

- in gene expression, cell death, and membrane transport of organic solutes. *J Hepatol* 34, 946–954.
- 3 Huang CS, He W, Meister A *et al.* (1995) Amino acid sequence of rat kidney glutathione synthetase. *Proc Natl Acad Sci USA* 92, 1232–1236.
 - 4 Fernández-Checa J, Lu SC, Ookhtens *et al.* (1992) The regulation of hepatic glutathione. In: Tavoloni N, Berk PD (eds) *Hepatic Anion Transport and Bile Secretion: Physiology and Pathophysiology*. New York: Marcel Dekker, pp. 363–395.
 - 5 Ballatori N, Hammond CL, Cunningham JB *et al.* (2005) Molecular mechanisms of reduced glutathione transport: role of the MRP/CFTR/ABCC and OATP/SLC21A families of membrane proteins. *Toxicol Appl Pharmacol* 204, 238–255.
 - 6 Meister A (1988) Glutathione. In: Aria IM, Jakoby WB, Popper H *et al.* (eds) *The Liver: Biology and Pathobiology*, 2nd edn. New York: Raven Press, pp. 401–417.
 - 7 Fernández-Checa JC, Kaplowitz N, Garcia-Ruiz C *et al.* (1997) GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect. *Am J Physiol* 273, G7–17.
 - 8 Fernandez-Checa JC, Kaplowitz N (2005) Hepatic mitochondrial glutathione: transport and role in disease and toxicity. *Toxicol Appl Pharmacol* 204, 263–273.
 - 9 Garcia-Ruiz C, Colell A, Morales A *et al.* (1995) Role of oxidative stress generated from the mitochondrial electron transport chain and mitochondrial glutathione status in loss of mitochondrial function and activation of transcription factor nuclear factor-kappa B: studies with isolated mitochondria and rat hepatocytes. *Mol Pharmacol* 825–834.
 - 10 Martensson J, Lai JC, Meister A (1990) High-affinity transport of glutathione is part of a multicomponent system essential for mitochondrial function. *Proc Natl Acad Sci USA* 87, 7185–7189.
 - 11 Coll O, Colell A, Garcia-Ruiz C *et al.* (2003) Sensitivity of the 2-oxoglutarate carrier to alcohol intake contributes to mitochondrial glutathione depletion. *Hepatology* 38, 692–702.
 - 12 Fernandez-Checa JC (2003) Redox regulation and signaling lipids in mitochondrial apoptosis. *Biochem Biophys Res Commun* 304, 471–479.
 - 13 Garcia-Ruiz C, Colell A, Mari M *et al.* (2003) Defective TNF- α -mediated hepatocellular apoptosis and liver damage in acidic sphingomyelinase knockout mice. *J Clin Invest* 111, 197–208.
 - 14 Samudio I, Konopleva M, Hail N *et al.* (2005) 2-cyano, 3, 12-dioxooleana, 1, 9, diene, 28-imidazolide (CDDO-IM) directly targets mitochondrial glutathione to induce apoptosis in pancreatic cancer. *J Biol Chem* 280, 36273–36282.
 - 15 Lluís JM, Morales A, Blasco C *et al.* (2005) Critical role of mitochondrial glutathione in the survival of hepatocytes during hypoxia. *J Biol Chem* 280, 3224–3232.
 - 16 Kuwana T, Mackey MR, Perkins G *et al.* (2002) Bid, Bax and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell* 111, 331–342.
 - 17 Gumprich E, Dahl R, Yerushalmi B *et al.* (2002) Nitric oxide ameliorates hydrophobic bile acid-induced apoptosis in isolated rat hepatocytes by non-mitochondrial pathways. *J Biol Chem* 277, 25823–25830.
 - 18 Otani K, Korenaga M, Beard MR *et al.* (2005) Hepatitis C virus core protein, cytochrome P450 2E1, and alcohol produce combined mitochondrial injury and cytotoxicity in hepatoma cells. *Gastroenterology* 128, 96–107.
 - 19 Fernandez-Checa JC, Colell A, Garcia-Ruiz C (2002) S-Adenosyl-L-methionine and mitochondrial reduced glutathione depletion in alcoholic liver disease. *Alcohol* 27, 179–183.
 - 20 Lluís JM, Colell A, Garcia-Ruiz C *et al.* (2003) Acetaldehyde impairs mitochondrial glutathione transport in HepG2 cells through endoplasmic reticulum stress. *Gastroenterology* 124, 708–724.
 - 21 Mari M, Colell A, Morales A *et al.* (2004) Acidic sphingomyelinase downregulates the liver-specific methionine adenosyltransferase 1A, contributing to tumor necrosis factor-induced lethal hepatitis. *J Clin Invest* 113, 895–904.
 - 22 Soccio RE, Breslow JL (2004) Intracellular cholesterol transport. *Arterioscler Thromb Vasc Biol* 24, 1150–1160.
 - 23 Hall EA, Ren S, Hylemon PB *et al.* (2005) Detection of the steroidogenic acute regulatory protein, StAR, in human liver cells. *Biochim Biophys Acta* 1733, 111–119.
 - 24 Kaufman RJ (1999) Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. *Genes Dev* 13, 1211–1233.
 - 25 Ji C, Kaplowitz N (2003) Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. *Gastroenterology* 124, 1488–1499.
 - 26 Han D, Canali R, Rettori D *et al.* (2003) Effect of glutathione depletion on sites and topology of superoxide and hydrogen peroxide production in mitochondria. *Mol Pharmacol* 64, 1136–1144.
 - 27 Vendemiale G, Grattagliano I, Altomare E *et al.* (1996) Effect of acetaminophen administration on hepatic glutathione compartmentation and mitochondrial energy metabolism in the rat. *Biochem Pharmacol* 52, 1147–1154.
 - 28 Zhao P, Slattery JT (2002) Effects of ethanol dose and ethanol withdrawal on rat liver mitochondrial glutathione: implication of potentiated acetaminophen toxicity in alcoholics. *Drug Metab Dispos* 30, 1413–1417.

2.3.10 Haem biosynthesis and excretion of porphyrins

Hervé Puy and Jean-Charles Deybach

Introduction

Synthesis of porphyrins occurs in nearly all living cells: in animals for haem production and in plants for chlorophyll production. Porphobilinogen (PBG) and δ -aminolaevulinic acid (ALA) are linear porphyrin precursors, whereas porphyrins are molecules that are cyclic precursors of haem. In mammalian cells, haem synthesis occurs mostly in liver and erythropoietic tissues. Eight enzymes are involved in haem synthesis from succinyl CoA and glycine; the biosynthetic pathway starts in the mitochondrion and, after passing through three cytoplasmic stages, re-enters the mitochondrion for the final steps of haem formation. Therefore, the first and last three enzymes are found in mitochondria and the others in the cytosol (Fig. 1). All these enzymes are encoded by nuclear genes, and their full-length human cDNA and genomic sequences have been isolated and characterized [1]. Haemoproteins, including haemoglobin or myoglobin, mitochondrial or microsomal cytochromes, catalase, peroxidase, nitric oxide synthase, prostaglandin endoperoxide

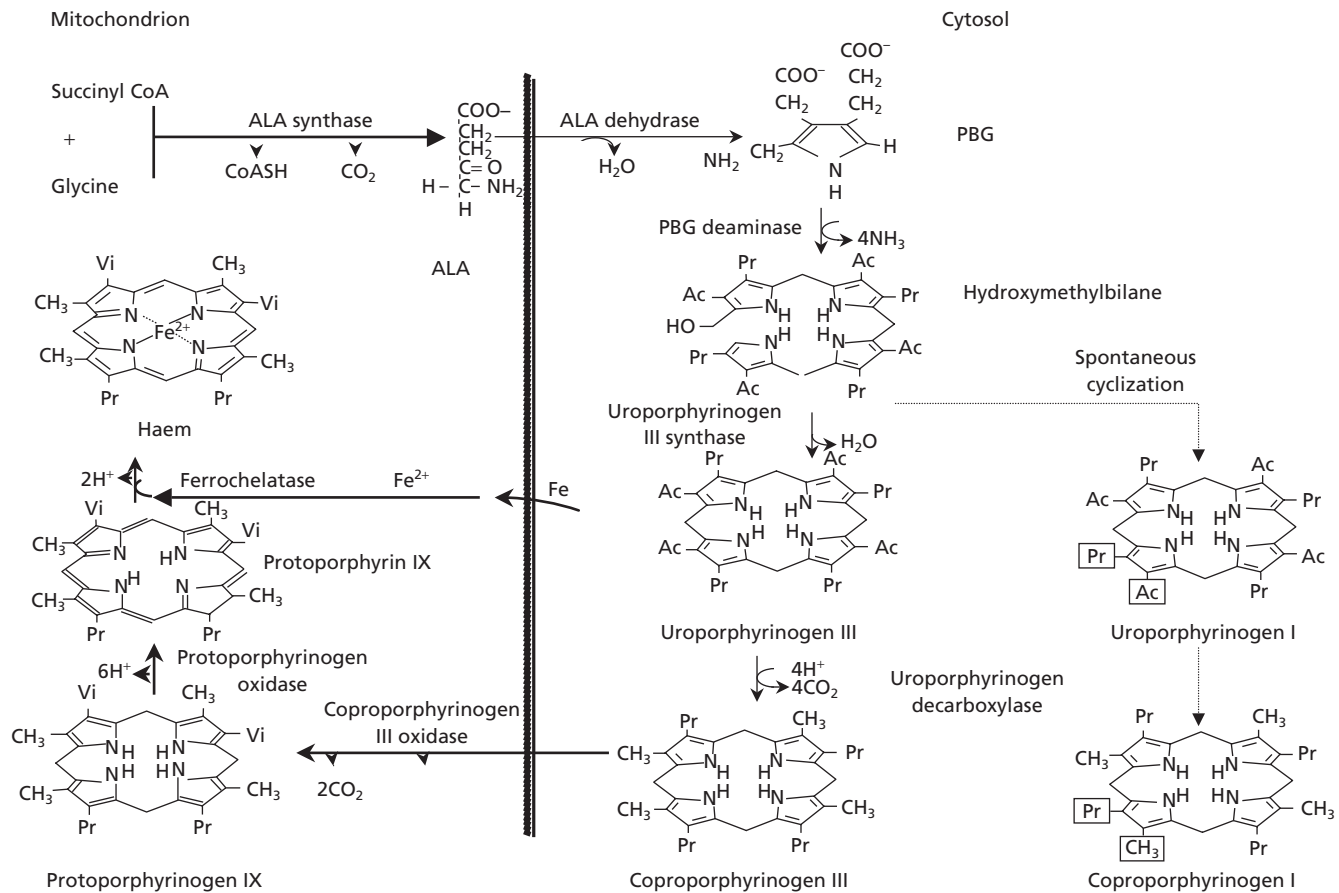


Fig. 1 The haem biosynthetic pathway. Subcellular distribution of enzymes and intermediates in the synthesis of haem is shown. ALA, δ -aminolaevulinic acid; PBG, porphobilinogen; Ac, acetyl ($\text{CH}_2\text{-COOH}$); CoASH, coenzyme A; Pr, propionyl ($\text{CH}_2\text{-CH}_2\text{-COOH}$); Vi, vinyl ($\text{CH}=\text{CH}_2$).

synthase, guanylate cyclase or tryptophan pyrrolase, play very important roles in electron and oxygen transport, in the activation of oxygen or hydrogen peroxide and, finally, in hydrogen peroxide decomposition.

Haem biosynthesis

Structure of porphyrins

Porphyrins are cyclic tetrapyrroles in which the pyrrole rings, conventionally designated as A, B, C and D, are linked through their carbon atoms by methene ($-\text{CH}=\text{}$) bridges (Fig. 1). All the cyclic tetrapyrrole intermediates of the biosynthetic pathway, with the exception of protoporphyrin IX, the last intermediate, are porphyrinogens, which are reduced forms that are rapidly oxidized to porphyrins when exposed to air with the loss of six protons. Porphyrins emit intense red fluorescence when exposed to light at around 400 nm. Thus, spectrofluorometric methods provide very sensitive detection and quantification of porphyrins (Soret band).

The naturally occurring porphyrins all have side-chains on the carbon atoms of the pyrrole rings. The porphyrin isomers differ in the arrangement of the side-chain substituents (e.g. acetyl and propionyl in uroporphyrins; methyl and propionyl in coproporphyrins). Water solubility of porphyrins is favoured by the presence of carboxylic acid side-chains. Uroporphyrin, the most water-soluble of the porphyrins, is excreted predominantly in urine, coproporphyrin mostly in urine and partly in bile, whereas protoporphyrin, the least soluble, is excreted only in the bile. There are four isomers of each of these porphyrins; only the I and III isomers occur in nature. In isomer I, the side-chains are arranged symmetrically around the ring; in isomer III, the substituents on ring D are reversed [2]. In animals, the complex between ferrous iron and protoporphyrins is usually called haem.

Biosynthesis

The two types of cells in the body that are responsible for synthesizing most of the haem are the erythropoietic cells (80%)

and the liver parenchymal cells (20%). The steps in the pathway are outlined in Figure 1.

Synthesis of porphyrin precursors:

δ -aminolaevulinic acid and porphobilinogen

ALA synthase (EC 2.3.1.37), the first enzyme in the pathway, is a mitochondrial protein that requires pyridoxal phosphate as a cofactor. Two different isoenzymes, erythroid-specific or house-keeping, are encoded by two separate genes on different chromosomes (see below). It catalyses the condensation of glycine and succinyl-CoA, which is produced by the tricarboxylic acid cycle, to form ALA, which is exclusively committed to the synthesis of haem. ALA dehydrase (EC 4.2.1.24) then catalyses the condensation of two molecules of ALA to form monopyrrole PBG. The ALA dehydrase gene, situated on chromosome 9, has two codominant alleles, 1 and 2 [3]. The isoenzymes produced in the liver and erythroid tissue through tissue-specific alternative splicing are identical, with the form of splicing determined by activation of an untranslated region. The ALA dehydrase-2 isoenzyme is more electronegatively charged than ALA dehydrase-1, and its affinity for lead, which inhibits its activity by competing with the zinc atoms needed for catalytic action, is therefore higher. As a consequence, individuals with the ALA dehydrase-2 genotype are more vulnerable to lead exposure.

Synthesis of coproporphyrinogen III

Two cytoplasmic enzymes, PBG deaminase (EC 4.3.1.8) and uroporphyrinogen III cosynthetase (cosynthetase; EC 4.2.1.75), convert four molecules of PBG to uroporphyrinogen III (with liberation of four molecules of ammonia). PBG deaminase catalyses the polymerization of four molecules of PBG, yielding a linear tetrapyrrole intermediate, hydroxymethylbilane (Fig. 1). In this reaction, four units of PBG are assembled head-to-tail by

the deaminase, starting with ring A and building round to ring D, to form the unrearranged bilane. The deaminase furnishes a straight-chain tetrapyrrole, hydroxymethylbilane, but it is not an enzyme for ring closure. The PBG deaminase contains, at its catalytic site, a dipyrrolomethane cofactor. The function of the dipyrrolomethane cofactor appears to be that of anchoring the substrate molecules at the catalytic site and directing the condensation of PBG to form the tetrapyrrole. Uroporphyrinogen III cosynthase then rapidly converts the intermediate into uroporphyrinogen III, a cyclic tetrapyrrole with eight carboxyl side-chains. In the absence of this ring-closing and side-chain-rearranging enzyme, an abortive I isomeric uroporphyrin is formed spontaneously, and, after partial decarboxylation to I isomeric coproporphyrinogen, is excreted via the hepatobiliary route as well as in urine. Uroporphyrinogen decarboxylase (EC 4.1.1.37), encoded on the short arm of chromosome 1, catalyses the stepwise decarboxylation of uroporphyrinogen III and converts the four acetic acid side-chains to methyl groups to form coproporphyrinogen III. The reactions, passing through 7-, 6- and 5-carboxylic states of the porphyrin molecule, take place at a single catalytic site. Only isomer III can be used in the remaining steps [3].

Synthesis of haem

Coproporphyrinogen oxidase (EC 1.3.3.3), encoded by a gene on chromosome 9, selectively converts two propionic acid chains to vinyl groups on the III isomeric form of coproporphyrinogen and then catalyses the stepwise decarboxylation to produce protoporphyrinogen IX. This mitochondrial enzyme, which requires copper for its action, is not membrane bound and was shown to be present in the intermembrane space. This localization implies that protoporphyrinogen oxidase (EC 1.3.3.4) must cross the inner membrane because haem is formed within the inner membrane (Fig. 2). Protoporphyrinogen

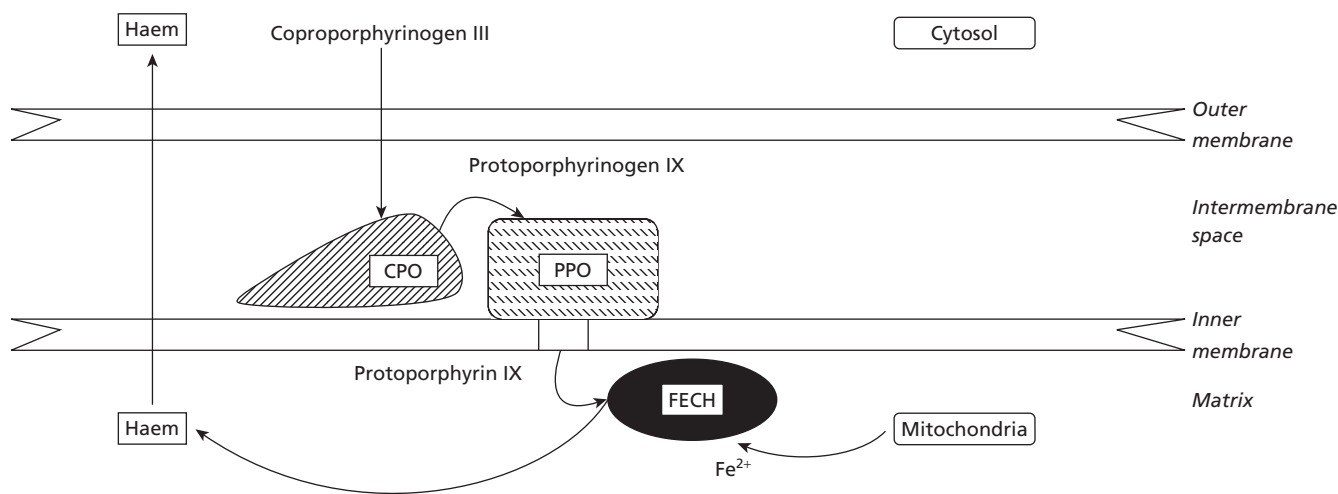


Fig. 2 Haem biosynthetic pathway. Association of the three terminal enzymes (coproporphyrinogen oxidase CPO, protoporphyrinogen oxidase PPO, ferrochelatase FECH) with the inner mitochondrial membrane.

oxidase, encoded by a gene on chromosome 1, catalyses the oxidation of protoporphyrinogen IX to protoporphyrin IX: six hydrogen atoms are removed (four from methylene bridges and two from pyrrole rings). Only oxidized molecules (porphyrins) are brightly coloured, whereas reduced porphyrins (porphyrinogens) are colourless. The final enzymatic step in haem synthesis is the insertion of iron by the enzyme ferrochelatase (EC 4.99.1.1), which is encoded by a gene on chromosome 18 and is localized to the inner membrane of mitochondria. Unlike the three preceding enzymes in the haem biosynthetic pathway, which use porphyrinogens as substrates, ferrochelatase uses protoporphyrin IX; other dicarboxyl porphyrins, such as deuterio- and mesoporphyrin IX, serve as good substrates for this enzyme *in vitro*. Only the reduced form of iron (Fe^{2+}), and not Fe^{3+} , is incorporated into protoporphyrin IX by the enzyme. Co^{2+} and Zn^{2+} are more efficient substrates for the enzyme than Fe^{2+} . Therefore, various rates of ferrochelatase activity can be obtained *in vitro*, depending upon which metal and porphyrin substrates are used. Moreover, in humans, ferrochelatase deficiency leads to free protoporphyrin accumulation, whereas iron deficiency leads to zinc protoporphyrin accumulation. The biosynthesis of haem requires 8 mol of glycine and 8 mol of succinic acid [3]. Each of the enzymes and their respective deficiency diseases are described in Chapter 16.5, Human hereditary porphyrias.

Haem catabolism

All cells are able to handle haem left over from the breakdown of haem proteins, thus preventing toxic accumulation of the compound. Haem oxygenase 1 (HO-1, EC 1.14.99.3), situated in the endoplasmic reticulum, is considered as a heat shock protein that is ubiquitously expressed, but is present in especially large amounts in liver and spleen, where the main degradation of haemoglobin takes place. Combined with nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) cytochrome 450 reductase, it cleaves one of the methene bridges of the porphyrin ring and generates carbon monoxide. The linear tetrapyrrol, biliverdin, is oxidized to bilirubin and excreted via the liver–bile route, whereas the liberated iron is reutilized. HO-1 activity is induced by a variety of stimuli, including haem itself, heavy metals, organic chemicals, endotoxins, hyperthermia, hypoglycaemia, burns and oxidative stress. Stimuli that increase HO-1 gene expression could accelerate the flux of metabolites through the haem biosynthetic pathway, and may thereby trigger clinical symptoms in some forms of hepatic porphyria by overload of the deficient enzyme [4]. The isoenzyme haem oxygenase 2 (HO-2), encoded by another gene, is not induced by the same agents that increase the transcription of the ubiquitous gene. This HO-2 gene is strongly expressed in brain, where it may serve to supply the tissue with carbon monoxide, a neuronal messenger that binds to the haem prosthetic group of guanylate cyclase generating cyclic GMP.

Regulation of haem synthesis

The mechanisms applied in the control of haem biosynthesis differ between liver and bone marrow, the two tissues that make haem in the largest amounts. Both erythroid-specific and non-erythroid or ‘housekeeping’ transcripts have been identified for each of the first four enzymes in the pathway. ALA is the first intermediate in the haem biosynthetic pathway exclusively committed to haem synthesis, and the rate of ALA synthesis is an important controlling step for haem formation. Erythroid-specific and housekeeping transcripts for the first step enzyme ALA synthase are encoded by two separate genes on different chromosomes: the erythroid-specific gene, expressed only in fetal liver and adult bone marrow (ALA synthase-2 on chromosome X), and the housekeeping gene, expressed in all cells (ALA synthase-1 on chromosome 3). For ALA dehydrase, porphobilinogen deaminase and uroporphyrinogen synthase, each transcript is activated from the same gene [5].

In the liver

In the liver, the haemoprotein enzymes formed, including cytochrome P450s, are rapidly turned over in response to current metabolic needs. ALA synthase-1 has many features of a rate-limiting enzyme in the production of haem. The rate of this enzyme turnover is very rapid; the half-life of mitochondrial ALA synthase-1 is among the shortest of all mitochondrial proteins. The ALA synthase-1 activity in normal liver is the lowest among all enzymes in the haem biosynthetic pathway. The free intracellular haem pool inhibits ALA synthase-1 activity via a negative feedback regulation (Fig. 3). Four potential targets for regulating the formation of the enzyme by haem have been identified: regulation of the production of ALA synthase at either (i) the transcriptional level or (ii) the translational level with a destabilization of ALA synthase mRNA by haem; (iii) modulation of the rate of entry of the enzyme into the mitochondria; or (iv) direct inhibition (Fig. 4). Basal or uninduced hepatic ALA synthase-1 provides sufficient ALA and haem to maintain normal levels of liver haemoproteins but, when the synthesis of the cytochrome P450 enzymes is induced and more haem synthesis is required, ALA synthase-1 is induced. The activity of PBG deaminase, the third enzyme in the pathway, is close to that of ALA synthase-1. Increased hepatic ALA synthase activity has been described during acute attacks in patients with acute porphyrias. ALA synthase induction is a secondary phenomenon; it is the result of exposure to several factors (such as drugs, hormones) that act on an enzyme more or less derepressed by haem deficiency. Under these conditions of ALA synthase-1 induction and increased ALA production, such as in an acute attack of an acute hepatic porphyria, PBG deaminase can become a rate-limiting metabolic step. This would account for the especially marked increases in ALA and PBG that are produced and excreted in acute intermittent porphyria (see Chapter 16.5). The activity of HO, the rate-limiting enzyme for

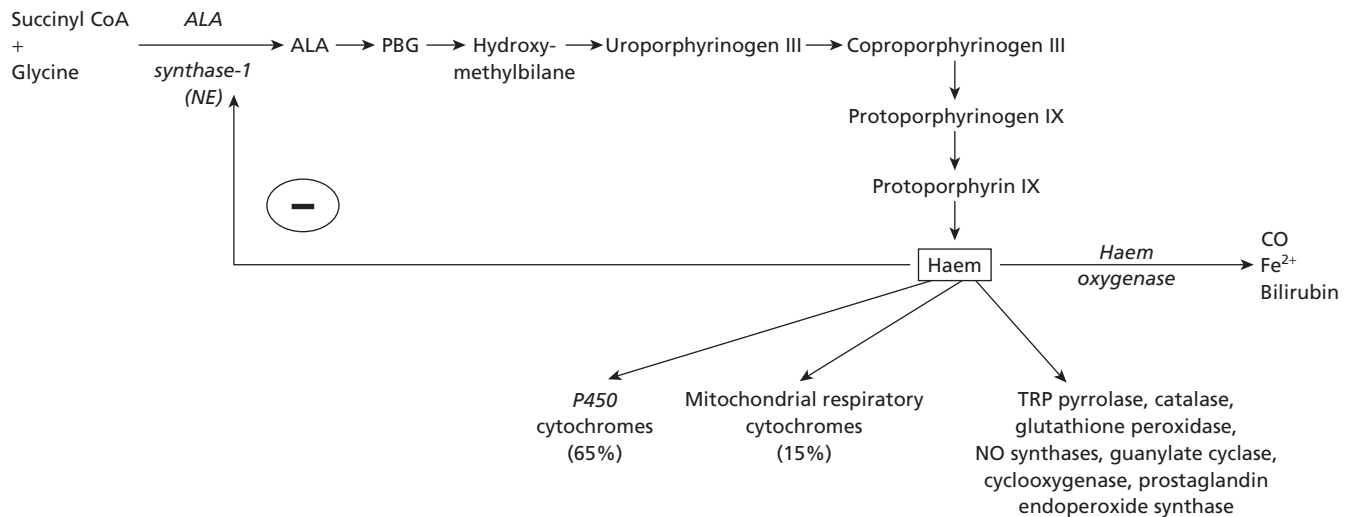
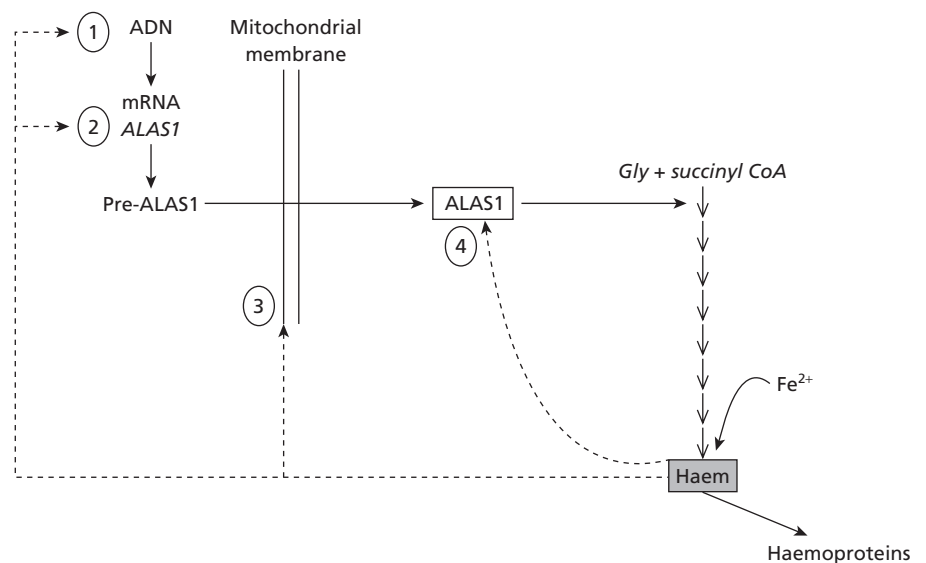


Fig. 3 Regulation of haem biosynthesis in the liver. ALA synthase-1 is rate limiting and feedback regulated by the intracellular concentration of haem. NE, non-erythroid form; TRP, tryptophan; NO, nitric oxide.

Fig. 4 Retroinhibition of hepatic ALA synthase-1 (ALAS1) activity via a negative feedback regulation mediated by the free intracellular haem pool. Four potential targets for regulating the formation of the enzyme by haem are identified: (1) the transcriptional level (*trans*-regulating factor haem responsive element: HRE), (2) the translational level with a destabilization of ALA synthase mRNA by haem, (3) modulation of the rate of entry of the enzyme into the mitochondria (mediated by a haem regulatory motif: HRM), (4) direct inhibition of the enzyme activity.



haem degradation to CO, iron and bile pigment, can also influence the level of regulatory free haem in hepatocytes. Starvation leads to increased HO activity, which may contribute to precipitating a crisis in acute hepatic porphyrias by depleting haem [6]. Conversely, attacks of these disorders can be prevented by a high carbohydrate diet. Thus, mechanisms for both haem synthesis and degradation can regulate haem formation.

In erythroid cells

In the erythroid cell, the synthesis of the enzymes participating in the formation of haem is under the control of iron and erythropoietin, formed under hypoxic conditions. Indeed,

regulatory influences in the erythrocyte act during cell differentiation and, in contrast to the liver, the erythrocyte also responds to stimuli for haem synthesis by increasing its cell numbers to meet changing requirements for haemoglobin. The haemoglobinization of the erythroid cell is controlled by the ALA synthase-2 isoenzyme, which exhibits a 75% identity in the C-terminal part with ALA synthase-1. This enzyme, produced by a gene on the X chromosome, is not inducible by the drugs that induce ALA synthase-1 in the liver and is not repressed by exogenous haem treatment. ALA synthase-2 activity is induced only during the period of active haem synthesis of the red cell, and its rate of formation, and thus its activity in the cell, is in the end regulated by the amount of free iron present. The erythroid ALA synthase-

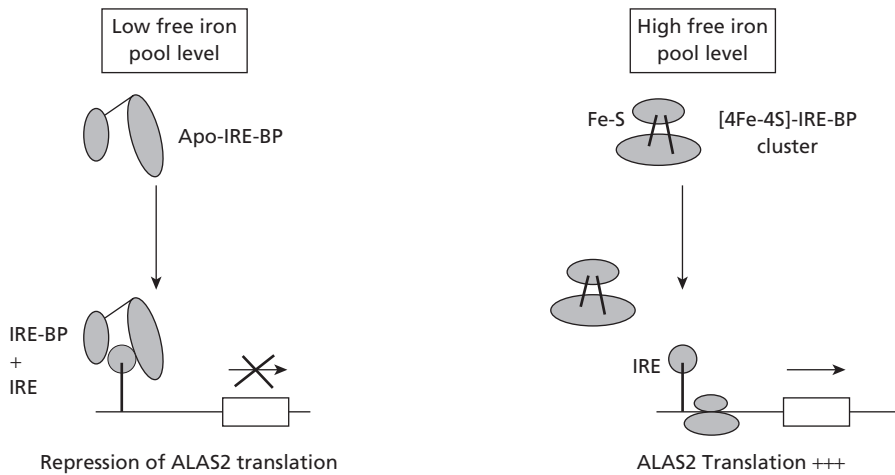


Fig. 5 Regulation of ALA synthase-2 translation by free iron/IRE/IRE-BP. In iron deficiency, the mRNA of erythroid ALA synthase-2 is blocked by attachment to an iron responsive element (IRE) of an IRE-binding protein (IRE-BP), and translation of this key enzyme is inhibited. In the presence of an excess of iron, the lack of attachment leads to an increase in the translation of the enzyme.

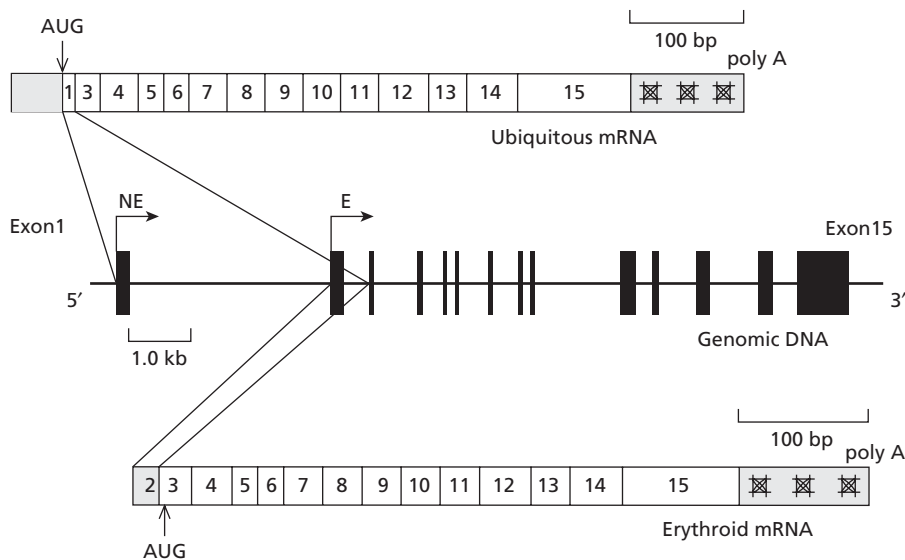


Fig. 6 Gene structure of the human porphobilinogen deaminase gene and its two transcripts produced by alternative splicing. NE, non-erythroid promoter; E, erythroid promoter.

2 gene encodes an iron-responsive element (IRE), which is a stable stem-loop structure on the mRNA with affinity for a specific cytosolic protein, the IRE-binding protein (IRE-BP). If there is enough free iron present in the erythroid cell, the IRE-BP is modified by the formation of sulphur/iron clusters; it then loses its ability to bind the IRE sequence. ALA synthase-2 translation is increased as a result, and the rate of formation of haem, and thus of globin chains as well, is activated. In iron deficiency, the unmodified IRE-BP blocks ALA synthase-2 translation by associating with its mRNA, and haem synthesis is consequently inhibited by lack of ALA synthase activity (Fig. 5). ALA dehydrase and PBG deaminase are other sites of regulation mediated by alternative splicing. The single PBG deaminase gene is located at chromosome 11q24.1–24.2 and contains 15 exons. It encodes erythroid-specific and ubiquitous isoforms of PBG deaminase that are generated by the use of separate promoters and alternative splicing of the two primary transcripts. The isoforms differ only at their NH₂ ends where the ubiquitous isoform extends for

an additional 17 residues, making a polypeptide of 361 amino acids (Fig. 6).

The upstream promoter is active in all tissues, and thus the enzyme encoded by the larger transcript was termed the 'house-keeping PBG deaminase'. The other promoter, located 3 kb downstream, is active only in erythroid cells. It displays some structural homology with the β -globin gene promoter, suggesting that some common *trans*-acting factors may coregulate the transcription of these genes during erythroid development. The expression of ALA synthase-2, ALA dehydrase and PBG deaminase erythroid isogenes is determined by *trans*-activation of nuclear factor GATA-1, CACC box and NF-E2 binding sites in the promoter areas [7]. Ferrochelatase, the final enzyme in haem biosynthesis, may also play a significant role in controlling the rate of haem formation in erythroid cells. Ferrochelatase deficiency in human protoporphyria results in the accumulation of protoporphyryn almost exclusively in erythroid tissue, even though ferrochelatase is deficient in all other tissues in these

patients. This finding suggests that ferrochelatase activity can become rate limiting in erythroid cells, but not in other tissues, when the enzyme itself or its substrate, iron, is partially deficient. Although protoporphyrin is excreted only in the bile and accumulates in the liver in some patients, it presumably originates in the bone marrow.

Excretion of porphyrins and of porphyrin precursors

Porphyrins and porphyrin precursors are excreted in urine and/or bile (Table 1). In relation to the total rate of haem synthesis, excretion of porphyrins is very small; in other words, few porphyrins (and porphyrin precursors, ALA, PBG) 'escape' during haem formation and therefore are not transformed into bilirubin. Each day, bone marrow and liver synthesize about 375 mg of haem. In humans, the mean level of ALA excreted in urine is ~ 3 mg/day; this means that less than 0.5% of ALA synthesized each day has not been used for haem synthesis [8].

Urine

Porphyrin precursors

ALA and PBG are excreted only in the urine. After injection of labelled precursors, a large fraction of labelled ALA is excreted unaltered in urine; a small fraction is much more efficiently incorporated into hepatic haem than into haemoglobin in erythropoietic cells, probably because of the relative impermeability of the erythropoietic cell membrane to ALA. Injection-labelled PBG cannot be demonstrated in the liver, probably because it also does not pass the liver cell membrane; the impermeability of the cell membrane is only relative as, in instances

where the amount of endogenous PBG is considerably increased (for instance acute porphyrias), huge amounts of PBG are found in urine. Moderately increased excretion of precursors is also observed in patients with hepatic diseases [8].

Porphyrins

Coproporphyrin is the predominant porphyrin in normal human urine (Table 1). However, urine contains only 30–35% of the total coproporphyrin; the remainder is found in bile. This urinary and faecal distribution is combined with a differential excretion of coproporphyrin isomers. The type 3 isomer predominates (70%) over the type 1 in urine, whereas the reverse is found in bile. It is now widely accepted that the unequal excretion rates of the isomers can be attributed to a hepatic carrier that favours the excretion of the I isomer [9]. In several human liver diseases with impaired hepatic excretory mechanisms, there is an increase in the total isomer ratio towards a predominance of the type 1 compound [2]. Other porphyrins present in normal human urine include uroporphyrin and traces of porphyrins with seven, six and five carboxyl groups. The intermediates (porphyrinogens) are unstable and rapidly oxidize to their corresponding porphyrins, and a large fraction of the porphyrins present in urine are excreted in this form of reduced colourless precursors. Freshly voided urine from patients with acute porphyria may have little if any increase in concentrations of preformed porphyrin; this probably explains why urine is frequently normal in colour when freshly passed, but darkens gradually on exposure to light and air. PBG can also be converted non-enzymatically to porphyrins (mostly uroporphyrin type 1): it is therefore very important to protect urine from light and to store it between 4 and 10°C if measurement of precursors has to be carried out.

Table 1 Normal values of haem pathway metabolites in humans: data from the Centre Français des Porphyries.

	Urine (per mmol creatinine)		Faeces (per g dry weight)		Red blood cell (per L)		Plasma (per L)	
	Normal value	Method	Normal value	Method	Normal value	Method	Normal value	Method
δ-Aminolaevulinic acid (µmol)	0–3	Ion-exchange chromatography	–	–	–	–	–	–
Porphobilinogen (µmol)	0–1	–	–	–	–	–	–	–
Total porphyrin (nmol)	–	–	10–200	Quantitative spectrophotometry	500–1900	Quantitative fluorimetric scanning	10–20	Quantitative fluorimetric scanning
Uroporphyrin (nmol)	0–10	Quantitative spectrophotometry	0–2	HPLC	0–6	HPLC	Lack of fluorescence emission spectroscopy (620–630 nm)	
Coproporphyrin (nmol)	0–20	–	0–60	HPLC	50–180	HPLC		
Protoporphyrin (nmol)	–	–	0–150	HPLC	300–1800	HPLC		

HPLC, high-pressure liquid chromatography.

Faeces

Significant amounts of porphyrins are excreted in faeces (mostly all of the protoporphyrin and 70% of coproporphyrin; Table 1). They may represent pigments that have reached the intestinal tract with the bile, except dicarboxylic porphyrins, which are mainly of dietary origin and may be derived from haem proteins of ingested food or intestinal haemorrhages; they may also be formed by intestinal microorganisms. Because of this, faecal porphyrins should be measured only if the patient's food has been devoid of bleeding or ingestion of meat in the past 3 days.

The mechanism by which protoporphyrin is excreted into bile has been studied mostly after description(s) of fatal liver disease in erythropoietic protoporphyria (see Chapter 16.5). Among haem-forming tissues, the bone marrow is the major source of protoporphyrin, a very poorly water-soluble compound: in the plasma, over 90% of protoporphyrin is bound to albumin with some bound to haemopexin. Hepatic uptake may occur through a process similar to that for other organic anions (such as bilirubin) that are bound to albumin. In the isolated, *in situ*-perfused rat liver, the overall disappearance of protoporphyrin follows first-order kinetics. Within the hepatocyte, protoporphyrin is associated with several proteins, among them one of the Z class of liver cytosolic proteins [10]. The rate-limiting step for the overall transport of protoporphyrin from plasma to bile appeared to be canalicular secretion, as less than 5% of the protoporphyrin extracted by liver was secreted into bile. This secretion should be mediated by the ABCG2/BCRP transporter, a member of the ATP-binding cassette (ABC) family [11]. The basal rate of bile secretion of porphyrins has been studied in healthy humans [12]: the flow of protoporphyrin is slightly higher than the flow of coproporphyrin; the flow of uroporphyrin is the lowest. Hepatic conjugation with glucuronic acid does not occur for protoporphyrin and coproporphyrin. Approximately 85% of hepatic protoporphyrin remains metabolically unaltered before being eliminated by bile secretion; 15% of protoporphyrin extracted by the liver may be converted to bilirubin, with non-haemoglobin haem species as intermediaries, and is also excreted in bile.

Hepatic infusion of micelle-forming bile acids facilitates canalicular protoporphyrin secretion [13]: the micelle-forming taurocholate increased biliary protoporphyrin concentration (by more than six times) and secretion (by more than 12 times) considerably more than dehydrocholate (a non-micelle-forming bile acid). Some bile acids (taurocholate and glycocholate) increase protoporphyrin metabolism 1.7- to 2.7-fold over control values. There are a number of direct and indirect ways in which bile acids might alter the metabolism of protoporphyrin, including either the stimulation of the activity of enzymes such as ferrochelatase and HO or the solubilization of protoporphyrin [14]. Before its final faecal excretion, a significant proportion of protoporphyrin is reabsorbed in the intestine and may circulate through the enterohepatic system [13]. However, it is not yet known how much intestinal microorganisms or food contribute to the total fecal porphyrin excretion.

References

- Anderson KE, Sassa S, Bishop DF *et al.* (2001) The porphyrias. In: Scriver CR, Beaudet AL, Sly WS *et al.* (eds) *The Metabolic Basis of Inherited Disease*, 8th edn, Vol. 1. New York: McGraw-Hill Publications, pp. 2991–3062.
- Mauzeral DC (1998) Evolution of porphyrins. *Clin Dermatol* 16, 195–201.
- Dailey HA (1997) Enzymes of heme biosynthesis. *J Biol Inorg Chem* 2, 411–417.
- Ponka P (1999) Cell biology of heme. *Am J Med Sci* 318, 241–256.
- May BK, Dogra SC, Sadlon TJ *et al.* (1995) Molecular regulation of heme biosynthesis in higher vertebrates. *Prog Nucleic Acid Res Mol Biol* 51, 1–51.
- Deybach JC, Puy H (2003) Acute intermittent porphyria from clinical to molecular aspects. In: Kadish KM, Smith KM, Guillard R (eds) *Porphyria Handbook*. San Diego, CA: Academic Press Publications, pp. 319–338.
- Thunell S, Harper P, Brock A *et al.* (2000) Porphyrins, porphyrin metabolism and porphyrias. *Scand J Clin Lab Invest* 60, 541–560.
- Doss MO, Sassa S (1994) The porphyrias. In: Noe DA, Rock RC (eds) *Laboratory Medicine. The Selection and Interpretation of Clinical Laboratory Studies*. Baltimore, MD: Williams & Wilkins Publications, pp. 535–553.
- Kaplowitz N, Javitt N, Kappas A (1972) Coproporphyrin I and 3 excretion in bile and urine. *J Clin Invest* 51, 2895–2891.
- Vincent SH, Muller-Eberhard U (1985) A protein of the Z class of liver cytosolic proteins in the rat that preferentially binds heme. *J Biol Chem* 260, 14521–14528.
- Zhou S, Zong Y, Ney PA *et al.* (2005) Increased expression of the Abcg2 transporter during erythroid maturation plays a role in decreasing cellular protoporphyrin IX levels. *Blood* 105, 2571–2576.
- McCormack LR, Liem HH, Strum WB *et al.* (1982) Effects of haem infusion on biliary secretion of porphyrins, haem and bilirubin in man. *Eur J Clin Invest* 12, 257–262.
- Ibrahim GW, Watson CJ (1968) Enterohepatic circulation and conversion of protoporphyrin to bile pigment in man. *Proc Soc Exp Biol Med* 127, 890–895.
- Berenson MM, Marin JJ, Larsen R *et al.* (1987) Effect of bile acids on hepatic protoporphyrin metabolism in perfused rat liver. *Gastroenterology* 93, 1086–1093.

2.3.11 Vitamins and the liver (A and D)

Masataka Okuno, Rie Matsushima-Nishiwaki and Soichi Kojima

Summary

The metabolism, pathological relevance and therapeutic applications of retinoids (vitamin A and its derivatives) and vitamin D are reviewed in human hepatic disorders. Both vitamins have profound effects on cell activities, including cell growth, differentiation and apoptosis. Retinoids consist of several molecular species, including retinoic acid (RA), retinol and retinylesters. Dietary retinoids are packed in nascent chylomicrons that are taken up by the liver, in which hepatic stellate cells (HSC) store