E. coli* and *S. typhimurium, cysG* expression is controlled by two promoters, namely, p*cysG* that is constitutively expressed, and *pnir* that also controls the expression of the *nirBDC* operon (encoding the nitrite reductase enzyme) and thus, induced by nitrate, nitrite and oxygen limiting conditions. This regulation involves NarL, NarP (two nitrate regulators), FNR, IHF, and Fis (factor for inversion stimulation) that bind to the p*nirB* promoter. Additionally, the *nir* operon is post-transcriptionally regulated, via increase of mRNA degradation under iron starvation and mediated by the iron stress-induced small RNA (RyhB) [95–100] (Table 1).

Klebsiella aerogenes contains two copies of *cysG*: one of the genes is co-transcribed with nitrite reductase genes, and therefore modulated by nitrate and nitrite under oxygen limiting conditions, while the other gene is regulated by the CysB transcriptional activator in response to sulphur starvation [78].

In *S. enterica typhimirium*, a regulatory mechanism involving the post-translational phosphorylation of residue Ser128 at $CysG^B$ active site inhibits the dehydrogenase and chelatase activities and, ultimately, impairs sirohaem synthesis, which may shift the metabolic flux to cobalamin formation [76,77].

In *P. denitrifi*cans, the *uroM* gene is included in the *nir* operon that is regulated by NNR (nitric oxide reductase regulator, a FNR-like transcriptional activator) and NirI (nitrite reductase transcriptional regulator), which is also under the control of NNR. Activation of NNR is dependent on nitric oxide [101,102]. Interestingly, studies in *P. denitrificans* suggested that UroM enzyme is tightly regulated by feedback inhibition by the substrate uro'gen III and the product S-adenosyl-L-homocysteine (SAH) [103].

In *S. aureus*, *uroM* and *sfhC* genes are located in the *nir* operon whose expression was shown to be positively regulated by the oxygen-responsive nitrogen regulation system (NreABC) in cells grown under anaerobic conditions [104,105].

In *B. subtilis*, UroM is encoded by the *nasF* gene that is upregulated under nitrogen and oxygen limiting conditions through the nitrogen regulator TnrA and the two-component response redox regulator ResDE, respectively [106,107]. Like in *P. denitrifi*cans, UroM activity is inhibited by the substrate uro'gen III. Hence, it is possible that formation of precorrin-2 constitutes a regulatory step for the production of cobalamin [92].

In *P. denitrificans*, the *shfC* gene was reported to be constitutively expressed [85], while in *Dichelobacter nodosus shfC* was shown to be overexpressed in the Δ*fur* strain, suggesting that Fur represses *shfC* when iron is available [108].

4. Synthesis of haem *b* from uroporphyrinogen III

Bacteria and archaea use one of three pathways to produce haem from uro'gen III, namely the sirohaem-dependent (SHD) pathway, the protoporphyrin-dependent (PPD) pathway, and the coproporphyrin-dependent (CPD) pathway (Figs. 1 and 4). In the following sections, we describe the enzymes of each respective pathway and their modes of regulation.

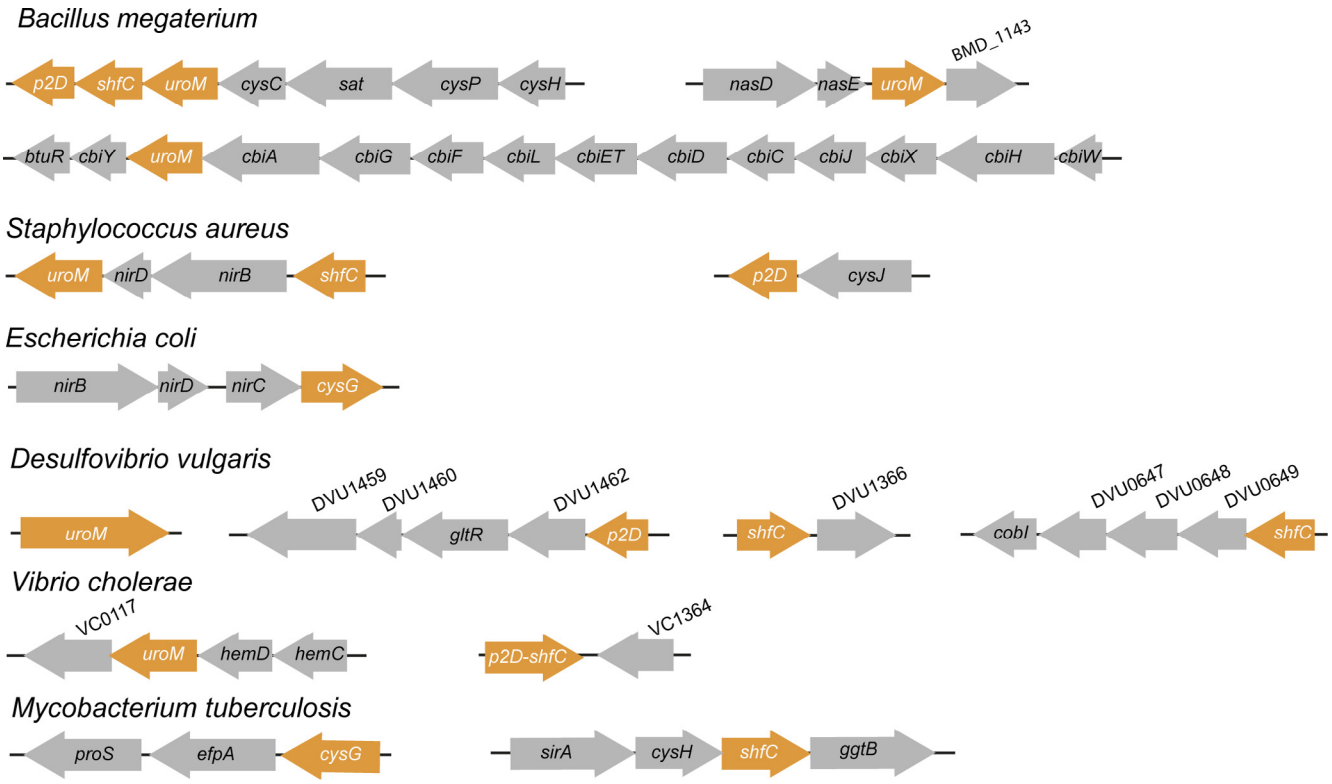

Fig. 3. Genome organisation of genes involved in sirohaem biosynthesis in sirohaem biosynthesis in selected bacteria. Genes required for sirohaem biosynthesis are marked in yellow, while genes in grey are named according to the KEGG database or by gene locus position. Adapted from [73].

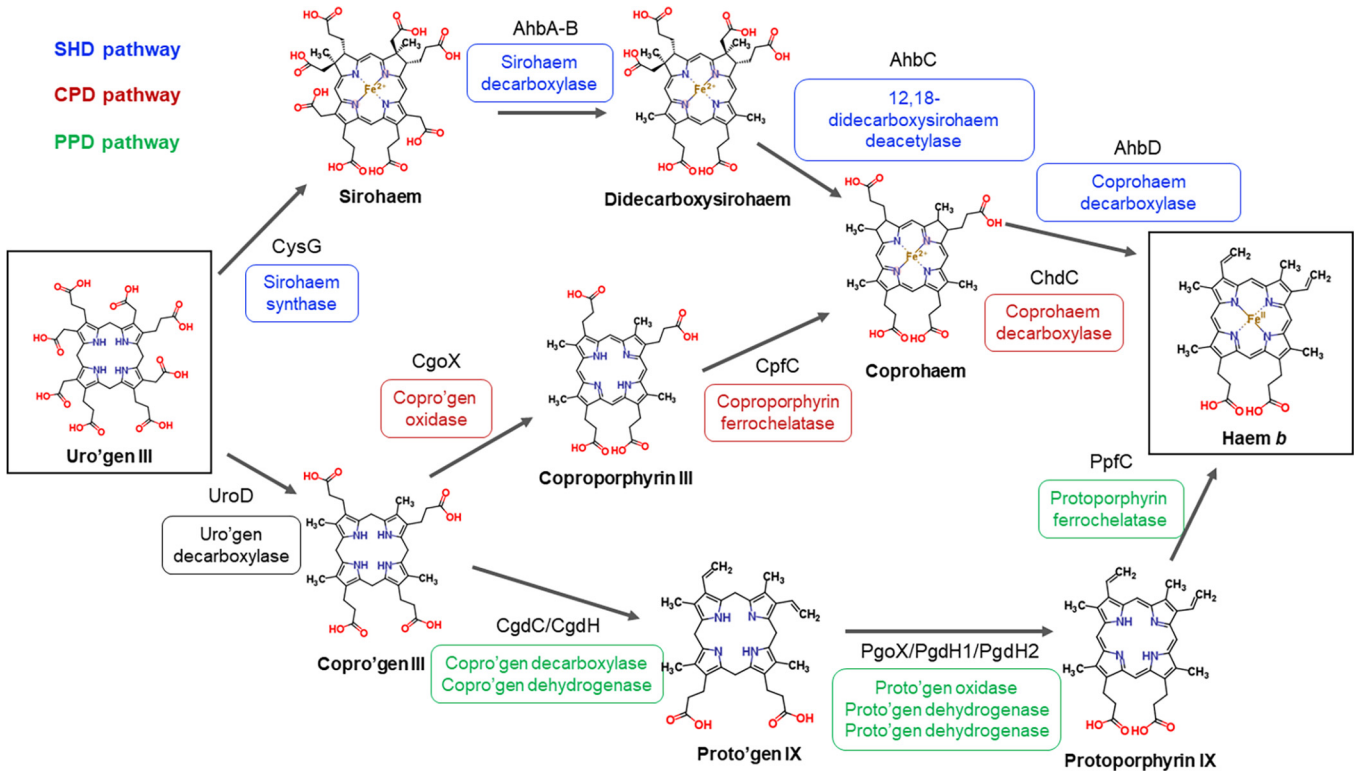

Fig. 4. Schematic representation of haem *b* synthesis from uroporphyrinogen III. Enzymes of the Sirohaem-Dependent (SHD) Pathway, Coproporphyrin-Dependent (CPD) Pathway and Protoporphyrin-Dependent (PPD) Pathway are depicted in blue, red and green, respectively. Enzymes are depicted in boxes and the intermediates/products are indicated in bold. Abbreviations: uro'gen III, uroporphyrinogen III; copro'gen III, coproporphyrinogen III; and proto'gen IX, protoporphyrinogen IX.

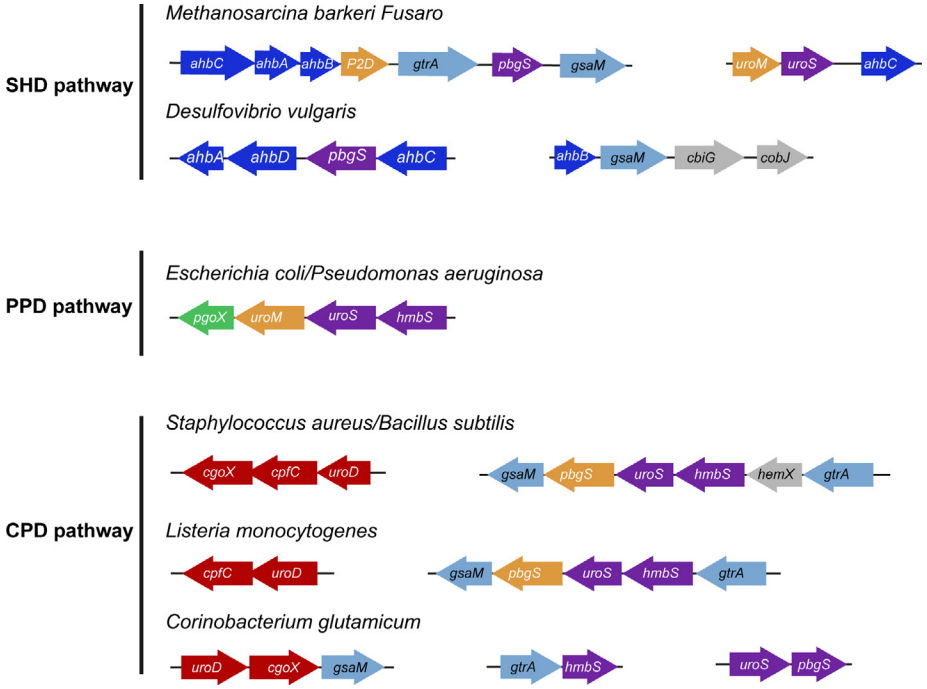

Fig. 5. Organisation of genes involved in SHD, PPD and CPD haem biosynthesis pathways. Arrangement of haem biosynthesis related genes as present in the genomes of selected bacteria. Genes are coloured according to the pathway: light blue for synthesis of 5-aminolaevulinic acid; purple for uro'gen III formation; dark blue for SHD pathway; green for PPD pathway; red for CPD pathway; yellow for sirohaem biosynthesis-related genes; and grey for those not associated with haem or sirohaem biosynthesis and that are named according to KEGG annotation.

4.1. Sirohaem-dependent pathway

The SHD pathway is considered the most ancient route for haem synthesis as it is present in archaea, sulphate-reducing and denitrifying bacteria (Figs. 1 and 4) [8]. This pathway is also known as the alternative haem biosynthesis pathway as reflected in the nomenclature of the respective enzymes. The existence of sirohaem intermediates was early reported by Ishida and colleagues when studying in *D. vulgaris* the formation of haem derived from precorrin, and the involvement of sirohaem was also deduced by analysis of several Archaea genomes [109,110]. Finally in 2011, the complete pathway, i.e. the enzymes and intermediates sustaining haem biosynthesis derived from sirohaem, was identified [111].

In the SHD pathway, sirohaem undergoes decarboxylation of the acetic acid side chains in rings C and D to methyl groups producing 12,18-didecarboxysirohaem (Fig. 4). This step is catalysed by an heterodimeric complex (~40 kDa), named sirohaem decarboxylase and formed by AhbA and AhbB enzymes [111–113]. The next two steps are catalysed by two radical *S*-adenosyl-L-methionine (SAM) enzymes, namely AhbC and AhbD. The AhbC protein is a 12,18-didecarboxysirohaem deacetylase that removes the acetic acid groups of rings A and B of didecarboxysirohaem generating coprohaem [111,114]. The haem *b* synthase AhbD transforms coprohaem to the final product haem *b*, through oxidative decarboxylation of coprohaem propionates side chains of rings A and B into vinyl groups (Fig. 4). *D. vulgaris*, AhbD was shown to be a monomeric cytoplasmic protein harbouring two [4Fe-4S]$^{2+/1+}$ clusters [115].

4.2. Regulation of the sirohaem-dependent pathway genes

The regulation of genes of the SHD pathway remains essentially unknown and cannot be deduced from the gene organisation that it is quite diverse amongst genomes.

In an apparently limited number of bacteria, the genes involved in the last steps are included in operons that contain gene products that are active in the common branch. This is the case of *D. vulgaris*, in which *ahbA* and *ahbD* are clustered together with *ahbC* and *pbgS*, and *ahbB* is closely located to *gsaM*. Also, in the archaeon *Methanosarcina barkeri*, *ahbA*, *ahbB* and *ahbD* are part of a single operon, whereas *ahbC* is located elsewhere in the genome but in the close vicinity of *uroS* (Fig. 5).

The *M. barkeri* AhbAB complex was shown to bind haem, raising the possibility that the heterodimeric complex might be subject to feedback regulation by the pathway's end-product [114].

4.2.1. Protoporphyrin-dependent pathway

The PPD pathway converts uro'gen III to haem in four steps involving the activity of UroD, CgdC/CgdH, PgdH1/PgdH2/PgoX and PpfC enzymes. It was the first route discovered for production of haem biosynthesis and, for this reason, used to be considered the classical pathway (Fig. 1 and Fig. 4) .

The uro'gen decarboxylase (UroD) catalyses decarboxylation of the four acetic acid side chains of uro'gen III, releasing four CO_2 molecules and forming coproporphyrinogen III (copro'gen III) [116–118]. This step is common to the PPD and the CPD pathways (see below). Next, copro'gen III undergoes oxidative decarboxylation of the two propionates side chains to vinyl groups generating protoporphyrinogen IX (proto'gen IX). Under aerobic conditions, the reaction is done by the oxygen-dependent copro'gen decarboxylase (CgdC), which catalyses the oxidative decarboxylation reaction with release of CO_2 and H_2O_2 as side-products [119–121]. Under anaerobiosis, the reaction occurs via the oxygen-independent copro'gen dehydrogenase protein (CgdH) that belongs to the radical SAM protein family and contains a [4Fe-4S]$^{2+}$ cluster. CgdH uses a still unknown electron donor to form proto'gen IX, releasing two CO_2 molecules as side-product [121–123].

The following step concerns the oxidation of proto'gen IX to protoporphyrin IX that may be done by three different enzymes:

the oxygen-dependent proto'gen oxidase PgoX or two dissimilar proto'gen dehydrogenases PgdH1 and PgdH2. PgoX belongs to the FAD-containing amine oxidase superfamily, and is found in few Gram-negative bacteria (< ~16%) [124,125]. PgdH1 and PgdH2 are FMN-containing enzymes. PgdH1 is membrane associated and linked to the respiratory chain through the quinone pool [126–130]. PgdH2 is the most common enzyme in Gram-negative bacteria and usually present in organisms lacking both PgoX and PgdH1 [131]. Since overexpression of PgdH2 does not overcome the haem auxotrophy of a Δ*pgdH1* mutant strain, PgdH1 and PgdH2 may have different mechanisms [132,133].

In the last step, protoporphyrin IX is converted to haem in a reaction catalysed by protoporphyrin ferrochelatase (PpfC), that promotes insertion of ferrous iron into the protoporphyrin scaffold [134–136]. Some ferrochelatases may contain one [2Fe-2S]$^{2+}$ cluster in the C-terminal region, as first shown for the *Caulobacter crescentus* and *Mycobacterium tuberculosis* enzymes [137]. *In silico* analysis revealed putative Fe-S coordination motifs in ferrochelatases of species belonging to Bacteroidetes, Planctomycetes, Chlamydiae, Verrucomicrobia, Cytophagaceae, and Flavobacteria [137–139], indicating a more spread occurrence of this type of ferrochelatase.

4.3. Regulation of the PPD pathway genes

In *E. coli* and *P. aeruginosa*, the *hmbS*, *uroS* and *pgoX* genes belong to the same operon (Fig. 5). However, this genomic organisation is not conserved in other organisms and the PPD pathway related genes usually occur disperse in the genomes.

The main factors modulating expression of the genes of the PPD pathway are oxidative stress, oxygen, iron, copper and haem availability. In *P. aeruginosa*, the *cgdC* and *cgdH* genes are mainly induced under anaerobic conditions, with *cgdH* being dependent on the transcriptional activators Anr and Dnr, as shown by DNA microarray and RNA-seq experiments [49,140–142]. Under aerobic conditions, there is also a Anr-dependent upregulation of *cgdH* that, however, is ~ 10 times lower than the induction observed under anoxic conditions. This is consistent with the increased level of the *P. aeruginosa gtrR* mRNA under anaerobic conditions, described above, and suggests that in this bacterium the same regulatory system controls several haem biosynthesis genes. In the genomes of some *Pseudomonas* species, Anr boxes are also present in the promoter regions of other haem biosynthetic genes, such as *uroD*, *pbgS*, and *hmbS* [49]. In *P. aeruginosa,* PPD-related genes appear to be regulated by the two-component system AlgZR, with AlgR repressing the *cgdH* expression along the logarithmic and stationary growth phases. However, it remains to be clarified to what type of signal the gene expression is responding and if this regulation occurs directly. Additionally, AlgZR regulates *hmbS* and *uroS*, which are co-transcribed with *algZR* genes [64,65,143]. In this bacterium, mutation of outer membrane haem receptors increases the haem biosynthesis rate, thus suggesting that external haem modulates expression of the haem biosynthesis related genes [46]. In *Neisseria gonorrhoeae,* addition of exogenous copper inhibits haem biosynthesis. As copper-treated cells accumulate coproporphyrin III (an auto-oxidized version of copro'gen III) and the growth defect was suppressed by addition of protoporphyrin IX to the medium, the authors proposed that CgdH is the haem biosynthetic enzyme targeted by copper toxicity [144].

Another example of oxygen dependent transcriptional regulation of the *cgdH* gene was observed in *E. coli*, in which *cgdH* expression was found to be higher under anaerobic conditions and lower under iron starvation, through a not yet known regulatory mechanism [145]. Also, in *Alcaligenes eutrophus, cgdH* was reported to be essential for anaerobic growth and possibly regulated by FNR as its promoter region contains a FNR binding site [146]. In *E. coli*, hydrogen peroxide induces the expression of *cgdC* and *ppfC* through OxyR, possibly to guarantee that sufficient haem is available for production of active catalase and peroxidase detoxifiers [147–149].

There is a general assumption based on experimental observations that ALA supplementation results in enhancement of haem synthesis, and that haem exerts feedback inhibition. This was first observed in *Rhodopseudomonas sphaeroids* and subsequently in *E. coli, P. aeruginosa,* and *Achromobacter metalcaligenes* [150–152]. In particular, *ppfC* was described to be slightly induced in the haem deficient *E. coli gtrR* mutant strain. Although in *E. coli* the *ppfC* gene is slightly upregulated under anaerobic conditions no modification of expression was observed in *fnr*/*anr*/*arcA* mutant strains [30], thus ruling out a role for these transcriptional factors in its regulation.

In the cyanobacterium *Anabaena* sp. PCC7120, iron limitation induces the expression of all haem biosynthetic genes through FurA. This regulator binds to the promoter regions of *pbgS*, *hmbS*, *pgoX*, *ppfC* genes and, overexpression of FurA increases the expression of *uroS*, *uroD*, *pgoX* and *ppfC* genes and decreases expression of *pbgS* and *hmbS*, as shown by qRT-PCR [68].

The cyanobacterium *Synechocystis* sp. PCC 6803 contains the chlorophyll regulator ChlR, a MarR (Multiple Antibiotic Resistance)-like regulator. The regulator controls the operon formed by *cgdH*, *acsFII* and *ho2*. The last two genes encode enzymes involved in the synthesis of chlorophyll *a* and biliverdin, respectively, and are induced under microaerobic conditions. Moreover, this regulation seems to be crucial for the cyanobacterium as a Δ*chlR* strain is unable to grow under microaerobic conditions [153]. *Synechococcus* sp. PCC 7002 ChlR contains an oxygen-sensitive [4Fe-4S]$^{2+/1+}$ cluster as shown by low temperature EPR and Mössbauer spectroscopies. Thus, the binding of holo-ChlR to promoter regions and consequent induction of the target genes occurs only under microaerobic conditions, as confirmed by RNA-seq and YFP transcriptional fusions [154].

4.3.1. Coproporphyrin-dependent pathway

The CPD pathway shares the first reaction (transformation of uro'gen III into copro'gen III catalysed by UroD) with the PPD pathway (Figs. 1 and 4) [155,156]. In the following step, copro'gen oxidase (CgoX) uses oxygen to remove six hydrogen atoms from copro'gen III yielding coproporphyrin III in a similar way to its PPD homolog enzyme (PgoX) [8]. In the CPD pathway, oxidation of copro'gen III, which generates the intermediate coproporphyrin III unique to this pathway, occurs at an earlier stage when compared with the PPD pathway. Interestingly, CgoX can utilise both copro'gen III and proto'gen IX as substrate [157]. Recently, it was proposed that *S. aureus* CgoX is also necessary for nitrate-dependent respiration sustained by haem containing enzymes, as the *ΔcgoX* mutant strain could not grow under anaerobic conditions unless haem was added to the medium [158].

In the next step of the CPD pathway, coproporphyrin ferrochelatase (CpfC) catalyses the insertion of ferrous iron into coproporphyrin III macrocycle to generate coprohaem, an intermediate that is shared with the SHD pathway. Although PpfC and CpfC ferrochelatases have high sequence similarity, only CpfC is able to use both coproporphyrin III and protoporphyrin IX substrates [8]. Interestingly, coprohaem is the cofactor of *D. desulfuricans* bacterioferritins, which shows that, when metallated, some intermediates are used as protein cofactors [159].

In the last step, the propionates of rings A and B of coprohaem are decarboxylated to vinyl groups by coprohaem decarboxylase (ChdC) generating haem [8]. In contrast with the AhbD enzyme of the SHD pathway that converts the same substrate to the same product using an anaerobic mechanism [112], the ChdC enzyme is proposed to use H_2O_2 to catalyse the reaction [160–163].

4.4. Regulation of CPD pathway genes

The organisation of the genes involved in the synthesis of haem via the CPD pathway is not conserved amongst organisms. However, some genes occur clustered in operons. For example, the *uroD* gene is part of an operon that encodes other enzymes involved in haem biosynthesis (Fig. 5). Moreover, *S. aureus*, *B. subtilis* and *Listeria monocytogenes* possess one operon that contains all the genes required to produce uro'gen III from glutamyl-tRNA. On the contrary, in *C. glutamicum*, only *gtrR* and *hmbS* are clustered together in a single operon (Fig. 5).

Information on regulation of the genes encoding CPD pathway-related enzymes is scarce and mainly focused on Gram-positive bacteria. *Listeria monocytogenes* SpxA1 (an oxidative stress sensor and redox regulator of Gram-positive bacteria [164]) activates *uroD* and *cpfC* gene expression in aerobic conditions [165]. However, this regulation is not conserved in all Gram-positive organisms. For example, although *B. subtilis* SpxA represses *katA* expression it has no apparent control on haem biosynthesis genes [166,167].

In *C. glutamicum*, the *cgoX* and *uroD* genes are regulated by intracellular levels of free haem and iron through HrrSA and the diphteria toxin receptor regulator DtxR, respectively [53,168]. HrrSA is activated by haem to repress transcription of *cgoX* and *uroD*, and induces the haem oxygenase (*hmuO*) and the haem efflux pump (*hrtAB*) genes [53]. On the other hand, the HrrSA system is regulated by DtxR that senses iron [169]. High intracellular iron levels trigger the DNA binding activity of DtxR repressing transcription of *hrrA*, thus alleviating repression of haem biosynthesis genes. This is an example of a cross-talk regulation in haem biosynthesis, as HrrA controls haem-related genes in response to both intracellular iron and haem levels [53,170].

Regulation of haem biosynthesis by intracellular metals has also been described in *S. aureus* and *B. subtilis*, albeit by different mechanisms. The *B. subtilis* ferrochelatase CpfC possesses a regulatory metal-binding site that upon ligation to Mg^{2+} increases the enzyme activity [171]. In *S. aureus*, Fe^{2+} binds to the catalytic site of CpfC, but as the iron levels raise Fe^{2+} binds to a second regulatory iron ion site causing inhibition of the ferrochelatase activity, i.e., in this case, iron levels directly modulate the ferrochelatase activity [156,172].

Interestingly, studies of *Listeria monocystogenes* CpfC revealed that the binding of coprohaem, the product of its reaction, stabilizes the protein which may represent a feedback mechanism of post-transcriptional regulation [173].

5. Protein-protein interactions supporting haem biosynthesis pathways

Protein–protein interactions are fundamental in many biological processes. These interactions may serve to modify the kinetic properties of enzymes, act as activators or suppressors of activities, and modify protein specificity. Interactions within protein complexes may also provide substrate channelling, avoiding the intracellular accumulation of highly unstable or toxic molecules, while achieving a more efficient transfer of metabolites. Moreover, several regulatory intracellular processes are based on protein–protein interactions [174,175].

In haem biosynthesis, a tight coordination of the pathway's enzymes is necessary to avoid the generation of highly unstable intermediates. For example, the intermediate hydroxymethylbilane, that is the substrate of UroS, may undergo spontaneous oxidation forming the non-physiological compound uro'gen I. While the haem biosynthesis associated enzymes and their catalytic activities are well documented [8,9,12], the current knowledge on protein–protein interactions (intracellular metabolons) that allow the traffic of the intermediates generated along the haem biosynthesis is very limited, and the little that is known refers mainly to eukaryotes.

The first protein complex proposed to act in haem biosynthesis was suggested for mitochondria [176] and involved a ternary complex formed by copro'gen oxidase, proto'gen oxidase and protoporphyrin ferrochelatase. The complex was deduced based on the cellular localization of copro'gen oxidase that, facing the outer face of the inner mitochondrial membrane, could interact with proto'gen oxidase (also located in the inner mitochondrial membrane). The active site of copro'gen oxidase also faces the cytosolic part, allowing direct interaction with ferrochelatase to channel protoporphyrin IX, which would prevent its toxic accumulation.

Later, Medlock and colleagues showed, by combining co-immunoprecipitation and mass spectrometry analyses, that mitochondria harbours a mega complex composed of proto'gen oxidase, ferrochelatase, aminolaevulinic acid synthase-2 and two iron metabolism related proteins namely, Abcb7 and Abcb10 [177]. Succinyl-CoA synthase is also included in this heteromeric-protein complex through its interaction with AlaS [177]. In the two cases, ferrochelatase constitutes the important key regulatory point of two cellular pathways namely, porphyrin synthesis and iron metabolism, as also shown latter by other authors [178]. Similar protein associations also occur in bacteria, which are described below and indicated in Table 2.

The archaeal *Methanopyrus kandleri* GtrR has a V-shaped structure forming a void that was initially proposed to accommodate the next enzyme of the biosynthetic pathway, namely GsaM [25]. This association may have beneficial effects for cells as the channelling avoids the intracellular diffusion of the highly unstable intermediate glutamate-1-semialdehyde. A GtrR-GsaM complex was shown in *E. coli* by co-immunoprecipitation and gel permeation chromatography experiments [179]. More recently, a similar GtrR-GsaM complex was isolated from cells of *Acinetobacter baumannii* when co-expressing the two proteins [180]. However, formation of this complex decreased the activity of GsaM probably due to a reduced accessibility to the GsaM active site [180].

In the cyanobacterium *Synechocystis* sp. PCC 6803, PgdH2 was isolated bound to haem *b*. The enzyme was also proposed to interact with CgdC based on the accumulation of harderoporphyrinogen (an intermediate of the CgdC reaction), which was observed when a *pgdH2* mutant strain was complemented with *E. coli* PgdH1 [181]. In the cyanobacterium *Synechococcus elongatus*, the highly conserved HtpG protein that responds to different stresses interacts with UroD. The interaction occurs via the N-terminal domain of HtpG, as shown by a yeast two-hybrid system experiment, causing

Table 2
Protein-protein interactions of enzymes involved in bacterial haem biosynthesis.

Protein*	Partner Protein	Function	Organism/References
PpfC	PgoX/ PgdH	Substrate channelling	*T. elongatus* [187]; *V. vulnificus* [188]
GtrR	GsaM	Substrate channelling	*E. coli* [179]; *A. baumannii* [180]
CgdC	PgdH2	Substrate channelling	*Synechocystis* sp. [181]
CpfC	ChdC	Substrate channelling	*S. aureus* [193]
PpfC	Irr	Regulation	*Rhizobiaceae, Bradyrhizobiaceae* and *Rhodobacteraceae* orders [183]
CpfC	Fra	Regulation	*B. subtilis* [189,190]
CpfC	IsdG	Regulation	*S. aureus* [194]
PpfC	PufQ	Regulation	*R. sphaeroides* [186]
UroD	HtpG	Regulation	*Synechococcus* sp. PCC 7002 [182]

* See abbreviations in the text.

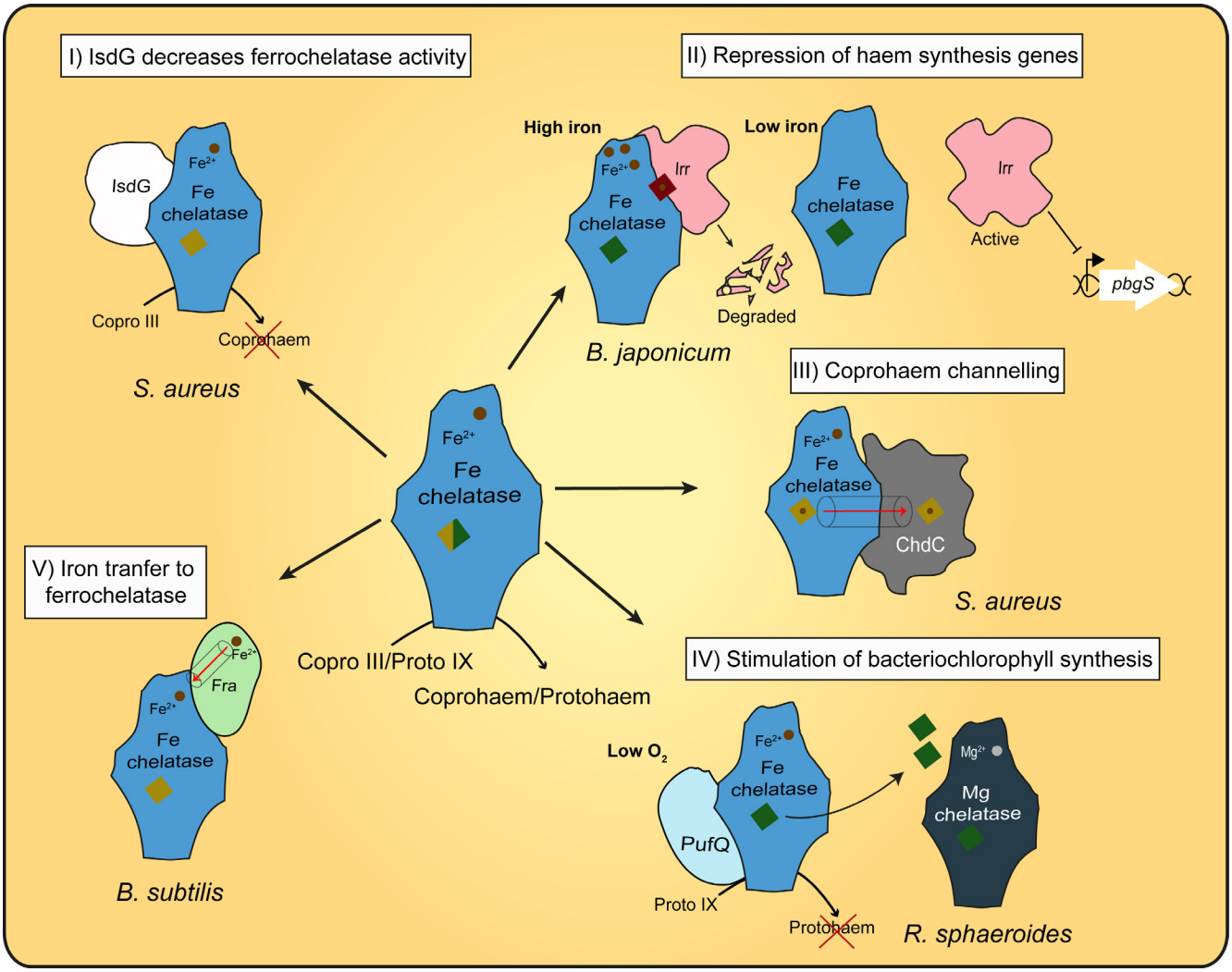

Fig. 6. Protein-protein interactions involving bacterial ferrochelatases. Ferrochelatase converts coproporphyrin III (copro III, dark yellow) or protoporphyrin IX (proto IX, green) to coprohaem or protohaem, respectively. A summary of the several protein interactions involving ferrochelatase described in the literature are schematised. I) in *S. aureus*, IsdG binds to ferrochelatase causing inactivation of the ferrochelatase activity [194]; II) in *B. japonicum*, at high iron concentrations the haem produced by ferrochelatase binds to Irr, triggering Irr degradation; alternatively under low iron levels, free Irr represses the *pbgS* gene of haem biosynthesis [184,185]; III) in *S. aureus*, ChdC interacts with ferrochelatase for substrate channelling of coprohaem that is the product of ferrochelatase [193]; IV) in *R. sphaeroides*, PufQ binds to ferrochelatase and impairs the use of protoporphyrin IX by ferrochelatase, leaving protoporphyrin IX available for magnesium insertion by magnesium chelatase [186]; and V) in *B. subtilis*, frataxin binds to ferrochelatase to facilitate iron transfer [189,190].

inhibition of UroD activity. Additionally, UroD activity is 2.5 times higher in a Δ*htpG* strain than in the wild type strain, and cell extracts overexpressing *htpG* exhibited ~ 3-fold lower UroD activity [182].

Several haem regulatory processes have been described to involve ferrochelatase the last or the penultimate enzyme of the PPD and CPD pathways, respectively (Fig. 6), and one of the best studied mechanisms implicates the alphaproteobacterial iron response regulator Irr that belongs to the Fur protein family [183]. Under iron replete conditions, the haem produced by ferrochelatase binds to Irr causing its degradation. At low iron levels, and consequently low haem content, no degradation occurs and Irr represses the transcription of *pbgS* and *alaS*, thus impairing the formation of toxic porphyrin intermediates [184,185].

More recently, it was reported for the purple photosynthetic bacterium *R. sphaeroides* a new regulatory mechanism of the porphyrin flux at the haem/bacteriochlorophyll branchpoint of the tetrapyrrole biosynthesis that occurs via interaction with ferrochelatase. In this organism, the synthesis of haem and bacteriochlorophylls uses protoporphyrin IX as precursor, which is the substrate of iron and magnesium chelatases. At low oxygen levels, the PufQ expressed from the *pufQBALMX* operon, which encodes the reaction centre light-collecting photosystem complex, sequesters ferrochelatase and provides sufficient protoporphyrin IX for bacteriochlorophyll synthesis, adjusting the metal insertion process into protoporphyrin IX [186].

In the cyanobacterium *Thermosynechococcus elongatus*, ferrochelatase interacts with proto'gen oxidase, as shown by co-immunoprecipitation and co-localisation assays [187].

In *Vibrio vulnificus*, a complex between ferrochelatase and proto'gen dehydrogenase in a 8:8 hetero-hexadecamer arrangement was also described [188].

In *B. subtilis*, in cell lysates CpfC co-purifies with Fra, a homolog of the eukaryotic iron chaperon frataxin [189,190]. Moreover, it has been shown that Fra transfers iron to CpfC, as the protein was able to convert protoporphyrin IX to haem when Fra was loaded with ferrous iron at several concentrations. Iron donation via Fra was assumed based on the observation that a Δ*fra* mutant strain has decreased nitric oxide synthase activity, an enzyme whose activity is haem dependent. Also, hydrogen–deuterium exchange-mass

spectrometry revealed that the two proteins undergo structural rearrangements that may facilitate iron transfer, which suggested that Fra is a specific chaperone of this CpfC [191]. Mutations of the conserved S54 residue in *B. subtilis* CpfC impaired haem synthesis and caused accumulation of coproporphyrin III. Located on the CpfC surface, this S54 residue is proposed to be important for binding of a cell component, e.g., a docking protein that could serve to deliver coproporphyrin III/iron or mobilize haem [192].

In *S. aureus*, a transient interaction between CpfC and ChdC, the last two enzymes of the CPD pathway, may allow substrate channelling of coprohaem [193]. *S. aureus* CpfC interacts with the haem oxygenase IsdG, an enzyme that belongs to the haem uptake system of this pathogen [194]. We showed that the CpfC activity decreases with increase of IsdG concentration, and binding of haem to IsdG stabilizes the protein [194,195]. Therefore, the interaction IsdG-CpfC would serve to impair the endogenous haem synthesis via the CPD pathway, and thus reduce the intracellular toxic haem accumulation. The interaction between ChdC, the last enzyme of the *S. aureus* haem synthesis pathway, with IsdG was also shown by FLIM-FRET experiments, but the physiological meaning of this interaction remains unclear [194].

6. Conclusions

Altogether, the currently published data indicate that sirohaem and haem biosynthesis are modulated mainly by oxygen availability and, in some cases, through feedback inhibition by the final product of the pathway, namely haem. As mentioned above, sirohaem biosynthesis is also sensitive to cellular nitrate and nitrite levels due to the occurrence of these genes in operons encoding nitrate and nitrite reductases, which in some bacteria are itself sirohaem-containing proteins. Also, protein–protein interactions, that regulate cell metabolism and provide the means for substrate channelling that endows a protective environment for highly reactive intermediates, seem to play a role in haem biosynthesis. An example is the formation of a heteromeric complex between PbgS and UroS that would impair the spontaneous formation of the non-physiological uroporphyrin I. However, the network of protein interactions is anticipated to be more extensive. In particular, interactions involving the last enzyme of the CPD pathway, ChdC, are predicted in light of what is already known about ferrochelatase PpfC. Additionally, interactions between the ferrochelatase and haem chaperones are also foreseen.

The multistep sirohaem and haem biosynthesis and the involvement of multiple substrates, that are also needed for other cellular functions, implies an intricate regulation that remains largely unknown. Therefore, new approaches such, as for example, high-throughput and machine learning methodologies will be important to advance our knowledge on haem homeostasis. Recently, Ulijasz and co-workers reported a novel method to identify genes that directly influence the intracellular haem levels. By using a Met-Seq (metabolite-coupled transposon sequencing) methodology that combines a fluorescent haem biosensor, fluorescence-activated cell sorting (FACS), and next-generation sequencing (NGS), they uncovered in *P. aeruginosa* over 80 haem-related genes, most of them involved in metabolic pathways not previously associated with haem, and that include secreted virulence effectors, predicted small RNAs and riboswitches [46]. Also, a machine learning implementation for the prediction and qualitative assignment of binding affinities was recently shown to be successful in identification and qualitative evaluation of haem-binding motifs in protein sequences [196]. A similar methodology could be applied to discover the regulatory processes of haem that sustain the balance between infection, haem uptake, and haem biosynthesis. These studies would provide basic knowledge of importance for the establishment of new targets for antimicrobial therapies for resistant infections, as well as for biotechnology purposes aimed at the production of fine chemicals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by Fundação para a Ciência e Tecnologia (Portugal) by grant PTDC/BIA-BQM/28642/2017 and through R&D unit LISBOA-01-0145-FEDER007660 (MostMicro) cofounded by FCT/MCTES and FEDER funds under the PT2020 Partnership Agreement. We also acknowledge funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 810856. JB is recipient of the MSCA-IF-2019 Individual Fellowship H2020-WF-02-2019, 101003441.

References

[1] M. Warren, A. Smith, eds., Tetrapyrroles: Birth, Life and Death, Springer-Verlag, New York, 2009. https://doi.org/10.1007/978-0-387-78518-9.
[2] H. Schlicke, A. Richter, M. Rothbart, P. Brzezowski, B. Hedtke, B. Grimm, Function of tetrapyrroles, regulation of tetrapyrrole metabolism and methods for analyses of tetrapyrroles, Procedia Chem. 14 (2015) 171–175, https://doi.org/10.1016/j.proche.2015.03.025.
[3] L. Thöny-Meyer, Biogenesis of respiratory cytochromes in bacteria, Microbiol Mol Biol Rev. 61 (3) (1997) 337–376, https://doi.org/10.1128/mmbr.61.3.337-376.1997.
[4] S.M. Mense, L. Zhang, Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases, Cell Res. 16 (8) (2006) 681–692, https://doi.org/10.1038/sj.cr.7310086.
[5] M.H. Vos, Ultrafast dynamics of ligands within heme proteins, Biochim. Biophys. Acta (BBA) – Bioenergetics. 1777 (1) (2008) 15–31, https://doi.org/10.1016/j.bbabio.2007.10.004.
[6] G. Layer, M.J. Warren, Biosynthesis of siroheme, cofactor F430 and heme *d1*, in: Handbook of Porphyrin Science, World Scientific Publishing Company, 2012: pp. 111–138. https://doi.org/10.1142/9789814335508_0015.
[7] M.R. O'Brian, L. Thöny-Meyer, Biochemistry, regulation and genomics of haem biosynthesis in prokaryotes, Adv Microb Physiol. 46 (2002) 257–318, https://doi.org/10.1016/s0065-2911(02)46006-7.
[8] H.A. Dailey, T.A. Dailey, S. Gerdes, D. Jahn, M. Jahn, M.R. O'Brian, M.J. Warren, Prokaryotic heme biosynthesis: multiple pathways to a common essential product, Microbiol. Mol. Biol. Rev. 81 (2017) 1–62, https://doi.org/10.1128/MMBR.00048-16.
[9] G. Layer, J. Reichelt, D. Jahn, D.W. Heinz, Structure and function of enzymes in heme biosynthesis, Protein Sci. 19 (6) (2010) 1137–1161, https://doi.org/10.1002/pro.405.
[10] D.A. Bryant, C.N. Hunter, M.J. Warren, Biosynthesis of the modified tetrapyrroles-the pigments of life, J. Biol. Chem. 295 (20) (2020) 6888–6925, https://doi.org/10.1074/jbc.REV120.006194.
[11] S.I. Beale, T.P. Begley, Biosynthesis of hemes, EcoSal Plus. 2 (2) (2007), https://doi.org/10.1128/ecosalplus.3.6.3.11.
[12] G. Layer, Heme biosynthesis in prokaryotes, Biochim. Biophys. Acta Mol. Cell Res. 2021 (1868) 1–13, https://doi.org/10.1016/j.bbamcr.2020.118861.
[13] T. Fan, B. Grimm, G. Layer, Chapter four – porphyrin and heme synthesis, in: B. Grimm (Ed.), Advances in Botanical Research, Academic Press, 2019, pp. 89–131, https://doi.org/10.1016/bs.abr.2019.06.001.
[14] A.I. Celis, J.L. DuBois, Making and breaking heme, Curr. Opin. Struct. Biol. 59 (2019) 19–28, https://doi.org/10.1016/j.sbi.2019.01.006.
[15] P. Brzezowski, A.S. Richter, B. Grimm, Regulation and function of tetrapyrrole biosynthesis in plants and algae, Biochim. Biophys. Acta 1847 (9) (2015) 968–985, https://doi.org/10.1016/j.bbabio.2015.05.007.
[16] J.E. Choby, E.P. Skaar, Heme synthesis and acquisition in bacterial pathogens, J. Mol. Biol. 428 (17) (2016) 3408–3428, https://doi.org/10.1016/j.jmb.2016.03.018.
[17] L.L. Anzaldi, E.P. Skaar, Overcoming the heme paradox: heme toxicity and tolerance in bacterial pathogens, Infect. Immun. 78 (12) (2010) 4977–4989, https://doi.org/10.1128/IAI.00613-10.
[18] M. Schobert, D. Jahn, Regulation of heme biosynthesis in non-phototrophic bacteria, J. Mol. Microbiol. Biotechnol. 4 (2002) 287–294, PMID: 11931560.
[19] K. Nakahigashi, K. Nishimura, K. Miyamoto, H. Inokuchi, Photosensitivity of a protoporphyrin-accumulating, light-sensitive mutant (*visA*) of *Escherichia coli* K-12, Proc. Natl. Acad. Sci. U.S.A. 88 (23) (1991) 10520–10524, https://doi.org/10.1073/pnas.88.23.10520.

[20] A.-L. Kaufholz, G.A. Hunter, G.C. Ferreira, T. Lendrihas, V. Hering, G. Layer, M. Jahn, D. Jahn, Aminolaevulinic acid synthase of *Rhodobacter capsulatus:* high-resolution kinetic investigation of the structural basis for substrate binding and catalysis, Biochem. J. 451 (2013) 205–216, https://doi.org/10.1042/BJ20121041.
[21] D.L. Nandi, δ-Aminolevulinic acid synthase of *Rhodopseudomonas spheroides:* binding of pyridoxal phosphate to the enzyme, Arch. Biochem. Biophys. 188 (2) (1978) 266–271, https://doi.org/10.1016/S0003-9861(78)80008-3.
[22] H. Ge, X. Lv, J. Fan, Y. Gao, M. Teng, L. Niu, Crystal structure of Glutamate1-semialdehyde aminotransferase from *Bacillus subtilis* with bound pyridoxamine-5′-phosphate, Biochem. Biophys. Res. Commun. 402 (2) (2010) 356–360, https://doi.org/10.1016/j.bbrc.2010.10.033.
[23] M. Hennig, B. Grimm, R. Contestabile, R.A. John, J.N. Jansonius, Crystal structure of glutamate-1-semialdehyde aminomutase: An α2-dimeric vitamin B6-dependent enzyme with asymmetry in structure and active site reactivity, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 4866–4871, https://doi.org/10.1073/pnas.94.10.4866.
[24] J. Moser, S. Lorenz, C. Hubschwerlen, A. Rompf, D. Jahn, *Methanopyrus kandleri* glutamyl-tRNA reductase*, J Biol Chem. 274 (43) (1999) 30679–30685, https://doi.org/10.1074/jbc.274.43.30679.
[25] J. Moser, W.-D. Schubert, V. Beier, I. Bringemeier, D. Jahn, D.W. Heinz, V-shaped structure of glutamyl-tRNA reductase, the first enzyme of tRNA-dependent tetrapyrrole biosynthesis, EMBO J. 20 (2001) 6583–6590, https://doi.org/10.1093/emboj/20.23.6583.
[26] S. Schauer, S. Chaturvedi, L. Randau, J. Moser, M. Kitabatake, S. Lorenz, E. Verkamp, W.-D. Schubert, T. Nakayashiki, M. Murai, K. Wall, H.-U. Thomann, D.W. Heinz, H. Inokuchi, D. Söll, D. Jahn, *Escherichia coli* glutamyl-tRNA reductase: trapping the thioester intermediate, J Biol Chem. 277 (50) (2002) 48657–48663, https://doi.org/10.1074/jbc.M206924200.
[27] S. Darie, R.P. Gunsalus, Effect of heme and oxygen availability on *hemA* gene expression in *Escherichia coli*: role of the *fnr*, *arcA*, and *himA* gene products, J. Bacteriol. 176 (17) (1994) 5270–5276, https://doi.org/10.1128/jb.176.17.5270-5276.1994.
[28] S.B. Melville, R.P. Gunsalus, Isolation of an oxygen-sensitive FNR protein of *Escherichia coli*: interaction at activator and repressor sites of FNR-controlled genes, Proc. Natl. Acad. Sci. U.S.A. 93 (3) (1996) 1226–1231, https://doi.org/10.1073/pnas.93.3.1226.
[29] P. Choi, L. Wang, C.D. Archer, T. Elliott, Transcription of the glutamyl-tRNA reductase (*hemA*) gene in *Salmonella typhimurium* and *Escherichia coli*: role of the *hemA* P1 promoter and the *arcA* gene product, J. Bacteriol. 178 (3) (1996) 638–646, https://doi.org/10.1128/jb.178.3.638-646.1996.
[30] P.M. McNicholas, G. Javor, S. Darie, R.P. Gunsalus, Expression of the heme biosynthetic pathway genes *hemCD*, *hemH*, *hemM*, and *hemA* of *Escherichia coli*, FEMS Microbiol. Lett. 146 (1997) 143–148, https://doi.org/10.1111/j.1574-6968.1997.tb10184.x.
[31] S. Iuchi, Z. Matsuda, T. Fujiwara, E.C.C. Lin, The *arcB* gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the *arc* modulon, Mol. Microbiol. 4 (5) (1990) 715–727, https://doi.org/10.1111/j.1365-2958.1990.tb00642.x.
[32] S. Iuchi, E.C. Lin, *arcA* (dye), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways, Proc. Natl. Acad. Sci. U.S.A. 85 (6) (1988) 1888–1892, https://doi.org/10.1073/pnas.85.6.1888.
[33] G. Unden, J. Schirawski, The oxygen-responsive transcriptional regulator FNR of *Escherichia coli*: the search for signals and reactions, Mol. Microbiol. 25 (1997) 205–210, https://doi.org/10.1046/j.1365-2958.1997.4731841.x.
[34] A.M. Jones, T. Elliott, A purified mutant HemA protein from *Salmonella enterica serovar Typhimurium* lacks bound heme and is defective for heme-mediated regulation *in vivo*, FEMS Microbiol. Lett. 307 (2010) 41–47, https://doi.org/10.1111/j.1574-6968.2010.01967.x.
[35] L. Wang, M. Elliott, T. Elliott, Conditional stability of the HemA protein (glutamyl-tRNA reductase) regulates heme biosynthesis in *Salmonella typhimurium*, J. Bacteriol. 181 (4) (1999) 1211–1219, https://doi.org/10.1128/JB.181.4.1211-1219.1999.
[36] L.Y. Wang, L. Brown, M. Elliott, T. Elliott, Regulation of heme biosynthesis in *Salmonella typhimurium*: activity of glutamyl-tRNA reductase (HemA) is greatly elevated during heme limitation by a mechanism which increases abundance of the protein, J. Bacteriol. 179 (9) (1997) 2907–2914, https://doi.org/10.1128/jb.179.9.2907-2914.1997.
[37] C.K. Smith, T.A. Baker, R.T. Sauer, Lon and Clp family proteases and chaperones share homologous substrate-recognition domains, Proc. Natl. Acad. Sci. U.S.A. 96 (12) (1999) 6678–6682, https://doi.org/10.1073/pnas.96.12.6678.
[38] L. Chen, L. Keramati, J.D. Helmann, Coordinate regulation of *Bacillus subtilis* peroxide stress genes by hydrogen peroxide and metal ions, Proc. Natl. Acad. Sci. U.S.A. 92 (18) (1995) 8190–8194, https://doi.org/10.1073/pnas.92.18.8190.
[39] M. Fuangthong, A.F. Herbig, N. Bsat, J.D. Helmann, Regulation of the *Bacillus subtilis fur* and *perR* genes by PerR: not all members of the PerR regulon are peroxide inducible, J. Bacteriol. 184 (12) (2002) 3276–3286, https://doi.org/10.1128/JB.184.12.3276-3286.2002.
[40] I. Schroder, P. Johansson, L. Rutberg, L. Hederstedt, The *hemX* gene of the *Bacillus subtilis hemAXCDBL* operon encodes a membrane protein, negatively affecting the steady-state cellular concentration of HemA (glutamyl-tRNA reductase), Microbiology 140 (4) (1994) 731–740, https://doi.org/10.1099/00221287-140-4-731.
[41] P. Johansson, L. Hederstedt, Organization of genes for tetrapyrrole biosynthesis in Gram-positive bacteria, Microbiology 145 (1999) 529–538, https://doi.org/10.1099/13500872-145-3-529.
[42] P. Johansson, Genetics of tetrapyrrole synthesis in Gram-positive bacteria, Thesis, Lund University, 1999. http://lup.lub.lu.se/record/39296.
[43] J.E. Choby, C.M. Grunenwald, A.I. Celis, S.Y. Gerdes, J.L. DuBois, E.P. Skaar, K.A. Kline, A. Richardson, D. Heinrichs, *Staphylococcus aureus* HemX modulates Glutamyl-tRNA Reductase abundance to regulate heme biosynthesis, MBio. 9 (1) (2018), https://doi.org/10.1128/mBio.02287-17.
[44] I. Delic-Attree, B. Toussaint, P.M. Vignais, Cloning and sequence analyses of the genes coding for the integration host factor (IHF) and HU proteins of *Pseudomonas aeruginosa*, Gene 154 (1) (1995) 61–64, https://doi.org/10.1016/0378-1119(94)00875-S.
[45] I. Delic-Attree, B. Toussaint, A. Froger, J.C. Willison, P.M. Vignais, Isolation of an IHF-deficient mutant of a *Pseudomonas aeruginosa* mucoid isolate and evaluation of the role of IHF in *algD* gene expression, Microbiology 142 (10) (1996) 2785–2793, https://doi.org/10.1099/13500872-142-10-2785.
[46] D.G. Glanville, C. Mullineaux-Sanders, C.J. Corcoran, B.T. Burger, S. Imam, T.J. Donohue, A.T. Ulijasz, M.F. Traxler, A high-throughput method for identifying novel genes that influence metabolic pathways reveals new iron and heme regulation in *Pseudomonas aeruginosa*, MSystems 6 (1) (2021), https://doi.org/10.1128/mSystems.00933-20.
[47] R. Krieger, A. Rompf, M. Schobert, D. Jahn, The *Pseudomonas aeruginosa hemA* promoter is regulated by Anr, Dnr, NarL and Integration Host Factor, Mol. Gen. Genomics 267 (3) (2002) 409–417, https://doi.org/10.1007/s00438-002-0672-7.
[48] E. Sevilla, M.T. Bes, A. González, M.L. Peleato, M.F. Fillat, Redox-based transcriptional regulation in prokaryotes: revisiting model mechanisms, Antioxid. Redox Signal. 30 (13) (2019) 1651–1696, https://doi.org/10.1089/ars.2017.7442.
[49] P.M. Tribelli, A.M. Lujan, A. Pardo, J.G. Ibarra, D. Fernández Do Porto, A. Smania, N.I. López, Core regulon of the global anaerobic regulator Anr targets central metabolism functions in *Pseudomonas* species, Sci. Rep. 9 (1) (2019), https://doi.org/10.1038/s41598-019-45541-0.
[50] L.A. Bibb, C.A. Kunkle, M.P. Schmitt, The ChrA-ChrS and HrrA-HrrS signal transduction systems are required for activation of the *hmuO* promoter and repression of the *hemA* promoter in *Corynebacterium diphtheriae*, Infect. Immun. 75 (5) (2007) 2421–2431, https://doi.org/10.1128/IAI.01821-06.
[51] M.P. Schmitt, Identification of a two-component signal transduction system from *Corynebacterium diphtheriae* that activates gene expression in response to the presence of heme and hemoglobin, J. Bacteriol. 181 (17) (1999) 5330–5340, https://doi.org/10.1128/JB.181.17.5330-5340.1999.
[52] J.M. Burgos, M.P. Schmitt, T.M. Henkin, The ChrSA and HrrSA two-component systems are required for transcriptional regulation of the *hemA* promoter in *Corynebacterium diphtheriae*, J. Bacteriol. 198 (18) (2016) 2419–2430, https://doi.org/10.1128/JB.00339-16.
[53] J. Frunzke, C. Gätgens, M. Brocker, M. Bott, Control of heme homeostasis in *Corynebacterium glutamicum* by the two-component system HrrSA, J. Bacteriol. 193 (5) (2011) 1212–1221, https://doi.org/10.1128/JB.01130-10.
[54] H. Ikushiro, A. Nagami, T. Takai, T. Sawai, Y. Shimeno, H. Hori, I. Miyahara, N. Kamiya, T. Yano, Heme-dependent inactivation of 5-aminolevulinate synthase from *Caulobacter crescentus*, Sci. Rep. 8 (2018) 1–13, https://doi.org/10.1038/s41598-018-32591-z.
[55] P.T. Erskine, R. Newbold, J. Roper, A. Coker, M.J. Warren, P.M. Shoolingin-Jordan, S.P. Wood, J.B. Cooper, The schiff base complex of yeast 5-aminolaevulinic acid dehydratase with laevulinic acid, Protein Sci. 8 (6) (1999) 1250–1256, https://doi.org/10.1110/(ISSN)1469-896X10.1110/ps.8.6.1250.
[56] N. Frankenberg, P.T. Erskine, J.B. Cooper, P.M. Shoolingin-Jordan, D. Jahn, D.W. Heinz, High resolution crystal structure of a Mg^{2+}-dependent porphobilinogen synthase, J. Mol. Biol. 289 (3) (1999) 591–602, https://doi.org/10.1006/jmbi.1999.2808.
[57] E.K. Jaffe, The porphobilinogen synthase family of metalloenzymes, Acta Cryst. D. 56 (2) (2000) 115–128, https://doi.org/10.1107/S0907444999014894.
[58] D.W. Bollivar, C. Clauson, R. Lighthall, S. Forbes, B. Kokona, R. Fairman, L. Kundrat, E.K. Jaffe, *Rhodobacter capsulatus* porphobilinogen synthase, a high activity metal ion independent hexamer, BMC Biochem. 5 (2004) 17, https://doi.org/10.1186/1471-2091-5-17.
[59] P.M. Jordan, A. Berry, Mechanism of action of porphobilinogen deaminase. The participation of stable enzyme substrate covalent intermediates between porphobilinogen and the porphobilinogen deaminase from Rhodopseudomonas spheroides, Biochem. J. 195 (1981) 177–181, https://doi.org/10.1042/bj1950177.
[60] P.M. Jordan, J.S. Seehra, The biosynthesis of uroporphyrinogen III: Order of assembly of the four porphobilinogen molecules in the formation of the tetrapyrrole ring, FEBS Lett. 104 (1979) 364–366, https://doi.org/10.1016/0014-5793(79)80853-4.
[61] H.L. Schubert, J.D. Phillips, A. Heroux, C.P. Hill, Structure and mechanistic implications of a uroporphyrinogen III synthase–product complex, Biochemistry 47 (33) (2008) 8648–8655, https://doi.org/10.1021/bi800635y.
[62] P.J. Silva, M.J. Ramos, Comparative density functional study of models for the reaction mechanism of uroporphyrinogen III synthase, J. Phys. Chem. B. 112 (10) (2008) 3144–3148, https://doi.org/10.1021/jp076235f.
[63] W.M. Stark, M.G. Baker, P.R. Raithby, F.J. Leeper, A.R. Battersby, The spiro intermediate proposed for biosynthesis of the natural porphyrins: synthesis

and properties of its macrocycle, J. Chem. Soc., Chem. Commun. (1985) 1294–1296, https://doi.org/10.1039/C39850001294.
[64] A.S. Little, Y. Okkotsu, A.A. Reinhart, F.H. Damron, M. Barbier, B. Barrett, A.G. Oglesby-Sherrouse, J.B. Goldberg, W.L. Cody, M.J. Schurr, M.L. Vasil, M.J. Schurr, R.J. Collier, D. Wozniak, G. Pier, *Pseudomonas aeruginosa* AlgR phosphorylation status differentially regulates pyocyanin and pyoverdine production, MBio 9 (1) (2018), https://doi.org/10.1128/mBio.02318-17.
[65] C.L. Pritchett, A.S. Little, Y. Okkotsu, A. Frisk, W.L. Cody, C.R. Covey, M.J. Schurr, G.A. O'Toole, Expression analysis of the *Pseudomonas aeruginosa* AlgZR two-component regulatory system, J Bacteriol. 197 (4) (2015) 736–748, https://doi.org/10.1128/JB.02290-14.
[66] T. Uchida, T. Funamizu, M. Chen, Y. Tanaka, K. Ishimori, Heme binding to porphobilinogen deaminase from *Vibrio cholerae* decelerates the formation of 1-Hydroxymethylbilane, ACS Chem Biol. 13 (3) (2018) 750–760, https://doi.org/10.1021/acschembio.7b009341.
[67] A. Pinochet-Barros, J.D. Helmann, Redox sensing by Fe^{2+} in bacterial Fur family metalloregulators, Antioxid. Redox Signal. 29 (18) (2018) 1858–1871, https://doi.org/10.1089/ars.2017.7359.
[68] A. González, M.T. Bes, A. Valladares, M.L. Peleato, M.F. Fillat, FurA is the master regulator of iron homeostasis and modulates the expression of tetrapyrrole biosynthesis genes in *Anabaena sp.* PCC 7120, Environ. Microbiol. 14 (12) (2012) 3175–3187, https://doi.org/10.1111/emi.2012.14.issue-12.
[69] M.J. Warren, N.J. Stolowich, P.J. Santander, C.A. Roessner, B.A. Sowa, A.I. Scott, Enzymatic synthesis of dihydrosirohydrochlorin (precorrin-2) and of a novel pyrrocorphin by uroporphyrinogen III methylase, FEBS Lett. 261 (1990) 76–80, https://doi.org/10.1016/0014-5793(90)80640-5.
[70] M.J. Warren, E. Raux, H.L. Schubert, J.C. Escalante-Semerena, The biosynthesis of adenosylcobalamin (vitamin B12), Nat Prod Rep. 19 (2002) 390–412, https://doi.org/10.1039/b108967f.
[71] S. Storbeck, J. Walther, J. Müller, V. Parmar, H.M. Schiebel, D. Kemken, T. Dülcks, M.J. Warren, G. Layer, The *Pseudomonas aeruginosa nirE* gene encodes the S-adenosyl-L-methionine-dependent uroporphyrinogen III methyltransferase required for heme *d1* biosynthesis, FEBS J. 276 (2009) 5973–5982, https://doi.org/10.1111/j.1742-4658.2009.07306.x.
[72] E. Raux, H.K. Leech, R. Beck, H.L. Schubert, P.J. Santander, C.A. Roessner, A.I. Scott, J.H. Martens, D. Jahn, C. Thermesr, A. Rambach, M.J. Warren, Identification and functional analysis of enzymes required for precorrin-2 dehydrogenation and metal ion insertion in the biosynthesis of sirohaem and cobalamin in *Bacillus megaterium*, Biochem. J. 370 (2003) 505–516, https://doi.org/10.1042/BJ20021443.
[73] M.A.M. Videira, S.A.L. Lobo, F.L. Sousa, L.M. Saraiva, Identification of the sirohaem biosynthesis pathway in *Staphylococcus aureus*, FEBS J. 287 (8) (2020) 1537–1553, https://doi.org/10.1111/febs.15091.
[74] M.J. Warren, E.L. Bolt, C.A. Roessner, A.I. Scott, J.B. Spencer, S.C. Woodcock, Gene dissection demonstrates that the *Escherichia coli cysG* gene encodes a multifunctional protein, Biochem. J. 302 (1994) 837–844, https://doi.org/10.1042/bj3020837.
[75] S.C. Woodcock, E. Raux, F. Levillayer, C. Thermes, A. Rambach, M.J. Warren, Effect of mutations in the transmethylase and dehydrogenase/chelatase domains of sirohaem synthase (CysG) on sirohaem and cobalamin biosynthesis, Biochem. J. 330 (1998) 121–129, https://doi.org/10.1042/bj3300121.
[76] M.E. Stroupe, H.K. Leech, D.S. Daniels, M.J. Warren, E.D. Getzoff, CysG structure reveals tetrapyrrole-binding features and novel regulation of siroheme biosynthesis, Nat. Struct. Biol. 10 (12) (2003) 1064–1073, https://doi.org/10.1038/nsb1007.
[77] J.M. Pennington, M. Kemp, L. McGarry, Y. Chen, M.E. Stroupe, Siroheme synthase orients substrates for dehydrogenase and chelatase activities in a common active site, Nat. Commun. 11 (2020) 1–11, https://doi.org/10.1038/s41467-020-14722-1.
[78] M.M. Kolko, L.A. Kapetanovich, J.G. Lawrence, Alternative pathways for siroheme synthesis in *Klebsiella aerogenes*, J. Bacteriol. 183 (1) (2001) 328–335, https://doi.org/10.1128/JB.183.1.328-335.2001.
[79] F. Blanche, L. Debussche, D. Thibaut, J. Crouzet, B. Cameron, Purification and characterization of S-adenosyl-L-methionine: uroporphyrinogen III methyltransferase from *Pseudomonas denitrificans*, J. Bacteriol. 171 (8) (1989) 4222–4231, https://doi.org/10.1128/jb.171.8.4222-4231.1989.
[80] S.A.L. Lobo, A. Brindley, M.J. Warren, L.M. Saraiva, Functional characterization of the early steps of tetrapyrrole biosynthesis and modification in *Desulfovibrio vulgaris Hildenborough*, Biochem. J. 420 (2009) 317–325, https://doi.org/10.1042/BJ20090151.
[81] P.J. Anderson, B. Entsch, D.B. McKay, A gene, *cobA + hemD*, from *Selenomonas ruminantium* encodes a bifunctional enzyme involved in the synthesis of vitamin B12, Gene 281 (1-2) (2001) 63–70, https://doi.org/10.1016/S0378-1119(01)00820-4.
[82] H.L. Schubert, R.S. Rose, H.K. Leech, A.A. Brindley, C.P. Hill, S.E.J. Rigby, M.J. Warren, Structure and function of SirC from *Bacillus megaterium*: a metal-binding precorrin-2 dehydrogenase, Biochem. J. 415 (2008) 257–263, https://doi.org/10.1042/BJ20080785.
[83] H.L. Schubert, E. Raux, A.A. Brindley, H.K. Leech, K.S. Wilson, C.P. Hill, M.J. Warren, The structure of *Saccharomyces cerevisiae* Met8p, a bifunctional dehydrogenase and ferrochelatase, EMBO J. 21 (2002) 2068–2075, https://doi.org/10.1093/emboj/21.9.2068.
[84] E. Raux, T. McVeigh, S.E. Peters, T. Leustek, M.J. Warren, The role of *Saccharomyces cerevisiae* Met1p and Met8p in sirohaem and cobalamin biosynthesis, Biochem J. 338 (1999) 701–708, https://doi.org/10.1042/bj3380701.
[85] S. Bali, S. Rollauer, P. Roversi, E. Raux-Deery, S.M. Lea, M.J. Warren, S.J. Ferguson, Identification and characterization of the 'missing' terminal enzyme for siroheme biosynthesis in α-proteobacteria, Mol. Microbiol. 92 (1) (2014) 153–163, https://doi.org/10.1111/mmi.2014.92.issue-110.1111/mmi.12542.
[86] T. Fujishiro, Y. Shimada, R. Nakamura, M. Ooi, Structure of sirohydrochlorin ferrochelatase SirB: the last of the structures of the class II chelatase family, Dalton Trans. 48 (18) (2019) 6083–6090, https://doi.org/10.1039/C8DT04727H.
[87] S.A.L. Lobo, M.A.M. Videira, I. Pacheco, M.N. Wass, M.J. Warren, M. Teixeira, P. M. Matias, C.V. Romão, L.M. Saraiva, *Desulfovibrio vulgaris* CbiKP cobaltochelatase: evolution of a haem binding protein orchestrated by the incorporation of two histidine residues, Environ. Microbiol. 19 (1) (2017) 106–118, https://doi.org/10.1111/1462-2920.13479.
[88] Célia.V. Romão, D. Ladakis, S.A.L. Lobo, M.A. Carrondo, A.A. Brindley, E. Deery, P.M. Matias, R.W. Pickersgill, L.M. Saraiva, M.J. Warren, Evolution in a family of chelatases facilitated by the introduction of active site asymmetry and protein oligomerization, Proc. Natl. Acad. Sci. U.S.A. 108 (1) (2011) 97–102, https://doi.org/10.1073/pnas.1014298108.
[89] E. Raux-Deery, H.K. Leech, K.-A. Nakrieko, K.J. McLean, A.W. Munro, P. Heathcote, S.E.J. Rigby, A.G. Smith, M.J. Warren, Identification and characterization of the terminal enzyme of siroheme biosynthesis from *Arabidopsis thaliana*: a plastid-located sirohydrochlorin ferrochelatase containing a 2Fe-2S center, J. Biol. Chem. 280 (6) (2005) 4713–4721, https://doi.org/10.1074/jbc.M411360200.
[90] K. Saha, M.E. Webb, S.E.J. Rigby, H.K. Leech, M.J. Warren, A.G. Smith, Characterization of the evolutionarily conserved iron-sulfur cluster of sirohydrochlorin ferrochelatase from *Arabidopsis thaliana*, Biochem. J. 444 (2012) 227–237, https://doi.org/10.1042/BJ20111993.
[91] S.A.L. Lobo, A.A. Brindley, C.V. Romão, H.K. Leech, M.J. Warren, L.M. Saraiva, Two distinct roles for two functional cobaltochelatases (CbiK) in *Desulfovibrio vulgaris* Hildenborough, Biochemistry 47 (21) (2008) 5851–5857, https://doi.org/10.1021/bi800342c.
[92] C. Robin, F. Blanche, L. Cauchois, B. Cameron, M. Couder, J. Crouzet, Primary structure, expression in *Escherichia coli*, and properties of S-adenosyl-L-methionine:uroporphyrinogen III methyltransferase from *Bacillus megaterium*, J. Bacteriol. 173 (15) (1991) 4893–4896, https://doi.org/10.1128/jb.173.15.4893-4896.1991.
[93] H.K. Leech, E. Raux-Deery, P. Heathcote, M.J. Warren, Production of cobalamin and sirohaem in *Bacillus megaterium*: an investigation into the role of the branchpoint chelatases sirohydrochlorin ferrochelatase (SirB) and sirohydrochlorin cobalt chelatase (CbiX), Biochem. Soc. Trans. 30 (2002) 610–613, https://doi.org/10.1042/bst0300610.
[94] S. Fuchs, J. Pané-Farré, C. Kohler, M. Hecker, S. Engelmann, Anaerobic gene expression in *Staphylococcus aureus*, J. Bacteriol. 189 (11) (2007) 4275–4289, https://doi.org/10.1128/JB.00081-07.
[95] P.R. Wright, A.S. Richter, K. Papenfort, M. Mann, J. Vogel, W.R. Hess, R. Backofen, J. Georg, Comparative genomics boosts target prediction for bacterial small RNAs, Proc. Natl. Acad. Sci. U.S.A. 110 (37) (2013) E3487–E3496, https://doi.org/10.1073/pnas.1303248110.
[96] T. Belyaeva, L. Griffiths, S. Minchin, J. Cole, S. Busby, The *Escherichia coli cysG* promoter belongs to the "extended -10" class of bacterial promoters, Biochem. J. 296 (3) (1993) 851–857, https://doi.org/10.1042/bj2960851.
[97] B.S. Goldman, J.R. Roth, Genetic structure and regulation of the *cysG* gene in *Salmonella typhimurium*, J. Bacteriol. 175 (5) (1993) 1457–1466, https://doi.org/10.1128/jb.175.5.1457-1466.1993.
[98] K.L. Tyson, J.A. Cole, S.J.W. Busby, Nitrite and nitrate regulation at the promoters of two *Escherichia coli* operons encoding nitrite reductase: identification of common target heptamers for both NarP- and NarL-dependent regulation, Mol. Microbiol. 13 (6) (1994) 1045–1055, https://doi.org/10.1111/j.1365-2958.1994.tb00495.x.
[99] D.F. Browning, J.A. Cole, S.J.W. Busby, Transcription activation by remodelling of a nucleoprotein assembly: the role of NarL at the FNR-dependent *Escherichia coli nir* promoter, Mol. Microbiol. 53 (2004) 203–215, https://doi.org/10.1111/j.1365-2958.2004.04104.x.
[100] E. Massé, F.E. Escorcia, S. Gottesman, Coupled degradation of a small regulatory RNA and its mRNA targets in *Escherichia coli*, Genes Dev. 17 (2003) 2374–2383, https://doi.org/10.1101/gad.1127103.
[101] N.F.W. Saunders, E.N.G. Houben, S. Koefoed, S. de Weert, W.N.M. Reijnders, H. V. Westerhoff, A.P.N. De Boer, R.J.M. Van Spanning, Transcription regulation of the nir gene cluster encoding nitrite reductase of *Paracoccus denitrificans* involves NNR and NirI, a novel type of membrane protein, Mol. Microbiol. 34 (1) (1999) 24–36, https://doi.org/10.1046/j.1365-2958.1999.01563.x.
[102] R.J.M. Van Spanning, E. Houben, W.N.M. Reijnders, S. Spiro, H.V. Westerhoff, N. Saunders, Nitric oxide is a signal for NNR-mediated transcription activation in *Paracoccus denitrificans*, J. Bacteriol. 181 (13) (1999) 4129–4132, https://doi.org/10.1128/JB.181.13.4129-4132.1999.
[103] J. Vévodová, R.M. Graham, E. Raux, H.L. Schubert, D.I. Roper, A.A. Brindley, A. Ian Scott, C.A. Roessner, N.P.J. Stamford, M. Elizabeth Stroupe, E.D. Getzoff, M. J. Warren, K.S. Wilson, Structure/function studies on a S-adenosyl-L-methionine-dependent uroporphyrinogen III C methyltransferase (SUMT), a key regulatory enzyme of tetrapyrrole biosynthesis, J. Mol. Biol. 344 (2) (2004) 419–433, https://doi.org/10.1016/j.jmb.2004.09.020.

[104] A. Kamps, S. Achebach, I. Fedtke, G. Unden, F. Götz, Staphylococcal NreB: an O2-sensing histidine protein kinase with an O2-labile iron–sulphur cluster of the FNR type, Mol. Microbiol. 52 (2004) 713–723, https://doi.org/10.1111/j.1365-2958.2004.04024.x.
[105] S. Schlag, S. Fuchs, C. Nerz, R. Gaupp, S. Engelmann, M. Liebeke, M. Lalk, M. Hecker, F. Gøtz, Characterization of the oxygen-responsive NreABC regulon of *Staphylococcus aureus*, J. Bacteriol. 190 (23) (2008) 7847–7858, https://doi.org/10.1128/JB.00905-08.
[106] M.M. Nakano, T. Hoffmann, Y. Zhu, D. Jahn, Nitrogen and oxygen regulation of *Bacillus subtilis nasDEF* encoding NADH-dependent nitrite reductase by TnrA and ResDE, J. Bacteriol. 180 (20) (1998) 5344–5350, https://doi.org/10.1128/JB.180.20.5344-5350.1998.
[107] L.V. Wray, A.E. Ferson, K. Rohrer, S.H. Fisher, TnrA, a transcription factor required for global nitrogen regulation in *Bacillus subtilis*, Proc. Natl. Acad. Sci. U.S.A. 93 (17) (1996) 8841–8845, https://doi.org/10.1073/pnas.93.17.8841.
[108] D. Parker, R.M. Kennan, G.S. Myers, I.T. Paulsen, J.I. Rood, Identification of a *Dichelobacter nodosus* ferric uptake regulator and determination of its regulatory targets, J. Bacteriol. 187 (1) (2005) 366–375, https://doi.org/10.1128/JB.187.1.366-375.2005.
[109] T. Ishida, L. Yu, H. Akutsu, K. Ozawa, S. Kawanishi, A. Seto, T. Inubushi, S. Sano, A primitive pathway of porphyrin biosynthesis and enzymology in *Desulfovibrio vulgaris*, Proc. Natl. Acad. Sci. U.S.A. 95 (9) (1998) 4853–4858, https://doi.org/10.1073/pnas.95.9.4853.
[110] S. Storbeck, S. Rolfes, E. Raux-Deery, M.J. Warren, D. Jahn, G. Layer, A novel pathway for the biosynthesis of heme in archaea: genome-based bioinformatic predictions and experimental evidence, Archaea 2010 (2010) 1–15, https://doi.org/10.1155/2010/175050.
[111] S. Bali, A.D. Lawrence, S.A. Lobo, L.M. Saraiva, B.T. Golding, D.J. Palmer, M.J. Howard, S.J. Ferguson, M.J. Warren, Molecular hijacking of siroheme for the synthesis of heme and *d1* heme, Proc. Natl. Acad. Sci. U.S.A. 108 (45) (2011) 18260–18265, https://doi.org/10.1073/pnas.1108228108.
[112] D.J. Palmer, S. Schroeder, A.D. Lawrence, E. Deery, S.A. Lobo, L.M. Saraiva, K.J. McLean, A.W. Munro, S.J. Ferguson, R.W. Pickersgill, D.G. Brown, M.J. Warren, The structure, function and properties of sirohaem decarboxylase - an enzyme with structural homology to a transcription factor family that is part of the alternative haem biosynthesis pathway, Mol. Microbiol. 93 (2) (2014) 247–261, https://doi.org/10.1111/mmi.12656.
[113] K. Haufschildt, S. Schmelz, T.M. Kriegler, A. Neumann, J. Streif, H. Arai, D.W. Heinz, G. Layer, The crystal structure of siroheme decarboxylase in complex with iron-uroporphyrin III reveals two essential histidine residues, J. Mol. Biol. 426 (19) (2014) 3272–3286, https://doi.org/10.1016/j.jmb.2014.07.021.
[114] M. Kühner, K. Haufschildt, A. Neumann, S. Storbeck, J. Streif, G. Layer, The alternative route to heme in the methanogenic archaeon *Methanosarcina barkeri*, Archaea 2014 (2014) 1–13, https://doi.org/10.1155/2014/327637.
[115] S.A.L. Lobo, A.D. Lawrence, C.V. Romão, M.J. Warren, M. Teixeira, L.M. Saraiva, Characterisation of Desulfovibrio vulgaris haem *b* synthase, a radical SAM family member, Biochim. Biophys. Acta Proteins Proteomics 1844 (7) (2014) 1238–1247, https://doi.org/10.1016/j.bbapap.2014.03.016.
[116] C.A. Lewis, R. Wolfenden, Uroporphyrinogen decarboxylation as a benchmark for the catalytic proficiency of enzymes, Proc. Natl. Acad. Sci. U.S.A. 105 (45) (2008) 17328–17333, https://doi.org/10.1073/pnas.0809838105.
[117] B.M. Martins, B. Grimm, H.-P. Mock, R. Huber, A. Messerschmidt, Crystal structure and substrate binding modeling of the uroporphyrinogen-III decarboxylase from *Nicotiana tabacum*: Implications for the catalytic mechanism, J. Biol. Chem. 276 (47) (2001) 44108–44116, https://doi.org/10.1074/jbc.M104759200.
[118] J.D. Phillips, F.G. Whitby, J.P. Kushner, C.P. Hill, Structural basis for tetrapyrrole coordination by uroporphyrinogen decarboxylase, EMBO J. 22 (2003) 6225–6233, https://doi.org/10.1093/emboj/cdg606.
[119] D. Breckau, E. Mahlitz, A. Sauerwald, G. Layer, D. Jahn, Oxygen-dependent coproporphyrinogen III oxidase (HemF) from *Escherichia coli* is stimulated by manganese, J. Biol. Chem. 278 (47) (2003) 46625–46631, https://doi.org/10.1074/jbc.M308553200.
[120] T.D. Lash, The enigma of coproporphyrinogen oxidase: how does this unusual enzyme carry out oxidative decarboxylations to afford vinyl groups?, Bioorg Med. Chem. Lett. 15 (20) (2005) 4506–4509, https://doi.org/10.1016/j.bmcl.2005.07.010.
[121] J.S. Seehra, P.M. Jordan, M. Akhtar, Anaerobic and aerobic coproporphyrinogen III oxidases of *Rhodopseudomonas spheroids*. Mechanism and stereochemistry of vinyl group formation, Biochem. J. 209 (1983) 709–718, https://doi.org/10.1042/bj2090709.
[122] X. Ji, T. Mo, W.-Q. Liu, W. Ding, Z. Deng, Q. Zhang, Revisiting the mechanism of the anaerobic coproporphyrinogen III oxidase HemN, Angew. Chem. Int. Ed. Engl. 58 (2019) 6235–6238, https://doi.org/10.1002/anie.201814708.
[123] K. Rand, C. Noll, H.M. Schiebel, D. Kemken, T. Dülcks, M. Kalesse, D.W. Heinz, G. Layer, The oxygen-independent coproporphyrinogen III oxidase HemN utilizes harderoporphyrinogen as a reaction intermediate during conversion of coproporphyrinogen III to protoporphyrinogen IX, Biol. Chem. 391 (2010) 55–63, https://doi.org/10.1515/bc.2010.006.
[124] H.R. Corradi, A.V. Corrigall, E. Boix, C.G. Mohan, E.D. Sturrock, P.N. Meissner, K.R. Acharya, Crystal structure of protoporphyrinogen oxidase from *Myxococcus xanthus* and its complex with the inhibitor acifluorfen, J. Biol. Chem. 281 (50) (2006) 38625–38633, https://doi.org/10.1074/jbc.M606640200.
[125] M. Koch, C. Breithaupt, R. Kiefersauer, Jörg Freigang, R. Huber, A. Messerschmidt, Crystal structure of protoporphyrinogen IX oxidase: a key enzyme in haem and chlorophyll biosynthesis, EMBO J. 23 (8) (2004) 1720–1728, https://doi.org/10.1038/sj.emboj.7600189.
[126] T.O. Boynton, L.E. Daugherty, T.A. Dailey, H.A. Dailey, Identification of *Escherichia coli* HemG as a novel, menadione-dependent flavodoxin with protoporphyrinogen oxidase activity, Biochemistry 48 (29) (2009) 6705–6711, https://doi.org/10.1021/bi900850y.
[127] A. Sasarman, J. Letowski, G. Czaika, V. Ramirez, M.A. Nead, J.M. Jacobs, R. Morais, Nucleotide sequence of the *hemG* gene involved in the protoporphyrinogen oxidase activity of *Escherichia coli* K12, Can. J. Microbiol. 39 (12) (1993) 1155–1161, https://doi.org/10.1139/m93-174.
[128] N.J. Jacobs, J.M. Jacobs, Nitrate, fumarate, and oxygen as electron acceptors for a late step in microbial heme synthesis, Biochim. Biophys. Acta 449 (1) (1976) 1–9, https://doi.org/10.1016/0005-2728(76)90002-5.
[129] N.J. Jacobs, J.M. Jacobs, Fumarate as alternate electron acceptor for the late steps of anaerobic heme synthesis in *Escherichia coli*, Biochem. Biophys. Res. Commun. 65 (1) (1975) 435–441, https://doi.org/10.1016/S0006-291X(75)80112-4.
[130] J.M. Jacobs, N.J. Jacobs, The late steps of anaerobic heme biosynthesis in *E. coli*: role for quinones in protoporphyrinogen oxidation, Biochem. Biophys. Res. Commun. 78 (1) (1977) 429–433, https://doi.org/10.1016/0006-291X(77)91272-4.
[131] K. Kobayashi, T. Masuda, N. Tajima, H. Wada, N. Sato, Molecular phylogeny and intricate evolutionary history of the three isofunctional enzymes involved in the oxidation of protoporphyrinogen IX, Genome Biol. Evol. 6 (2014) 2141–2155, https://doi.org/10.1093/gbe/evu170.
[132] T.O. Boynton, S. Gerdes, S.H. Craven, E.L. Neidle, J.D. Phillips, H.A. Dailey, Discovery of a gene involved in a third bacterial protoporphyrinogen oxidase activity through comparative genomic analysis and functional complementation, Appl. Environ. Microbiol. 77 (14) (2011) 4795–4801, https://doi.org/10.1128/AEM.00171-11.
[133] K. Kato, R. Tanaka, S. Sano, A. Tanaka, H. Hosaka, Identification of a gene essential for protoporphyrinogen IX oxidase activity in the cyanobacterium *Synechocystis sp.* PCC6803, Proc. Natl. Acad. Sci. U.S.A. 107 (38) (2010) 16649–16654, https://doi.org/10.1073/pnas.1000771107.
[134] H.A. Dailey, J.E. Fleming, B.M. Harbin, Ferrochelatase from *Rhodopseudomonas sphaeroides*: substrate specificity and role of sulfhydryl and arginyl residues, J. Bacteriol. 165 (1) (1986) 1–5, https://doi.org/10.1128/jb.165.1.1-5.1986.
[135] J.M. Frustaci, M.R. O'Brian, Characterization of a *Bradyrhizobium japonicum* ferrochelatase mutant and isolation of the *hemH* gene, J. Bacteriol. 174 (13) (1992) 4223–4229, https://doi.org/10.1128/jb.174.13.4223-4229.1992.
[136] M. Hoggins, H.A. Dailey, C.N. Hunter, J.D. Reid, Direct measurement of metal ion chelation in the active site of human ferrochelatase, Biochemistry 46 (27) (2007) 8121–8127, https://doi.org/10.1021/bi602418e.
[137] T.A. Dailey, H.A. Dailey, Identification of [2Fe-2S] clusters in microbial ferrochelatases, J. Bacteriol. 184 (9) (2002) 2460–2464, https://doi.org/10.1128/JB.184.9.2460-2464.2002.
[138] M. Shepherd, T.A. Dailey, H.A. Dailey, A new class of [2Fe-2S]-cluster-containing protoporphyrin (IX) ferrochelatases, Biochem. J. 397 (2006) 47–52, https://doi.org/10.1042/BJ20051967.
[139] H.A. Dailey*, TA. Dailey, C.-K. Wu**, A.E. Medlock, J.P. Rose, K.-F. Wang, Ferrochelatase at the millennium: structures, mechanisms and [2Fe-2S] clusters, CMLS, Cell Mol. Life Sci. 57 (13) (2000) 1909–1926, https://doi.org/10.1007/PL00000672.
[140] J.H. Hammond, E.F. Dolben, T.J. Smith, S. Bhuju, D.A. Hogan, J.P. Armitage, Links between Anr and quorum sensing in *Pseudomonas aeruginosa* biofilms, J. Bacteriol. 197 (17) (2015) 2810–2820, https://doi.org/10.1128/JB.00182-15.
[141] A. Rompf, C. Hungerer, T. Hoffmann, M. Lindenmeyer, U. Römling, U. Gross, M.O. Doss, H. Arai, Y. Igarashi, D. Jahn, Regulation of *Pseudomonas aeruginosa hemF* and *hemN* by the dual action of the redox response regulators Anr and Dnr, Mol. Microbiol. 29 (1998) 985–997, https://doi.org/10.1046/j.1365-2958.1998.00980.x.
[142] K. Trunk, B. Benkert, N. Quäck, R. Münch, M. Scheer, J. Garbe, L. Jänsch, M. Trost, J. Wehland, J. Buer, M. Jahn, M. Schobert, D. Jahn, Anaerobic adaptation in *Pseudomonas aeruginosa:* definition of the Anr and Dnr regulons, Environ. Microbiol. 12 (2010) 1719–1733, https://doi.org/10.1111/j.1462-2920.2010.02252.x.
[143] S.E. Lizewski, J.R. Schurr, D.W. Jackson, A. Frisk, A.J. Carterson, M.J. Schurr, Identification of AlgR-regulated genes in *Pseudomonas aeruginosa* by use of microarray analysis, J. Bacteriol. 186 (17) (2004) 5672–5684, https://doi.org/10.1128/JB.186.17.5672-5684.2004.
[144] K.Y. Djoko, A.G. McEwan, Antimicrobial action of copper is amplified via inhibition of heme biosynthesis, ACS Chem. Biol. 8 (10) (2013) 2217–2223, https://doi.org/10.1021/cb4002443.
[145] B. Troup, C. Hungerer, D. Jahn, Cloning and characterization of the *Escherichia coli hemN* gene encoding the oxygen-independent coproporphyrinogen III oxidase, J. Bacteriol. 177 (11) (1995) 3326–3331, https://doi.org/10.1128/jb.177.11.3326-3331.1995.
[146] C. Lieb, R.A. Siddiqui, B. Hippler, D. Jahn, B. Friedrich, The *Alcaligenes eutrophus hemN* gene encoding the oxygen-independent coproporphyrinogen III oxidase, is required for heme biosynthesis during anaerobic growth, Arch. Microbiol. 169 (1) (1997) 52–60, https://doi.org/10.1007/s002030050540.
[147] S. Mancini, J.A. Imlay, The induction of two biosynthetic enzymes helps *Escherichia coli* sustain heme synthesis and activate catalase during hydrogen peroxide stress, Mol. Microbiol. 96 (4) (2015) 744–763, https://doi.org/10.1111/mmi.12967.

[148] S. Mukhopadhyay, H.E. Schellhorn, Identification and characterization of hydrogen peroxide-sensitive mutants of *Escherichia coli*: genes that require OxyR for expression, J. Bacteriol. 179 (2) (1997) 330–338, https://doi.org/10.1128/jb.179.2.330-338.1997.
[149] M. Zheng, X. Wang, L.J. Templeton, D.R. Smulski, R.A. LaRossa, G. Storz, DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide, J. Bacteriol. 183 (15) (2001) 4562–4570, https://doi.org/10.1128/JB.183.15.4562-4570.2001.
[150] B.F. Burnham, W.S. Pierce, K.R. Williams, M.H. Boyer, C.K. Kirby, Control of porphyrin biosynthesis through a negative-feedback mechanism. Studies with preparations of δ-aminolaevulate synthetase and δ-aminolaevulate dehydratase from Rhodopseudomonas spheroides, Biochem. J. 87 (3) (1963) 462–472, https://doi.org/10.1042/bj0870462.
[151] W.K. Philipp-Dormston, M. Doss, Over-production of porphyrins and heme in heterotrophic bacteria, Z Naturforsch C Biosci. 30 (1975) 425–426, https://doi.org/10.1515/znc-1975-5-624.
[152] G.T. Javor, E.F. Febre, Enzymatic basis of thiol-stimulated secretion of porphyrins by *Escherichia coli*, J. Bacteriol. 174 (3) (1992) 1072–1075, https://doi.org/10.1128/jb.174.3.1072-1075.1992.
[153] R. Aoki, T. Takeda, T. Omata, K. Ihara, Y. Fujita, MarR-type transcriptional regulator ChlR activates expression of tetrapyrrole biosynthesis genes in response to low-oxygen conditions in cyanobacteria, J. Biol. Chem. 287 (16) (2012) 13500–13507, https://doi.org/10.1074/jbc.M112.346205.
[154] M. Ludwig, M.-E. Pandelia, C.Y. Chew, B. Zhang, J.H. Golbeck, C. Krebs, D.A. Bryant, ChlR protein of *Synechococcus sp.* PCC 7002 is a transcription activator that uses an oxygen-sensitive [4Fe-4S] cluster to control genes involved in pigment biosynthesis, J. Biol. Chem. 289 (24) (2014) 16624–16639, https://doi.org/10.1074/jbc.M114.561233.
[155] H.A. Dailey, S. Gerdes, T.A. Dailey, J.S. Burch, J.D. Phillips, Noncanonical coproporphyrin-dependent bacterial heme biosynthesis pathway that does not use protoporphyrin, Proc. Natl. Acad. Sci. U.S.A. 112 (7) (2015) 2210–2215, https://doi.org/10.1073/pnas.1416285112.
[156] S.A.L. Lobo, A. Scott, M.A.M. Videira, D. Winpenny, M. Gardner, M.J. Palmer, S. Schroeder, A.D. Lawrence, T. Parkinson, M.J. Warren, L.M. Saraiva, *Staphylococcus aureus* haem biosynthesis: characterisation of the enzymes involved in final steps of the pathway, Mol. Microbiol. 97 (3) (2015) 472–487, https://doi.org/10.1111/mmi.13041.
[157] M. Hansson, L. Hederstedt, *Bacillus subtilis* HemY is a peripheral membrane protein essential for protoheme IX synthesis which can oxidize coproporphyrinogen III and protoporphyrinogen IX, J Bacteriol. 176 (19) (1994) 5962–5970, https://doi.org/10.1128/jb.176.19.5962-5970.1994.
[158] J.E. Choby, E.P. Skaar, P.D. Fey, *Staphylococcus aureus* coproporphyrinogen III oxidase is required for aerobic and anaerobic heme synthesis, MSphere 4 (4) (2019), https://doi.org/10.1128/mSphere.00235-19.
[159] C.V. Romão, R. Louro, R. Timkovich, M. Lübben, M.-Y. Liu, J. LeGall, A.V. Xavier, M. Teixeira, Iron-coproporphyrin III is a natural cofactor in bacterioferritin from the anaerobic bacterium *Desulfovibrio desulfuricans*, FEBS Lett. 480 (2000) 213–216, https://doi.org/10.1016/S0014-5793(00)01939-6.
[160] A.I. Celis, G.H. Gauss, B.R. Streit, K. Shisler, G.C. Moraski, K.R. Rodgers, G.S. Lukat-Rodgers, J.W. Peters, J.L. DuBois, Structure-based mechanism for oxidative decarboxylation reactions mediated by amino acids and heme propionates in coproheme decarboxylase (HemQ), J. Am. Chem. Soc. 139 (5) (2017) 1900–1911, https://doi.org/10.1021/jacs.6b11324.
[161] S. Hofbauer, M. Dalla Sega, S. Scheiblbrandner, Z. Jandova, I. Schaffner, G. Mlynek, K. Djinović-Carugo, G. Battistuzzi, P.G. Furtmüller, C. Oostenbrink, C. Obinger, Chemistry and molecular dynamics simulations of heme *b*-HemQ and coproheme-HemQ, Biochemistry 55 (38) (2016) 5398–5412, https://doi.org/10.1021/acs.biochem.6b00701.
[162] S. Hofbauer, A. Hagmüller, I. Schaffner, G. Mlynek, M. Krutzler, G. Stadlmayr, K.F. Pirker, C. Obinger, H. Daims, K. Djinović-Carugo, P.G. Furtmüller, Structure and heme-binding properties of HemQ (chlorite dismutase-like protein) from *Listeria monocytogenes*, Arch. Biochem. Biophys. 574 (2015) 36–48, https://doi.org/10.1016/j.abb.2015.01.010.
[163] S. Hofbauer, G. Mlynek, L. Milazzo, D. Pühringer, D. Maresch, I. Schaffner, P.G. Furtmüller, G. Smulevich, K. Djinović-Carugo, C. Obinger, Hydrogen peroxide-mediated conversion of coproheme to heme *b* by HemQ-lessons from the first crystal structure and kinetic studies, FEBS J. 283 (23) (2016) 4386–4401, https://doi.org/10.1111/febs.13930.
[164] S. Nakano, K.N. Erwin, M. Ralle, P. Zuber, Redox-sensitive transcriptional control by a thiol/disulphide switch in the global regulator, Spx, Mol. Microbiol. 55 (2005) 498–510, https://doi.org/10.1111/j.1365-2958.2004.04395.x.
[165] M.R. Cesinger, M.K. Thomason, M.B. Edrozo, C.R. Halsey, M.L. Reniere, *Listeria monocytogenes* SpxA1 is a global regulator required to activate genes encoding catalase and heme biosynthesis enzymes for aerobic growth, Mol. Microbiol. 114 (2) (2020) 230–243, https://doi.org/10.1111/mmi.v114.210.1111/mmi.14508.
[166] S. Nakano, E. Kuster-Schock, A.D. Grossman, P. Zuber, Spx-dependent global transcriptional control is induced by thiol-specific oxidative stress in *Bacillus subtilis*, Proc. Natl. Acad. Sci. U.S.A. 100 (23) (2003) 13603–13608, https://doi.org/10.1073/pnas.2235180100.
[167] T. Rochat, P. Nicolas, O. Delumeau, A. Rabatinová, J. Korelusová, A. Leduc, P. Bessières, E. Dervyn, L. Krásny, P. Noirot, Genome-wide identification of genes directly regulated by the pleiotropic transcription factor Spx in *Bacillus subtilis*, Nucleic Acids Res. 40 (2012) 9571–9583, https://doi.org/10.1093/nar/gks755.
[168] J. Wennerhold, M. Bott, The DtxR regulon of *Corynebacterium glutamicum*, J. Bacteriol. 188 (8) (2006) 2907–2918, https://doi.org/10.1128/JB.188.8.2907-2918.2006.
[169] J.A. D'Aquino, J. Tetenbaum-Novatt, A. White, F. Berkovitch, D. Ringe, Mechanism of metal ion activation of the diphtheria toxin repressor DtxR, Proc. Natl. Acad. Sci. U.S.A. 102 (51) (2005) 18408–18413, https://doi.org/10.1073/pnas.0500908102.
[170] Y.J. Ko, Y.-C. Joo, J.E. Hyeon, E. Lee, M.-E. Lee, J. Seok, S.W. Kim, C. Park, S.O. Han, Biosynthesis of organic photosensitizer Zn-porphyrin by diphtheria toxin repressor (DtxR)-mediated global upregulation of engineered heme biosynthesis pathway in *Corynebacterium glutamicum*, Sci. Rep. 8 (1) (2018), https://doi.org/10.1038/s41598-018-32854-9.
[171] D. Lecerof, M.N. Fodje, R. Alvarez León, U. Olsson, A. Hansson, E. Sigfridsson, U. Ryde, M. Hansson, S. Al-Karadaghi, Metal binding to *Bacillus subtilis* ferrochelatase and interaction between metal sites, J. Biol. Inorg. Chem. 8 (4) (2003) 452–458, https://doi.org/10.1007/s00775-002-0436-1.
[172] C. Hobbs, J.D. Reid, M. Shepherd, The coproporphyrin ferrochelatase of *Staphylococcus aureus*: mechanistic insights into a regulatory iron-binding site, Biochem. J. 474 (2017) 3513–3522, https://doi.org/10.1042/BCJ20170362.
[173] S. Hofbauer, J. Helm, C. Obinger, K. Djinović-Carugo, P.G. Furtmüller, Crystal structures and calorimetry reveal catalytically relevant binding mode of coproporphyrin and coproheme in coproporphyrin ferrochelatase, FEBS J. 287 (13) (2020) 2779–2796, https://doi.org/10.1111/febs.v287.1310.1111/febs.15164.
[174] X. Peng, J. Wang, W. Peng, F.-X. Wu, Y. Pan, Protein–protein interactions: detection, reliability assessment and applications, Brief Bioinform. 18 (2017) 798–819, https://doi.org/10.1093/bib/bbw066.
[175] J. De Las Rivas, C. Fontanillo, Protein–protein interaction networks: unraveling the wiring of molecular machines within the cell, Brief Funct. Genomics 11 (6) (2012) 489–496, https://doi.org/10.1093/bfgp/els036.
[176] G.C. Ferreira, T.L. Andrew, S.W. Karr, H.A. Dailey, Organization of the terminal two enzymes of the heme biosynthetic pathway. Orientation of protoporphyrinogen oxidase and evidence for a membrane complex, J. Biol. Chem. 263 (8) (1988) 3835–3839, https://doi.org/10.1016/S0021-9258(18)69000-3.
[177] A.E. Medlock, M.T. Shiferaw, J.R. Marcero, A.A. Vashisht, J.A. Wohlschlegel, J.D. Phillips, H.A. Dailey, M. Liesa, Identification of the mitochondrial heme metabolism complex, PLoS ONE 10 (8) (2015) e0135896, https://doi.org/10.1371/journal.pone.0135896.
[178] N. Maio, K.S. Kim, G. Holmes-Hampton, A. Singh, T.A. Rouault, Dimeric ferrochelatase bridges ABCB7 and ABCB10 homodimers in an architecturally defined molecular complex required for heme biosynthesis, Haematologica 104 (9) (2019) 1756–1767, https://doi.org/10.3324/haematol.2018.214320.
[179] C. Lüer, S. Schauer, K. Möbius, J. Schulze, W.-D. Schubert, D.W. Heinz, D. Jahn, J. Moser, Complex formation between glutamyl-tRNA reductase and glutamate-1-semialdehyde 2,1-aminomutase in *Escherichia coli* during the initial reactions of porphyrin biosynthesis, J. Biol. Chem. 280 (19) (2005) 18568–18572, https://doi.org/10.1074/jbc.M500440200.
[180] C. Nardella, D. Boi, M.L. di Salvo, A. Barile, J. Stetefeld, A. Tramonti, R. Contestabile, Isolation of a complex formed between *Acinetobacter baumannii* HemA and HemL, key enzymes of tetrapyrroles biosynthesis, Front. Mol. Biosci. 6 (2019) 6, https://doi.org/10.3389/fmolb.2019.00006.
[181] P. Skotnicová, R. Sobotka, M. Shepherd, J. Hájek, P. Hrouzek, M. Tichý, The cyanobacterial protoporphyrinogen oxidase HemJ is a new *b*-type heme protein functionally coupled with coproporphyrinogen III oxidase, J. Biol. Chem. 293 (32) (2018) 12394–12404, https://doi.org/10.1074/jbc.RA118.003441.
[182] S. Watanabe, T. Kobayashi, M. Saito, M. Sato, K. Nimura-Matsune, T. Chibazakura, S. Taketani, H. Nakamoto, H. Yoshikawa, Studies on the role of HtpG in the tetrapyrrole biosynthesis pathway of the cyanobacterium *Synechococcus elongatus* PCC 7942, Biochem. Biophys. Res. Commun. 352 (1) (2007) 36–41, https://doi.org/10.1016/j.bbrc.2006.10.144.
[183] M.R. O'Brian, Perception and homeostatic control of iron in the rhizobia and related bacteria, Annu. Rev. Microbiol. 69 (1) (2015) 229–245, https://doi.org/10.1146/annurev-micro-091014-104432.
[184] Z. Qi, M.R. O'Brian, Interaction between the bacterial iron response regulator and ferrochelatase mediates genetic control of heme biosynthesis, Mol. Cell. 9 (1) (2002) 155–162, https://doi.org/10.1016/S1097-2765(01)00431-2.
[185] I. Hamza, S. Chauhan, R. Hassett, M.R. O'Brian, The bacterial irr protein is required for coordination of heme biosynthesis with iron availability, J. Biol. Chem. 273 (34) (1998) 21669–21674, https://doi.org/10.1074/jbc.273.34.21669.
[186] J.W. Chidgey, P.J. Jackson, M.J. Dickman, C.N. Hunter, PufQ regulates porphyrin flux at the haem/bacteriochlorophyll branchpoint of tetrapyrrole biosynthesis via interactions with ferrochelatase, Mol. Microbiol. 106 (6) (2017) 961–975, https://doi.org/10.1111/mmi.13861.
[187] A. Masoumi, I.U. Heinemann, M. Rohde, M. Koch, M. Jahn, D. Jahn, Complex formation between protoporphyrinogen IX oxidase and ferrochelatase during haem biosynthesis in *Thermosynechococcus elongatus*, Microbiology 154 (2008) 3707–3714, https://doi.org/10.1099/mic.0.2008/018705-0.
[188] H. Kim, H. Kim, J.K. Lee, Biochemical characterization of protoporphyrinogen dehydrogenase and protoporphyrin ferrochelatase of *Vibrio vulnificus* and the critical complex formation between these enzymes, Biochim. Biophys. Acta Gen. Subj. 1862 (12) (2018) 2674–2687, https://doi.org/10.1016/j.bbagen.2018.08.012.

[189] T. Yoon, J.A. Cowan, Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis, J. Biol. Chem. 279 (25) (2004) 25943–25946, https://doi.org/10.1074/jbc.C400107200.
[190] K.Z. Bencze, K.C. Kondapalli, J.D. Cook, S. McMahon, C. Millán-Pacheco, N. Pastor, T.L. Stemmler, The structure and function of frataxin, Crit. Rev. Biochem. Mol. Biol. 41 (5) (2006) 269–291, https://doi.org/10.1080/10409230600846058.
[191] A. Mielcarek, B. Blauenburg, M. Miethke, M.A. Marahiel, S. Adinolfi, Molecular insights into frataxin-mediated iron supply for heme biosynthesis in *Bacillus subtilis*, PLoS ONE 10 (3) (2015) e0122538, https://doi.org/10.1371/journal.pone.0122538.
[192] U. Olsson, A. Billberg, S. Sjøvall, S. Al-Karadaghi, M. Hansson, *In vivo* and *in vitro* studies of *Bacillus subtilis* ferrochelatase mutants suggest substrate channeling in the heme biosynthesis pathway, J. Bacteriol. 184 (14) (2002) 4018–4024, https://doi.org/10.1128/JB.184.14.4018-4024.2002.
[193] A.I. Celis, J.E. Choby, J. Kentro, E.P. Skaar, J.L. DuBois, Control of metabolite flux during the final steps of heme *b* biosynthesis in Gram-positive bacteria, Biochemistry 58 (52) (2019) 5259–5270, https://doi.org/10.1021/acs.biochem.9b00140.
[194] M.A.M. Videira, S.A.L. Lobo, L.S.O. Silva, D.J. Palmer, M.J. Warren, M. Prieto, A. Coutinho, F.L. Sousa, F. Fernandes, L.M. Saraiva, *Staphylococcus aureus* haem biosynthesis and acquisition pathways are linked through haem monooxygenase IsdG, Mol. Microbiol. 109 (3) (2018) 385–400, https://doi.org/10.1111/mmi.14060.
[195] M.L. Reniere, K.P. Haley, E.P. Skaar, The flexible loop of *Staphylococcus aureus* IsdG is required for its degradation in the absence of heme, Biochemistry 50 (31) (2011) 6730–6737, https://doi.org/10.1021/bi200999q.
[196] A.A. Paul George, M. Lacerda, B.F. Syllwasschy, M.-T. Hopp, A. Wißbrock, D. Imhof, HeMoQuest: a webserver for qualitative prediction of transient heme binding to protein motifs, BMC Bioinf. 21 (2020) 124, https://doi.org/10.1186/s12859-020-3420-2.