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Heme Biosynthesis: Biochemistry, Molecular Biology, and Relationship to Disease

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INTRODUCTION

This volume of *Journal of Bioenergetics and Biomembranes* features a minireview series on heme biosynthesis and disorders associated with enzymes of the heme biosynthetic pathway. It is the first time that such a series has been covered in this journal, and it represents great timing. In the past few years, tremendous progress at the molecular level has been made on the different enzymes of the pathway (e.g., enzyme mechanisms have been unravelled, structural motifs with putative functional roles have been proposed, one three-dimensional structure has been determined), new developments in the "gene frontier" have been achieved (e.g., genes of the heme biosynthetic pathway have been cloned and characterized, mutations associated with sideroblastic anemia and certain porphyrias have been identified, regulatory mechanisms have been advanced), exciting findings in the pathogenesis and treatment of porphyrias have been accomplished (e.g., animal models for certain porphyrias have been created, gene transfer experiments have been performed, and the results indicate that gene therapy may soon become a therapeutic alternative for porphyrias), and new challenges await.

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The heme biosynthetic pathway comprises eight enzymes. In eukaryotes, four enzymes (i.e., 5-aminolevulinate synthase, coproporphyrinogen oxidase, protoporphyrinogen oxidase, and ferrochelatase) are associated with mitochondria, while four are cytosolic enzymes (i.e., porphobilinogen synthase, prophobilinogen deaminase, uroporphyrinogen III synthase, and uroprophyrinogen decarboxylase) (Fig. 1). The first enzyme of the heme biosynthetic pathway in animals, fungi, and some bacteria is 5-aminolevulinate synthase, which catalyzes the condensation of glycine and succinyl-CoA to form 5-aminolevulinic acid. Ferreira and Gong, in the first article of the series, review structure–function studies of 5-aminolevulinate synthase. The article contains a review of the progress made in the development of heterologous 5-aminolevulinate synthase expression systems and a discussion on translational regulatory mechanisms for the erythroid 5-aminolevulinate synthase mRNA. In addition, the structural and functional roles of the pyridoxal 5′-phosphate-binding lysine and of a structural motif, proposed to be at the cofactor's binding site, are examined.

In the second article, S. Bottomley *et al.* discuss "Molecular Defects of Erythroid 5-Aminolevulinate Synthase in X-linked Sideroblastic Anemia." The human genetic disorder X-linked sideroblastic anemia is associated with defects in the aminolevulinate synthase erythroid-specific gene. The authors discuss a heterogeneous series of point mutations, found in the catalytic domain, as the cause of the disorder. This article is particularly interesting, not

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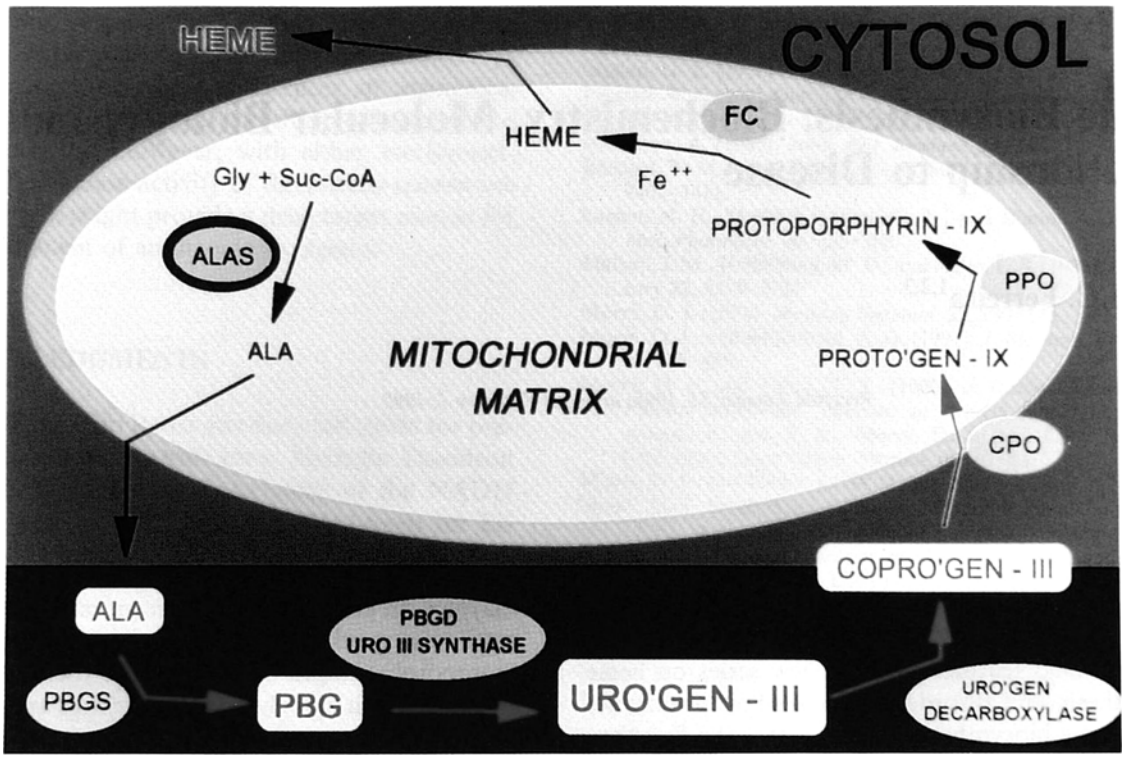

Fig. 1. Heme biosynthetic pathway in eukaryotic animal cells. Depicted are the eight enzymes of the pathway. This minireview series concentrates on seven of the eight enzymes (see text for description of their roles). Abbreviations: ALAS, 5-aminolevulinate synthase; PBGS, porphobilinogen synthase; PBGD, porphobilinogen deaminase; Uro III synthase, uroporphyrinogen III synthase; Uro'gen decarboxylase, uroporphyrinogen III decarboxylase; CPO, coproporphyrinogen oxidase; PPO, protoporphyrinogen oxidase; FC, ferrochelatase.

only because of the studies and results gathered by the authors, but also because it involves a collaborative perspective from three of the major laboratories working on 5-aminolevulinate synthase.

Porphobilinogen synthase is the second enzyme of the heme biosynthetic pathway (Fig. 1) and it catalyzes the asymmetric condensation of two molecules of 5-aminolevulinic acid to yield the monopyrrole, porphobilinogen. In the third article, E. K. Jaffe examines, in detail, the mechanism of the porphobilinogen synthase-catalyzed reaction. The roles of zinc and magnesium ions at the active site and/or as an allosteric effector are discussed in terms of the mechanism of the enzyme and the evolutionary pattern. The article also includes a section on the importance of porphobilinogen synthase to human health; trace levels of lead inhibit catalytic activity. The mechanism of inhibition is examined.

The third and fourth enzymes of the heme biosynthetic pathway, porphobilinogen deaminase and uroporphyrinogen III synthase (Fig. 1), catalyze the conversion of four molecules of porphobilinogen into uroporphyrinogen III. In the fourth article, P. M. Jordan reviews the recent breakthroughs in protein chemistry and DNA recombinant technology made by the author and his coworkers towards elucidating the mechanisms of the two enzymes. In addition, Jordan examines the X-ray structure of *Escherichia coli* porphobilinogen deaminase, the only enzyme of the pathway for which the three-dimensional structure has been determined, as a model for the human deaminase structure and as the framework for the molecular understanding of the mutations found in acute intermittent porphyria.

In the fifth article, Deybach and Puy examine the "Porphobilinogen Deaminase Gene Structure and Molecular Defects." Decreased (50%) porphobilinogen deaminase activities are a characteristic of cells from acute intermittent porphyria patients and asymptomatic carriers of the gene defect. This porphyria is the most common type of acute hepatic porphyria. Clinical expression of the disease generally occurs after puberty, but environmental or acquired factors may precipitate acute attacks. In this article,

Deybach and Puy review the molecular biology of the human porphobilinogen deaminase gene (e.g., structure and chromosomal localization) and evaluate all of the so-far identified mutations and polymorphisms at the porphobilinogen deaminase locus, which could be used for identification of the gene defect carriers.

Uroporphyrinogen decarboxylase catalyzes the decarboxylation of uroporphyrinogen III to coproporphyrinogen III (Fig. 1). In the sixth article, G. Elder reviews, completely, the enzyme structure, the mechanism, and the molecular genetic aspects associated with the enzyme. In particular, the author examines the effects of inherited and aquired factors in the pathogenesis of two human porphyrias (i.e., porphyria cutanea tarda and hepatoerythropoietic porphyria), which are associated with decreased uroporphyrinogen decarboxylase activity in the liver.

Coproporphyrinogen oxidase, the antepenultimate enzyme of the heme biosynthetic pathway (Fig. 1), catalyzes the conversion of two propionate groups at positions 2 and 4 of coproporphyrinogen III to two vinyl groups. The product of the coproporphyrinogen oxidase-catalyzed reaction is, therefore, protoporphyrinogen IX. In the seventh article, B. Grandchamp and colleagues discuss "Molecular Abnormalities of Coproporphyrinogen Oxidase in Patients with Hereditary Coproporphyria." The authors review the recent cloning of the human coproporphyrinogen oxidase cDNA and gene, the primary structure of the enzyme, and the structure of the gene. In this manuscript, the authors also analyze molecular defects of coproporphyrinogen oxidase associated with coproporphyria manifested in some families.

Protoporphyrinogen oxidase is the penultimate enzyme of the heme biosynthetic pathway (Fig. 1). It catalyzes the six-electron oxidation of protoporphyrinogen IX into protoporphyrin IX. In addition, reduced protoporphyrinogen oxidase activity is a hallmark of variegate porphyria. However, while recent progress has been made in the development of purification methods for the enzyme[1–4] and in cloning the *Bacillus subtillis* protoporphyrinogen oxidase gene,[5] the corresponding human gene or cDNA remain to be cloned. Only with the cloning of human cDNAs and gene encoding protoporphyrinogen oxidase will it be possible to elucidate the molecular basis of variegate porphyria. Because of the lack of space and the lack of genetic information to interpret, at a molecular level, variegate porphyria (at least, some clinical cases), this enzyme was put "on hold" until a next minireview series.

Ferrochelatase is the terminal enzyme of the heme biosynthetic pathway (Fig. 1). It catalyzes the insertion of ferrous iron into the protoporphyrin IX to form protoheme IX. In the eighth article, G. Ferreira *et al.* evaluate the recent progress in the cloning of ferrochelatase cDNAs and genes, determination of the protein primary and secondary structures, and development of expression systems. Of significance, the authors discuss the identification of an iron-sulfur cluster, of yet unknown function, in mammalian ferrochelatase. In the ninth article, Taketani and Fujita discuss "The Ferrochelatase Gene Structure and Molecular Defects Associated with Erythropoietic Protoporphyria." The authors review in detail the gene structure of the human ferrochelatase gene and analyze different point-mutations found in patients with erythropoietic protoporphyria.

Finally, in the tenth article, H. De Verneuil *et al.* discuss "Porphyrias: Animal Models and Prospects for Cellular and Gene Therapy." Gene therapy may soon become a treatment alternative for porphyrias. De Verneuil and colleagues provide an analysis of the progress made in obtaining animal models for porphyrias (e.g., the PBGD knock-out mouse as a model of acute intermittent porphyria and the protoporphyric mouse $Fech^{m1Pas}/Fech^{m1Pas}$ obtained after chemical mutagenesis), and in gene transfer in different porphyria disease cells. The results of their gene transfer *in vitro* experiments indicate that certain erythropoietic porphyrias (e.g., congenital erythropoietic porphyria) are good candidates for treatment by gene therapy in hematopoietic stem cells.

This minireview series represents an up-to-date overview of the enzymes and genes of the heme biosynthetic pathway, at the structural, functional and regulatory levels. It also focuses on the disorders associated with the human heme biosynthetic pathway and on the recent advances toward their treatment. Hopefully, this series will create also an incentive for *Journal of Bioenergetics and Biomembranes* readers to follow this exciting area and, perhaps, to become involved in this area of research.

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