

Heme Synthesis

G C Ferreira, University of South Florida, Tampa, FL, USA

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Glossary

Erythroblast Erythroid cell precursor; nucleated cell in bone marrow that develops into an erythrocyte.

Erythrocyte A red blood cell; non-nucleated, disk-shaped blood cell that contains hemoglobin.

Erythroid Erythrocyte related.

Erythropoiesis Formation and production of erythrocytes.

Tetrapyrrole A general term that refers to molecules with four rings of the pyrrole type, most often linked together by single-atom bridges between the α -positions of the five-membered pyrrole rings. The arrangement of the four rings is macrocyclic in porphyrins.

Introduction

Heme, a four-ring organic compound with a central iron atom, plays multiple roles in cellular processes. For example, it is the crucial component in hemoglobin, a red blood cell protein responsible, in vertebrates, for the transport of oxygen from the lungs to the different tissues and the transport of carbon dioxide from the tissues to the lungs. It is heme that gives the characteristic red color to blood. Given its importance in many vital functions in organisms ranging from bacteria to man, it is not surprising that most cells synthesize heme. However, in humans, differentiating erythrocytes represent the major site of heme production due to the synthesis of hemoglobin, which accounts for ~85% of the total heme.

Heme Structure

The observation that hemoglobin can be split into two components, with the smaller unit corresponding to the compound which today is recognized as heme, was made by L R LeCanu in 1837. While the correct structure of heme was initially proposed by Kuster in 1912, it only received general acceptance 16 years later upon its chemical synthesis. The Nobel prize-winning organic chemist Hans Fischer developed syntheses leading to the assembly of four rings, or pyrroles, into a macrocyclic structure with a purple–red color, which he appropriately named ‘porphyrin’ (from the Greek porphuros, meaning purple). The chemically synthesized porphyrin, which had properties identical to those of the natural porphyrin (derived from heme upon the release of the iron atom), was designated protoporphyrin IX. Thus, heme consists of four rings (pyrroles), composed of carbon, nitrogen, and hydrogen atoms, linked together in a cyclic array and containing an atom of iron in its center (Figure 1).

Heme is now recognized as a member of a large family of metalated tetrapyrroles, which are essential for life on Earth. They include chlorophylls, cobalamin (vitamin B₁₂), siroheme, and coenzyme F₄₃₀. Chlorophylls trap the light energy required in photosynthesis. Cobalamin-containing enzymes catalyze intramolecular rearrangements, methylations, and reduction of ribonucleotides to deoxyribonucleotides. Siroheme is an essential component of enzymes involved in

sulfur and nitrogen metabolisms. Coenzyme F₄₃₀, present in methanogenic bacteria, functions in methane formation. While the central metal ions in these tetrapyrroles differ – Fe²⁺ in the case of heme and siroheme, Mg²⁺ in chlorophyll and bacteriochlorophyll, Co²⁺ in cobalamin, and Ni²⁺ in coenzyme F₄₃₀ – the unifying feature is the presence of a complexed metal ion, which promotes a remarkable number of different biochemical reactions. Further, the protein scaffold binding the metalloporphyrin modulates the chemistry (redox state and coordination properties) of the metal ion, such that the spectrum of the reactions expands beyond the normal properties of the metal ion. It is this interplay between the protein moiety and the metalloporphyrin that provides the plethora of functions associated with metalloporphyrin-containing proteins.

The Heme Biosynthetic Pathway

Formation of 5-Aminolevulinate

The biosynthesis of tetrapyrroles is initiated from simple molecules and comprises only a few enzymatic steps (Figure 2). The first committed precursor, 5-aminolevulinic acid (ALA), is synthesized from either succinyl-CoA or glutamate, and thus the names C4 and C5 (based on the number of carbon atoms in the precursor), for the two ALA biosynthetic pathways evolved in nature. In most nonphotosynthetic eukaryotes and a few prokaryotes (i.e., the α -proteobacteria), ALA results from the condensation between glycine and succinyl CoA (C4 pathway), while in plants and most bacteria, ALA is derived from glutamate (C5 pathway). The C4 pathway requires a single enzyme, ALA synthase (ALAS); by contrast, three enzymes are involved in the conversion of glutamate into ALA. Specifically, glutamyl-transfer RNA (tRNA) synthetase converts glutamate to glutamyl-tRNA, which is reduced by glutamyl-tRNA reductase to glutamyl-1-semialdehyde (GSA); this product is then transformed by GSA aminomutase or GSA aminotransferase (GSA-AT) to ALA. Pyridoxal 5'-phosphate, a vitamin B₆-derivative, is an essential component (cofactor) of both ALAS and GSA-AT, as these enzymes cannot function relying solely on the protein moiety. Among prokaryotes, ALAS is only found in the α -proteobacteria, which are considered to be the most closely related, free-living organisms to the

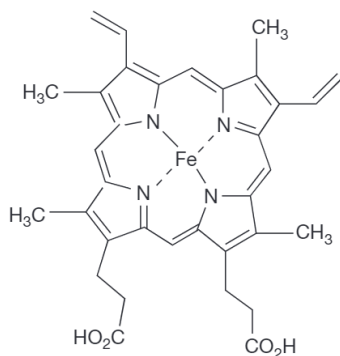


Figure 1 Iron–protoporphyrin IX, which is commonly designated as heme. Iron–protoporphyrin IX (or heme) has an iron ion complexed with the conjugated, aromatic porphyrin ring and an asymmetrical side-chain substitution. Note the $-\text{CH}_3$ (methyl), $-\text{CH}=\text{CH}_2$ (vinyl), and $-\text{CH}_2-\text{CH}_2-\text{COOH}$ (propionate) groups as side chains.

ancestral progenitor of the mitochondrion. Thus, it is conceivable that eukaryotic ALASs were acquired from a free-living α -proteobacterium during its transformation, first to an endosymbiont and subsequently to a mitochondrion.

From ALA to Uroporphyrinogen III

In eukaryotes, the first and three final enzymatic steps of heme biosynthesis occur within mitochondria, while the intervening four occur in the cytosol (**Figure 2**). Once ALA is made and transported to the cytosol, through most likely a specialized mitochondrial transporter, two molecules of ALA are asymmetrically condensed to form the monopyrrole porphobilinogen (PBG) by PBG synthase (PBGS; also known as ALA dehydratase). PBG constitutes the building unit of tetrapyrroles and hence eight molecules of ALA are required to form one heme molecule. Crystal structures of PBGSs from different species have confirmed the presence of two distinct binding sites for each ALA molecule, termed A and P to distinguish the two ALA substrate molecules. The A-side of ALA forms the acetyl half of PBG, leaving its amino group intact, while the P-side of ALA

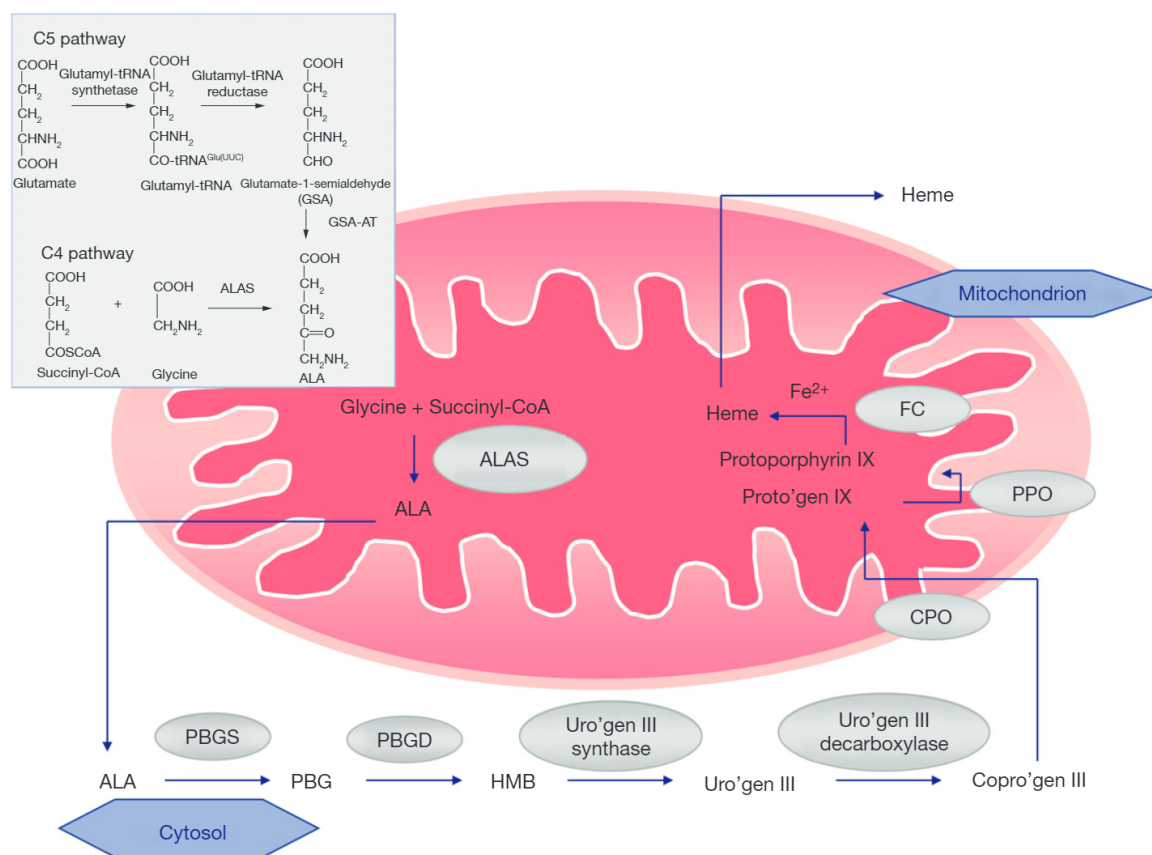


Figure 2 Diagrammatic representation of the heme biosynthetic pathway in eukaryotic animal cells. ALA, 5-aminolevulinate; ALAS, ALA synthase; PBG, porphobilinogen; PBGS, PBG synthase; PBGD, PBG deaminase; HMB, hydroxymethylbilane; Uro'gen III, uroporphyrinogen III; copro'gen III, coproporphyrinogen III; CPO, coproporphyrinogen III oxidase; proto'gen IX, protoporphyrinogen IX; PPO, protoporphyrinogen IX oxidase; FC, ferrochelatase. Inset: The two paths to ALA.

forms the propionyl half of PBG with the amino group becoming part of the PBG ring. PBGS has a homo-octomeric structure composed of four dimers, and each subunit contains an active site metal ion (zinc or magnesium and/or potassium depending on the species) and in many cases an allosteric metal ion (magnesium). The next step in heme biosynthesis involves the deamination and stepwise polymerization of four PBG molecules by PBG deaminase to give the linear tetrapyrrole 1-hydroxymethylbilane. Then, uroporphyrinogen III synthase catalyzes the cyclization of the linear tetrapyrrole, whereby the D ring of hydroxymethylbilane is inverted to generate the asymmetrical macrocycle, uroporphyrinogen III. In the absence of uroporphyrinogen III synthase, 1-hydroxymethylbilane cyclizes into the nonphysiological relevant tetrapyrrole, uroporphyrinogen I. The incredible acrobatics that must occur with the flipping of the D ring and closure of hydroxymethylbilane speak for the importance of uroporphyrinogen III synthase in making uroporphyrinogen III synthesis reaction. In fact, disastrous consequences emerge with the malfunction of uroporphyrinogen III synthase, as observed in patients with the blood disorder congenital erythropoietic porphyria, who typically have high levels of uroporphyrinogen I and derivatives thereof.

From Uroporphyrinogen III to Protoporphyrinogen IX

For most eukaryotes, the last cytosolic step is the sequential decarboxylation of the four acetate side chains of uroporphyrinogen III to methyl groups by uroporphyrinogen decarboxylase (Figure 2). This enzyme is a (α/β)₈ barrel protein, which appears to be functional as a homodimer and not to require prosthetic groups or cofactors. Remarkably, uroporphyrinogen decarboxylase enhances the rate of substrate decarboxylation by a factor of 1.2×10^{17} , one of the largest rate enhancements reported for an enzyme that functions without the help of cofactors. The uroporphyrinogen decarboxylase-catalyzed reaction gives coproporphyrinogen III, which is the substrate of coproporphyrinogen oxidase (CPO). The oxidative decarboxylation of the propionate side chains of the rings A and B of coproporphyrinogen III yields protoporphyrinogen IX. While eukaryotic CPO activity depends on oxygen, prokaryotic organisms, under anaerobic conditions, use alternative electron acceptors in the oxidative decarboxylation reaction. Hence, oxygen-independent CPOs (HemN and HemZ, i.e., the products of the *hemN* and *hemZ* genes) are found in bacteria; indeed, facultatively anaerobic bacteria, such as *Escherichia coli*, possess both oxygen-dependent CPO (HemF) and oxygen-independent CPO (HemN). HemN contains an iron-sulfur center ([4Fe-4S]), which is essential for the activity of the enzyme in conjunction with nicotinamide adenine dinucleotide (phosphate), reduced (NAD(P)H) and S-adenosyl-L-methionine (SAM). As one would expect, the enzyme appears to have a distinct reaction mechanism with the involvement of SAM in the formation of a radical, and thus HemN has been aptly identified as a radical SAM enzyme. As far as the subcellular location of the oxygen-dependent CPO goes, it seems to vary! In mammals and other higher eukaryotes, it appears to be associated with the inner side of the mitochondrial outer membrane, whereas in the yeast *Saccharomyces cerevisiae* and bacteria it is found in the cytosol.

From Protoporphyrinogen IX to Heme

In the penultimate step, protoporphyrinogen oxidase (PPO) catalyzes the complete aromatization of protoporphyrinogen IX ring into protoporphyrin IX, which involves a six-electron oxidation (Figure 2). Similar to CPO, anaerobic and facultative anaerobic bacteria have an oxygen-independent PPO; however, in contrast to CPO, facultative anaerobic bacteria possess solely an oxygen-independent PPO (and not in complementation with an oxygen-dependent PPO). All other organisms appear to have PPOs, which use oxygen as the terminal electron acceptor. Ferrochelatase catalyzes the terminal step of the heme biosynthetic pathway, the insertion of ferrous iron into the protoporphyrin IX macrocyclic ring. Eukaryotic ferrochelatases associate with the inner side of the mitochondrial inner membrane, and vertebrate enzymes have a [2Fe-2S] cluster (although this is not an exclusive structural feature of vertebrate ferrochelatases). The mechanism of the ferrochelatase reaction involves an out-of-plane distortion of the porphyrin, which facilitates the reaction by exposing the pyrrole nitrogens to the incoming ferrous iron substrate.

Regulation of the Heme Biosynthetic Pathway/Heme Biosynthesis

There is not a single ubiquitous regulatory mechanism for heme biosynthesis, and so the discussion in this article focuses solely on the regulation of the pathway in mammalian cells. Considering that heme is predominantly synthesized in bone marrow (i.e., in erythroblasts and reticulocytes that still contain mitochondria) and liver cells, most of the studies on regulation have focused on these two types of cells. Predominance of heme synthesis in bone marrow and liver cells reflects the greater demands due to the syntheses of the heme-containing proteins, hemoglobin, and cytochrome P-450 enzymes, respectively. The major regulatory and rate-limiting step of the pathway corresponds to the reaction catalyzed by ALAS, the first enzyme of the pathway. Two genes, located in two different chromosomes in humans, encode the two ALAS forms of the enzyme (or isozymes): ALAS1 and ALAS2. The gene encoding ALAS1 is expressed in every cell, whereas that for ALAS2 is specifically expressed in erythroid cells. Importantly, the regulatory mechanisms for the expression of the two ALAS genes and the posttranscriptional events seem to be distinct in differentiating erythrocytes from those in nonerythroid cells. ALAS1 controls the production of heme for basic cellular functions and is feedback regulated by heme through (1) repression of transcription of the ALAS1 gene, (2) inhibition of translation of the ALAS1 message, (3) destabilization of the ALAS1 message, and (4) inhibition of import of the cytosolic precursor form of the enzyme into mitochondria. By contrast, certain drugs and hormones induce liver cells to make more ALAS, and consequently heme and cytochrome P-450, by activating transcription of the ALAS1 gene.

In differentiating erythroid cells, the syntheses of heme and globin need to be coordinated, as the production of hemoglobin requires that neither the protein (globin) component nor the nonprotein component (heme) will be in excess. And indeed, identical transcriptional elements have been identified

in the genes of ALAS2 and globin. Another level of regulation of the erythroid heme biosynthetic pathway is centered at the stage of translation, which responds to the cellular iron levels. This response is mediated through specific interactions between an iron-responsive element (IRE), present in the ALAS2 messenger RNA (mRNA), and IRE-binding proteins, such that under iron limitation, the translation of ALAS2 mRNA is inhibited, while the presence of iron above the cellular threshold relieves the repression caused by IRE-binding proteins. The essential requirement for ALAS2 in heme biosynthesis and erythropoiesis was clearly demonstrated with targeted disruption of ALAS2 in the mouse, which led to no hemoglobin in cells, the accumulation of iron (albeit in the cytoplasm and not in mitochondria) and, ultimately, embryonic death.

Heme Function

The known roles of heme are many and variable, and most probably many other functions await discovery. As referred to above, heme is a prosthetic group of proteins involved in oxygen transport such as hemoglobins and myoglobins, which are the major blood and muscle proteins, respectively. In addition, heme participates in other cellular processes including anaerobic and aerobic respiration, sensing of diatomic gases (e.g., nitric oxide and carbon monoxide), detoxification of foreign substances (e.g., drugs) by oxidative metabolism, and signal transduction. Some of these functions might also be coupled with the regulatory roles of heme in gene transcription, translation, microRNA (miRNA) processing, mitochondrial protein import, protein stability, and differentiation. A transcription factor that regulates the mammalian biological clock was also discovered to use heme as a cofactor to sense, and react to, changes in the cell, thus linking cellular metabolism with the biological clock. Another area of research has led to the emergence of a new proposal that heme deficiency or dysregulation is intimately associated with the aging process, including neural decay.

Disorders of the Heme Biosynthetic Pathway

Malfunction of the heme biosynthetic pathway can have disastrous consequences, and in humans it can be manifested in porphyrias. Briefly, porphyrias are disorders that result from inherited or acquired defects in the heme biosynthetic pathway. The first reports of porphyrias were in 1874. Since then, eight types of porphyrias associated with defects in each of the eight enzymes of the pathway have been identified (Table 1), and depending on the major site of expression of the enzymatic defect, they have been classified into hepatic or erythropoietic porphyria. Although the molecular mechanisms behind the clinical manifestations are not well understood, the two major symptoms include cutaneous photosensitivity and central nervous system disturbances, and thus the other classification of either photosensitive or neurological porphyrias. Some porphyrias, however, manifest overlapping symptoms. Photosensitivity is a result of porphyrin accumulation, which typically occurs when the defective enzyme is

Table 1 Heme biosynthesis-associated disorders

Disorder	Defective enzyme
X-linked sideroblastic anemia	5-Aminolevulinate synthase (erythroid)
X-linked dominant protoporphyria	5-Aminolevulinate synthase (erythroid)
ALA dehydratase, or Doss, porphyria	Porphobilinogen synthase (or ALA dehydratase)
Acute intermittent porphyria	Porphobilinogen deaminase
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase
Coproporphyria	Coproporphyrinogen oxidase
Variegate porphyria	Protoporphyrinogen oxidase
Erythropoietic protoporphyria	Ferrochelatase

anywhere in the pathway beyond the third step of the pathway (i.e., the PBG deaminase-catalyzed reaction), whereas the neurologic manifestations are associated with overproduction of ALA and PBG. However, the first porphyria associated with erythroid ALAS, named X-linked dominant protoporphyria (XLDPP), was recently identified, and elevated concentration of ALA-derived protoporphyrin IX and Zn-protoporphyrin were observed in the erythrocytes of the XLDPP patients. Since the 1980s, many of the genetic mutations responsible for the enzymatic defects have been identified and mouse models, targeted to correct the defective enzyme, have been developed by engineering functional enzyme expression in bone marrow cells. Zebrafish have also provided powerful molecular models for erythropoietic porphyrias. For example, mutations in the ferrochelatase gene resulted in photosensitive zebrafish with autofluorescent blood. Finally, mutations in the gene encoding for the erythroid ALAS can cause X-linked sideroblastic anemia (Table 1), a blood disorder characterized by mitochondrial iron accumulation due to a reduction in heme biosynthesis destined for hemoglobin production in erythroblasts.

See also: **Metabolism** Vitamins and Hormones: Porphyrin Metabolism; **Protein/Enzyme** Structure Function and Degradation: Heme Proteins; **Signaling**: Hematopoietin Receptors.

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