

Chapter 2

When Did Hemes Enter the Scene of Life? On the Natural History of Heme Cofactors and Heme-Containing Enzymes

Anne-Lise Ducluzeau^a and Wolfgang Nitschke^{b,*}

^a*School of Fisheries and Ocean Sciences, University of
Alaska-Fairbanks, 245 O'Neill Bldg., Fairbanks, AK 757220, USA*

^b*Laboratoire de Bioénergétique et Ingénierie des Protéines UMR
7281 CNRS/AMU FR3479, F-13402 Marseille Cedex 20, France*

Summary.....	13
I. Introduction.....	14
II. Hemes in LUCA; Pros and Cons.....	15
A. Does Ubiquity Automatically Translate into Deep Ancestry?.....	15
B. Phylogenies of Heme-Bearing Bioenergetic Enzymes Suggest a Red LUCA.....	15
C. The Dichotomy of Heme Biosynthesis Enzymes.....	16
1. Does the Existence of Two Distinct Pathways Necessarily Argue for an Anemic LUCA?.....	18
III. An Alternative Scenario.....	19
A. A Scenario Potentially Reconciling Molecular Phylogeny of Heme Enzymes and Pathway Dichotomy.....	19
B. Is the Heme-Biosynthesis-Pathway Dichotomy Related to Other Major Pathway Dichotomies?.....	20
IV. Why All the Fuzz?.....	21
Acknowledgments.....	22
References.....	22

Summary

Heme proteins are almost ubiquitous both in Archaea and in Bacteria. The last universal common ancestor (LUCA) of the two prokaryotic domains was, therefore, assumed until recently to already have made use of heme cofactors, a notion bolstered by molecular phylogenies of several heme-bearing enzymes. The discovery of a second pathway for heme biosynthesis, predominantly present in Archaea, was subsequently interpreted to indicate independent origins of heme biosynthesis in each of the two prokaryotic domains (Lane and Martin (Cell 151:1406–1416, 2012)), implying that the LUCA might have been entirely devoid of hemes and heme proteins. In this contribution, we outline the presently

*Author for correspondence, e-mail: nitschke@imm.cnrs.fr

available evidence in favour of either scenario and propose a new model reconciling the seemingly contradictory messages sent by molecular phylogeny of heme-bearing enzymes and the biosynthesis pathway dichotomy. A possible relation to other biosynthesis pathway dichotomies is suggested, and the far-reaching repercussions of the ultimate resolution of the controversy on our understanding of free energy conversion in the LUCA and at life's origin are emphasized.

I. Introduction

From the point of view of thermodynamics, living organisms fundamentally are free energy converting systems transforming environmental redox disequilibria into the extraordinary entropy decrease that characterizes cellular life (Branscomb and Russell 2013). One of the quintessential attributes of life therefore is its ability to perform redox reactions and to channel reducing equivalents from the environmental electron donating to the accepting substrates. Redox compounds therefore can safely be assumed to be crucial elements of life in general and thus must have already played a paramount role at its origin. Obvious candidates for such redox compounds at life's inception are centres made up from environmental transition metals such as iron, nickel, molybdenum, tungsten, manganese, cobalt etc.

Indeed, several redox cofactors found in extant life have been proposed based on molecular phylogeny to be as old as life itself, that is, iron sulphur clusters (Eck and Dayhoff 1966), nickel-iron centres (Vignais et al. 2001) and molybdenum/tungsten compounds (Schoepp-Cothenet et al. 2012). Iron-sulphur clusters and Mo/W-centres are indeed basically ubiquitous in extant life. However, another iron-based redox centre, i.e. heme, likely is almost as widely distributed in living organisms as are iron-sulphur and Mo/W-centres.

The heme cofactors in heme proteins overwhelmingly serve as electron transferring redox centres operating in an impressively wide range of electrochemical potentials. The major part of contributions to this volume indeed deals with electron transferring heme proteins. However, hemes can also play the roles of small-molecule transporters (e.g. Suzuki and Imai 1998) and sensors (Cutruzzolà et al. 2014; Martinkova et al. 2013; Pokkuluri et al. 2008) or even directly participate in catalysis (e.g. P₄₅₀, O₂-reductase *alias* cytochrome oxidase or *cd*₁-type nitrite reductase). The listed examples imply that in the context of this contribution, the term “heme” will refer to substituted Fe-tetrapyrroles in general, encompassing not only Fe-protoporphyrin IX (FePPIX), i.e. heme *b* and its derived heme *c*, but also differently substituted Fe-tetrapyrroles such as sirohemes or heme *a*, heme *o*, etc. This extension of scope becomes ineluctable in the evolutionary context since, as we will see, the synthesis pathways of differently substituted protoporphyrins are heavily convoluted and individual pathways can therefore not be dealt with separately.

Until a few years ago, the occurrence of heme proteins and hence necessarily of hemes in the oldest cellular entity which we can define, i.e. the last universal common ancestor (LUCA) of Bacteria and Archaea, was tacitly taken for granted by a large part of the heme protein community. A number of recent articles have challenged this view (Lane and Martin 2012; Sousa et al. 2013, Sousa and Martin 2014), and the previously held opinion on the evolutionary ancestry of all kinds of heme proteins are consequently called into question. Our contribution aims at providing the readers with a better understanding of the pros and cons of both

Abbreviations: Ahb-pathway – Alternative heme biosynthesis pathway; (B)Chl – (Bacterio)Chlorophyll; FePPIX – Iron-protoporphyrin IX; GOE – Great oxidation event; HGT – Horizontal gene transfer; LUCA – Last universal common ancestor of Archaea and Bacteria; Mo/W – Molybdenum/tungsten; NOR – NO reductase; O₂R – O₂ reductase

the “heme-bearing” and the “heme-free LUCA” paradigms and of the evolutionary consequences at stake in either scenario.

II. Hemes in LUCA; Pros and Cons

A. Does Ubiquity Automatically Translate into Deep Ancestry?

In the realm of the prokaryotes, only a small number of taxonomic groups are known thus far, the members of which are devoid of heme and the most conspicuous examples likely are certain methanogenic Archaea, the homoacetogenic Bacteria from the clostridial phylum or strict fermenters such as the Thermotogales (Sousa et al. 2013). Pervasive presence of a specific trait in both Archaea and Bacteria is frequently taken as indicating the presence of this trait already prior to the Archaea/Bacteria divergence (see for example the case made for aerobic respiration in the LUCA by Brochier-Armanet et al. 2009). As we have argued in the past (van Lis et al. 2011; Nitschke and Russell 2013; Ducluzeau et al. 2014a), we consider this line of reasoning as fraught with problems. The existence of horizontal gene transfer (HGT) between the prokaryotic domains is a well-established fact (haloarchaeal genomes provide an extreme example, see Nelson-Sathi et al. 2012) and a novel trait providing a substantial increase in evolutionary fitness is likely to be widely distributed within the prokaryotes via this mechanism. The evolutionary driving force for trait-dissipation is further augmented if the novel trait is specifically adapted to altered ambient conditions following large-scale environmental transitions. Such transitions have almost certainly occurred several times during the roughly four billion years of life’s history on planet Earth and the most conspicuous and best-studied example is the so-called Great-Oxidation-Event (GOE) occurring about 2.3 billion years ago. The GOE has indeed turned the basically O₂-free primordial planet into an “aerobic”

world. Whether the GOE had resulted in a persistent oxygenation of the biosphere or was followed by a substantial drop in O₂-levels between 1.9 and about one billion years ago is presently debated (Partin et al. 2013). While the “phylogenomic distribution argument” thus considers that scarce traits have a poor chance of having been present in the LUCA whereas ubiquitous ones are good candidates, we would argue that the undisputable occurrence of profound, one-way, changes of the planet’s geo-environment substantially diminishes the logical basis of this argument. In our view, evolutionary traits favourable during life’s infancy have a non-negligible chance of becoming outcompeted following major changes in the environment by traits evolving as a result of such environmental overturning and subsequently being widely dispersed through HGT.

All this thus means that the mere pervasive presence of heme cofactors in both Archaea and Bacteria likely is insufficient to conclude on a heme-bearing LUCA. A more stringent approach, albeit prone to a number of experimental difficulties (as discussed in Ducluzeau et al. 2014a) consists in the application of molecular phylogeny (Zuckermandl and Pauling 1965). The obvious difficulty here is that hemes as organic, transition-metal bearing, cofactors are not (directly) encoded by genes and therefore don’t feature (gene- or amino acid-) sequences. However, enzymes using hemes as crucial cofactors as well as the proteins involved in heme biosynthesis do. Which kind of messages can be extracted from the molecular memories of these systems?

B. Phylogenies of Heme-Bearing Bioenergetic Enzymes Suggest a Red LUCA

In the following, we will restrict our discussion to cover only enzymes participating in bioenergetic electron transfer. To the best of our knowledge, heme-dependent enzymes from other types of cellular processes have so far not been studied in great detail with

respect to their deep evolutionary history. Furthermore, the paramount importance for life of free energy converting mechanisms guarantees a good species coverage for individual enzymes and thus improves the reliability of derived phylogenetic trees. Last but not least, the bioenergetic cytochrome systems are what readers of this volume will be most interested in.

Examples for bioenergetic enzymes which involve heme cofactors in a functionally crucial manner are provided by the Rieske/cytb complexes (encompassing the *bc*₁ and *b*₆*L* complexes as well as their homologs), the superfamily of O₂- and NO-reductases and the *cd*₁-type nitrite reductases. Other enzymes feature heme-containing subunits involved in electron transfer from or to the catalytic centre while their reaction turnover is not or only marginally dependent on the presence or absence of these heme proteins. Representatives of the latter class are for instance Group 1 [Ni-Fe]-hydrogenases (Pandelia et al. 2012), the superfamily of Mo/W-bisPGD-enzymes (Schoepp-Cothenet et al. 2012), succinate dehydrogenases/fumarate reductases (Lemos et al. 2002), certain types of heterodisulphide reductases (Thauer et al. 2008) or several enzymes involved in the bioenergetics of sulphur compounds (Grein et al. 2013). The heme subunits in these enzymes typically serve to link redox reactions in the soluble phase (i.e. the peri- or cytoplasm) to membrane-integral lipophilic hydrogen carriers such as quinones and methanophenazines (Schoepp-Cothenet et al. 2013). The vast majority of these heme-subunits belong to one of three structural groups (Baymann et al. 2003; Schoepp-Cothenet et al. 2013; Grimaldi et al. 2013).

Only a few of the proteins mentioned have been studied by molecular phylogeny so far. Prominent examples are the Rieske/cytb complexes (Schütz et al. 2000; Dibrova et al. 2013; Kao and Hunte 2014), the O₂R/NOR superfamily (Pereira et al. 2001; Ducluzeau et al. 2009, 2014; Gribaldo et al. 2009; Sousa et al. 2012), the cytochrome subunits of Group 1 [Ni-Fe]-hydrogenases (Pandelia et al. 2012) and *cd*₁-type nitrite reductases

(van Lis et al. 2011). For the majority of these heme proteins, the reconstructed phylogenetic trees support their presence prior to the Archaea/Bacteria divergence and they have consequently been proposed to be “pre-LUCA” enzymes. For the case of the O₂R/NOR superfamily, a controversy persists as to which of the subfamilies is the most ancient (Gribaldo et al. 2009 vs. Ducluzeau et al. 2014b) while all studies advocate the presence of ancestral members of the superfamily in the LUCA (as discussed in Ducluzeau et al. 2014a). For the case of the Rieske/cytb complexes, one article (Dibrova et al. 2013) challenges the conclusion on a pre-LUCA presence repeatedly arrived at over the last two decades (Castresana et al. 1995; Schütz et al. 2000; Lebrun et al. 2006; Ducluzeau et al. 2009; ten Brink et al. 2013) as well as most recently (Kao and Hunte 2014).

As we have pointed out in the past (Ducluzeau et al. 2014a), molecular phylogeny is prone to a number of methodological and database problems and the results obtained (i.e. the phylogenetic trees) from this approach represent likelihoods rather than certainties. With this caveat having been clearly stated, we would nevertheless hold that molecular phylogenies of heme-carrying proteins and enzymes are by and large in favour of the presence of heme proteins in the LUCA.

C. The Dichotomy of Heme Biosynthesis Enzymes

Two distinct pathways have been found enabling prokaryotes to synthesize FePPIX (heme *b*) (Fig. 2.1). The earlier discovered pathway was elucidated mainly in crown-group proteobacteria (and in particular in *E. coli*) and in Firmicutes (Bacilli) (for a recent review, see Bali et al. 2014). More recently, a distinct pathway was discovered in sulphate-reducing δ -proteobacteria (Ishida et al. 1998; Bali et al. 2011) and was shown to be abundant among Archaea (Bali et al. 2011). The earlier deciphered pathway is now frequently referred to as the

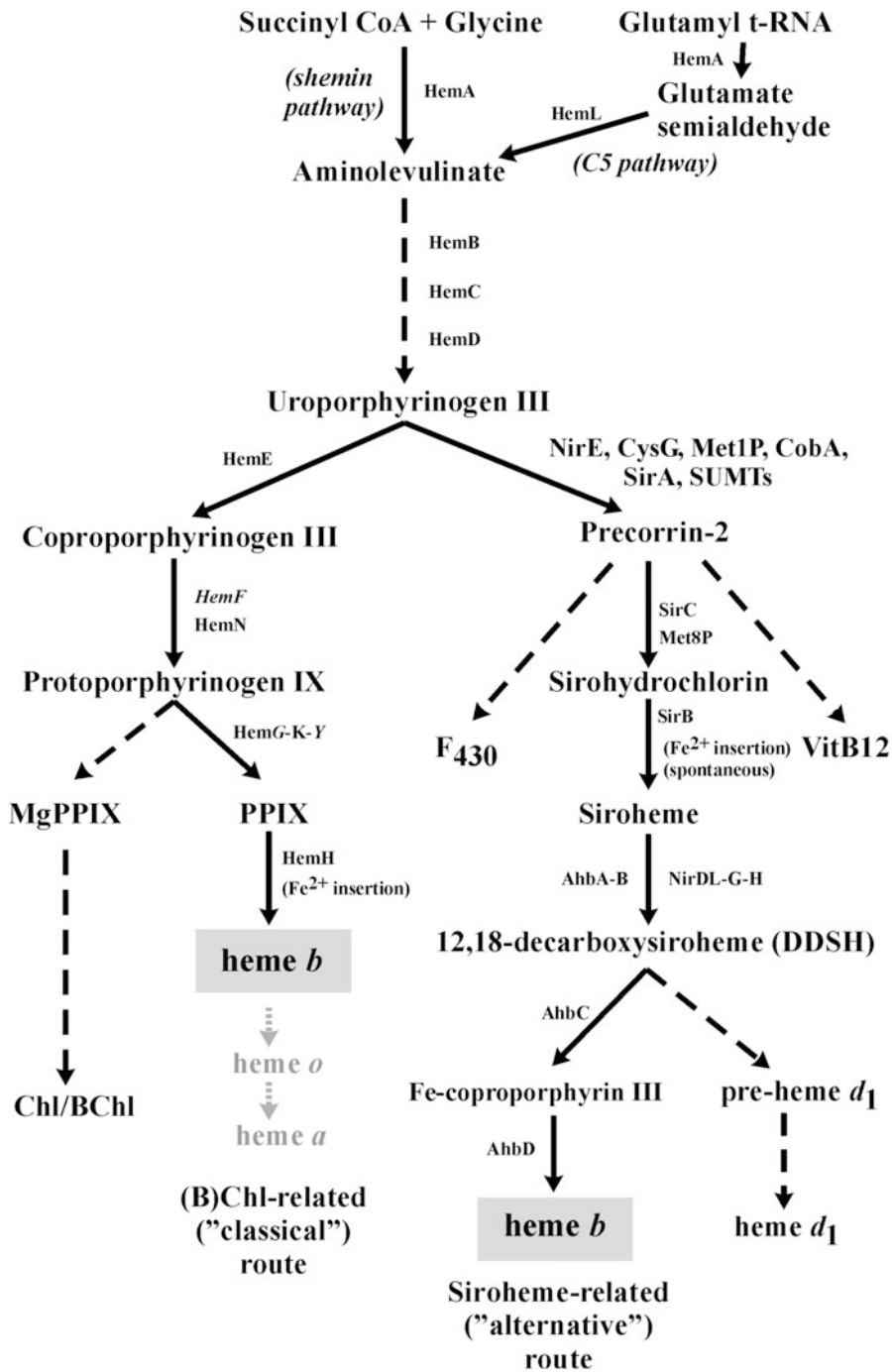


Fig. 2.1. Schematic representation of the two biosynthetic pathways yielding heme *b* and its close relatives together with their relationship to the biosynthesis routes for (B)Chl- and corrin-derived cofactors. Dashed arrows denote shortcuts summarising several consecutive steps. The corresponding enzymes catalysing specific conversions are marked next to the arrows. O₂-dependent enzymes are highlighted in italics.

“classical” route (Bali et al. 2011, 2014). It is closely connected to the biosynthesis of (bacterio)chlorophylls and branches from the (bacterio)chlorophyll-pathways at the level of protoporphyrinogen IX (Fig. 2.1, left branch). The second route, now generally termed the “alternative heme biosynthesis” (Ahb-) pathway (Fig. 2.1, right branch), sequentially features corrins and sirohemes as intermediates on the way to heme *b* (Bali et al. 2011) rather than porphyrins as in the traditional pathway. Both routes share the common intermediate uroporphyrinogen III and all steps leading up to this compound (see Fig. 2.1).

1. Does the Existence of Two Distinct Pathways Necessarily Argue for an Anemic LUCA?

Several recent articles have interpreted the dichotomy of heme biosynthesis pathways as indicating that LUCA had not yet invented hemes and hence must have been devoid of heme-carrying enzymes (Lane and Martin 2012; Sousa et al. 2013; Sousa and Martin 2014). In the line of these authors’ argu-

ments, the fact that Archaea mainly synthesize hemes via the Ahb-pathway while the majority of Bacteria utilize the classical one, means that the two routes must have evolved independently, one in Archaea and the other one in Bacteria (Fig. 2.2a) and that the LUCA therefore must have operated on types of metabolism not calling upon the catalytic or electron transfer properties of hemes. An inventory of genes coding for enzymes involved in one or the other pathway over all available genomes (Sousa et al. 2013) confirmed the predominance of the Ahb-route in Archaea and the classical pathway in Bacteria. Exceptions to this rule detected in this study, some of which have already been pointed out previously (Bali et al. 2011), were taken to be due to HGT events.

The apparent overall correlation between type of prokaryotic domain and type of biosynthesis pathway certainly is intriguing. However, are these observations sufficient to unambiguously draw a conclusion about a heme-free LUCA? A number of general considerations and a few details of the pathways indicate that the story may be more complicated:

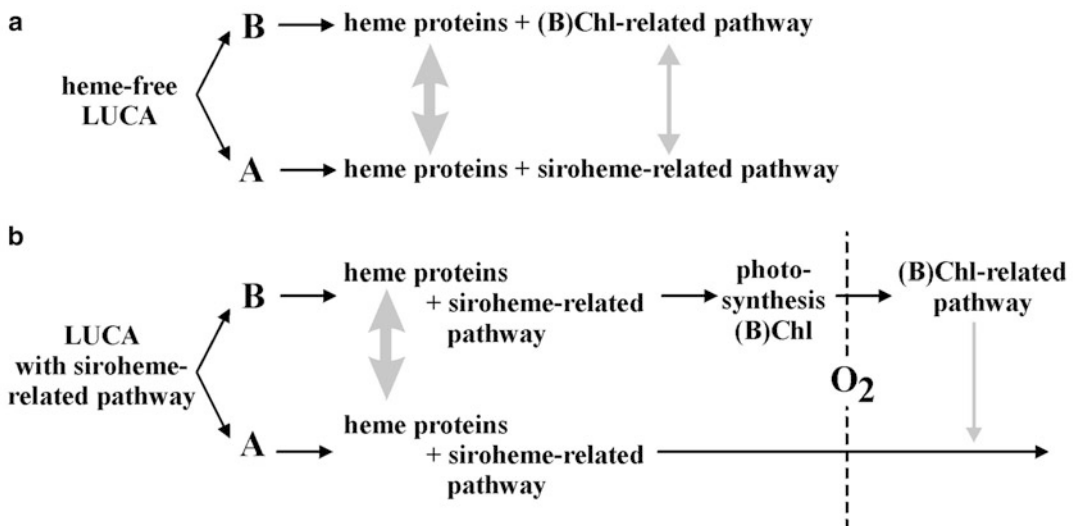


Fig. 2.2. Schematic representation of (a) the evolutionary scenario accounting for pathway dichotomy as proposed by Lane and Martin (2012), Sousa et al. (2013) and (b) our “alternative” model as described in the text. A and B stand for Archaea and Bacteria, respectively. The (B)Chl- and the siroheme-related pathways correspond to the “classical” and the “Ahb”-routes as defined by Bali et al. (2014), respectively.

- The Ahb-pathway appears substantially more widely distributed over both Archaea and Bacteria whereas the traditional pathway, abundant in several bacterial phyla, is only very rarely present in Archaea (see Fig. 7 in Sousa et al. 2013).
- The classical pathway is related to the synthesis of (B)Chl. In the past, the standard interpretation of this relationship was that (B)Chl synthesis evolved from the heme-biosynthetic pathway. This was based on the consideration that hemes are likely to be evolutionarily older than (B)Chls. Photosynthesis indeed appears to have originated “relatively” late within the bacterial domain (Baymann et al. 2001). This scenario appeared inevitable while the classical pathway was still thought to be life’s only way to make hemes, i.e. before the discovery of the Ahb route. The present picture of the topology of reaction schemes shown in Fig. 2.1, however, allows alternative, plausible scenarios. Given the existence of a parallel heme-biosynthesis pathway, the evolutionary sequence of a (B)Chl route growing out of an ancestral Ahb-pathway, and a second route for heme synthesis evolving only later as a derivative of the (B)Chl scheme, is at least as plausible as the original “(B)Chl-from-heme” model. The plausibility of this latter scenario is reinforced by the fact (Fig. 2.1) that the Ahb-pathway is part of the route providing not only heme *b* but also vitamin B₁₂, siroheme, and cofactor F₄₃₀ (a molecule involved in crucial reaction steps of archaeal methanogenesis, Thauer et al. 2008). The credentials of a “good-for-everything” pathway as the more ancestral appear much stronger to us than those of a system specialized to making only heme *b* (plus *a* and *o*) such as the classical route. An analogous set of arguments has already been considered by Bali et al. (2014).
- Counting from their common precursor, that is, uroporphyrinogen III, the classical pathway goes through three major intermediates before reaching FePPIX (heme *b*) while the Ahb pathway passes through five such reaction intermediates.
- Intriguingly, the classical pathway features several steps which are dependent on the presence of O₂ (the respective enzymes are

denoted by italics in Fig. 2.1). In the absence of O₂, alternative enzymes catalyse these conversions through chemically differing reaction schemes (for a review, see Bali et al. 2014).

III. An Alternative Scenario

A. A Scenario Potentially Reconciling Molecular Phylogeny of Heme Enzymes and Pathway Dichotomy

In addition to the above detailed “independent and domain-inherent origins”-model, at least one additional distinct scenario is thus in principle possible. In this scenario, one of the two pathways would have been present in the LUCA and consequently been inherited by both Archaea and Bacteria. The other pathway would have been a later innovation featuring some kind of evolutionary advantage over the ancestral pathway. This novel pathway might have appeared either in Bacteria or in Archaea and then have supplanted the ancestral pathway in many species due to its evolutionary edge over the other route. An analogous sequence of events has, for example, been invoked to rationalize the evolutionary history of O₂- and NO-reductases (Ducluzeau et al. 2014b).

In the light of the above listed pathway idiosyncrasies we will in the following tentatively outline a more detailed version of this alternative scenario of the evolutionary history of heme biosynthesis. In this scenario, the Ahb-pathway is the ancestral route for the biosynthesis of heme *b*, siroheme, Vit B₁₂, F₄₃₀ and likely further related cofactors (Fig. 2.2b). This pathway was already present in the LUCA and operated under strictly anaerobic conditions which likely characterized the primordial planet. It was subsequently vertically inherited into both Archaea and Bacteria. At some point in evolutionary history, certainly prior to 2.5 billion years ago, i.e. prior to the GOE, certain Bacteria evolved photosynthesis with tetrapyrroles as light-responsive cofactors (in contrast

to archaeal photosynthesis which is based on light-induced conformational changes of opsins and which eventually gave “vision” to life). Optimization of tetrapyrrol-based photosynthesis towards higher absorption efficiency for solar photons in the wavelength window not filtered out by the atmosphere led to the emergence of the (B)Chl branch of Fig. 2.1. Hemes, by contrast, were still synthesized via the Ahb-route. The ultimate advent of non-negligible amounts of O₂ in the biosphere (Fig. 2.2b) induced by oxygenic photosynthesis (although probably only after a significant time lag due to geochemical buffering of the O₂ produced) allowed the emergence of a plethora of biochemical pathways making profitable use of the newly available, highly reactive and strongly oxidizing molecular oxygen (Raymond and Segrè 2006). Among them was an energetically more economical pathway for heme *b* biosynthesis. Since the oxygenic photosynthesizers likely were among the very first to encounter increasing levels of O₂, it seems plausible that this novel heme biosynthesis pathway evolved within these species and was related to the (B)Chl route due to co-regulation. When O₂ had become pervasive in the biosphere (i.e. after 2.3 billion years ago), this pathway became strongly favourable even for non-O₂-producers generating an evolutionary driving force for its dissemination towards other aerobic organisms throughout the prokaryotes. The Ahb-route was increasingly overgrown by the more modern “classical” pathway except in organisms that remained confined to the shrinking anaerobic niches and never ventured out into oxygenated environments. Aerobic species having entirely lost the Ahb-route, however, were obliged to develop alternative O₂-independent enzymes if they were to recolonise O₂-poor or anaerobic habitats, which rationalizes the presence of O₂-dependent and O₂-independent enzymes at certain biosynthesis steps in the (B)Chl-related pathway (Fig. 2.1).

The wording of the preceding paragraph highlights the psychological hurdles entailed

by the current pathway nomenclature. Referring to a “classical” and an “alternative” route subliminally opposes the notion that the “alternative” pathway might have been THE ancestral route while the “classical” one might in fact represent the actual alternative route that had emerged only at a later time. As will be discussed below, the same nomenclature scheme is in use in the field of menaquinone biosynthesis (Hiratsuka et al. 2008). We do think that it is time to adopt less suggestive terms to identify these pathways. For obvious reasons (see Fig. 2.1), we propose the terms “(B)Chl-related pathway” for the “classical” route and “siroheme-related pathway” for the “alternative” one. We have used this scheme to denote pathways in Fig. 2.2.

It seems obvious to us that both the above sketched “heme-free-LUCA” and the “Ahb-in-LUCA” scenarios are generally in line with presently available evidence. To decide between these two alternatives, global phylogenies of enzymes involved in all (i.e. encompassing heme-, (B)Chl-, siroheme-) biosynthesis pathways are required. Such a study would represent an impressive and highly time-consuming task, but we would argue that it will be indispensable for progressing on the question of the evolutionary history of hemes and heme proteins.

B. Is the Heme-Biosynthesis-Pathway Dichotomy Related to Other Major Pathway Dichotomies?

The scenario of an Ahb-pathway in the LUCA which was later outcompeted (likely after the onset of oxygenic photosynthesis some 2.7 billion years ago) by a more recently emerged system was stimulated by the fact that several other intriguingly analogous pathway dichotomies may find an explanation in the same manner. Two prominent ones are those of the menaquinone- and the iron-sulphur cluster biosynthesis systems. Menaquinones (MK), likely the most ancestral of respiratory quinones (Schoepp-Cothenet et al. 2009, 2013) are

synthesized in prokaryotes via two distinct routes, i.e. the (once again) “classical” Men-pathway (predominantly studied in *E. coli* (Bentley and Meganathan 1983) and the “alternative” futasine-pathway (Hiratsuka et al. 2008). The dichotomy of MK-biosynthetic routes has been argued to again indicate the absence of quinones in the LUCA (Lane and Martin 2012; Sousa et al. 2013). Phylogenies of several enzymes involved in both pathways have been reconstructed and appear to show that the futasine pathway is more ancestral than the Men-route (Zhi et al. 2014). However, when it comes to deciding whether the futasine pathway was present in the LUCA, the published data so far are inconclusive. Phylogenetic tree reconstruction based on different algorithms yields divergent results and no clear answer is therefore possible at present.

A further pathway dichotomy has been found for iron-sulphur cluster biosynthesis. The quite distinct “Isc”- and “Suf”- pathways are scattered over the realm of the prokaryotes with some species containing either one or the other, while others use both pathways differentially as a function of growth conditions and predominantly of oxidative stress (Roche et al. 2013). To the best of our knowledge, no detailed and evolutionarily deep molecular phylogenies of the involved enzymes have been reported so far. However, while heme- and quinone-biosynthesis may indeed be argued to have been absent from the LUCA, we would hold that an iron-sulphur-cluster-free LUCA does not figure in any of the presently proposed scenarios. Parallel, independent, origins of the Isc- and Suf-systems in Archaea and Bacteria, as proposed by Lane and Martin (2012) for heme and quinone biosynthesis, therefore seem highly unlikely. Both in the Fe-S and in the MK-biosynthesis pathways, O₂-dependent/resistant enzymes are asymmetrically distributed and seem to be more frequent in the Men- and the Suf-routes. Since Fe-S centres are highly O₂-labile, it seems more likely to us that emergence of an “aerobic” system (likely the Suf-pathway) was

driven by the need to harden the Fe-S-cluster transporting biosynthesis enzymes against the deleterious effects of O₂ rather than by favourable O₂-mediated biosynthetic steps.

The occurrence of all these pathway dichotomies therefore may well indicate the late origins of O₂-dependent routes supplanting anaerobic ones instead of the independent emergences of two pathways in Archaea and Bacteria, respectively. We have proposed in the past that a very similar scenario perfectly rationalizes the convoluted evolutionary history of O₂- and NO-reductases (Ducluzeau et al. 2014). The advent of molecular O₂ on planet Earth certainly represented one of the most profound geochemical revolutions of the environment. It seems to make perfect sense to us that the appearance of the novel and powerful reactant O₂ would drive the emergence of pathways that reap benefits from the opportunity of unprecedented reaction schemes. If the mentioned pathway dichotomies should indeed reflect the late emergence of O₂-related systems, a better understanding of the source organisms, i.e., the species wherein these novel pathways emerged, will substantially further our understanding of life’s ways to weather the biochemical turmoil that must have been associated with the GOE.

IV. Why All the Fuzz?

The controversy concerning the detailed evolutionary scenario accounting for the origin of heme cofactors may appear purely academic and of interest only to a very restricted community of molecular phylogeny geeks. However, as we have discussed recently (Ducluzeau et al. 2014a), the resolution to this problem will have substantial consequences for our understanding of energy conversion at the origin of life. Approaches attempting to retrodict bioenergetic mechanisms operating in the LUCA from what we see in extant life to our mind represents a much more “empirical” way to study the origin of life than the conceiving of “*ab-initio*” hypotheses based

solely on chemical and geochemical plausibility arguments (discussed in Schoepp-Cothenet et al. 2013). A scenario remarkably successful in rationalizing the emergence of chemiosmotic free energy conversion, while being perfectly in agreement with thermodynamic requirements, was proposed by Martin and Russell (2003, 2007) as a biochemical incarnation of the seminal hypothesis stipulating alkaline hydrothermal vents as the cradle of life (Russell and Hall 1997). This scenario stipulates Wood-Ljungdahl-type energy conversion (Martin and Russell 2007) and carbon fixation as the ancestral types of metabolism in the LUCA and inorganic versions thereof back to the very origin of life. The Wood-Ljungdahl pathways mainly rely on Fe-S- and Ni-containing cofactors for catalysis but are devoid of quinones and hemes (Lane and Martin 2012). More recently, a variant of this scenario was proposed based on a re-evaluation of the inventory of likely redox substrates present in presumed locales for life's origin (Nitschke and Russell 2013). This scenario still maintains the importance of catalytic steps featuring in the Wood-Ljungdahl pathway (if partially in the reverse direction of catalysis), but adds an oxidative branch resembling anaerobic respiration necessarily implicating quinones and hemes. Whereas the first scenario stipulates only H_2 and CO_2 as providing the environmental redox disequilibrium which drove the emergence of life, the second one envisages a wider cocktail of substrates involving at least H_2 and CH_4 as reductants and CO_2 , nitrate, nitrite and potentially Fe^{3+} , Mn^{4+} etc. (Russell et al. 2014) as oxidants resulting in a substantially stronger and multifaceted environmental redox disequilibrium tapped by nascent life. Whereas quinones and hemes are conceivable in the LUCA, they are more difficult to envisage at life's very origin. Inorganic metal-complexes able to have played the roles later taken over by quinones and hemes have therefore been proposed (Nitschke et al. 2013). However, if the LUCA had been devoid of hemes and quinones altogether, the types of energy metabolism pro-

posed to have fuelled the LUCA in the more recent scenario (Nitschke and Russell 2013) are precluded and by extension have little chance of having operated at life's origin.

The question whether the LUCA was red or "anemic" therefore by far exceeds the mere evolutionary history of heme proteins and has crucial repercussions for our understanding of life's origin.

Acknowledgments

We thank Shilpa Bali (Oxford/UK) for stimulating discussions and for providing manuscripts prior to publication. We furthermore are grateful to Mike Russell (Pasadena/USA) and Bill Cramer (Purdue/USA) for critical reading of our manuscript. Innumerable stimulating discussions with Barbara Schoepp-Cothenet and Frauke Baymann (both Marseille/France) are gratefully acknowledged. ALD is supported by the Moore Foundation and by NSF's Division of Polar Programs (grant number 1203262).

References

- Bali S, Lawrence AD, Lobo SA, Saraiva LM, Golding BT, Palmer DJ, Howard MJ, . . . , Warren MJ (2011) Molecular hijacking of siroheme for the synthesis of heme and d1 heme. *Proc Natl Acad Sci USA* 108:18260–18265
- Bali S, Palmer DJ, Schroeder S, Ferguson SJ, Warren MJ (2014) Recent advances in the biosynthesis of modified tetrapyrroles: the discovery of an alternative pathway for the formation of heme and heme d_1 . *Cell Mol Life Sci* 71:2837–2863
- Baymann F, Brugna M, Mühlenhoff U, Nitschke W (2001) Daddy, where did (PS) I come from? *Biochim Biophys Acta Bioenerg* 1507:291–310
- Baymann F, Lebrun E, Brugna M, Schoepp-Cothenet B, Giudici-Orticoni M-T, Nitschke W (2003) The redox protein construction kit: pre last universal common ancestor evolution of energy-conserving enzymes. *Philos Trans R Soc Lond B Biol Sci* 358:267–274
- Bentley R, Meganathan R (1983) Biosynthesis of vitamin K (menaquinone) in bacteria. *Microbiol Rev* 46:241–280

- Branscomb E, Russell ML (2013) Turnstiles and bifurcators: the disequilibrium converting engines that put metabolism on the road. *Biochim Biophys Acta Bioenerg* 1827:62–78
- Brochier-Armanet C, Talla E, Gribaldo S (2009) The multiple evolutionary histories of dioxygen reductases: implications for the origin and evolution of aerobic respiration. *Mol Biol Evol* 26:285–297
- Castresana J, Lübken M, Saraste M (1995) New archaeobacterial genes coding for redox proteins: implications for the evolution of aerobic metabolism. *J Mol Biol* 250:202–210
- Cutruzzola F, Arcovito A, Giardina G, della Longa S, D'Angelo P, Rinaldo S (2014) Distal-proximal crosstalk in the heme binding pocket of the NO sensor DNR. *Biomaterials* 27:736–773
- Dibrova DV, Cherepanov DA, Galperin MY, Skulachev VP, Mulikidjanian AY (2013) Evolution of cytochrome *bc* complexes: from membrane-anchored dehydrogenases of ancient bacteria to triggers of apoptosis in vertebrates. *Biochim Biophys Acta Bioenerg* 1827:1407–1427
- Ducluzeau A-L, van Lis R, Duval S, Schoepp-Cothenet B, Russell MJ, Nitschke W (2009) Was nitric oxide the first strongly oxidizing terminal electron sink? *Trends Biochem Sci* 34:9–15
- Ducluzeau A-L, Schoepp-Cothenet B, Baymann F, Russell MJ, Nitschke W (2014a) Free energy conversion in the LUCA: Quo vadis? *Biochim Biophys Acta Bioenerg* 1837:982–988
- Ducluzeau A-L, Schoepp-Cothenet B, van Lis R, Baymann F, Russell MJ, Nitschke W (2014b) The evolution of the respiratory O₂/NO reductases; an out-of-the-phylogenetic-box perspective. *J R Soc Interface* 11:20140196
- Eck RV, Dayhoff MO (1966) Evolution of the structure of ferredoxin based on living relics of primitive amino acid sequences. *Science* 152:363–366
- Grein F, Ramos AR, Venceslau SS, Pereira IAC (2013) Unifying concepts in anaerobic respiration: insights from dissimilatory sulfur metabolism. *Biochim Biophys Acta Bioenerg* 1827:145–160
- Gribaldo S, Talla E, Brochier-Armanet C (2009) Evolution of the haem copper oxidases superfamily: a rooting tale. *Trends Biochem Sci* 34:375–381
- Grimaldi S, Cécaldi P, Schoepp-Cothenet B, Guigliarelli B, Magalon A (2013) The prokaryotic Mo/W-bisPGD enzymes family: a catalytic workhorse in bioenergetics. *Biochim Biophys Acta Bioenerg* 1827:1048–1085
- Hiratsuka T, Furihata K, Ishikawa J, Yamashita H, Itoh N, Seto H, Dairi T (2008) An alternative menaquinone biosynthetic pathway operating in microorganisms. *Science* 321:1670–1673
- Ishida T, Yu L, Akutsu H, Ozawa K, Kawanishi S, Seto A, Inubushi T, Sano S (1998) A primitive pathway of porphyrin biosynthesis and enzymology in *D. vulgaris*. *Proc Natl Acad Sci USA* 95:4853–4858
- Kao W-C, Hunte C (2014) The molecular evolution of the Qo motif. *Genome Biol Evol* 6:1894–1910
- Lane N, Martin WF (2012) The origin of membrane bioenergetics. *Cell* 151:1406–1416
- Lebrun E, Santini JM, Brugna M, Ducluzeau A-L, Ouchane S, Schoepp-Cothenet B, Baymann F, Nitschke W (2006) The Rieske protein: a case study on the pitfalls of multiple sequence alignments and phylogenetic reconstruction. *Mol Biol Evol* 23:1180–1191
- Lemos RC, Fernandes AS, Pereira MM, Gomes CM, Teixeira M (2002) Quinol:fumarate oxidoreductases and succinate:quinone oxidoreductases: phylogenetic relationships, metal centres and membrane attachment. *Biochim Biophys Acta Bioenerg* 1553:158–170
- Martin W, Russell MJ (2003) On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Philos Trans R Soc Lond B Biol Sci* 358:59–83
- Martin WF, Russell MJ (2007) On the origin of biochemistry at an alkaline hydrothermal vent. *Philos Trans R Soc Lond B Biol Sci* 362:1887–1925
- Martinkova M, Kitanishi K, Shimizu T (2013) Heme-based globin-coupled oxygen sensors: linking oxygen binding to functional regulation of diguanylate cyclase, histidine kinase and methyl-accepting chemotaxis. *J Biol Chem* 288:27702–27711
- Nelson-Sathi S, Dagan T, Landan G, Janssen A, Steel M, McInerney JO, Deppenmeier U, Martin WF (2012) Acquisition of 1,000 eubacterial genes physiologically transformed a methanogen at the origin of Haloarchaea. *Proc Natl Acad Sci USA* 109:20537–20542
- Nitschke W, Russell MJ (2013) Beating the acetyl-CoA pathway to the origin of life. *Philos Trans R Soc Lond B Biol Sci* 368:20120258
- Nitschke W, McGlynn S, Milner-White J, Russell MJ (2013) On the antiquity of metalloenzymes and their substrates in bioenergetics. *Biochim Biophys Acta Bioenerg* 1827:871–881
- Pandelia ME, Lubitz W, Nitschke W (2012) Evolution and diversification of Group 1 [NiFe] hydrogenases. Is there a phylogenetic marker for O₂ tolerance? *Biochim Biophys Acta Bioenerg* 1817:1565–1575

- Partin CA, Bekker A, Planavsky NJ, Scott CT, Gill BC, Li C, Podkovyrov V, . . . , Lyons TW (2013) Large-scale fluctuations in Precambrian atmosphere and oceanic oxygen levels from the record of U in shales. *Earth Planet Sci Lett* 369–370:284–293
- Pereira MM, Santana M, Teixeira M (2001) A novel scenario for the evolution of haem-copper oxygen reductases. *Biochim Biophys Acta Bioenerg* 1505:185–208
- Pokkuluri PR, Pessanha M, Londer YY, Wood SJ, Duke NEC, Wilton R, Catarino T, . . . , Schiffer M (2008) Structures and solution properties of two novel periplasmic sensor domains with c-type heme from chemotaxis proteins of *Geobacter sulfurreducens*: implications for signal transduction. *J Mol Biol* 377:1498–1517
- Raymond J, Segrè D (2006) The effect of oxygen on biochemical networks and the evolution of complex life. *Science* 311:1764–1767
- Roche B, Aussel L, Ezraty B, Mandin P, Py B, Barras F (2013) Iron/sulfur proteins biogenesis in prokaryotes: formation, regulation and diversity. *Biochim Biophys Acta Bioenerg* 1827:455–469
- Russell MJ, Hall AJ (1997) The emergence of life from iron-monosulphide bubbles at a submarine hydrothermal redox and pH front. *J Geol Soc Lond* 154:377–402
- Russell MJ, Barge LM, Bhartia R, Bocanegra D, Bracher PJ, Branscomb E, Kidd R, . . . , Kanik I (2014) The drive to life on wet and icy worlds. *Astrobiology* 14:308–343
- Schoepp-Cothenet B, Lieutaud C, Baymann F, Verméglio DD, Friedrich T, Kramer DM, Nitschke W (2009) Menaquinone as pool quinone in a purple bacterium. *Proc Natl Acad Sci USA* 106: 8549–8554
- Schoepp-Cothenet B, van Lis R, Philippot P, Magalon A, Russell MJ, Nitschke W (2012) The ineluctable requirement for the trans-iron elements molybdenum and/or tungsten in the origin of life. *Sci Rep* 2:263
- Schoepp-Cothenet B, van Lis R, Atteia A, Baymann F, Capowiez L, Ducluzeau A-L, Duval S, . . . , Nitschke W (2013) On the universal core of bioenergetics. *Biochim Biophys Acta Bioenerg* 1827:79–93
- Schütz M, Brugna M, Lebrun E, Baymann F, Huber R, Stetter K-O, Hauska G, . . . , Nitschke W (2000) Early evolution of cytochrome bc complexes. *J Mol Biol* 300:663–675
- Sousa FL, Alves RJ, Ribeiro MA, Pereira-Leal JB, Teixeira M, Pereira MM (2012) The superfamily of heme-copper oxygen reductases: types and evolutionary considerations. *Biochim Biophys Acta Bioenerg* 1817:629–637
- Sousa FL, Thiergart T, Landan G, Nelson-Sathi S, Pereira IA, Allen JF, Lane N, Martin WF (2013) Early bioenergetic evolution. *Phil Trans R Soc London* 368:1622
- Sousa FL, Martin WF (2014) Biochemical fossils of the ancient transition from geoenergetics to bioenergetics in prokaryotic one carbon compound metabolism. *Biochim Biophys Acta, Bioenerg* 1837:964–981
- Suzuki T, Imai K (1998) Evolution of myoglobin. *Cell Mol Life Sci* 54:979–1004
- ten Brink F, Schoepp-Cothenet B, van Lis R, Nitschke W, Baymann F (2013) Multiple Rieske/cytb complexes in a single organism. *Biochim Biophys Acta Bioenerg* 1827:1392–1406
- Thauer RK, Kaster AK, Seedorf H, Buckel W, Hedderich R (2008) Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat Rev Microbiol* 6:579–591
- van Lis R, Ducluzeau A-L, Nitschke W, Schoepp-Cothenet B (2011) The nitrogen cycle in the Archaea; an intricate interplay of enzymatic and abiotic reactions. In: Moir JWB (ed) *Nitrogen Cycling in Bacteria: Molecular Analysis*. Caister Academic, Norfolk, pp 1–21
- Vignais PM, Billoud B, Meyer J (2001) Classification and phylogeny of hydrogenases. *FEMS Microbiol Rev* 25:455–501
- Zhi X-Y, Yao J-C, Tang S-K, Huang Y, Li H-W, Li W-J (2014) The futasoline pathway played an important role in menaquinone biosynthesis during early prokaryote evolution. *Genome Biol Evol* 6: 149–160
- Zuckerkandl E, Pauling L (1965) Molecules as documents of evolutionary history. *J Theor Biol* 8: 357–366

Cytochrome Complexes: Evolution, Structures, Energy
Transduction, and Signaling

Cramer, W.A.; Kallas, T. (Eds.)

2016, XLV, 739 p. 178 illus., 138 illus. in color.,

Hardcover

ISBN: 978-94-017-7479-6