Chapter 26

Cofactor Assembly of Cytochrome bc_1-b_6f Complexes

Sara Guenthner Cline[a,*], Stéphane Thierry Gabilly[b], Nitya Subrahmanian[c], and Patrice Paul Hamel[c,*]

[a]*Department of Mathematics, Computer and Natural Sciences, Athens State University, S303C Waters Hall, 300 N. Beaty Street, Athens, AL 35611, USA*

[b]*Department of Plant and Microbial Biology, University of California, Berkeley, 441 Koshland Hall, Berkeley, CA 94720-3120, USA*

[c]*Department of Molecular Genetics and Department of Biological Chemistry and Pharmacology, The Ohio State University, 500 Aronoff Laboratory, 318 W. 12th Avenue, Columbus, OH 43210, USA*

*Author for correspondence, e-mail: sara.cline@athens.edu; hamel.16@osu.edu

W.A. Cramer and T. Kallas (eds.), Cytochrome Complexes: Evolution, Structures, Energy Transduction, and Signaling, Advances in Photosynthesis and Respiration 41, pp. 501–525,
DOI 10.1007/978-94-017-7481-9_26,

Summary

Cytochromes bc_1 and b_6f are enzyme complexes catalyzing essential electron transfer reactions in bacterial and organellar energy-transducing membranes. Electron transport relies on heme and Fe-S redox cofactors contained in the three catalytic subunits common to bc_1 and b_6f complexes: a *b*-type cytochrome (cytochrome *b*), a *c*-type cytochrome (cytochrome c_1 or *f*) and a [2Fe-2S]-containing protein (Rieske). Maturation of the *b*-type and *c*-type cytochromes requires conversion of the subunit from the apoform to the holoform by the incorporation of two hemes (b_L and b_H) in cytochrome *b* of the bc_1 complex, three hemes (b_L, b_H and c_i) in cytochrome b_6 of the b_6f complex, and a single heme (*c*) in both cytochromes c_1 and *f*. Assembly of heme *c* requires the formation of two thioether bonds at a C*XX*CH motif in cytochrome c_1 and *f* on the *p*-side, and for c_i, a single thioether bond in cytochrome b_6 on the *n*-side of the membrane, respectively. It is not known how non-covalently bound hemes (b_L and b_H) are inserted into cytochrome *b* and b_6, but covalent linkage of heme is known to be a catalyzed process. The chemistry of thioether linkage formation appears, on the surface, to be simple (i.e. addition of a sulfhydryl to the α carbon of the vinyl group of heme). However, experimental investigations in bacteria, mitochondria and plastids have revealed an unsuspected biochemical complexity for this process. At least six different pathways for thioether bond formation have been described. Four of these, System I, II, III (on the *p*-side) and IV (on the *n*-side), are defined by prototypical components and have been extensively studied.

I. Introduction

Cytochrome bc_1 and cytochrome b_6f complexes (hereafter referred to as bc_1 and b_6f) are multimeric enzymes responsible for electron transfer reactions in bacterial and eukaryotic energy-transducing membranes (Dibrova et al. 2013; Hasan et al. 2013). Cytochrome bc_1 complexes are quinol-cytochrome *c* oxido-reductases that function in aerobic respiration in diverse bacterial phyla and all mitochondria, with the exception of some protozoan parasites and one photosynthetic alveolate (Nawathean and Maslov 2000; Stechmann et al. 2008; Flegontov et al. 2015). In photosynthetic bacteria, bc_1 are also key enzymes in anoxygenic photosynthesis (see Chap. 10). On the other hand, cytochrome b_6f complexes are essential to oxygenic photosynthesis and operate as quinol-plastocyanin (or cytochrome c_6) oxido-reductases in all cyanobacteria and plastids of both algae and land plants (see Chap. 9). In cyanobacteria, b_6f is also required for the respiratory electron transfer chain (see Chap. 17). Variations of the typical bc_1/b_6f enzymes also exist in several eubacterial and archeal lineages. Such a variant is the *bc* complex, an enzyme related to the b_6f, and

Abbreviations: BN-PAGE – Blue native polyacrylamide gel electrophoresis; Fe-S – Iron-sulfur; HHP – Heme handling protein; HO – Heme oxygenase; HRM – Heme regulatory motif; IMS – Intermembrane space; ISP – Iron-sulfur protein; OPR – Octotricopeptide repeat; PSI – Photosystem I; TDOR – Thiol-disulfide oxido-reductase; TMD – Transmembrane domain; Y2H – Yeast two-hybrid; WWD – tryptophan rich domain containing the tryptophan-tryptophan-aspartic acid signature

present in both anoxygenic phototrophic and non-phototrophic bacteria of the Firmicutes phylum (see Chap. 3).

Common to bc_1 and b_6f are three catalytic subunits, a *b*-type cytochrome, a *c*-type cytochrome (c_1 or *f*), and a Rieske iron-sulfur protein (ISP), which harbor the prosthetic groups required for catalysis (Dibrova et al. 2013; Hasan et al. 2013). In addition to the catalytic subunits, bc_1 and b_6f contain subunits which do not participate directly in electron transfer, but are required for assembly/activity of a functional complex. While mitochondrial bc_1 contains additional seven or eight subunits that are absent in bacterial bc_1 complexes (see Chap. 10), the four additional subunits found in the chloroplast b_6f have counterparts in the cyanobacterial complex (see Chap. 9). The biogenesis of bc_1 and b_6f is an intricate process, requiring the synthesis of the structural subunits, the maturation of the redox subunits by attachment/incorporation of the prosthetic groups and the stepwise integration of the different subunits in the membrane. In this chapter, we will focus on the pathways controlling prosthetic group attachment to the catalytic subunits of bc_1 and b_6f, with emphasis on the heme attachment reaction.

II. Prosthetic Groups in bc_1 and b_6f

A. Common and Unique Prosthetic Groups in bc_1/b_6f *Complexes*

Iron-sulfur (Fe-S) cluster and heme (ferroprotoporphyrin IX) are the prosthetic groups common to both bc_1 and b_6f complexes and are present in the three conserved catalytic subunits (Dibrova et al. 2013; Hasan et al. 2013). The membrane-anchored Rieske subunit, present in both bc_1 and b_6f, contains a single [2Fe-2S] cluster in a domain facing the *p*-side[1] of the membrane (Dibrova et al. 2013; Hasan et al. 2013). Rieske ISPs are characterized by unique ligand coordination of their Fe-S cluster, with one of the two iron centers being coordinated by two cysteine residues and the other by two histidine residues (Schmidt and Shaw 2001). In bc_1/b_6f, cytochromes *b* and b_6 are membrane-embedded proteins containing two *b*-hemes, b_L and b_H, on the *p*- and *n*-side of the membrane, respectively. Both hemes of cytochromes *b* and b_6 are coordinated by two conserved histidines in a four-helix bundle. Cytochromes c_1 and *f* are membrane-anchored, *c*-type cytochromes, also generically referred to as cytochromes *c*. Cytochromes of the *c*-type are a large family of hemoproteins with one or several heme moieties covalently attached to a $CX_nC(H/K)$[2] motif, known as the heme-binding site (Thony-Meyer 1997). Axial ligands of covalently linked heme in *c*-type cytochromes are provided by the H/K residue in the heme-binding site and an amino acid in the protein, usually a methionine residue (Thony-Meyer 1997). Histidine in the heme-binding site is the most common axial ligand and lysine is only found in nitrate-reducing enzymes (Einsle et al. 1999). In cytochrome c_1 and cytochrome *f*, a single heme *c* is attached on the *p*-side of the membrane. This attachment occurs via two thioether bonds between the vinyl-2 and -4 groups of heme and the sulfhydryl groups on the first and second cysteines occurring in a C*XX*CH motif of the protein. A notable exception occurs in the phylum Euglenozoa where the vinyl-4 of heme is linked to a single cysteine contained within a F*XX*CH motif in mitochondrial cytochrome c_1 (Priest and Hajduk 1992).

[1] *p*- and *n*-sides refer to the positive and negative side of the energy-transducing membrane system, respectively. The *p*-side corresponds to the bacterial periplasm, thylakoid lumen and mitochondria IMS while the *n*-side is the bacterial cytoplasm, plastid stroma and mitochondrial matrix.

[2] Where *X* can be any amino-acid except cysteine. The number of intervening residues (n) is usually 2, with the rare exception of some bacterial cytochromes where n = 3, 4 or 15 (Jungst et al. 1991; Jentzen et al. 1998; Aragão et al. 2003; Hartshorne et al. 2007).

Another type of covalent linkage of a single heme, referred to as heme c_i (or c_n), has been found in cytochrome b_6. This heme is covalently linked via one thioether bond between the α carbon of the vinyl-2 group of heme and a cysteine residue (Kurisu et al. 2003; Stroebel et al. 2003). Heme c_i was also detected as covalently linked to the cytochrome *b* subunit of the *bc* complexes in *Bacilli* and *Heliobacteria* representatives of the Firmicutes phylum (Kutoh and Sone 1988; Yu and Le Brun 1998; Ducluzeau et al. 2008). Unlike heme *c* in cytochromes c_1 and *f*, heme c_i is not attached to a recognizable consensus motif and is localized on the *n*-side of the membrane (de Vitry 2011). Because *b*-type and *c*-type cytochromes refer to cytochromes with non-covalently and covalently bound heme(s), respectively, cytochrome b_6, which was initially defined as a *b*-type based on b_L and b_H hemes is now also referred to as a *c*-type cytochrome because of the presence of heme c_i (Thony-Meyer 1997; Kuras et al. 2007).

In addition to Fe-S and heme, subunit IV of the b_6f contains two non-covalently bound pigments, one molecule of chlorophyll *a* (Huang et al. 1994; Pierre et al. 1997) and one molecule of β-carotene (Zhang et al. 1999), which are absent in the bc_1. The typical carotenoid in b_6f is 9-*cis*-β-carotene but other carotenoids, such as 9-*cis*-α-carotene and echinenone have been found in some algal and cyanobacterial enzymes (Boronowsky et al. 2001; Li et al. 2005). The function of chlorophyll *a* and β-carotene remains enigmatic as light is not required for electron transfer catalyzed by the b_6f complex.

B. Incorporation of Prosthetic Groups in bc_1 *and* b_6f *Subunits*

Maturation of the redox subunits of the bc_1 and b_6f requires apo- to holoform conversion of the subunit by the incorporation of one or several prosthetic groups. Failure to incorporate the prosthetic group yields a bc_1/b_6f assembly defect as the apoform of the subunit is usually unstable and degraded. One notable exception is the Rieske ISP in *Saccharomyces cerevisiae*, whose apoform was shown to assemble in the bc_1 in the absence of Fe-S cluster incorporation (Graham and Trumpower 1991).

While the requirement for catalysis in the covalent attachment of heme has long been acknowledged, it is not known if incorporation of non-covalently bound cofactors, such as *b*-hemes into apocytochrome b/b_6, chlorophyll *a* and β-carotene into subunit IV and the Fe-S cluster in the Rieske ISP are also enzymatically assisted in vivo. The fact that these cofactors are retained by non-covalent interactions, and were shown, in some cases, to be correctly inserted into their corresponding apoprotein(s) in vitro, has led to the assumption that incorporation of non-covalently bound cofactors is not a catalyzed reaction in vivo (Robertson et al. 1994; Holton et al. 1996; Gubernator et al. 2006). The recent discovery that different assembly factors of mitochondrial bc_1 associate with cytochrome *b* intermediates containing either b_L only or b_L and b_H suggests that incorporation of non-covalent heme might also be an assisted process (Hildenbeutel et al. 2014). Another example, highlighting the role of factors in assisting the delivery of non-covalently bound heme, is that of NO synthase. Its biogenesis requires thioredoxin, the Hsp90 chaperone, and glycerolphosphate-dehydrogenase for insertion of *b*-heme into the apoenzyme (Chakravarti et al. 2010; Ghosh et al. 2011; Hannibal et al. 2012). The concept of protein-assisted incorporation of non-covalently bound prosthetic group extends to molecules other than heme, as shown by the description of dedicated carriers facilitating Fe-S cluster insertion in a subset of apoprotein target(s) (Balk and Schaedler 2014). Hence, it is possible that incorporation of the Fe-S cluster in the apoform of bc_1/b_6f Rieske ISP also requires dedicated factor(s).

III. Pathways for the Covalent Attachment of Heme

A. Six Distinct Pathways for Heme Attachment in bc_1/b_6f

Covalent heme attachment to apocytochromes c_1 and f is a post-translational modification, occurring on the *p*-side of the energy-transducing membrane of bacteria, mitochondria and plastids (Kranz et al. 2009; Allen 2011; Mavridou et al. 2013; Verissimo and Daldal 2014). In cyanobacteria and plastids, heme c_i is formed by covalent linkage of heme to apocytochrome b_6 on the *n*-side of the thylakoid membrane. By analogy, it is reasonable to assume that heme c_i in Firmicute *bc* is also attached on the cytoplasmic side of the membrane (de Vitry 2011). Heme attachment can take place independently of the presence of other bc_1/b_6f complex subunits but the steady-state level of the holoprotein is usually dependent upon association/assembly with other subunits (Kuras et al. 1997; Zara et al. 2007).

At least three distinct mechanisms for the covalent attachment of heme to the apoforms of cytochromes c_1 and *f*, the so-called Systems I, II and III, have been described (Kranz et al. 2009; Allen 2011; Mavridou et al. 2013; Verissimo and Daldal 2014; Babbitt et al. 2015). All of these systems are not unique to the maturation of cytochrome c_1 and *f*, but some also control the heme ligation reaction to multiple apocytochrome *c* substrates in the bacterial periplasm, soluble apocytochrome *c* in the mitochondrial IMS (intermembrane space) and soluble apocytochrome c_6 in the thylakoid lumen of green algae. A fourth pathway for covalent heme attachment, System IV, is solely dedicated to the attachment of heme c_i to apocytochrome b_6, and hence is specific to the biogenesis of the b_6f (de Vitry 2011).

Because each system can be recognized on the basis of unique assembly factors, the distribution of the different maturation pathways was analyzed in the three domains of life. While the occurrence of a heme attachment pathway cannot be entirely predicted, a few generalities about the distribution of the three systems in energy-transducing membranes have been noted (Bertini et al. 2007; Allen et al. 2008a; Giegé et al. 2008; Allen 2011; Babbitt et al. 2015). While all bacteria utilize System I or System II, archaea have so far been found to have only System I. All plastids appear to use System II and mitochondria possess System I or System III.

Mitochondrial cytochromes *c* and c_1, with a heme attached via a single thioether bond, were found to occur in all three major taxonomic groups of the Euglenozoa (diplonemids, kinetoplastids and euglenids), even in the absence of any component of the known *c*-type cytochrome maturation system. This was taken as an indication that a novel pathway for cytochrome *c* maturation (also referred to as System V) must operate in mitochondria of organisms belonging to this phylum (Allen et al. 2008a; Allen 2011). Similarly, as no prototypical component of System IV can be detected in the genomes of Firmicutes, a novel pathway for thioether bond formation in cytochrome *b*, System VI, was postulated (Allen 2011; Mavridou et al. 2013). Note that because Systems V and VI are yet to be experimentally investigated, their designations as novel systems still await confirmation.

B. Biochemical Requirements for Thioether Bond Formation

The biochemical requirements to complete the covalent attachment of heme on the *p*-side are presumed to be universal and common to all energy-transducing membranes (Hamel et al. 2009; Bonnard et al. 2010). Covalent heme attachment is dependent on several steps including: (1) transport of the apocytochrome *c* and heme substrates to the *p*-side of the energy-transducing membrane, (2) maintenance of both the sulfhydryls of the $CX_nC(H/K)$ motif and the heme iron in a reduced form, and (3) formation of two thioether linkages between the vinyl-2 and -4 of heme, with the N- and C-terminal cysteines of the heme-binding motif, respectively.

1. Transport of Pre-apocytochrome c *and Heme*

The transport of bacterial and organellar apocytochromes *c* from the site of their synthesis (cytoplasm or plastid stroma) to their final sub-cellular location (periplasm, mitochondrial IMS or thylakoid lumen) has been examined in many experimental systems. In most cases, this requires transport of both the heme and the protein via independent pathways, and pre-apoprotein cleavage prior to heme attachment.

Most *c*-type cytochromes are synthesized in a precursor form with appropriate targeting sequences that direct them, as unfolded polypeptides across at least one biological membrane, via general translocation routes such as Sec in bacteria, Toc/Tic/Sec in the plastid and Tom/Tim in the mitochondria (Thöny-Meyer and Künzler 1997; Nakamoto et al. 2000; Hamel et al. 2009). The presequences are cleaved during translocation to yield the apoform of the protein, which is typically the substrate for the heme attachment reaction. Although less common, heme attachment has been found to occur first as a prerequisite for processing of the pre-cytochrome c_1 in *S. cerevisiae* mitochondria (Zollner et al. 1992; Steiner et al. 1996).

The final two steps of heme synthesis, catalyzed by protoporphyrinogen oxidase and ferrochelatase, take place on the *n*-side of the membrane (Ajioka et al. 2006). Ferrochelatase is required for the incorporation of reduced iron in protoporphyrin IX and seems to be membrane-associated, facing the *n*-side (Ajioka et al. 2006). This orientation implies that a mechanism for transmembrane "delivery" of heme from the *n*-side to the *p*-side must operate, since mature heme is produced on the *n*-side. Exceptions occur in organisms where heme is not synthesized in the mitochondria. Such organisms are either parasites relying on dietary heme, like helminths and some trypanosomes, or eukaryotes producing heme within the plastid (Atteia et al. 2005; Hamza and Dailey 2012; Koreny et al. 2013). In both cases, heme is assumed to be transported from the cytoplasm or the plastid to the mitochondrial IMS. With the exception of System II, where a transmembrane heme delivery route was shown to be required for cytochrome *c* assembly (see Sect. III.D.2), the pathways controlling heme distribution from its site of synthesis are unknown.

2. Maintenance of Sulfhydryls and Heme Iron in the Reduced Form

In vitro, uncatalyzed conversion of apocytochrome *c* to its holoform can be achieved in the presence of heme, provided that a reducing agent is added to the reaction (Daltrop et al. 2002; Daltrop and Ferguson 2003, 2004). In the absence of a reductant, the apocytochrome *c* heme-binding site is disulfide-bonded and heme attachment does not proceed. This indicates that reduced apocytochrome *c* is the only competent substrate for the heme ligation reaction. In the case of heme, the need of reduced iron for thioether bond formation is less intuitive and was deduced from reconstitution experiments (Barker et al. 1993; Daltrop et al. 2002). In these experiments, iron in the ferric form was postulated to initiate a radical-based chemistry, yielding side products with incorrectly attached heme. Hence, the initial oxidation state of the heme iron, when it is presented to the apoprotein, is critical to the formation of the thioether bond linkage. Mechanisms for heme reduction have been postulated for System I (see Chap. 27 by Khalfaoui-Hassani et al. in this volume), System II (see Sect. II.D.2), and System III (see Sect. III.E.2). There are known mechanisms for maintaining the heme-binding site sulfhydryls in a reduced form in Systems I and II (see Sects. III.C.2 and III.D.3), but not in System III. It is likely that System V does not require a mechanism to maintain sulfhydryls in a reduced form, considering that there is a single thioether bond forming cysteine in the A/F*XX*CH heme-binding motif (Allen et al. 2008b).

3. Stereospecific Formation of Thioether Bond Formation

Chemical reaction of the heme-binding site sulfhydryls with the vinyl side chains of heme can yield eight possible combinations. Yet only one combination is found in all cytochromes *c*, where the α carbons of vinyl-2 and -4 of heme are linked to the amino (N)- and carboxyl (C)-terminal cysteines in the C*(X)*$_n$CH/K motif (Barker and Ferguson 1999). This stereospecificity of heme attachment also extends to mitochondrial cytochrome *c* of Euglenozoa, where the cysteine in the A/F*XX*CH motif is forming a thioether bond with the α-carbon of the vinyl-4 of heme (Vilmos et al. 2009). The mechanisms controlling this stereoselectivity in the heme ligation reaction still remain undeciphered.

C. System I or CCM Pathway (Cytochrome C Maturation)

1. Overview of the CCM Pathway in Bacteria

The most complex "CCM" (for *C*ytochrome *C M*aturation) pathway, is currently the best characterized in terms of identity of the components, their biochemical activities and the sequence of events (Kranz et al. 2009; Sanders et al. 2010; Verissimo and Daldal 2014). The CCM was initially discovered in α- and γ-proteobacteria. Additionally, it was also assumed to operate in the mitochondria of vascular plants and some protozoa (e.g. *Paramecium* spp.), based on the occurrence of mitochondria-encoded Ccm-like proteins (Allen et al. 2003). Extensive functional studies in various microbial models, including *Escherichia coli* and *Rhodobacter capsulatus*, led to the identification of multiple cytochrome *c* assembly factors that can be categorized in three functional modules (Fig. 26.1): (1) heme handling/delivery, (2) preparation of apocytochrome *c* substrate via maintenance of the heme-linking sulfhydryls in a reduced form, and (3) thioether bond formation. The bacterial CCM pathway is detailed in the Chap. 27 by Khalfaoui-Hassani et al. in this volume. In the following section, we review the findings of the mitochondrial CCM pathway from experimental investigation in the *Arabidopsis* plant model (Fig. 26.1).

2. A Variant of Bacterial System I in Land Plant Mitochondria

(1) Heme Handling and Delivery. In bacteria, the heme relay pathway includes several steps in the periplasm: (i) loading of heme onto CcmC and transfer onto heme chaperone CcmE, (ii) formation of holoCcmE by covalent attachment of heme to a conserved histidine, and (iii) release of holoCcmE from interaction with CcmC by the ABC-transporter CcmAB and CcmD (see the Chap. 27 by Khalfaoui-Hassani et al.). In land plant mitochondria, the components controlling the delivery and relay of heme can be recognized on the basis to their sequence similarity to bacterial CcmABCE, with the exception of CcmD (Giegé et al. 2008). CcmD, a small membrane-anchored protein, is part of the bacterial CcmABC-holoCcmE complex and is required for the release of holoCcmE (Feissner et al. 2006a; Richard-Fogal et al. 2008, 2009). It is likely that CcmD cannot be identified in plants because there is little conservation of its primary sequence in System I in bacteria and archaea.

Bacterial CcmC is a polytopic membrane HHP (Heme Handling Protein). HHPs are heme-interacting proteins containing heme-coordinating residues (such as histidine) and hydrophobic amino acids that can interact with the porphyrin ring (such as tryptophan) (Li et al. 2011). Bacterial CcmC displays a characteristic tryptophan-rich "WWD" heme-binding motif, flanked by two conserved histidines which are essential for heme loading onto CcmE (Schulz et al. 1999, 2000; Richard-Fogal and Kranz 2010). Compatible with a function in mitochondrial cytochrome *c* assembly, the tryptophan-rich "WWD" domain and flanking histidines of

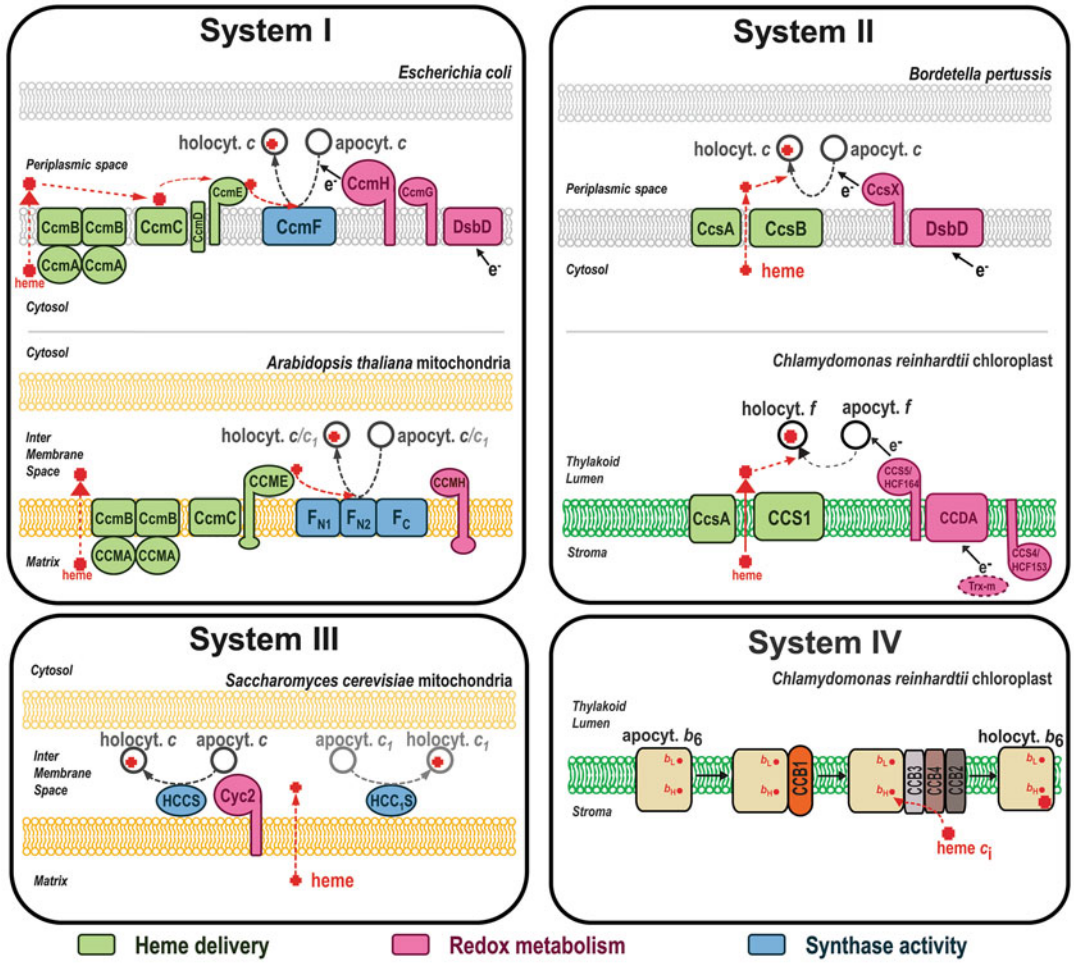

Fig. 26.1. Overview of pathways (Systems I, II, III and IV) for covalent heme attachment. The figure is adapted from Hamel et al. 2009. Bacterial membranes are shown in *gray*, thylakoid membranes are shown in *green* and mitochondrial membranes are shown in *orange*, the outer membrane (*light orange*) and the inner membrane (*dark orange*). For Systems I, II and III, the components shown in *green* are involved in heme handling/delivery, the ones in *pink* are involved in redox metabolism, and the *blue* components are postulated to have a holocytochrome *c* synthase activity. For System II, CcsA and CcsB/CCS1 display heme handling/delivery but also act as holocytochrome *c* synthase. System I operates in mitochondria of land plants and some protists, and Gram$^-$ (α, γ) bacteria. System II occurs in chloroplasts, Gram$^+$ and Gram$^-$ (β, δ, ε) bacteria, aquificales and cyanobacteria. System III is restricted to mitochondria of fungi, animals, apicomplexan parasites, *red* and *green* algae and diatoms. CcdA or DsbD occur in bacterial System I and in System II and are Thiol-disulfide oxido-reductases (TDORs) of the DsbD family. Cyc2p is restricted to organisms where both HCCS and HCC_1S are present. In animals, only one holocytochrome *c* synthase, HCCS is present. For System IV, a simplified pathway for the CCB-dependent heme attachment is shown (adapted from Saint-Marcoux et al. 2009).

plant CcmC are predicted to be exposed to the mitochondrial IMS (Giegé et al. 2008). Plant CcmC is mitochondria-encoded and detected in mitochondrial membrane fractions (Raczynska et al. 2006). A severe reduction of CcmC abundance, in a mutant deficient for the processing of the *ccmC* transcript, has no impact on the activities of the bc_1 and cytochrome *c* oxidase complexes (Jonietz et al. 2011). However, the abundance of mitochondrial holocytochrome c_1 and *c* was not documented and the role of CcmC

in cytochrome *c* maturation remains to be demonstrated.

Arabidopsis CCME is a nuclear-encoded peripheral protein of the mitochondrial inner membrane with its heme-binding domain exposed to the mitochondrial IMS (Spielewoy et al. 2001). When expressed in the *E. coli* periplasm, mitochondrial CCME inserts correctly in the membrane but is unable to substitute for the function of bacterial CcmE in cytochrome *c* maturation (Spielewoy et al. 2001). However the fact that mitochondrial CCME binds heme covalently, via its conserved histidine when expressed in bacteria, was taken as an indication that its function as a heme chaperone is probably conserved (Spielewoy et al. 2001).

Nuclear-encoded CCMA, the ATP binding component of the ABC-transporter, localizes to the mitochondrial matrix, and was shown to be associated with the inner membrane (Rayapuram et al. 2007). In agreement with its sequence similarity to bacterial CcmA, a recombinant form of mitochondrial CCMA exhibits ATPase activity (Rayapuram et al. 2007). Interaction of CCMA at the inner membrane with CcmB, the mitochondria-encoded membrane component of the ABC transporter, is supported by yeast two-hybrid (Y2H) experiments (Rayapuram et al. 2007). ATP/Mg^{2+} was shown to facilitate CCMA dissociation from the mitochondrial membrane, most likely because ATP hydrolysis induced conformational changes that modify the interaction with the transmembrane domains of CcmB (Rayapuram et al. 2007). Mitochondrial CCMA occurs in a 480 kDa complex that does not contain other Ccm proteins such as CcmF, CCME or CCMH (see below) (Rayapuram et al. 2007). It is likely that this complex contains CcmB but this could not be tested due to the lack of an antibody against this protein.

(2) Preparation of the Apocytochrome c Substrate. Maintenance of the heme-linking cysteines in a reduced form is essential for covalent attachment of heme to apocytochrome *c*. Bacterial CcmH is a membrane-anchored, periplasm-facing thiol-disulfide reductase proposed to be involved in maintaining the heme-binding motif of apocytochromes *c* in a reduced form (Sanders et al. 2010; Verissimo and Daldal 2014). However, its mechanism of action still remains obscure (Verissimo and Daldal 2014) (see the Chap. 27 by Khalfaoui-Hassani et al.). In plants, the ortholog of CcmH is a nuclear-encoded, integral inner membrane protein with an IMS-facing domain containing a R*CXX*C redox motif (Meyer et al. 2005). Y2H experiments show an interaction between the IMS-facing domain of mitochondrial CCMH and apocytochrome *c*. Moreover, in vitro redox assays with a recombinant form of mitochondrial CCMH show that the R*CXX*C motif can reduce a disulfide in a model apocytochrome *c* peptide (Meyer et al. 2005). The thioredoxin-like CcmG and a TDOR (Thiol-disulfide oxido-reductase) of the DsbD family (see Fig. 26.1), which are proposed to maintain bacterial CcmH in a reduced form, cannot be identified in land plant genomes (Giegé et al. 2008; Sanders et al. 2010; Cho and Collet 2013). Hence, it is unclear how mitochondrial CCMH is maintained reduced in the IMS (Giegé et al. 2008; Bonnard et al. 2010).

(3) Thioether Bond Formation. CcmF was long considered to be the component catalyzing thioether bond formation but experimental evidence for this claim was only provided recently (Ren et al. 2002; Sanders et al. 2008; Richard-Fogal et al. 2009; San Francisco et al. 2014). Bacterial CcmF is a heme-handling protein (HHP) with a "WWD" domain and two flanking histidines on the *p*-side and two transmembrane histidines facing the *n*-side of the membrane (Richard-Fogal et al. 2009; San Francisco et al. 2011). The histidines on the *p*-side are proposed to bind the CcmE-attached heme while histidines on the *n*-side are ligands of a structural *b*-heme. The transmembrane *b*-heme was proposed to be involved in transferring electrons from the cytoplasm to the periplasmic side in order to maintain the heme substrate in a reduced form. *Arabidopsis* CcmF is encoded by the mitochondrial genome in the form of three

genes (*ccmF*$_{N1}$, *ccmF*$_{N2}$, *ccmF*$_{C}$), each of which encodes an integral inner membrane protein with similarity to a corresponding domain of bacterial CcmF (Giegé et al. 2004; Rayapuram et al. 2008). Histidines on the *p*-side are in CcmF$_{N1}$ and CcmF$_{N2}$ while the *n*-side histidines are provided by CcmF$_{N2}$ and CcmF$_{C}$. The occurrence of CcmF$_{N1}$, −F$_{N2}$, −F$_{C}$ in a mitochondrial membrane complex and Y2H-based interaction of CcmF$_{N2}$ with CcmF$_{N1}$ and CcmF$_{C}$ suggest that all three CcmF proteins constitute a functional unit in plant mitochondria (Giegé et al. 2004; Rayapuram et al. 2008). CcmF$_{N2}$ also contains the WWD domain that was shown to face the IMS and interact with apoforms of both cytochrome *c* and c_1 in an Y2H assay (Rayapuram et al. 2008). CcmF$_{N2}$ interacts with CCMH in Y2H assays and both proteins are also detected in a complex at the inner membrane (Rayapuram et al. 2008). It is likely that mitochondrial CcmF and CCMH form a complex controlling the heme ligation reaction, similar to the bacterial CcmFH, which was shown to carry the cytochrome *c* synthase activity (San Francisco et al. 2014). A role for *Arabidopsis* CcmF$_{C}$ in *c*-type cytochrome maturation was evidenced through the identification of a splicing defect of the *ccmF*$_{C}$ transcript that results in decreased accumulation of holoforms of cytochromes *c* and c_1 (Francs-Small et al. 2011). Similarly, an editing defect at a single position in the *ccmF*$_{N}$ transcript results in loss of CcmF$_{N}$ and severe reduction of mitochondrial *c*-type holocytochromes in maize (Sun et al. 2015).

D. System II or CCS (Cytochrome C Synthesis) Pathway

1. Discovery of System II in Plastids

System II was originally discovered in the green alga *Chlamydomonas reinhardtii* through genetic studies of the *ccs* mutants (*ccs* for *c*ytochrome *c* *s*ynthesis), a class of photosynthesis-minus mutants specifically deficient for the accumulation of the holoforms of plastid *c*-type cytochromes (Howe and Merchant 1992; Howe et al. 1995; Xie et al. 1998). The *ccs* mutants are deficient for the thylakoid membrane-bound *c*-type cytochrome *f*, which is required for cytochrome b_6f assembly, and also for soluble cytochrome c_6, the functional replacement of plastocyanin in Cu-deficient conditions (see the Chaps. 30 and 31 by Bendall and Howe, and Diaz-Moreno et al. in this volume). All *ccs* mutants display a block in the conversion of apo- to holoform of plastid *c*-type cytochromes because the heme attachment to apocytochromes, a chemical reaction taking place in the thylakoid lumen, is compromised (Howe and Merchant 1993, 1994; Xie et al. 1998). It is also expected that lumen resident cytochrome c_{6A}, the novel *c*-type cytochrome conserved in the green lineage (see the Chap. 33 by Howe et al.) is not assembled in the *ccs* mutants. However, this could not be determined because its holoform could not be detected in wild-type strains.

At least 7 loci, plastid *ccsA* and nuclear *CCS1* to *CCS6* were uncovered through genetic analysis of *ccs* mutants (Howe and Merchant 1992; Xie et al. 1998; Dreyfuss and Merchant 1999; Page et al. 2004). Sequence analysis of the cloned *ccsA* and *CCS1* genes (Xie and Merchant 1996; Inoue et al. 1997) revealed that the predicted protein products were novel integral membrane proteins co-occurring in cyanobacteria, the majority of the Gram-positive bacteria (e.g. *Bacillus subtilis*), proteobacteria of the β- (e.g. *Bordetella pertussis*), δ − (e.g. *Desulfovibrio desulfuricans*), and ε groups (e.g. *Helicobacter pylori*, *Wolinella succinogenes*), and aquificales (Simon and Hederstedt 2011). Forward and reverse genetics studies in several bacterial and cyanobacterial models have confirmed the roles of CcsA and CCS1-like proteins in the assembly of *c*-type cytochromes. This led to the definition of a distinct pathway for thioether bond formation in apocytochrome *c* (Tichy and Vermaas 1999; Beckett et al. 2000; Le Brun et al. 2000; Kern et al. 2010a, b). A representation of bacterial and plastid System II components is shown on Fig. 26.1.

2. A Transmembrane Heme Channel with Cytochrome c Synthase Activity

While CcsA was recognized as a member of the HHP family, based on the presence of the "WWD" domain and conserved histidines also found in System I CcmC and CcmF, there was no motif in CCS1 suggestive of a biochemical function in thioether bond formation (Inoue et al. 1997; Goldman et al. 1998; Hamel et al. 2003). Functional investigation of plastid CcsA and CCS1 proteins established that they are polytopic membrane proteins with functional domains exposed to the lumen and essential histidinyl residues on both the lumen and stromal side of the thylakoid membrane (Dreyfuss et al. 2003; Hamel et al. 2003). CcsA and CCS1 require each other for accumulation in vivo and were therefore proposed to function as a transmembrane heme delivery system from stroma to lumen in a complex, possibly with the products of some of the other *CCS* loci (Dreyfuss et al. 2003; Hamel et al. 2003). Experimental support for the biochemical activity of CcsA and CCS1 came with the discovery that naturally occurring Ccs1-CcsA fusion proteins called CcsBA, from several ε-proteobacteria, could assemble reporter cytochrome(s) *c* in an *E. coli* strain lacking all of the Ccm components (Feissner et al. 2006b; Frawley and Kranz 2009; Goddard et al. 2010; Kern et al. 2010a; Richard-Fogal et al. 2012). The finding that CcsBA could substitute for all Ccm components indicated that the fusion protein was necessary and sufficient for thioether bond formation. The function of CcsBA as a transmembrane heme delivery system was confirmed from spectroscopic analysis of the purified CcsBA protein. This analysis revealed that heme can be trapped at an external binding domain where periplasmic localized histidines, provided by the CcsA moiety, act as axial ligands (Frawley and Kranz 2009). Histidines in transmembrane domains (TMD) of CcsB and CcsA, close to the cytoplasmic surface of the membrane, were postulated to act as an entry site for heme. This conclusion was based on the fact that heme was no longer detected at the external binding domain when these residues are mutated. Imidazole-dependent rescue of the cytochrome *c* synthase activity of CcsBA carrying mutations in the TMD histidines also supported their implied involvement in providing a route for heme transport from the cytoplasm to the periplasm (Frawley and Kranz 2009). The mechanism by which heme is maintained reduced prior to the ligation reaction remains to be established. However, heme is bound to CcsBA in the oxidized form when either one of the heme-interacting histidines facing the periplasm is mutated (Frawley and Kranz 2009). This observation led to the proposal that histidine ligands act by protecting reduced heme from oxidation.

3. Operation of a Transmembrane Disulfide Reducing Pathway

(1) A Two-Component Redox Pathway in Bacteria. In System II, the involvement of thiol-based chemistry in the heme attachment reaction was first demonstrated in bacteria through the discovery of redox factors controlling cytochrome *c* assembly. Genetic screens in System II bacterial models led to the identification of a thiol-disulfide oxidoreductase (TDOR) from the DsbD family at the plasma membrane and a membrane-anchored, periplasm-facing thioredoxin-like ResA/CcsX, which are required for the covalent linkage of heme to apoforms of cytochromes *c* (Schiott et al. 1997a, b; Beckett et al. 2000; Erlendsson et al. 2003). Loss of function of the redox cytochrome *c* assembly factors can be bypassed by provision of exogenous reduced thiols in the medium, a finding that led to the proposal that these components function in a disulfide reducing pathway (Beckett et al. 2000; Erlendsson and Hederstedt 2002; Erlendsson et al. 2003; Feissner et al. 2005; Small et al. 2013). One interpretation is that the thiols act as a substitute for the reducing activity of the assembly factors. A possible mechanism is that the thiols reduce a disulfide in the apocytochrome *c* heme-binding motif to free sulfhydryls. In

agreement with this view is the fact that the redox cytochrome *c* assembly factors are no longer required for heme attachment when the components of the disulfide forming pathway are inactivated (Erlendsson and Hederstedt 2002; Erlendsson et al. 2003; Small et al. 2013). One likely explanation is that disulfide bonds in apocytochromes *c* are formed by the disulfide forming pathway during their translocation to the periplasm. When the disulfide forming pathway no longer operates, sulfhydryls remain reduced and apocytochrome *c* is in the competent form to be acted upon by heme. So it appears that in bacteria the disulfide reducing pathway, in the context of thioether bond formation, is only needed to counter sulfhydryl oxidation of the heme-binding cysteines. In System I bacteria, a similar transmembrane disulfide reducing pathway (consisting of the thioredoxin-like CcmG and a TDOR) is also required for cytochrome *c* assembly (Fig. 26.1). As in System II, this pathway is only necessary for holocytochrome *c* maturation when the disulfide bond forming enzymes operate (Monika et al. 1997; Deshmukh et al. 2000, 2003; Bardischewsky and Friedrich 2001; Turkarslan et al. 2008).

In vivo, the disulfide bond in the heme-binding site of apocytochrome *c* is reduced to sulfhydryls by action of the thioredoxin-like ResA/CcsX. This is supported by extensive biochemical and structural studies of *B. subtilis* ResA, which reveal that the enzyme undergoes drastic conformational change and consequent increase in reactivity upon binding of oxidized apocytochrome *c* (Crow et al. 2004; Colbert et al. 2006; Lewin et al. 2006, 2008; Hodson et al. 2008). In the periplasm, ResA is maintained reduced by the activity of a TDOR of the DsbD family, which conveys the reducing power across the cytoplasmic membrane via thiol-disulfide exchange reactions. The primary source of electrons for this pathway is provided in the form of NADPH, via TrxA, the cytoplasmic thioredoxin (Möller and Hederstedt 2008).

(2) A Bacterial-Like Redox Pathway in Plastids. In plastids, the implication of thiol-based chemistry in the assembly of plastid cytochrome *c* was inferred from (1) the observation that the *Chlamydomonas ccs4* and *ccs5* mutants could be phenotypically rescued by application of exogenous reductants, and (2) the occurrence in *Arabidopsis* of CCDA, another TDOR of the DsbD family at the thylakoid membrane, and HCF164, a membrane-anchored thioredoxin-like protein facing the lumen (Lennartz et al. 2001; Page et al. 2004; Motohashi and Hisabori 2010). Loss of function of CCDA and HCF164 yields a b_6f assembly defect, an expected phenotype if heme attachment to apocytochrome *f* is compromised. However, this phenotype could not be attributed to a specific defect in cytochrome *c* assembly because the heme attachment reaction was not monitored in the *ccda* and *hcf164* mutants in *Arabidopsis* (Lennartz et al. 2001; Page et al. 2004). Molecular identification of the *Chlamydomonas CCS5* gene showed that the corresponding gene product is the ortholog of *Arabidopsis* HCF164. CCS5 was shown to interact with plastid apocytochromes *c* in a Y2H assay and a recombinant form of the molecule was active in reducing a disulfide-bonded C*XX*CH motif in a soluble form of apocytochrome *f* in vitro (Gabilly et al. 2010). Based on these findings, a role for CCS5/HCF164 as an apocytochrome *c* disulfide reductase in the heme attachment reaction was formulated. Oxidation of apocytochrome *c* C*XX*CH motif in the thylakoid lumen remains to be demonstrated, but the fact that the *ccs5*-null mutant is rescued in vivo by application of exogenous thiols is in agreement with the proposed disulfide reductase activity of CCS5/HCF164 (Gabilly et al. 2010). It is possible that, as in bacteria, apocytochrome *c* is a target of the disulfide bond forming pathway operating in the thylakoid lumen, but this remains to be experimentally tested (Karamoko et al. 2011; Lu et al. 2013).

The identification of CCS4 is intriguing, because this component does not display any motif suggesting a role in thiol-based redox chemistry (Gabilly et al. 2011). CCS4 displays limited similarity to *Arabidopsis*

HCF153, a thylakoid membrane-anchored protein required for the assembly of the cytochrome b_6f complex (Lennartz et al. 2006). However, the activity and site of HCF153 action in the assembly process has so far remained undeciphered. The thiol-dependent photosynthetic rescue of the *ccs4* mutant, and the suppression of the *ccs4* phenotype by ectopic expression of CCDA, confirms the activity of CCS4 in a disulfide-reducing pathway for cytochrome *c* assembly (Gabilly et al. 2011). Moreover, the CCDA-dependent suppression of *ccs4* solidifies the placement of CCDA in plastid cytochrome *c* maturation, as earlier studies in *Arabidopsis* supported, but did not establish the requirement of plastid CCDA in the conversion of apo- to holocytochromes *c* (Page et al. 2004). By analogy to the bacterial disulfide reducing pathway, the proposed model is that CCDA and CCS5/HCF164 define a trans-thylakoid pathway for the delivery of reductants from stroma to lumen via thiol-disulfide exchanges (Page et al. 2004; Motohashi and Hisabori 2006, 2010; Gabilly et al. 2010, 2011). The involvement of CCS4 in the disulfide bond reducing pathway remains unclear, but a role in regulating the activity/stability of CCDA was postulated (Gabilly et al. 2011). The source of reductant is expected to originate from the stroma and *in organello* experiments support the role of the stromal thioredoxin Trx-*m* as a possible electron donor to both CCDA and HCF164 (Motohashi and Hisabori 2006, 2010).

4. Additional System II Components in Plastids?

Saturating screens in System II bacteria have established the involvement of four assembly components CcsA, Ccs1/CcsB, a TDOR of the DsbD family and the thioredoxin-like ResA/CcsX (Beckett et al. 2000; Le Brun et al. 2000). In plastids, at least four additional CCS factors were revealed through genetic studies of *Chlamydomonas ccs* mutants, out of which, CCS3 and CCS6 still remain uncharacterized at the molecular level (Xie et al. 1998; Page et al. 2004). The *CCS2* locus was cloned and the corresponding gene shown to encode a plastid-resident OPR (octotricopeptide repeat) protein (Cline et al., unpublished). Because all the OPR proteins characterized to date regulate translation, stability or maturation of chloroplast transcripts (Auchincloss et al. 2002; Balczun et al. 2005; Merendino et al. 2006; Eberhard et al. 2011; Rahire et al. 2012; Kleinknecht et al. 2014; Marx et al. 2015; Wang et al. 2015), the CCS2 protein is probably not directly involved in the heme attachment reaction. One possible function is that it controls the translation of the plastid-encoded *ccsA* transcript. It is plausible that cytochrome *c* maturation in the thylakoid lumen is more complicated than in bacteria and relies on additional components. The discovery that CCS4 functions in the disulfide reducing pathway and appears to be restricted to photosynthetic eukaryotes supports this view (Karamoko et al. 2013). The fact that the *Chlamydomonas CCS3, CCS4, CCS5* and *CCS6* loci are only defined by single alleles suggests that screens for plastid cytochrome *c* deficient mutants are not saturated and that additional CCS loci could be uncovered (Howe and Merchant 1992; Xie et al. 1998; Dreyfuss and Merchant 1999; Page et al. 2004).

*E. System III or HCCS (*HoloCytochrome C Synthase*) Pathway*

1. Discovery of HCCS, the Signature Component of System III

System III, the first pathway for thioether bond formation to be described is defined by a single component, the prototypical HCCS or holocytochrome *c* synthase. This enzyme is responsible for the terminal step in the holocytochrome *c* synthesis pathway, that is, the formation of the thioether bonds in apocytochromes *c* and c_1 (Allen 2011; Babbitt et al. 2015). The HCCS pathway seems to be restricted to mitochondria of fungi, animals, apicomplexan parasites, red and green algae, and some protists (e.g. *Dictyostelium discoideum*), as gauged

from genome sequence analysis (Giegé et al. 2008; Allen 2011; Mavridou et al. 2013; Babbitt et al. 2015). Remarkably, the existence of two distinct mitochondrial enzymes, HCCS and HCC_1S, catalyzing heme attachment onto apocytochrome *c* and c_1, respectively, was first discovered more than 30 years ago from studies of holocytochrome *c* reconstitution using apoprotein, heme and mitochondrial extracts from fungi (Korb and Neupert 1978; Basile et al. 1980; Hennig and Neupert 1983; Visco et al. 1985). Identification of the HCCS and HCC_1S-encoding genes came from later genetic studies of mutants deficient in mitochondrial cytochromes *c* and c_1 in fungi *S. cerevisiae*, *Neurospora crassa*, and *Candida albicans*, and these organisms have remained the focus of attention for the analysis of System III (Dumont et al. 1987; Nargang et al. 1988; Drygas et al. 1989; Zollner et al. 1992; Cervera et al. 1998). Genetic studies in *S. cerevisiae* established that apocytochrome *c* can only be assembled by HCCS, while apocytochrome c_1 is a substrate of both HCCS and HCC_1S (Bernard et al. 2003). The holocytochrome c_1 synthase activity of HCCS is weak but can be enhanced if the substrate-enzyme interactions are modified via over-expression of the HCCS-encoding gene or point mutations in either HCCS or apocytochrome c_1 (Bernard et al. 2003). However, animals are the only System III organisms with a single HCCS, which was shown to act on both apocytochrome *c* and c_1 substrates (Schwarz and Cox 2002; Bernard et al. 2003).

Both HCCS and HCC_1S are peripherally bound to the mitochondrial inner membrane and face the IMS, the compartment where the heme attachment reaction takes place (Dumont et al. 1991; Steiner et al. 1995; Bernard et al. 2005). All HCCSs are related in sequence (less than 20 % amino acid sequence identity in pairwise comparisons) and display zero to four typical HRMs (heme regulatory motif), also present in several other heme-binding proteins, such as the transcription factor Hap1p and HO (Heme oxygenase) (Li et al. 2011). In intact or detergent-solubilized mitochondria, HCCSs were shown to interact with apocytochromes *c* (Nicholson et al. 1988; Nicholson and Neupert 1989; Mayer et al. 1995) and also with heme via the HRM (Steiner et al. 1996). Nevertheless, the HRMs are not strictly essential for formation of holocytochrome *c* and c_1, suggesting that other domains of the HCCS must be important for their enzymatic activity (Steiner et al. 1996; Moore et al. 2011).

Because mitochondrial cytochrome *c* could be correctly matured in the bacterial periplasm or cytoplasm upon expression of HCCS alone, and did not require co-expression of any other assembly factors, HCCSs were proposed to participate in the biogenesis of *c*-type cytochromes by catalyzing thioether bond formation (Dumont et al. 1988; Pollock et al. 1998; Sanders and Lill 2000; Richard-Fogal et al. 2012).

However, despite the fact that HCCSs were the first *c*-type cytochrome assembly factors to be discovered, the specific enzymatic function of the proteins had remained elusive for a long time until recently. An important breakthrough in detailing the enzymology of HCCS came with the purification and spectroscopic analysis of the human enzyme co-expressed with its cognate apocytochrome *c* substrate in the bacterial cytoplasm (San Francisco et al. 2013; Babbitt et al. 2014a, b). Furthermore, the use of mutant forms of the enzyme and apocytochrome substrate enabled the trapping of intermediate complexes, which led to the delineation of possible steps in the heme attachment reaction. The first step in the HCCS-dependent heme attachment reaction is the binding of heme to the enzyme via two domains (I and II) containing conserved residues. One of these, a histidine in domain II, acts as an axial ligand of heme. Formation of the heme-HCCS complex triggers the recognition of the apocytochrome *c* substrate. The determinants for substrate recognition by HCCS were established using cytochrome *c* and found

to lie within a 9 residue sequence upstream the C*XX*CH motif (Stevens et al. 2011; Asher and Bren 2012; Verissimo et al. 2012; San Francisco et al. 2013). It is likely that the sequence requirements for recognition of cytochrome c_1 are similar but this awaits experimental testing.

The heme-HCCS-apocytochrome *c* together forms a ternary complex, where heme is coordinated by one histidine in domain II and another histidine in the C*XX*CH motif on apocytochrome *c* (San Francisco et al. 2013). The mechanism for thioether bond formation is still obscure, but bis-histidinyl coordination of heme in the heme-HCCS-apocytochrome *c* complex is postulated to provide the ideal geometry for stereospecific thioether bond formation. In addition, the observation that some residues in HCCS control the retention of holocytochrome *c* in HCCS suggests that a mechanism for releasing the mature holoform from the enzyme is probably required for optimal cytochrome *c* synthesis (San Francisco et al. 2013; Babbitt et al. 2014a, b). Interestingly, none of the domains defined as important for the HCCS enzymatic activity include the HRMs initially postulated to be required for HCCS function (Allen 2011). In HO, another enzyme containing HRM motifs, the HRM cysteine sulfhydryls can switch between an oxidized or reduced form depending on the cellular redox state. Furthermore, thiol-disulfide interconversion at the HRMs was shown to regulate the heme-binding activity of HO and it is possible HRMs in HCCSs perform the same function (Yi and Ragsdale 2007; Yi et al. 2009).

2. Missing Components in System III?

System III appears deceptively simple in terms of composition, in contrast to the complexity of Systems I and II, because extensive genetic screens in *S. cerevisiae* and *N. crassa* have so far revealed only HCCS and HCC_1S as assembly factors for mitochondrial cytochromes *c* (Lang and Kaudewitz 1982; Dumont et al. 1987; Drygas et al. 1989; Sherman 1990; Zollner et al. 1992). Important future avenues for research include investigation into the mechanisms of heme delivery to the mitochondrial IMS. Furthermore, by analogy to System I and II, mechanisms for maintenance of the sulfhydryls and chemical reduction of heme prior to the ligation reaction must also operate. There is evidence that both of these are still biochemical requirements of the pathway from *in organello* reconstitution of holocytochromes *c* and c_1. In the former instance, the C*XX*CH sulfhydryls were shown to be maintained reduced by an unknown mechanism until the heme ligation reaction (Nicholson et al. 1987). In the latter instance, both pyrimidine and flavin nucleotides were shown to be required for chemical reduction of heme iron (Basile et al. 1980; Nicholson and Neupert 1989; Nicholson et al. 1989; Tong and Margoliash 1998).

One candidate protein for the reduction of heme is Cyc2p, a mitochondrial protein first discovered in a screen for cytochrome *c* deficient mutants in yeast and initially assigned a function in the import of apocytochrome *c* (Dumont et al. 1993; Pearce et al. 1998; Sanchez et al. 2001). Cyc2p is an inner membrane anchored, IMS-facing, NAD(P)H-dependent flavoprotein and was re-isolated as a factor enhancing the holocytochrome c_1 synthase activity of HCCS through a multicopy suppressor screen (Bernard et al. 2003, 2005). An in vivo indication that Cyc2p controls a reductive step in the heme attachment reaction is the finding that the requirement for its function can be bypassed by exogenous reductants (Corvest et al. 2012), similar to what was observed for plastid and bacterial cytochrome *c* assembly mutants deficient in the disulfide reducing pathway (Deshmukh et al. 2000, 2003; Bardischewsky and Friedrich 2001; Turkarslan et al. 2008; Gabilly et al. 2010, 2011). However, although redox titrations of Cyc2p flavin indicate that reduction of a disulfide at the C*XX*CH site of apocytochrome *c* is a thermodynamically favorable reaction, Cyc2p does not act as an apocytochrome *c* C*XX*CH disulfide

reductase in vitro. Instead, Cyc2p is able to catalyze the NAD(P)H-dependent reduction of hemin in vitro, indicating a possible role in reducing the heme iron prior to its attachment (Corvest et al. 2010, 2012). Y2H analysis showing that Cyc2p interacts with HCCS and also with apocytochromes *c* and c_1 led to the proposal that Cyc2p, possibly in a complex with HCCS, reduces the heme iron prior to co-factor attachment to the apoforms of cytochrome *c* and c_1. Operation of a distinct heme reductase dedicated to the HCC_1S enzyme was postulated based on the fact that Cyc2p is only required for the HCCS-dependent assembly of cytochrome *c* and c_1 and does not control the HCC_1S-catalyzed heme attachment to apocytochrome c_1 (Corvest et al. 2010). Because Cyc2p appears to be restricted to fungi, additional mechanism(s) of heme reduction in System III mitochondria must exist in other organisms.

F. System IV or CCB (Cofactor Assembly on Complex C Subunit B)

1. A Multi-step Pathway for Heme c_i Formation in the b_6f Complex

Covalently attached heme in cytochrome b_6 in the b_6f and cytochrome *b* in the *bc* of Firmicutes was suspected from spectroscopic measurement and the observation that heme/cytochrome *b* association was unusually resistant to denaturing treatment (Lavergne 1983; Joliot and Joliot 1988; Kuras et al. 1997; Yu and Le Brun 1998) However, b_L or b_H heme was believed to be attached covalently at the time and the discovery of an additional heme (heme c_i) bound via a single thioether bond in the X-ray structures of chloroplast and cyanobacterial b_6f came as a surprise (Kurisu et al. 2003; Stroebel et al. 2003). Heme c_i is located in close vicinity to heme b_H and unlike heme *c* in canonical *c*-type cytochromes, does not appear to have any amino acid axial ligands (Kurisu et al. 2003; Stroebel et al. 2003; de Vitry et al. 2004). Heme c_i has a unique axial ligand, a water molecule (or possibly a hydroxide ion) also interacting with a propionate group of heme b_H. A pathway for apo- to holocytochrome b_6 conversion was deduced from the analysis of *Chlamydomonas* strains depleted for heme or carrying mutations of the histidines coordinating b_L and b_H hemes (Kuras et al. 1997). While apocytochrome b_6 could be immunodetected in heme-deprived cells and mutants unable to ligate b_L, a distinct immunoreactive species of cytochrome b_6 was present in mutants altered for b_H ligation. The same species is also detected when the thioether bond forming cysteine in cytochrome b_6 is mutated (de Vitry et al. 2004). This indicates that loss of b_H ligation prevents formation of heme c_i and, reciprocally, that, in the absence of covalent attachment of heme, heme b_H is presumably no longer retained. Spectroscopic analysis of the purified b_6f complex altered for b_H ligation showed that heme b_L was present while hemes b_H and c_i were lost (Malnoë et al. 2011). Collectively, these results demonstrate that holocytochrome b_6 assembly proceeds through a b_L-containing intermediate, followed by incorporation of b_H and formation of heme c_i.

2. Identification of Novel Proteins Required for Heme c_i Assembly

The accumulation of a b_L-containing intermediate was exploited as a signature to identify assembly factors controlling heme c_i formation, referred to as CCB (for Cofactor assembly of complex C subunit B[3]) (Kuras et al. 1997; de Vitry et al. 2004). In *Chlamydomonas*, four *CCB* loci were defined from the genetic analysis of b_6f-deficient mutants that no longer assemble holocytochrome b_6 but still accumulate the b_L-containing cytochrome b_6 intermediate (Gumpel et al. 1995; Kuras et al. 1997, 2007). The *ccb* mutants are unable to grow photosynthetically but still retain low levels of b_6f (Lyska et al. 2007; Saint-Marcoux

[3]B is for petB (or cytochrome b_6) of the cytochrome b_6f complex (complex C).

et al. 2009). Wild-type levels of b_6f lacking heme c_i can be restored in *ccb* mutants with suppressor mutations attenuating the activity of the thylakoid FtsH protease (Malnoë et al. 2014). This result demonstrates that heme c_i is not absolutely required for complex assembly/activity and that b_6f lacking heme c_i becomes prone to proteolytic degradation. The photosynthetic growth of the suppressed strains is light-sensitive, an indication that the presence of heme c_i is required for optimal activity of the b_6f complex (Malnoë et al. 2014).

The *CCB* genes were cloned and the corresponding gene products were shown to be novel thylakoid transmembrane proteins conserved in all organisms performing oxygenic photosynthesis (Kuras et al. 2007). In *Arabidopsis*, loss of function of CCB proteins yields a defect in heme c_i formation (Lyska et al. 2007; Lezhneva et al. 2008). In cyanobacteria, *ccb* mutants are impaired for photosynthesis, but the function of CCB in heme c_i biogenesis could not be ascertained because the impact on the b_6f complex was not examined (Ishikawa et al. 2009).

The following pathway of action for the CCB machinery can be deduced from genetic and biochemical studies (Fig. 26.1). Interaction of CCB proteins with cytochrome b_6 is supported by BN-PAGE and co-immunoprecipitation experiments (Saint-Marcoux et al. 2009). Because subunit IV is not detected in association with the CCB factors, it was deduced that unassembled cytochrome b_6, presumably a form containing both b_L and b_H hemes, is the substrate of the CCB machinery. The detection of a CCB1/cytochrome b_6 complex in *ccb2*, *ccb*3 and *ccb4* mutants indicates that this complex is the first intermediate in the pathway and may chaperone the cytochrome b_6 substrate before the heme ligation step. This step is believed to be catalyzed by a CCB2/CCB4/CCB3 complex, also detected in association with cytochrome b_6 in a mutant altered for the thioether bond-forming cysteine. The presence of a stable CCB2/CCB4 heterodimer and a CCB3/cytochrome b_6 complex indicates that these might also be intermediates in the assembly pathway. A model was postulated where CCB3/cytochrome b_6 is the second intermediate in the pathway and recruits the CCB2/CCB4 complex to form the CCB3/CCB2/CCB4 heme ligation complex (Fig. 26.1). Although the biochemical activity of the CCB factors remains to be elaborated, it is reasonable to postulate that some of the protein domains must interact with both heme and cytochrome b_6. Moreover a mechanism for maintaining heme in a reduced form must exist, considering that the redox state of the heme iron is critical for the chemistry of thioether bond formation. Typical heme-binding motifs found in System I and II cytochrome *c* assembly factors, or conserved histidines, are not present in the CCB factors (Kuras et al. 2007). Occurrence of conserved, heme-interacting residues, such as tryptophan, tyrosine and/or phenylalanine, in regions exposed to the *n*-side of the membrane speaks to a possible heme handling/relay function, but this remains to be experimentally tested.

Acknowledgments

This work is supported by a National Science Foundation grant (MCB-0920062), a U.S. Department of Energy (DOE), Office of Science, Basic Energy Sciences (BES) (DE-SC0014562) grant and a Muscular Dystrophy Assocation grant (MDA#4247) to P.H. We thank Dr. A. Simcox for critical reading of the manuscript and Dr. R. Kranz, Dr. A. Malnoë, Dr. J. Stevens, Dr. S. Ferguson and Dr. G. Bonnard for their valuable insights into cytochrome *c* maturation.

References

Ajioka RS, Phillips JD, Kushner JP (2006) Biosynthesis of heme in mammals. Biochim Biophys Acta 1763:723–736

Allen JWA (2011) Cytochrome *c* biogenesis in mitochondria – Systems III and V. FEBS J 278: 4198–4216

Allen JW, Daltrop O, Stevens JM, Ferguson SJ (2003) C-type cytochromes: diverse structures and biogenesis systems pose evolutionary problems. Philos Trans R Soc Lond B Biol Sci 358:255–266

Allen JW, Jackson AP, Rigden DJ, Willis AC, Ferguson SJ, Ginger ML (2008a) Order within a mosaic distribution of mitochondrial *c*-type cytochrome biogenesis systems? FEBS J 275:2385–2402

Allen JWA, Ferguson SJ, Ginger ML (2008b) Distinctive biochemistry in the trypanosome mitochondrial intermembrane space suggests a model for stepwise evolution of the MIA pathway for import of cysteine-rich proteins. FEBS Lett 582:2817–2825

Aragão D, Frazão C, Sieker L, Sheldrick GM, LeGall J, Carrondo MA (2003) Structure of dimeric cytochrome c_3 from *Desulfovibrio gigas* at 1.2 A resolution. Acta Crystallogr D Biol Crystallogr 59:644–653

Asher WB, Bren KL (2012) Cytochrome *c* heme lyase can mature a fusion peptide composed of the amino-terminal residues of horse cytochrome *c*. Chem Commun (Camb) 48:8344–8346

Atteia A, van Lis R, Beale SI (2005) Enzymes of the heme biosynthetic pathway in the nonphotosynthetic alga *Polytomella* sp. Eukaryot Cell 4:2087–2097

Auchincloss AH, Zerges W, Perron K, Girard-Bascou J, Rochaix JD (2002) Characterization of Tbc2, a nucleus-encoded factor specifically required for translation of the chloroplast *psbC* mRNA in *Chlamydomonas reinhardtii*. J Cell Biol 157:953–962

Babbitt SE, San Francisco B, Bretsnyder EC, Kranz RG (2014a) Conserved residues of the human mitochondrial holocytochrome c synthase mediate interactions with heme. Biochemistry 53:5261–5271

Babbitt SE, San Francisco B, Mendez DL, Lukat-Rodgers GS, Rodgers KR, Bretsnyder EC, Kranz RG (2014b) Mechanisms of mitochondrial holocytochrome c synthase and the key roles played by cysteines and histidine of the heme attachment site, Cys-XX-Cys-His. J Biol Chem 289:28795–28807

Babbitt SE, Sutherland MC, Francisco BS, Mendez DL, Kranz RG (2015) Mitochondrial cytochrome c biogenesis: no longer an enigma. Trends Biochem Sci 40:446–455

Balczun C, Bunse A, Hahn D, Bennoun P, Nickelsen J, Kuck U (2005) Two adjacent nuclear genes are required for functional complementation of a chloroplast trans-splicing mutant from *Chlamydomonas reinhardtii*. Plant J 43:636–648

Balk J, Schaedler TA (2014) Iron cofactor assembly in plants. Annu Rev Plant Biol 65:125–153

Bardischewsky F, Friedrich CG (2001) Identification of *ccdA* in *Paracoccus pantotrophus* GB17: disruption of *ccdA* causes complete deficiency in *c*-type cytochromes. J Bacteriol 183:257–263

Barker PD, Ferguson SJ (1999) Still a puzzle: why is haem covalently attached in c-type cytochromes? Struct Fold Des 7:281–290

Barker PD, Ferrer JC, Mylrajan M, Loehr TM, Feng R, Konishi Y, Funk, WD, ..., Mauk AG (1993) Transmutation of a heme protein. Proc Natl Acad Sci U S A 90:6542–6546

Basile G, Di Bello C, Taniuchi H (1980) Formation of an iso-1-cytochrome *c*-like species containing a covalently bonded heme group from the apoprotein by a yeast cell-free system in the presence of hemin. J Biol Chem 255:7181–7191

Beckett CS, Loughman JA, Karberg KA, Donato GM, Goldman WE, Kranz RG (2000) Four genes are required for the system II cytochrome *c* biogenesis pathway in *Bordetella pertussis*, a unique bacterial model. Mol Microbiol 38:465–481

Bernard DG, Gabilly ST, Dujardin G, Merchant S, Hamel PP (2003) Overlapping specificities of the mitochondrial cytochrome *c* and c_1 heme lyases. J Biol Chem 278:49732–49742

Bernard DG, Quevillon-Cheruel S, Merchant S, Guiard B, Hamel PP (2005) Cyc2p, a membrane-bound flavoprotein involved in the maturation of mitochondrial *c*-type cytochromes. J Biol Chem 280:39852–39859

Bertini I, Cavallaro G, Rosato A (2007) Evolution of mitochondrial-type cytochrome *c* domains and of the protein machinery for their assembly. J Inorg Biochem 101:1798–1811

Bonnard G, Corvest V, Meyer EH, Hamel PP (2010) Redox processes controlling the biogenesis of *c*-type cytochromes. Antioxid Redox Signal 13: 1385–1401

Boronowsky U, Wenk S, Schneider D, Jager C, Rogner M (2001) Isolation of membrane protein subunits in their native state: evidence for selective binding of chlorophyll and carotenoid to the b_6 subunit of the cytochrome b_6f complex. Biochim Biophys Acta 1506:55–66

Cervera AM, Gozalbo D, McCreath KJ, Gow NA, Martinez JP, Casanova M (1998) Molecular cloning and characterization of a *Candida albicans* gene coding for cytochrome *c* haem lyase and a cell wall-related protein. Mol Microbiol 30:67–81

Chakravarti R, Aulak KS, Fox PL, Stuehr DJ (2010) GAPDH regulates cellular heme insertion into inducible nitric oxide synthase. Proc Natl Acad Sci U S A 107:18004–18009

Cho SH, Collet JF (2013) Many roles of the bacterial envelope reducing pathways. Antioxid Redox Signal 18:1690–1698

Colbert CL, Wu Q, Erbel PJ, Gardner KH, Deisenhofer J (2006) Mechanism of substrate specificity in *Bacillus subtilis* ResA, a thioredoxin-like protein involved in cytochrome *c* maturation. Proc Natl Acad Sci U S A 103:4410–4415

Corvest V, Murrey DA, Bernard DG, Knaff DB, Guiard B, Hamel PP (2010) *c*-type cytochrome assembly in *Saccharomyces cerevisiae*: a key residue for apocytochrome c_1/lyase interaction. Genetics 186:561–571

Corvest V, Murrey DA, Hirasawa M, Knaff DB, Guiard B, Hamel PP (2012) The flavoprotein Cyc2p, a mitochondrial cytochrome c assembly factor, is a NAD(P)H-dependent haem reductase. Mol Microbiol 83:968–980

Crow A, Acheson RM, Le Brun NE, Oubrie A (2004) Structural basis of Redox-coupled protein substrate selection by the cytochrome *c* biosynthesis protein ResA. J Biol Chem 279:23654–23660

Daltrop O, Ferguson SJ (2003) Cytochrome *c* maturation. The in vitro reactions of horse heart apocytochrome *c* and *Paracoccus dentrificans* apocytochrome c_{550} with heme. J Biol Chem 278:4404–4409

Daltrop O, Ferguson SJ (2004) In vitro studies on thioether bond formation between *Hydrogenobacter thermophilus* apocytochrome c_{552} with metalloprotoporphyrin derivatives. J Biol Chem 279:45347–45353

Daltrop O, Allen JW, Willis AC, Ferguson SJ (2002) In vitro formation of a *c*-type cytochrome. Proc Natl Acad Sci U S A 99:7872–7876

de Vitry C (2011) Cytochrome *c* maturation system on the negative side of bioenergetic membranes: CCB or System IV. FEBS J 278:4189–4197

de Vitry C, Desbois A, Redeker V, Zito F, Wollman FA (2004) Biochemical and spectroscopic characterization of the covalent binding of heme to cytochrome b_6. Biochemistry 43:3956–3968

Deshmukh M, Brasseur G, Daldal F (2000) Novel *Rhodobacter capsulatus* genes required for the biogenesis of various *c*-type cytochromes. Mol Microbiol 35:123–138

Deshmukh M, Turkarslan S, Astor D, Valkova-Valchanova M, Daldal F (2003) The dithiol:disulfide oxidoreductases DsbA and DsbB of *Rhodobacter capsulatus* are not directly involved in cytochrome *c* biogenesis, but their inactivation restores the cytochrome *c* biogenesis defect of *CcdA*-null mutants. J Bacteriol 185:3361–3372

Dibrova DV, Cherepanov DA, Galperin MY, Skulachev VP, Mulkidjanian AY (2013) Evolution of cytochrome *bc* complexes: from membrane-anchored dehydrogenases of ancient bacteria to triggers of apoptosis in vertebrates. Biochim Biophys Acta 1827:1407–1427

Dreyfuss BW, Merchant S (1999) *CCS5*, a new locus required for chloroplast *c*-type synthesis. In: Pusztai J, Garab G (eds) Proceedings of the XIth International Congress on Photosynthesis. Kluwer, Dordrecht, pp 3139–3142

Dreyfuss BW, Hamel PP, Nakamoto SS, Merchant S (2003) Functional analysis of a divergent system II protein, Ccs1, involved in *c*-type cytochrome biogenesis. J Biol Chem 278:2604–2613

Drygas ME, Lambowitz AM, Nargang FE (1989) Cloning and analysis of the *Neurospora crassa* gene for cytochrome *c* heme lyase. J Biol Chem 264:17897–17906

Ducluzeau AL, Chenu E, Capowiez L, Baymann F (2008) The Rieske/cytochrome *b* complex of Heliobacteria. Biochim Biophys Acta 1777:1140–1146

Dumont ME, Ernst JF, Hampsey DM, Sherman F (1987) Identification and sequence of the gene encoding cytochrome *c* heme lyase in the yeast *Saccharomyces cerevisiae*. EMBO J 6:235–241

Dumont ME, Ernst JF, Sherman F (1988) Coupling of heme attachment to import of cytochrome *c* into yeast mitochondria. Studies with heme lyase-deficient mitochondria and altered apocytochromes *c*. J Biol Chem 263:15928–15937

Dumont ME, Cardillo TS, Hayes MK, Sherman F (1991) Role of cytochrome *c* heme lyase in mitochondrial import and accumulation of cytochrome *c* in *Saccharomyces cerevisiae*. Mol Cell Biol 11:5487–5496

Dumont ME, Schlichter JB, Cardillo TS, Hayes MK, Bethlendy G, Sherman F (1993) *CYC2* encodes a factor involved in mitochondrial import of yeast cytochrome *c*. Mol Cell Biol 13:6442–6451

Eberhard S, Loiselay C, Drapier D, Bujaldon S, Girard-Bascou J, Kuras R, Choquet Y, Wollman FA (2011) Dual functions of the nucleus-encoded factor TDA1 in trapping and translation activation of *atpA* transcripts in *Chlamydomonas reinhardtii* chloroplasts. Plant J 67:1055–1066

Einsle O, Messerschmidt A, Stach P, Bourenkov GP, Bartunik HD, Huber R, Kroneck PM (1999) Structure of cytochrome c nitrite reductase. Nature 400:476–480

Erlendsson LS, Hederstedt L (2002) Mutations in the thiol-disulfide oxidoreductases BdbC and BdbD can suppress cytochrome *c* deficiency of CcdA-defective *Bacillus subtilis* cells. J Bacteriol 184:1423–1429

Erlendsson LS, Acheson RM, Hederstedt L, Le Brun NE (2003) *Bacillus subtilis* ResA is a thiol-disulfide oxidoreductase involved in cytochrome *c* synthesis. J Biol Chem 278:17852–17858

Feissner RE, Beckett CS, Loughman JA, Kranz RG (2005) Mutations in cytochrome assembly and periplasmic redox pathways in *Bordetella pertussis*. J Bacteriol 187:3941–3949

Feissner RE, Richard-Fogal CL, Frawley ER, Kranz RG (2006a) ABC transporter-mediated release of a haem chaperone allows cytochrome *c* biogenesis. Mol Microbiol 61:219–231

Feissner RE, Richard-Fogal CL, Frawley ER, Loughman JA, Earley KW, Kranz RG (2006b) Recombinant cytochromes *c* biogenesis systems I and II and analysis of haem delivery pathways in *Escherichia coli*. Mol Microbiol 60:563–577

Flegontov P, Michalek J, Janouskovec J, Lai DH, Jirku M, Hajduskova E, Tomčala A, . . . , Lukes J (2015) Divergent mitochondrial respiratory chains in phototrophic relatives of apicomplexan parasites. Mol Biol Evol 32:1115–1131

Francs-Small CC, Kroeger T, Zmudjak M, Ostersetzer-Biran O, Rahimi N, Small I, Barkan A (2011) A PORR domain protein required for *rpl2* and *ccmF*$_C$ intron splicing and for the biogenesis of *c*-type cytochromes in *Arabidopsis* mitochondria. Plant J 69:996–1005

Frawley ER, Kranz RG (2009) CcsBA is a cytochrome *c* synthetase that also functions in heme transport. Proc Natl Acad Sci U S A 106:10201–10206

Gabilly ST, Dreyfuss BW, Karamoko M, Corvest V, Kropat J, Page MD, Merchant SS, Hamel PP (2010) CCS5, a thioredoxin-like protein involved in the assembly of plastid *c*-type cytochromes. J Biol Chem 285:29738–29749

Gabilly ST, Kropat J, Karamoko M, Page MD, Nakamoto SS, Merchant SS, Hamel PP (2011) A novel component of the disulfide-reducing pathway required for cytochrome *c* assembly in plastids. Genetics 187:793–802

Ghosh A, Chawla-Sarkar M, Stuehr DJ (2011) Hsp90 interacts with inducible NO synthase client protein in its heme-free state and then drives heme insertion by an ATP-dependent process. FASEB J 25:2049–2060

Giegé P, Rayapuram N, Meyer EH, Grienenberger JM, Bonnard G (2004) CcmF$_C$ involved in cytochrome *c* maturation is present in a large sized complex in wheat mitochondria. FEBS Lett 563:165–169

Giegé P, Grienenberger JM, Bonnard G (2008) Cytochrome *c* biogenesis in mitochondria. Mitochondrion 8:61–73

Goddard AD, Stevens JM, Rondelet A, Nomerotskaia E, Allen JW, Ferguson SJ (2010) Comparing the substrate specificities of cytochrome *c* biogenesis Systems I and II: bioenergetics. FEBS J 277:726–737

Goldman BS, Beck DL, Monika EM, Kranz RG (1998) Transmembrane heme delivery systems. Proc Natl Acad Sci U S A 95:5003–5008

Graham LA, Trumpower BL (1991) Mutational analysis of the mitochondrial Rieske iron-sulfur protein of *Saccharomyces cerevisiae*. III. Import, protease processing, and assembly into the cytochrome bc_1 complex of iron-sulfur protein lacking the iron-sulfur cluster. J Biol Chem 266:22485–22492

Gubernator B, Kroliczewski J, Kallas T, Szczepaniak A (2006) Iron-sulfur cluster reconstitution of spinach chloroplast Rieske protein requires a partially prefolded apoprotein. Biochim Biophys Acta 1764:735–742

Gumpel NJ, Ralley L, Girard-Bascou J, Wollman F-A, Nugent JHA, Purton S (1995) Nuclear mutants of *Chlamydomonas reinhardtii* defective in the biogenesis of the cytochrome b_6f complex. Plant Mol Biol 29:921–932

Hamel PP, Dreyfuss BW, Xie Z, Gabilly ST, Merchant S (2003) Essential histidine and tryptophan residues in CcsA, a system II polytopic cytochrome *c* biogenesis protein. J Biol Chem 278:2593–2603

Hamel P, Corvest V, Giege P, Bonnard G (2009) Biochemical requirements for the maturation of mitochondrial *c*-type cytochromes. Biochim Biophys Acta 1793:125–138

Hamza I, Dailey HA (2012) One ring to rule them all: trafficking of heme and heme synthesis intermediates in the metazoans. Biochim Biophys Acta 1823:1617–1632

Hannibal L, Collins D, Brassard J, Chakravarti R, Vempati R, Dorlet P, Santolini J, . . . , Stuehr DJ (2012) Heme binding properties of glyceraldehyde-3-phosphate dehydrogenase. Biochemistry 51:8514–8529

Hartshorne RS, Kern M, Meyer B, Clarke TA, Karas M, Richardson DJ, Simon J (2007) A dedicated haem lyase is required for the maturation of a novel bacterial cytochrome *c* with unconventional covalent haem binding. Mol Microbiol 64:1049–1060

Hasan SS, Yamashita E, Cramer WA (2013) Transmembrane signaling and assembly of the cytochrome b6f-lipidic charge transfer complex. Biochim Biophys Acta 1827:1295–1308

Hennig B, Neupert W (1983) Biogenesis of cytochrome *c* in *Neurospora crassa*. Methods Enzymol 97:261–274

Hildenbeutel M, Hegg EL, Stephan K, Gruschke S, Meunier B, Ott M (2014) Assembly factors monitor sequential hemylation of cytochrome *b* to regulate mitochondrial translation. J Cell Biol 205:511–524

Hodson CT, Lewin A, Hederstedt L, Le Brun NE (2008) The active-site cysteinyls and hydropho-

bic cavity residues of ResA are important for cytochrome *c* maturation in *Bacillus subtilis*. J Bacteriol 190:4697–4705

Holton B, Wu X, Tsapin AI, Kramer DM, Malkin R, Kallas T (1996) Reconstitution of the 2Fe-2S center and g = 1.89 electron paramagnetic resonance signal into overproduced Nostoc sp. PCC 7906 Rieske protein. Biochemistry 35:15485–15493

Howe G, Merchant S (1992) The biosynthesis of membrane and soluble plastidic *c*-type cytochromes of *Chlamydomonas reinhardtii* is dependent on multiple common gene products. EMBO J 11:2789–2801

Howe G, Merchant S (1993) Maturation of thylakoid lumen proteins proceeds post-translationally through an intermediate in vivo. Proc Natl Acad Sci U S A 90:1862–1866

Howe G, Merchant S (1994) Role of heme in the biosynthesis of cytochrome c_6. J Biol Chem 269:5824–5832

Howe G, Mets L, Merchant S (1995) Biosynthesis of cytochrome *f* in *Chlamydomonas reinhardtii*: analysis of the pathway in gabaculine-treated cells and in the heme attachment mutant B6. Mol Gen Genet 246:156–165

Huang D, Everly RM, Cheng RH, Heymann JB, Schagger H, Sled V, Ohnishi T, ..., Cramer WA (1994) Characterization of the chloroplast cytochrome *b6f* complex as a structural and functional dimer. Biochemistry 33:4401–4409

Inoue K, Dreyfuss BW, Kindle KL, Stern DB, Merchant S, Sodeinde OA (1997) *CCS1*, a nuclear gene required for the post-translational assembly of chloroplast *c*-type cytochromes. J Biol Chem 272:31747–31754

Ishikawa M, Fujiwara M, Sonoike K, Sato N (2009) Orthogenomics of photosynthetic organisms: bioinformatic and experimental analysis of chloroplast proteins of endosymbiont origin in *Arabidopsis* and their counterparts in *Synechocystis*. Plant Cell Physiol 50:773–788

Jentzen W, Ma JG, Shelnutt JA (1998) Conservation of the conformation of the porphyrin macrocycle in hemoproteins. Biophys J 74:753–763

Joliot P, Joliot A (1988) The low-potential electron-transfer chain in the cytochrome *b/f* complex. Biochim Biophys Acta 933:319–333

Jonietz C, Forner J, Hildebrandt T, Binder S (2011) RNA PROCESSING FACTOR3 is crucial for the accumulation of mature *ccmC* transcripts in mitochondria of *Arabidopsis* accession Columbia. Plant Physiol 157:1430–1439

Jungst A, Wakabayashi S, Matsubara H, Zumft WG (1991) The nirSTBM region coding for cytochrome cd_1-dependent nitrite respiration of *Pseudomonas stutzeri* consists of a cluster of mono-, di-, and tetraheme proteins. FEBS Lett 279:205–209

Karamoko M, Cline S, Redding K, Ruiz N, Hamel PP (2011) Lumen Thiol Oxidoreductase1, a disulfide bond-forming catalyst, is required for the assembly of photosystem II in Arabidopsis. Plant Cell 23:4462–4475

Karamoko M, Gabilly ST, Hamel PP (2013) Operation of trans-thylakoid thiol-metabolizing pathways in photosynthesis. Front Plant Sci 4:476

Kern M, Eisel F, Scheithauer J, Kranz RG, Simon J (2010a) Substrate specificity of three cytochrome *c* haem lyase isoenzymes from *Wolinella succinogenes*: unconventional haem *c* binding motifs are not sufficient for haem *c* attachment by NrfI and CcsA1. Mol Microbiol 75:122–137

Kern M, Scheithauer J, Kranz RG, Simon J (2010b) Essential histidine pairs indicate conserved haem binding in epsilon proteobacterial cytochrome *c* haem lyases. Microbiology 156:3773–3781

Kleinknecht L, Wang F, Stube R, Philippar K, Nickelsen J, Bohne AV (2014) RAP, the sole octotricopeptide repeat protein in *Arabidopsis*, is required for chloroplast 16S rRNA maturation. Plant Cell 26:777–787

Korb H, Neupert W (1978) Biogenesis of cytochrome *c* in *Neurospora crassa*. Synthesis of apocytochrome *c*, transfer to mitochondria and conversion to holocytochrome *c*. Eur J Biochem 91:609–620

Koreny L, Obornik M, Lukes J (2013) Make it, take it, or leave it: heme metabolism of parasites. PLoS Pathog 9, e1003088

Kranz RG, Richard-Fogal C, Taylor J-S, Frawley ER (2009) Cytochrome *c* biogenesis: mechanisms for covalent modifications and trafficking of heme and for heme-iron redox control. Microbiol Mol Biol Rev 73:510–528

Kuras R, de Vitry C, Choquet Y, Girard-Bascou J, Culler D, Buschlen S, Merchant S, Wollman FA (1997) Molecular genetic identification of a pathway for heme binding to cytochrome b_6. J Biol Chem 272:32427–32435

Kuras R, Saint-Marcoux D, Wollman FA, de Vitry C (2007) A specific *c*-type cytochrome maturation system is required for oxygenic photosynthesis. Proc Natl Acad Sci U S A 104:9906–9910

Kurisu G, Zhang H, Smith JL, Cramer WA (2003) Structure of the cytochrome b_6f complex of oxygenic photosynthesis: tuning the cavity. Science 302:1009–1014

Kutoh E, Sone N (1988) Quinol-cytochrome *c* oxidoreductase from the thermophilic bacterium PS3. Purification and properties of a cytochrome $bc_1(b_6f)$ complex. J Biol Chem 263:9020–9026

Lang BF, Kaudewitz F (1982) Cytochrome c_1-deficient mutants in *Saccharomyces cerevisiae*. Curr Genet 6:229–235

Lavergne J (1983) Membrane potential-dependent reduction of cytochrome *b*-6 in an algal mutant lacking photosystem I centers. Biochim Biophys Acta 725:25–33

Le Brun NE, Bengtsson J, Hederstedt L (2000) Genes required for cytochrome *c* synthesis in *Bacillus subtilis*. Mol Microbiol 36:638–650

Lennartz K, Plücken H, Seidler A, Westhoff P, Bechtold N, Meierhoff K (2001) *HCF164* encodes a thioredoxin-like protein involved in the biogenesis of the cytochrome b_6f complex in *Arabidopsis*. Plant Cell 13:2539–2551

Lennartz K, Bossmann S, Westhoff P, Bechtold N, Meierhoff K (2006) HCF153, a novel nuclear-encoded factor necessary during a post-translational step in biogenesis of the cytochrome b_6f complex. Plant J 45:101–112

Lewin A, Crow A, Oubrie A, Le Brun NE (2006) Molecular basis for specificity of the extracytoplasmic thioredoxin ResA. J Biol Chem 281:35467–35477

Lewin A, Crow A, Hodson CT, Hederstedt L, Le Brun NE (2008) Effects of substitutions in the CXXC active-site motif of the extracytoplasmic thioredoxin ResA. Biochem J 414:81–91

Lezhneva L, Kuras R, Ephritikhine G, de Vitry C (2008) A novel pathway of cytochrome *c* biogenesis is involved in the assembly of the cytochrome b_6f complex in *arabidopsis* chloroplasts. J Biol Chem 283:24608–24616

Li B, Mao D, Liu Y, Li L, Kuang T (2005) Characterization of the cytochrome b_6f complex from marine green alga, *Bryopsis corticulans*. Photosynth Res 83:297–305

Li T, Bonkovsky HL, Guo JT (2011) Structural analysis of heme proteins: implications for design and prediction. BMC Struct Biol 11:13

Lu Y, Wang HR, Li H, Cui HR, Feng YG, Wang XY (2013) A chloroplast membrane protein LTO1/AtVKOR involving in redox regulation and ROS homeostasis. Plant Cell Rep 32:1427–1440

Lyska D, Paradies S, Meierhoff K, Westhoff P (2007) HCF208, a homolog of *Chlamydomonas* CCB2, is required for accumulation of native cytochrome b_6 in *Arabidopsis thaliana*. Plant Cell Physiol 48:1737–1746

Malnoë A, Wollman FA, de Vitry C, Rappaport F (2011) Photosynthetic growth despite a broken Q-cycle. Nat Commun 2:301

Malnoë A, Wang F, Girard-Bascou J, Wollman FA, de Vitry C (2014) Thylakoid FtsH protease contributes to photosystem II and cytochrome b_6f remodeling in *Chlamydomonas reinhardtii* under stress conditions. Plant Cell 26:373–390

Marx C, Wunsch C, Kuck U (2015) The octatricopeptide repeat (OPR) protein Raa8 is required for chloroplast *trans*-splicing. Eukaryot Cell 14:998–1005

Mavridou DAI, Ferguson SJ, Stevens JM (2013) Cytochrome *c* assembly. IUBMB Life 65:209–216

Mayer A, Neupert W, Lill R (1995) Translocation of apocytochrome *c* across the outer membrane of mitochondria. J Biol Chem 270:12390–12397

Merendino L, Perron K, Rahire M, Howald I, Rochaix JD, Goldschmidt-Clermont M (2006) A novel multifunctional factor involved in trans-splicing of chloroplast introns in *Chlamydomonas*. Nucleic Acids Res 34:262–274

Meyer EH, Giege P, Gelhaye E, Rayapuram N, Ahuja U, Thony-Meyer L, Grienenberger JM, Bonnard G (2005) AtCCMH, an essential component of the *c*-type cytochrome maturation pathway in *Arabidopsis* mitochondria, interacts with apocytochrome *c*. Proc Natl Acad Sci U S A 102:16113–16118

Möller MC, Hederstedt L (2008) Extracytoplasmic processes impaired by inactivation of trxA (thioredoxin gene) in Bacillus subtilis. J Bacteriol 190:4660–4665

Monika EM, Goldman BS, Beckman DL, Kranz RG (1997) A thioreduction pathway tethered to the membrane for periplasmic cytochromes *c* biogenesis; in vitro and in vivo studies. J Mol Biol 271:679–692

Moore RL, Stevens JM, Ferguson SJ (2011) Mitochondrial cytochrome *c* synthase: CP motifs are not necessary for heme attachment to apocytochrome *c*. FEBS Lett 585:3415–3419

Motohashi K, Hisabori T (2006) HCF164 receives reducing equivalents from stromal thioredoxin across the thylakoid membrane and mediates reduction of target proteins in the thylakoid lumen. J Biol Chem 281:35039–35047

Motohashi K, Hisabori T (2010) CcdA is a thylakoid membrane protein required for the transfer of reducing equivalents from stroma to thylakoid lumen in the higher plant chloroplast. Antioxid Redox Signal 13:1169–1176

Nakamoto SS, Hamel P, Merchant S (2000) Assembly of chloroplast cytochromes *b* and *c*. Biochimie 82:603–614

Nargang FE, Drygas ME, Kwong PL, Nicholson DW, Neupert W (1988) A mutant of *Neurospora crassa* deficient in cytochrome *c* heme lyase activity cannot import cytochrome *c* into mitochondria. J Biol Chem 263:9388–9394

Nawathean P, Maslov DA (2000) The absence of genes for cytochrome *c* oxidase and reductase subunits in maxicircle kinetoplast DNA of the respiration-deficient plant trypanosomatid *Phytomonas serpens*. Curr Genet 38:95–103

Nicholson DW, Neupert W (1989) Import of cytochrome *c* into mitochondria: reduction of heme, mediated by NADH and flavin nucleotides, is obligatory for its covalent linkage to apocytochrome *c*. Proc Natl Acad Sci U S A 86:4340–4344

Nicholson DW, Kohler H, Neupert W (1987) Import of cytochrome *c* into mitochondria. Cytochrome *c* heme lyase. Eur J Biochem 164:147–157

Nicholson DW, Hergersberg C, Neupert W (1988) Role of cytochrome *c* heme lyase in the import of cytochrome *c* into mitochondria. J Biol Chem 263:19034–19042

Nicholson DW, Stuart RA, Neupert W (1989) Biogenesis of cytochrome c_1. Role of cytochrome c_1 heme lyase and of the two proteolytic processing steps during import into mitochondria. J Biol Chem 264:10156–10168

Page MLD, Hamel PP, Gabilly ST, Zegzouti H, Perea JV, Alonso JM, Ecker JR, . . . , Merchant S (2004) A homolog of prokaryotic thiol disulfide transporter CcdA is required for the assembly of the cytochrome b_6f complex in *Arabidopsis* chloroplasts. J Biol Chem 279:32474–32482

Pearce DA, Cardillo TS, Sherman F (1998) Cyc2p is required for maintaining ionic stability and efficient cytochrome *c* import and mitochondrial function in *Saccharomyces cerevisiae*. FEBS Lett 439:307–311

Pierre Y, Breyton C, Lemoine Y, Robert B, Vernotte C, Popot JL (1997) On the presence and role of a molecule of chlorophyll *a* in the cytochrome b_6f complex. J Biol Chem 272:21901–21908

Pollock WB, Rosell FI, Twitchett MB, Dumont ME, Mauk AG (1998) Bacterial expression of a mitochondrial cytochrome *c*. Trimethylation of lys72 in yeast iso-1-cytochrome c and the alkaline conformational transition. Biochemistry 37:6124–6131

Priest JW, Hajduk SL (1992) Cytochrome *c* reductase purified from *Crithidia fasciculata* contains an atypical cytochrome c_1. J Biol Chem 267:20188–20195

Raczynska KD, Le Ret M, Rurek M, Bonnard G, Augustyniak H, Gualberto JM (2006) Plant mitochondrial genes can be expressed from mRNAs lacking stop codons. FEBS Lett 580:5641–5646

Rahire M, Laroche F, Cerutti L, Rochaix JD (2012) Identification of an OPR protein involved in the translation initiation of the PsaB subunit of photosystem I. Plant J 72:652–661

Rayapuram N, Hagenmuller J, Grienenberger JM, Giegé P, Bonnard G (2007) AtCCMA interacts with AtCcmB to form a novel mitochondrial ABC transporter involved in cytochrome *c* maturation in *Arabidopsis*. J Biol Chem 282:21015–21023

Rayapuram N, Hagenmuller J, Grienenberger JM, Bonnard G, Giege P (2008) The three mitochondrial encoded CcmF proteins form a complex that interacts with CCMH and *c*-type apocytochromes in *Arabidopsis*. J Biol Chem 283:25200–25208

Ren Q, Ahuja U, Thony-Meyer L (2002) A bacterial cytochrome c heme lyase. CcmF forms a complex with the heme chaperone CcmE and CcmH but not with apocytochrome c. J Biol Chem 277:7657–7663

Richard-Fogal C, Kranz RG (2010) The CcmC:heme:CcmE complex in heme trafficking and cytochrome *c* biosynthesis. J Mol Biol 401:350–362

Richard-Fogal CL, Frawley ER, Kranz RG (2008) Topology and function of CcmD in cytochrome *c* maturation. J Bacteriol 190:3489–3493

Richard-Fogal CL, Frawley ER, Bonner ER, Zhu H, San Francisco B, Kranz RG (2009) A conserved haem redox and trafficking pathway for cofactor attachment. EMBO J 28:2349–2359

Richard-Fogal CL, San Francisco B, Frawley ER, Kranz RG (2012) Thiol redox requirements and substrate specificities of recombinant cytochrome *c* assembly systems II and III. Biochim Biophys Acta 1817:911–919

Robertson DE, Farid RS, Moser CC, Urbauer JL, Mulholland SE, Pidikiti R, Lear JD, . . . , Dutton PL (1994) Design and synthesis of multi-haem proteins. Nature 368:425–432

Saint-Marcoux D, Wollman FA, de Vitry C (2009) Biogenesis of cytochrome b_6 in photosynthetic membranes. J Cell Biol 185:1195–1207

San Francisco B, Bretsnyder EC, Rodgers KR, Kranz RG (2011) Heme ligand identification and redox properties of the cytochrome *c* synthetase, CcmF. Biochemistry 50:10974–10985

San Francisco B, Bretsnyder EC, Kranz RG (2013) Human mitochondrial holocytochrome *c* synthase's heme binding, maturation determinants, and complex formation with cytochrome *c*. Proc Natl Acad Sci U S A 110:E788–E797

San Francisco B, Sutherland MC, Kranz RG (2014) The CcmFH complex is the system I holocytochrome *c* synthetase: engineering cytochrome *c* maturation independent of CcmABCDE. Mol Microbiol 91:996–1008

Sanchez NS, Pearce DA, Cardillo TS, Uribe S, Sherman F (2001) Requirements of Cyc2p and the porin, Por1p, for ionic stability and mitochondrial integrity in *Saccharomyces cerevisiae*. Arch Biochem Biophys 392:326–332

Sanders C, Lill H (2000) Expression of prokaryotic and eukaryotic cytochromes *c* in *Escherichia coli*. Biochim Biophys Acta 1459:131–138

Sanders C, Turkarslan S, Lee DW, Onder O, Kranz RG, Daldal F (2008) The cytochrome *c* maturation components CcmF, CcmH, and CcmI form a membrane-integral multisubunit heme ligation complex. J Biol Chem 283:29715–29722

Sanders C, Turkarslan S, Lee D-W, Daldal F (2010) Cytochrome *c* biogenesis: the Ccm system. Trends Microbiol 18:266–274

Schiott T, Throne-Holst M, Hederstedt L (1997a) *Bacillus subtilis* CcdA-defective mutants are blocked in a late step of cytochrome *c* biogenesis. J Bacteriol 179:4523–4529

Schiott T, von Wachenfeldt C, Hederstedt L (1997b) Identification and characterization of the *ccdA* gene, required for cytochrome *c* synthesis in *Bacillus subtilis*. J Bacteriol 179:1962–1973

Schmidt CL, Shaw L (2001) A comprehensive phylogenetic analysis of Rieske and Rieske-type iron-sulfur proteins. J Bioenerg Biomembr 33:9–26

Schulz H, Fabianek RA, Pellicioli EC, Hennecke H, Thöny-Meyer L (1999) Heme transfer to the heme chaperone CcmE during cytochrome c maturation requires the CcmC protein, which may function independently of the ABC-transporter CcmAB. Proc Natl Acad Sci U S A 96:6462–6467

Schulz H, Pellicioli EC, Thöny-Meyer L (2000) New insights into the role of CcmC, CcmD and CcmE in the haem delivery pathway during cytochrome c maturation by a complete mutational analysis of the conserved tryptophan-rich motif of CcmC. Mol Microbiol 37:1379–1388

Schwarz QP, Cox TC (2002) Complementation of a yeast *CYC3* deficiency identifies an X-linked mammalian activator of apocytochrome *c*. Genomics 79:51–57

Sherman F (1990) Studies of yeast cytochrome *c*: how and why they started and why they continued. Genetics 125:9–12

Simon J, Hederstedt L (2011) Composition and function of cytochrome *c* biogenesis System II. FEBS J 278:4179–4188

Small JL, Park SW, Kana BD, Ioerger TR, Sacchettini JC, Ehrt S (2013) Perturbation of cytochrome *c* maturation reveals adaptability of the respiratory chain in *Mycobacterium tuberculosis*. mBio 4, e00475-13

Spielewoy N, Schulz H, Grienenberger JM, Thony-Meyer L, Bonnard G (2001) CCME, a nuclear-encoded heme-binding protein involved in cytochrome *c* maturation in plant mitochondria. J Biol Chem 276:5491–5497

Stechmann A, Hamblin K, Perez-Brocal V, Gaston D, Richmond GS, van der Giezen M, Clark CG, Roger AJ (2008) Organelles in *Blastocystis* that blur the distinction between mitochondria and hydrogenosomes. Curr Biol 18:580–585

Steiner H, Zollner A, Haid A, Neupert W, Lill R (1995) Biogenesis of mitochondrial heme lyases in yeast. Import and folding in the intermembrane space. J Biol Chem 270:22842–22849

Steiner H, Kispal G, Zollner A, Haid A, Neupert W, Lill R (1996) Heme binding to a conserved Cys-Pro-Val motif is crucial for the catalytic function of mitochondrial heme lyases. J Biol Chem 271:32605–32611

Stevens JM, Zhang Y, Muthuvel G, Sam KA, Allen JW, Ferguson SJ (2011) The mitochondrial cytochrome *c* N-terminal region is critical for maturation by holocytochrome *c* synthase. FEBS Lett 585:1891–1896

Stroebel D, Choquet Y, Popot JL, Picot D (2003) An atypical haem in the cytochrome b_6f complex. Nature 426:413–418

Sun F, Wang X, Bonnard G, Shen Y, Xiu Z, Li X, Gao D, . . . , Tan BC (2015) *Empty pericarp7* encodes a mitochondrial E-subgroup pentatricopeptide repeat protein that is required for $ccmF_N$ editing, mitochondrial function and seed development in maize. Plant J 84:283–295

Thony-Meyer L (1997) Biogenesis of respiratory cytochromes in bacteria. Microbiol Mol Biol Rev 61:337–376

Thöny-Meyer L, Künzler P (1997) Translocation to the periplasm and signal sequence cleavage of preapocytochrome *c* depend on *Sec* and *Lep*, but not on the *ccm* gene products. Eur J Biochem 246:794–799

Tichy M, Vermaas W (1999) Accumulation of preapocytochrome *f* in a *Synechocystis* sp. PCC 6803 mutant impaired in cytochrome *c* maturation. J Biol Chem 274:32396–32401

Tong J, Margoliash E (1998) Cytochrome *c* heme lyase activity of yeast mitochondria. J Biol Chem 273:25695–25702

Turkarslan S, Sanders C, Ekici S, Daldal F (2008) Compensatory thio-redox interactions between DsbA, CcdA and CcmG unveil the apocytochrome *c* holdase role of CcmG during cytochrome *c* maturation. Mol Microbiol 70:652–666

Verissimo AF, Daldal F (2014) Cytochrome *c* biogenesis System I: an intricate process catalyzed by a maturase supercomplex? Biochim Biophys Acta 1837:989–998

Verissimo AF, Sanders J, Daldal F, Sanders C (2012) Engineering a prokaryotic apocytochrome *c* as an efficient substrate for *Saccharomyces cerevisiae* cytochrome *c* heme lyase. Biochem Biophys Res Commun 424:130–135

Vilmos F, Katharine AS, Stuart JF, Michael LG, James WAA (2009) Structure of a trypanosomatid mitochondrial cytochrome *c* with heme attached via only one thioether bond and implications for the substrate

recognition requirements of heme lyase. FEBS J 276:2822–2832

Visco C, Taniuchi H, Berlett BS (1985) On the specificity of cytochrome *c* synthetase in recognition of the amino acid sequence of apocytochrome *c*. J Biol Chem 260:6133–6138

Wang F, Johnson X, Cavaiuolo M, Bohne A-V, Nickelsen J, Vallon O (2015) Two *Chlamydomonas* OPR proteins stabilize chloroplast mRNAs encoding small subunits of photosystem II and cytochrome b_6f. Plant J 82:861–873

Xie Z, Merchant S (1996) The plastid-encoded *ccsA* gene is required for heme attachment to chloroplast *c*-type cytochromes. J Biol Chem 271:4632–4639

Xie Z, Culler D, Dreyfuss BW, Kuras R, Wollman FA, Girard-Bascou J, Merchant S (1998) Genetic analysis of chloroplast *c*-type cytochrome assembly in *Chlamydomonas reinhardtii*: one chloroplast locus and at least four nuclear loci are required for heme attachment. Genetics 148:681–692

Yi L, Ragsdale SW (2007) Evidence that the heme regulatory motifs in heme oxygenase-2 serve as a thiol/disulfide redox switch regulating heme binding. J Biol Chem 282:21056–21067

Yi L, Jenkins PM, Leichert LI, Jakob U, Martens JR, Ragsdale SW (2009) Heme regulatory motifs in heme xygenase-2 form a thiol/disulfide redox switch that responds to the cellular redox state. J Biol Chem 284:20556–20561

Yu J, Le Brun NE (1998) Studies of the cytochrome subunits of menaquinone:cytochrome *c* reductase (*bc* complex) of *Bacillus subtilis*. Evidence for the covalent attachment of heme to the cytochrome *b* subunit. J Biol Chem 273:8860–8866

Zara V, Conte L, Trumpower BL (2007) Identification and characterization of cytochrome bc_1 subcomplexes in mitochondria from yeast with single and double deletions of genes encoding cytochrome bc_1 subunits. FEBS J 274:4526–4539

Zhang H, Huang D, Cramer WA (1999) Stoichiometrically bound beta-carotene in the cytochrome b_6f complex of oxygenic photosynthesis protects against oxygen damage. J Biol Chem 274: 1581–1587

Zollner A, Rodel G, Haid A (1992) Molecular cloning and characterization of the *Saccharomyces cerevisiae CYT2* gene encoding cytochrome-c_1-heme lyase. Eur J Biochem 207:1093–1100