Intracellular Trafficking of Porphyrins

Iqbal Hamza*

Department of Animal and Avian Sciences and Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742

ABSTRACT Hemes are porphyrins that play a critical role in diverse biological processes. Heme synthesis culminates in the mitochondrial matrix, but the eight-step biosynthetic pathway is spatially shared between the mitochondria and cytoplasm. A recent paper describes the nature of the transporter that translocates the heme precursor coproporphyrinogen III into the mitochondria for heme synthesis. The identification of ABCB6 (ATP-binding cassette, subfamily B, member 6) and future studies aimed at precisely delineating the mechanism and the physiological nature of its ligand(s) will further enhance our current understanding of the intracellular movement of tetrapyrroles in eukaryotes.

With a few exceptions, heme, a metalloporphyrin, is synthesized *via* a multistep biosynthetic pathway with well-defined intermediates that are highly conserved throughout evolution. Depending upon the organelle and cell type, heme pathway intermediates are utilized for the synthesis of other tetrapyrrole compounds, including bilins, chlorophylls, and corrins (*1–3*). Despite our extensive knowledge of heme biosynthesis, the intracellular trafficking of heme and porphyrins are not well understood (Figure 1). In a recent issue of *Nature*, Krishnamurthy *et al.* (*4*) report on the identification of ABCB6 (ATP-binding cassette, subfamily B, member 6), a mitochondrial outer membrane (OM) transporter, which was shown by different experimental approaches to be able to transport porphyrins. ABCB6 is proposed to translocate coproporphyrinogen III (CPgenIII), a heme precursor, from the cytoplasm to the mitochondria for synthesis of heme. Their findings therefore represent a significant advancement in our understanding of intraorganellar porphyrin transport in mammalian cells.

Porphyrins are heterocyclic organic rings made from four pyrrole subunits linked *via* methine bridges. The name porphyrin is derived from the Greek word *prophura* for purple because the extensive conjugation of these tetrapyrroles gives them their violet-red hues. Heme is an iron-containing porphyrin and serves as a prosthetic group for many biological processes, including oxidative metabolism, xenobiotic detoxification, the synthesis and sensing of diatomic gases, cellular differentiation, gene regulation at the level of transcription, protein translation and targeting, and protein stability.

Heme synthesis culminates in the mitochondrial matrix, but the eight sequential enzymatic steps are spatially separated between the cytoplasm and the mitochondria. CPgenIII is a product of the fifth enzyme in the heme pathway, uroporphyrinogen III decarboxylase, and enters the mitochondria to undergo three additional enzymatic reactions to generate heme (Figure 1, panel a) (*5*). The pathway culminates when ferrous iron is catalytically inserted into the protoporphyrin IX (PPIX) ring by ferrochelatase (FECH), a mitochondrial inner-membrane (IM)-associated enzyme (*6, 7*). Importantly, iron and porphyrins are toxic to cells; iron generates hydroxyl radicals from Fenton-based reactions, and PPIX catalyzes light-dependent generation of oxygen radicals. Moreover, the end product of the synthetic pathway is heme, a cytotoxic macrocycle with peroxidase activity. Consequently, cellular heme synthesis is coupled with iron availability and apo-protein synthesis to prevent uncoordinated accumulation of iron, porphyrin intermediates, and heme (*8, 9*).

Two unanswered questions are pertinent to heme synthesis in eukaryotic cells: how does δ-aminolevulinic acid (ALA), the first intermediate of the pathway, exit from the mitochondrial matrix into the cytoplasm, and how does CPgenIII translocate from the cytoplasm into the intermembrane mitochondrial space (IMS)? The results from Krishnamurthy *et al.* (*4*) provide evidence that ABCB6 may be responsible for
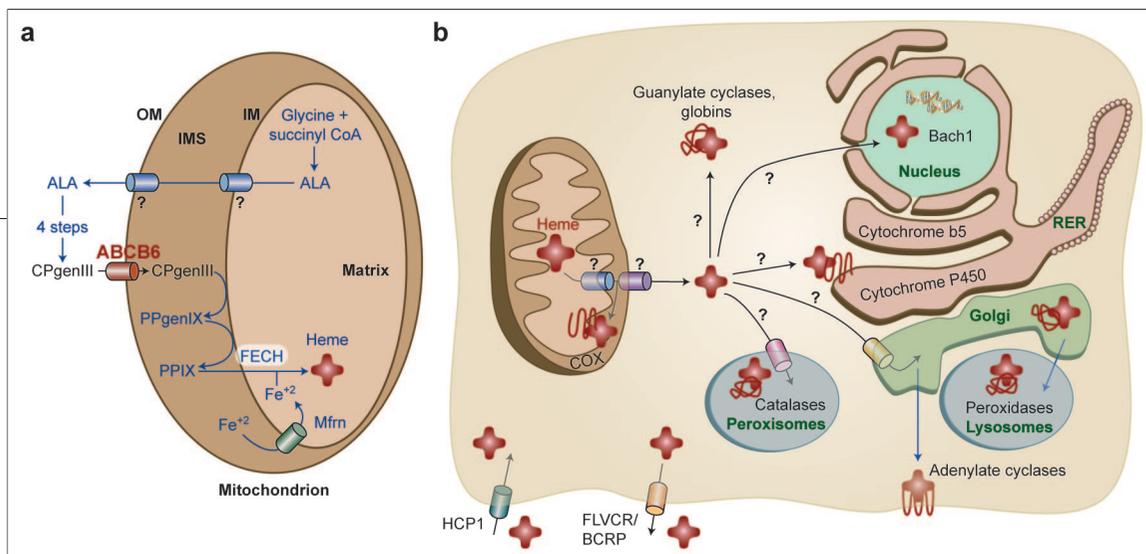
*Corresponding author, hamza@umd.edu.

Figure 1. Schematic model of mammalian heme homeostasis. Presumptive heme pathways that are currently unknown are marked with a question mark. a) In eukaryotic cells, heme is synthesized *via* a multistep pathway that is spatially separated between the cytosol and the mitochondria. The mechanism of export of ALA from the mitochondria is unknown. ALA is converted to CPgenIII *via* four enzymatic reactions, and CPgenIII is imported into the mitochondria, presumably by ABCB6, an OM transporter, or by OGC. CPgenIII is converted to protoporphyrinogen (PPgenIX) and PPIX on the IMS side of the IM. The final step of heme synthesis occurs by the enzymatic chelation of ferrous iron (Fe^{+2}) into PPIX catalyzed by FECH located on the matrix side of the IM. Mitoferrin (Mfrn)/Mrs 3/4 is predicted to import Fe^{+2} into the mitochondria. CoA = coenzyme A. b) The nascent heme moiety is somehow transported through two mitochondrial membranes and incorporated into a multitude of hemoproteins, presumably by hemochaperones, in different cellular compartments. Recent studies have identified HCP1 as the heme importer, primarily in intestinal cells, whereas FLVCR and BCRP are predicted to export heme in erythrocytes. COX = cytochrome oxidase, RER = rough endoplasmic reticulum.

CPgenIII transport. However, the recent work by Kabe *et al.* (*10*) suggests that the 2-oxoglutarate carrier (OGC) may also perform the same function. Thus, additional work is required to firmly resolve the identity of the transporter and the biological nature of its ligand. Both studies use the commercially available oxidized planar conjugated macrocycle, coproporphyrin III (CPIII), rather than the physiologically relevant substrate CPgenIII, a reduced nonplanar porphyrin.

The findings that ABCB6 expression is coordinated with heme synthesis and cellular function provide further proof of the importance of orchestrated timing of biological processes during development. As erythrocytes mature, the primary function of these oxygen-carrying cells is hemoglobin synthesis, which is underscored by a dramatic and highly coordinated up-regulation of heme and globin production. Using biochemical and cell culture assays with wild-type and mutant forms of ABCB6, the authors provide evidence that *Abcb6* messenger RNA (mRNA) and protein are up-regulated in erythroleukemia and in G1ER cells, which are an immature red cell precursor line (*4*). They show that ABCB6 binds porphyrins, including heme, but

binding and competition assays suggest that the substrate is likely to be CPIII. ABCB6 activity was found to be highly regulated by cellular heme levels. Inhibition of heme synthesis by succinyl acetone resulted in down-regulation of *Abcb6*, whereas ectopic expression of *Abcb6* resulted in increased PPIX accumulation and up-regulation of mRNA for several heme biosynthesis genes. Notably, knock-down of *Abcb6* by RNA-mediated interference resulted in diminished heme synthesis, further proof that *Abcb6* expression is coordinated with heme synthesis. Furthermore, genetic ablation of a single copy of *Abcb6* in mouse embryonic stem cells revealed a haploid insufficiency phenotype; $Abcb6^{+/-}$ heterozygous cells had accumulated half the levels of PPIX compared with wild-type cells when treated with the heme precursor, ALA. Altogether, the paper provides evidence that ABCB6 plays an important role in regulating heme synthesis either by directly channeling CPgenIII to the mitochondria (Figure 1, panel a) or by indirectly regulating another step in the pathway.

The molecules and the mechanisms involved in heme transport across biological membranes to various cellular destinations

are poorly understood, partly because of the use of *in vitro* biochemical approaches, static microscopic techniques, and inappropriate genetic model systems. Given the well-established paradigm for intracellular copper trafficking pathways (*11*), it is likely that specific pathways also exist for transport, trafficking, sequestration, and egress of heme in cells (Figure 1, panel b). Although several parallels exist between copper and heme, the most noteworthy is that both are essential cofactors that participate in electron transfer reactions but are toxic compounds when found in excess. A major difference, however, is that nutritional copper is acquired exogenously, whereas heme is produced endogenously by a defined and regulated pathway. Although the pathway and intermediates for heme synthesis have been well defined, the handling of heme from its point of synthesis in the mitochondria to its insertion into hemoproteins remains poorly understood (Figure 1, panel b). Heme is a hydrophobic molecule and is insoluble in the aqueous cellular milieu. Free heme is toxic to biological macromolecules. How then is heme transported through the mitochondrial IMs to specific hemoproteins that reside in the cytoplasm,

peroxisomes, mitochondrial IMS, secretory pathway, and nucleus? What are the mechanisms for incorporating heme into apo-hemoproteins? Are these mechanisms specific to certain target proteins or to the milieu of a subcellular compartment? Humans have intracellular hemoproteins, such as hemo-, myo-, neuro-, and cytoglobins, as well as heme enzymes, including cytochrome P450s, adenylate cyclases, soluble guanylate cyclases, peroxidases, catalases, and respiratory cytochromes. These enzymes are located in different cellular organelles, and they perform diverse biological functions that depend upon heme as a cofactor. Thus, in principle, specific intracellular pathways are likely to also exist for the safe, efficient, and accurate transfer of heme from the mitochondrial IM to distinct hemoproteins that are present in various subcellular compartments (Figure 1, panel b).

Researchers have recently demonstrated that the breast cancer resistance protein (BCRP) and the feline leukemia virus subgroup C receptor (FLVCR) are potential heme exporters in developing erythroid cells and that the heme carrier protein 1 (HCP1) is the intestinal heme importer in mammals (*12–14*) (Figure 1, panel b). Although it is unclear why heme export would be necessary in red blood cells given the overwhelming requirement for heme in hemoglobin synthesis, these studies underscore the necessity for translocation of heme between membrane compartments.

A conceptual setback in identifying heme trafficking pathways has been the difficulty in dissociating biosynthesis from downstream trafficking events for three main reasons: (i) organisms normally make endogenous heme *via* a highly regulated pathway, (ii) defects in the heme synthesis pathway are usually lethal or have pleiotropic effects, and (iii) exogenous heme/porphyrins are poorly utilized by organisms that normally make heme. Although hemes are found in all phyla, certain prokaryotic organisms neither make heme nor contain hemo-

proteins, and the protozoa *Leishmania spp.* appear to lack seven of the eight enzymes of the heme biosynthetic pathway (*2, 15*). An exception is *Caenorhabditis elegans*, a free-living nematode that does not synthesize heme but ingests dietary heme to fulfill its heme auxotrophy. *C. elegans* has the repertoire of hemoproteins that humans have (*16*). It represents a unique genetic model system for dissecting the cellular and molecular determinants of heme homeostasis because it has a clean genetic background devoid of endogenous heme. Worms will therefore permit external control over the flux of heme and intracellular trafficking pathways, an advantage not attainable in other model systems.

The practical implications of discoveries in porphyrin transport are far-reaching. Identification of mammalian heme transporters, including HCP1, will allow the design of more bioavailable forms of iron or porphyrin-based "nutraceuticals" to deliver iron more effectively to iron-deficient populations. Identification of the mechanisms by which enzymes such as the cytochrome P450s and guanylate cyclases acquire heme will provide novel insights into modulating biologic responses to pharmaceuticals, xenobiotics, and gases such as nitric oxide. Finally, characterization of how heme is transported in organisms may lead to the discovery of parallel trafficking pathways for other tetrapyrroles such as vitamin B_{12}.

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