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Review

Reprint of: Biogenesis of the cytochrome bc_1 complex and role of assembly factors ☆

Pamela M. Smith, Jennifer L. Fox, Dennis R. Winge *

University of Utah Health Sciences Center, Department of Biochemistry, Salt Lake City, Utah 84132, USA
University of Utah Health Sciences Center, Department of Medicine, Salt Lake City, Utah 84132, USA

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ABSTRACT

The cytochrome bc_1 complex is an essential component of the electron transport chain in most prokaryotes and in eukaryotic mitochondria. The catalytic subunits of the complex that are responsible for its redox functions are largely conserved across kingdoms. In eukarya, the bc_1 complex contains supernumerary subunits in addition to the catalytic core, and the biogenesis of the functional bc_1 complex occurs as a modular assembly pathway. Individual steps of this biogenesis have been recently investigated and are discussed in this review with an emphasis on the assembly of the bc_1 complex in the model eukaryote *Saccharomyces cerevisiae*. Additionally, a number of assembly factors have been recently identified. Their roles in bc_1 complex biogenesis are described, with special emphasis on the maturation and topogenesis of the yeast Rieske iron–sulfur protein and its role in completing the assembly of functional bc_1 complex. This article is part of a Special Issue entitled: Biogenesis/Assembly of Respiratory Enzyme Complexes.

1. Introduction

All living organisms must produce chemical energy in the form of adenosine triphosphate (ATP) to utilize in cellular metabolism. ATP production relies on electron transport chains, which are essential cellular mechanisms capable of extracting energy from sunlight (photosynthesis) or chemical redox reactions (respiration). Prosthetic groups (flavins, iron–sulfur clusters, hemes, metal ions and quinones) located within the protein complexes of an electron transport chain act as redox carriers to terminal electron acceptors. The passage of electrons that is facilitated by these redox carriers is coupled to the translocation of protons across the membrane in which the electron transport chain resides. This process establishes a proton gradient between membrane compartments, which is used to drive ATP formation by ATP synthase.

Ubiquinol–cytochrome *c* oxidoreductase (E.C. 1.10.2.2, cytochrome bc_1 complex, Complex III) is a central component of the respiratory electron transport chain embedded in the inner membrane of mitochondria in eukaryotes. The bc_1 complex is also present in the plasma membrane of many bacteria, and its structural and functional counterpart, the cytochrome b_6f complex, is located in the thylakoid membrane of chloroplasts in plants and cyanobacteria. The protonmotive Q cycle is the definitive mechanism of energy transduction by bc_1 complexes, whereby oxidation of a membrane-localized quinol results in reduction of a diffusible *c*-type cytochrome and protons are translocated across the impermeable lipid bilayer in which the complex resides.

2. Prokaryotic bc_1 complexes

Whereas the homodimeric bc_1 complexes of eukaryotes contain up to 11 protein subunits per monomer [1–3], the bacterial versions are smaller. Prokaryotic bc_1 complexes typically consist of only the three essential catalytic subunits: cytochrome *b*, cytochrome c_1, and the Rieske iron–sulfur (Fe/S) protein, which all exhibit high sequence similarity to their mitochondrial counterparts [4]. Cytochrome *b* is an integral membrane protein that contains eight hydrophobic α-helices and two heme *b* molecules with distinct redox chemistry. Cytochrome c_1 and the Rieske Fe/S protein are structurally and topographically similar in that each protein consists of a single transmembrane α-helix attached to a hydrophilic globular domain containing the cofactor (a covalently bound heme *c* and a 2Fe–2S cluster, respectively). The globular domain of each protein is exposed to the periplasmic side of the plasma membrane, although this cofactor-containing domain comprises the N-terminus of cytochrome c_1 versus the C-terminus of the Rieske Fe/S protein.

The biogenesis of bacterial bc_1 complexes involves assembly of the catalytic subunits in addition to their individual maturation. Maturation of cofactor-containing subunits requires several deliberate steps, including cofactor delivery to the appropriate cellular compartment, preservation of the apoprotein in a conformation amenable to cofactor insertion, and ligation of the cofactor to the specific functional groups of the subunit polypeptide. In bacterial bc_1 complex biogenesis, an early step is the delivery of apo-cytochrome c_1 to the periplasm

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* Corresponding author. Tel.: +1 801 585 5103; fax: +1 801 585 3432.
E-mail address: dennis.winge@hsc.utah.edu (D.R. Winge).

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by the Sec translocon, followed by covalent attachment of heme *c* by the system I machinery [5]. Deletion of the cytochrome c_1 gene or mutagenesis of the heme-binding site of cytochrome c_1 protein results in the loss of both the cytochrome *b* and Rieske protein subunits [6,7]. Additionally, removal of the C-terminal membrane anchor in *Rhodobacter sphaeroides* resulted in detectable amounts of the soluble holo-protein in the periplasm, while the other complex subunits remained unassociated in the membrane [8]. These results suggest that insertion of heme *c* can occur independently of cytochrome c_1 membrane insertion and, significantly, that a functional holo-cytochrome c_1 is required for the assembly and association of the remaining bc_1 complex subunits.

Similarly, mutagenesis of the ligating histidine residues in cytochrome *b* to prevent its cofactor insertion results in a nonfunctional enzyme that impairs bc_1 complex assembly [9]. Since there are no known catalysts for the association between apo-cytochrome *b* and heme and because the interaction is non-covalent, it has been suggested that heme is spontaneously inserted into the apo-protein. This postulate is supported by in vitro experiments in which heme is able to bind synthetic peptides mimicking two cytochrome *b* helices [10]. Therefore, while the model for bacterial bc_1 complex assembly assumes that the *b* hemes are inserted either very soon after or concurrent with the folding of the cytochrome *b* polypeptide into the membrane, there is no experimental evidence for this prediction and no information exists regarding from which side of the plasma membrane the hemes are added to cytochrome *b*.

The cytochrome *b* and cytochrome c_1 proteins form a protease-resistant primary complex that permits a later association of the Rieske Fe/S protein [11,12]. This addition of the Rieske protein to complete the assembly of the bacterial bc_1 complex appears to be dependent on the acquisition of its 2Fe–2S cluster [13]. Mutations in the Rieske Fe/S protein to prevent its cofactor insertion have no effect on the assembly or cofactor insertion of the cytochrome *b* and c_1 subunits [13].

3. Eukaryotic bc_1 complexes

In addition to the catalytic subunits found in the prokaryotic ancestors of mitochondria, crystallographic studies of eukaryotic bc_1 complexes depict a dimeric enzyme complex composed of as many as 11 non-redundant subunits [14–16]. Structures have been determined for yeast, chicken, and bovine bc_1 complexes [14–18]. A summary of the nomenclature for equivalent subunits in mammals and fungi is given in Table 1. Much of the work discussed herein has utilized *Saccharomyces cerevisiae* as a model system due to its valuable ability to survive by fermentation in the absence of functional bc_1 complex, permitting gene deletion and mutagenesis studies. Therefore, yeast nomenclature will be used except where specified.

Table 1
Comparison of eukaryotic bc_1 complex subunit nomenclature.

Name	Mammal (bovine[a])	Fungi (*S. cerevisiae*[b])
Cor 1	SU1 (*UQCRC1*)	Qcr1 (*COR1*)
Cor 2	SU2 (*UQCRC2*)	Qcr2 (*COR2*)
Cyt *b*	SU3 (*MT-CYB*)	Qcr3 (*COB*)
Cyt *c*	SU4 (*CYC1*)	Qcr4 (*CYT1*)
Rieske Fe/S protein	SU5 (*UQCRFS1*)	Qcr5 (*RIP1*)
–	SU6 (*UQCRQ*)	Qcr7 (*QCR7*)
–	SU7 (*UQCRB*)	Qcr8 (*QCR8*)
Acidic hinge	SU8 (*UQCRH*)	Qcr6 (*QCR6*)
–	SU10 (*UQCR10*)	Qcr9 (*QCR9*)
–	SU11 (*UQCR11*)	Qcr10 (*QCR10*)
Rieske presequence	SU9 (*UQCRFS1*)	–

[a] PDB accession number 1BGY [14,18,20]. Human gene names are in italics.
[b] PDB accession number 3CX5 [17]. Gene names are listed in italics.

The catalytic core of each bc_1 monomer in eukaryotes is analogous to prokaryotic bc_1 complexes: it consists of the eight transmembrane helices of cytochrome *b* (Cob), along with cytochrome c_1 (Cyt1) and the Rieske Fe/S protein (Rip1). Cyt1 and Rip1 are anchored in the inner membrane by their single transmembrane domains, exposing their globular domains containing the heme *c* and 2Fe–2S prosthetic groups, respectively, to the intermembrane space (IMS). In addition to this catalytic core, eukaryotic bc_1 complexes contain seven or eight proteins that do not possess prosthetic groups and do not directly participate in electron transport or proton pumping (Fig. 1). These subunits consist of the two core proteins, Cor1 and Cor2, and the supernumerary subunits Qcr6–Qcr10. The yeast crystal structure does not contain Qcr10, yet Qcr10 is an authentic subunit of the complex, likely analogous to mammalian SU11, which is seen in the bovine structure [14,19]. Mammalian complexes contain an additional subunit not present in *S. cerevisiae*, SU9, which is embedded between Cor1 and Cor2 and corresponds to the presequence of the Rieske Fe/S protein [14,20]. Despite the absence of SU9, the structural similarity of mammalian and yeast bc_1 complexes makes yeast an informative paradigm for bc_1 complex assembly.

3.1. Subunit composition and maturation

3.1.1. Cytochrome b

In addition to the homology between prokaryotic and eukaryotic bc_1 complexes, another consequence of the endosymbiotic theory of mitochondrial origins is the dual genetic origin of the proteins that comprise the respiratory complexes. The respiratory chain is unique in that its assembly requires coordinated expression of subunits from both the nuclear and mitochondrial genomes. The mitochondrial genome in yeast encodes seven hydrophobic proteins that comprise the catalytic cores of the respiratory complexes and which are often the nucleating proteins for biogenesis of their respective complex. This nucleating role is true of cytochrome *b* (Cob), which is the sole bc_1 subunit encoded by the mitochondrial genome in all known sequenced eukaryotic genomes [21].

COB pre-mRNA is processed by the matrix protein Cbp2, and translation is activated by the mitochondrial proteins Cbs1 and Cbs2, which interact with the 5′ untranslated region of *COB* mRNA and the mitochondrial ribosome [22,23] (Fig. 2). Cbp6 was also initially identified as a translational activator of *COB* mRNA, but was more recently found to form a complex with the matrix assembly protein Cbp3 at the ribosomal exit tunnel, where the two proteins interact with the newly synthesized cytochrome *b*. This Cob–Cbp6–Cbp3 complex persists independently of the ribosome and recruits the additional IMS assembly factor Cbp4 [24]. The exact function of this assembly intermediate is unknown, but it presumably stabilizes the newly synthesized cytochrome *b* while it receives its two heme *b* cofactors and forms interactions with additional bc_1 subunits.

The mature Cob protein consists of 385 amino acid residues (43.6 kDa), migrates aberrantly by SDS-PAGE (32 kDa), and will aggregate upon boiling in SDS [25]. A four-helix bundle accommodates the low- and high-potential heme moieties (hemes b_L and b_H, respectively). These hemes are non-covalently bound by conserved histidine residues in the second and fourth transmembrane helices [26], with the help of conserved glycines and hydrogen bond contacts in the first and third helices that provide spatial and structural stability for ligation [27–32]. Heme b_L is located close to the IMS side of the inner membrane (IM), but it is protected from solvent by the position of Cyt1. Cob is functionally important for interaction with Rip1 and ubiquinol in catalysis (see Section 3.1.4). The heme b_H is located near the matrix surface of Cob in a solvent-filled cavity accessible from the matrix through Cor1, Cor2 and Qcr8 [16], where it can receive electrons from heme b_L, which are used to replenish the Q cycle.

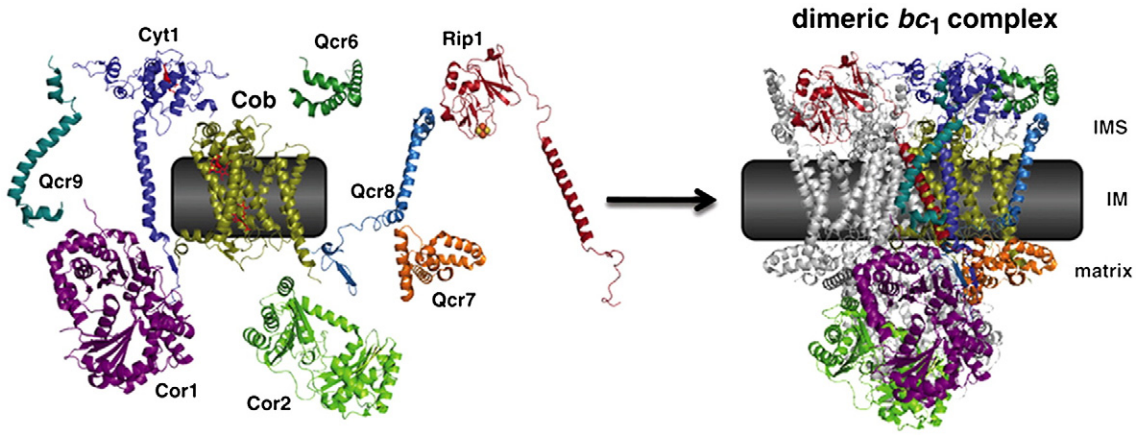

Fig. 1. Subunits of the bc_1 complex in *S. cerevisiae*. Individual subunits of one monomer are shown surrounding Cob, the integral membrane protein that initiates bc_1 complex biogenesis (left). The location of the subunits in the mature dimeric complex is shown on the right. The structure from PDB accession number 3CX5 was used to generate the figure.

3.1.2. Cor proteins 1 and 2 and Qcr7

While it is now apparent that Cob is the nucleus of the bc_1 complex, the two largest protein subunits were mistakenly identified as the central proteins of the complex and named accordingly [33]. These Cor1 and Cor2 proteins are actually located within the mitochondrial matrix, with Cor1 bound to the IM. Interestingly, the Cor proteins are highly homologous to the Zn-binding matrix processing peptidases (MPP). In yeast, they do not possess proteolytic activity due to an incomplete active site [34]; however, proteolytic activity is present in plants, where the Cor proteins serve as the matrix processing peptidase [35].

The Cor proteins are encoded by the nuclear genome and targeted to the mitochondrial matrix by a traditional mitochondrial targeting signal [36]. The mature Cor1 is slightly larger (431 residues) than Cor2 (352 residues). Cor1 shares an interface with Cob in the same monomer and anchors the matrix-localized ends of the single transmembrane helices from Cyt1, Rip1, Qcr8, and Qcr9 [17,36,37]. The Cor2 subunits of each monomer comprise an extensive dimeric interface of the bc_1 complex [1]. Cor2 is anchored to the complex only by its interaction with Cor1 in the same monomer and by an interaction in the opposing monomer with the small subunit Qcr7 (126 residues), which is the third bc_1 subunit located in the mitochondrial matrix and is situated at the interface of the matrix and IM [38].

3.1.3. Cytochrome c_1 and Qcr6

Cytochrome c_1 (Cyt1) is also nuclear-encoded, and thus must be post-translationally imported into mitochondria as an unfolded precursor polypeptide (of 309 residues). However, Cyt1 contains a bipartite targeting signal sequence to direct its intra-mitochondrial sorting [39,40]. The N-terminal portion of the targeting sequence is proteolytically removed by MPP in the matrix, resulting in an intermediate Cyt1 pre-protein. The second domain consisting of a C-terminal internal hydrophobic sorting sequence directs the insertion of Cyt1 into the IM leading to a helical hairpin structure with both termini within the matrix [40]. Following membrane insertion, the Imp2 signal peptidase catalyzes a second cleavage of Cyt1 in the IMS, resulting in a mature protein containing 248 amino acid residues and an N_{out}–C_{in} topology. Cyt1 is the only bc_1 complex subunit with this orientation, where the C-terminus is directed towards the matrix and the N-terminus is located in the IMS.

Heme c_1 cofactor attachment to this IMS-localized N-terminal domain of Cyt1 occurs prior to proteolytic processing by the inner membrane peptidase complex Imp2, as observed by positive heme staining of the intermediate Cyt1 [41]. The covalent attachment of heme *c* to apo-Cyt1 is mediated by either of the two heme lyases in the IMS, CCHL (Cyc3) and CC_1HL (Cyt2). However, no direct evidence exists that these assembly factors catalyze thioether bond formation directly. Furthermore, these lyases in general are not strictly specific for their substrates. Therefore, it remains possible that CCHL and CC_1HL are actually part of a larger complex that is required for covalent bond formation in cytochrome *c*-type proteins. In mammals, the activities of both CCHL and CC_1HL are possessed by the HCCS heme synthetase, which functions to assemble both cytochrome c_1 and the mobile IMS protein cytochrome *c* [41]. An additional protein involved in Cyt1 maturation is Cyc2, which has a proposed redox role in the CCHL pathway, but this protein is essential only when the

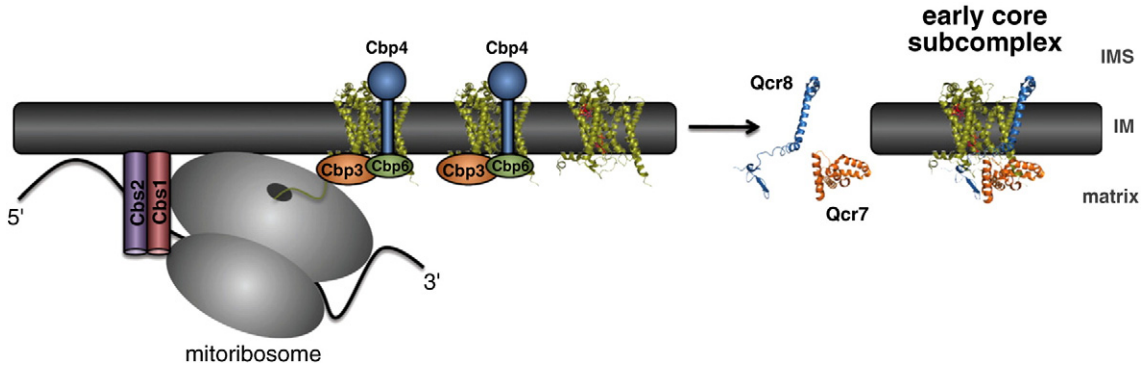

Fig. 2. Biogenesis of the bc_1 complex through formation of the early core subcomplex. Biogenesis of the bc_1 complex is initiated by translation of the mitochondrial-encoded Cob subunit (yellow). See text for details and description of individual proteins. The figure was generated using the yeast structure from PDB accession number 3CX5 and details recently described in [24].

CXXCH heme-binding motif of Cyt1 is mutated such that the redox chemistry of the cysteinyl thiols is affected [42].

The heme *c*-containing, IMS-localized domain of Cyt1 interacts with both the soluble redox carrier cytochrome *c* and the small acidic subunit Qcr6 in the IMS. The interaction between Cyt1 and cytochrome *c* is facilitated by a loop between the heme-binding CXXCH motif and the heme-bracing residues of Cyt1; the loop is also important for bc_1 complex dimerization [18]. Cyt1 interacts with Qcr6 through another long extension that is present prior to the first of its five N-terminal helices (three of which it shares with other class I cytochromes) [18], and in bovine mitochondria, SU8 (the homolog of yeast Qcr6) has been co-purified with Cyt1.

Qcr6 bears structural similarity to the twin CX_9C proteins that are IMS import substrates of the Erv1-Mia40 disulfide relay system (for review, see [43]). However, the two canonical α-helices are joined by only one disulfide bond in the yeast Qcr6. The bovine SU8 contains two disulfide linkages, but the cysteines are separated by $CX_{15}C$ and $CX_{13}C$ motifs. Qcr6 contains a cleavable presequence of 25 amino acids, but that sequence is dispensable for import and assembly into the mature bc_1 complex [44], perhaps consistent with an IMS-localized subunit where oxidative folding is a determinant of subcompartment retention. Early experiments suggested that deletion of Qcr6 impairs proteolytic maturation of Cyt1 (which in turn impairs Rip1 maturation), especially at elevated temperatures [45]. Qcr6 is a very acidic protein, and this may contribute to functional roles in preserving the heme environment of Cyt1 and promoting proper interaction with cytochrome *c* [45,46]. Consistent with this, cells lacking Qcr6 have attenuated bc_1 catalytic activity [45]. However, a bc_1 complex containing all of the other subunits can be formed in the absence of Qcr6 and this complex is competent to form supercomplexes with cytochrome oxidase [47–49]. Mutant *qcr6* cells appear to be enriched in supercomplex formation [48,49], yet the abundance of the supercomplex would be restricted to the level of processed Cyt1.

3.1.4. Rieske Fe/S protein

The Rieske-type Fe/S protein (Rip1) precursor of 215 amino acid residues bears a N-terminal mitochondrial targeting sequence, which results in full translocation to the mitochondrial matrix, despite its final location in the IM with its C-terminal catalytic center residing in the IMS [50,51]. Upon import into the matrix, MPP proteolytically cleaves 22 amino acids of the precursor protein to form an intermediate preprotein. In yeast, a second cleavage occurs after this intermediate Rip1 is inserted into the bc_1 complex with its Fe/S cluster [52–54], which removes an additional eight residues to produce a mature protein of 185 amino acid residues [55]. The purpose of this second proteolysis step is unknown, but a yeast mutant that is processed in a single step results in functional Rip1 in the bc_1 complex [56]. In mammals, the Fe/S protein (SU5) presequence is cleaved in a single step and retained as a subunit (SU9) of the mature bc_1 complex [20].

The import of Rip1 precursor protein into the mitochondrial matrix may be required because of the matrix localization of the Fe/S cluster (ISC) assembly machinery (for review, see [57,58]), which is the presumed source of the Rip1 2Fe–2S cluster. In the matrix, Fe/S clusters are assembled on scaffold proteins, ISCU (humans) or Isu1/2 (yeast), and are subsequently transferred to target apoproteins via the assistance of specific ISC chaperones [58–60]. The sulfur for cluster assembly originates from the cysteine desulfurase activity of Nfs1 and its effector protein Isd11 [61–65]. The source of iron for cluster formation is less obvious, but the small protein frataxin (Yfh1 in yeast) has been previously suggested as the iron donor [66–68]. Recombinant frataxin was also demonstrated in vitro as an allosteric activator for Fe/S cluster biogenesis via complex formation with Isu1, Nfs1, and Isd11, and the transfer of sulfur from Nfs1 to Isu2 was accelerated upon iron binding [69]. However, a recent observation that a mutation in Isu1 can bypass the requirement for frataxin in yeast [70] suggests that further studies are necessary to determine the complete mechanism of ISC biogenesis. The localization of this ISC machinery in the mitochondrial matrix and the import of apo-Rip1 into the matrix suggest that Rip1 receives its Fe/S cluster in the matrix prior to being incorporated into the bc_1 complex.

The 2Fe–2S cluster of Rip1 is coordinated by two cysteine and two histidine residues (C159, H161, C178, and H181 in yeast) within a C-terminal globular domain comprised of three antiparallel β-sheet layers located in the mitochondrial IMS [71]. In the absence of this Fe/S cluster, Rip1 is often susceptible to protease degradation, although there are examples of apo-Rip1 that can be inserted into the bc_1 complex (with the resulting complex being nonfunctional) [72–74]. The C-terminal domain also contains two additional cysteine residues (C164 and C180 in yeast) that form a disulfide bond responsible for stabilizing the Fe/S cluster [75]. This disulfide bond is proposed to form after Rip1 is installed into the bc_1 complex, as the IMS is characteristically a more oxidizing environment than the matrix [76]. Due to its essential function in poising the redox potential of the Fe/S cluster, the sequence and structure of the C-terminus are highly conserved [14,18,19]. It is connected by a short linker (7–9 amino acids) to a single membrane-spanning α-helix followed by the extreme N-terminus, which is exposed in the matrix.

These Rip1 structural elements are critical to its function in the Q cycle of the bc_1 complex. That the bc_1 complex exists as an obligate homodimer can be directly attributed to the Rip1 subunits: each subunit crosses the homodimeric interface of the complex, resulting in an interaction of its globular domain with the catalytic subunits in one monomer, while its transmembrane helix resides in the adjacent monomer. Another defining feature of Rip1 essential to the function of the bc_1 complex is the conformational flexibility of the C-terminal globular domain. This catalytic domain is able to move between cytochrome *b* and cytochrome c_1 in the electron transfer process [18,77]; for review, see [78]. This movement of the Rip1 C-terminus is dependent on the highly conserved linker domain, T^{85}ADVLA-MAK^{93}, whose three small alanine residues are especially conserved and may contribute to its enhanced mobility. Mutations that restrict the flexibility of the linker region severely affect catalytic activity of the complex, and changes to the length of the linker region also impair activity, likely by changing the distance between Rip1 and Cob for interaction at the Q binding site [79–83].

3.1.5. Small subunits

Qcr8 [84], Qcr9 [85] and Qcr10 [86] are small subunits under 100 residues with a single transmembrane domain and are located at the periphery of the complex. The three proteins have their N-termini within the matrix. Qcr8 and Qcr9 are conserved from yeast to man, whereas Qcr10 is a fungal-specific bc_1 subunit. Whereas mammalian bc_1 complexes lack Qcr10, they contain another small subunit (SU11) with a single transmembrane domain that is conserved within the animal kingdom and which may be structurally analogous to Qcr10. Qcr9 and Qcr10 are likely imported into the yeast mitochondrial inner membrane by a stop-transfer process. In contrast, Qcr8 contains three prolines and limited charged residues within its transmembrane domain and may be transferred into the matrix for re-insertion by the conservative sorting pathway [87]. Qcr8 associates with and stabilizes Cob within an early step in bc_1 biogenesis [88]. In the absence of Qcr8, no Cob assembly intermediates are seen [89]. Qcr9 is required for formation of the mature dimeric bc_1 complex [85]. Cells lacking Qcr9 form a stalled assembly intermediate with hemylated Cob and show only residual bc_1 activity. In yeast, Qcr10 appears to be only loosely associated with the bc_1 complex; this subunit is lost in the protein preparation of the yeast crystal structure and is the only subunit not essential for catalytic activity of the enzyme [86]. In yeast lacking Qcr10, the activity is decreased to 60% of wild-type [86]. In bovine, activity of bc_1 complex lacking SU11 can be restored simply by adding a mixture of phospholipids [90].

3.2. Assembly of the bc_1 complex

The additional supernumerary subunits in eukaryotic bc_1 complexes complicate the process of biogenesis compared to formation of the simple complex in prokaryotes, but the overall assembly still proceeds as a modular process. This similarity is perhaps not surprising given the bacterial ancestry of mitochondria. In the modular assembly model of the eukaryotic bc_1 complex, subunits form subcomplexes, which come together in an ordered manner to amass the functional homodimer.

Analysis of the bc_1 complex components in mitochondria purified from strains lacking genes for the different bc_1 subunits has suggested that biogenesis of the bc_1 complex occurs via a multiple-step pathway involving both subunit and non-subunit proteins in distinct complexes [88]. This model was substantiated by Drs. Zara and Trumpower with the addition of two-dimensional polyacrylamide gel electrophoretic analysis (2D-PAGE) to verify the components of each sub-complex [47,91]. In 2D-PAGE, intact protein complexes are resolved in one dimension by non-denaturing blue native polyacrylamide gel electrophoresis (BN-PAGE), followed by separation into the individual, denatured protein components in a second dimension (SDS-PAGE) [92].

3.2.1. Early core subcomplex

As previously discussed, Cob is recognized as the anchor subunit in bc_1 complex biogenesis, and its eight transmembrane helices are located in the core of the complex. Four additional transmembrane helices from Cyt1, Rip1, Qcr8, and Qcr9 surround this central core, as does the Qcr6 subunit (Fig. 1). In a *rho*^{o} strain (completely lacking mitochondrial DNA, and thus, Cob) all of the tested subunits were either diminished (Cyt1, Rip1, Cor1/2, and Qcr9) or absent altogether (Qcr6/7/8) [89]. Further analysis determined that the deletion of any of the three genes encoding Cob, Qcr7, and Qcr8 leads to the absence of the other two proteins, suggesting that Cob, Qcr7, and Qcr8 may functionally interact [88,89]. Indeed, a 230-kDa complex containing these three proteins was identified in both *cor1*Δ and *cor2*Δ deletion strains [47]. Furthermore, the extreme N-terminus of Qcr7 was independently and specifically shown to be important for association and/or stabilization of Qcr8 and Cob [93]. Thus, an initial core assembly complex contains the mitochondrial-encoded Cob associated with the two nuclear-encoded subunits Qcr7 and Qcr8 (Fig. 2).

Interestingly, in the same 230-kDa subcomplex, the Cox12 subunit of complex IV was also detected. Cox12 is required for the assembly of functional complex IV, but its continued presence is not essential for enzymatic activity following complex assembly [94]. Also, Cox12 contains a CX_9C–$CX_{10}C$ motif that is structurally similar to the twin CX_9C protein family, whose members are proposed to act as scaffolds of macromolecular structures in the IMS [95]. In this capacity, Cox12 may mediate the formation of the bc_1/complex IV supercomplex. In complex IV structures, Cox12 sits on top of the CIV core subunit Cox1 and forms a depression with neighboring Cox2 and Cox13, which could be a docking site for the cytochrome *c* redox carrier [96,97]. Perhaps Cox12 released from complex IV could interact with cytochrome *c*-associated proteins of the bc_1 complex (i.e., Cyt1 or Qcr6).

3.2.2. Cyt1/Cor protein subcomplexes

In the aforementioned *rho*^{o} strain studies, one curious observation is that Cor1, Cor2, and Cyt1 were the bc_1 subunits least affected by the absence of Cob, leading Zara and colleagues to investigate another possible subcomplex between these subunits [47]. Cor1 and Cor2 are detected in high molecular weight (400–800 kDa) complexes, which may represent aggregates and/or multimeric species and which can be extracted from the purified bc_1 complex in the presence of salt and detergent. However, no subcomplex has been detected containing Cor1, Cor2, and Cyt1; rather, it was observed that Cyt1 forms a complex with either Cor1 or Cor2 independently. Cyt1 and Cor2 associate in a small 100-kDa complex whenever the central core complex is disrupted, while Cyt1 and Cor1 form a 78-kDa complex only in the absence of Cor2 [47]. In the fully assembled bc_1 complex, Cyt1 is spatially distant from both Cor proteins and, furthermore, has only a few residues in the matrix compartment, so the biological implication for the observed subcomplexes is not obvious. One possibility is that each subcomplex is actually the association of the preproteins of one or both subunits prior to processing and insertion into the bc_1 complex. In plants, such complexes would be expected to form during Cyt1 processing because the Cor proteins serve as the mitochondrial processing peptidase [35], but the yeast Cor proteins do not possess proteolytic activity.

3.2.3. Late core subcomplex

A significant subcomplex was identified by BN-PAGE of detergent-solubilized mitochondria from a *qcr9*Δ yeast strain. This subassembly intermediate consists of the central core complex (Cob, Qcr7, and Qcr8), along with Cyt1, Cor1, and Cor2 [91]. The bc_1 complex assembly factor Bcs1 (discussed in detail in Section 3.4.2) is also associated with this subcomplex, as is Qcr6, which does not appear to be present in any earlier subcomplex. Therefore, the late core subcomplex lacks only Qcr10 and Rip1, suggesting that Qcr9 is required for the incorporation of these two subunits. In fact, mitochondria from *rip1*Δ and *qcr10*Δ strains, as well as strains lacking the non-subunit assembly factors Bcs1 and Mzm1 (addressed in Sections 3.2.4 and 3.4.2 and 3.4.3), also exhibit assembly that is stalled at the stable late core subcomplex, which in these cases contains Qcr9 [91,98]. With the exception of Rip1, the late core subcomplex amasses all of the essential bc_1 complex subunits into a single protease-resistant assembly intermediate (Fig. 3). However, since Rip1 contains the essential 2Fe–2S center that facilitates electron transfer to Cyt1, the late core complex lacking Rip1 is devoid of function.

3.2.4. Rieske Fe/S protein subcomplexes

The incorporation of Rip1 into the bc_1 complex is notable because Rip1 is the last essential subunit to be added to the complex. Prior to incorporation, precursor Rip1 is imported into the mitochondrial matrix, where it is processed and presumably receives its 2Fe–2S cluster cofactor. Unincorporated bc_1 complex subunits are subject to proteolytic degradation [99], so it is hypothesized that Rip1 may form a subcomplex with other proteins in defense of proteolysis. Alternatively, Rip1 could form subcomplexes in an effort to protect its susceptible domains (e.g., the Fe/S cluster or the hydrophobic transmembrane helix) from deleterious interactions (e.g., unwanted redox chemistry or aggregation, respectively). Qcr10 is an obvious candidate for interaction with Rip1 as the only other remaining subunit to be added to the maturing complex, but Rip1 and Qcr10 are functionally distinct in that Rip1 is a catalytic subunit and Qcr10 is a dispensable supernumerary subunit [86]. Instead, Qcr9 and Rip1 have both been observed to migrate in a complex of approximately 66 kDa in deletion strains where assembly could not proceed past the early core subcomplex (*cor1*Δ, *cor2*Δ, and *cyt1*Δ). In the *qcr9*Δ strain, Rip1 was detected only as “free” Rip1. Thus, Rip1 and Qcr9 potentially interact apart from the bc_1 complex only when neither protein can be inserted into the complex.

A second physical interaction of Rip1 prior to its incorporation in the bc_1 complex was also recently reported, involving the assembly factor Mzm1 [98]. This Rip1–Mzm1 complex migrates near 66 kDa on BN-PAGE (our unpublished data), and it is possible that Mzm1 actually represents a third protein component of the Qcr9–Rip1 complex in the matrix rather than a novel subcomplex of similar size. Qcr9 and Mzm1 may both interact with Rip1 in the matrix of mitochondria when bc_1 complex assembly is stalled; the hydrophobic transmembrane domains of Qcr9 and Rip1 would hypothetically interact, while Mzm1 interacts with the C-terminal globular domain of Rip1 (our unpublished results).

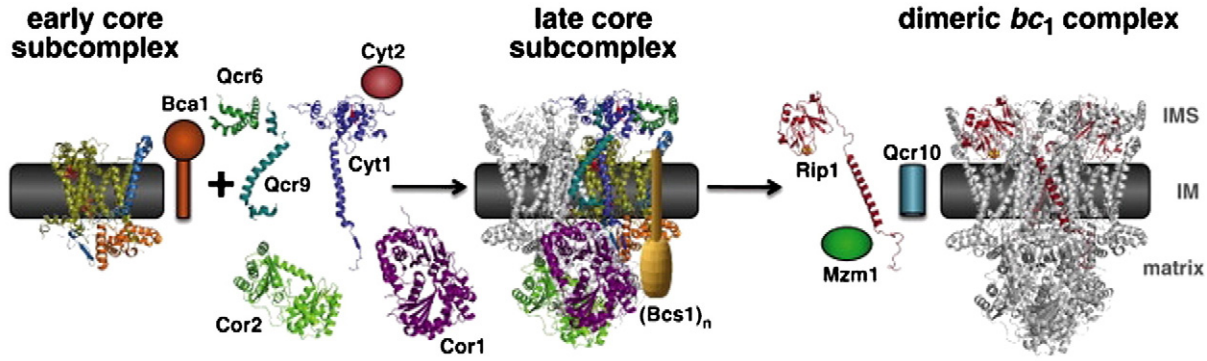

Fig. 3. Biogenesis of functional bc_1 complex. Biogenesis of the bc_1 complex proceeds from the early core subcomplex to the late core subcomplex via addition of the catalytic Cyt1 subunit and several supernumerary subunits. The subunits interact in a variety of smaller subcomplexes (see text for details) and with the assembly factors Bca1 and Cyt2. The assembly factor Bcs1 associates with the late core complex and, in conjunction with the additional assembly factor Mzm1, is essential for incorporation of the catalytic Rip1 subunit. The Qcr10 subunit is not present in the yeast crystal structure, but it is the final subunit to be inserted into the bc_1 complex. The structure from PDB accession number 3CX5 was used to generate the figure.

3.2.5. Insertion of Rip1 into the late core subcomplex

Although Qcr9 and Rip1 seem to interact when assembly of the bc_1 complex is stalled, the insertion of Qcr9 into the complex precedes and is required for the binding of Rip1. In addition, the ATP-dependent assembly factor Bcs1 is an essential requirement for Rip1 insertion [100]. Neither Qcr9 nor Bcs1 can compensate for the absence of each other, which implies that the two proteins have different roles in Rip1 insertion. The proposed roles of Bcs1 are discussed in Section 3.4.2. Qcr9 functionally interacts with Rip1 in terms of bc_1 complex stability, where the transmembrane α-helix of Rip1 contacts the transmembrane α-helix of Qcr9 in the same monomer [19,101]. Rip1 is highly labile in *qcr9*Δ cells, but it is unclear whether this instability arises from a failure of Rip1 to be incorporated into the bc_1 complex or from a weak association of Rip1 with the bc_1 complex in the absence of Qcr9.

3.3. Dimerization and supercomplex formation

An unresolved question in bc_1 complex biogenesis is when the complex dimerizes. The 500-kDa size of the late core subcomplex could represent either a monomeric bc_1 complex in association with various unidentified assembly factors or a dimeric bc_1 complex perhaps associated with monomeric Bcs1. Integration of Rip1 and Qcr10 into the late core complex triggers a size shift from 500 to 670 kDa, which is too large to be attributed to the simple addition of the Rip1 and Qcr10 subunits. However, the change in size is too small to indicate that insertion of Rip1 alone triggers dimerization of the bc_1 complex. Therefore, unresolved questions regarding Rip1 topogenesis and any role it may have in bc_1 complex dimerization remain to be investigated, including the possibility that unidentified assembly factors contribute to bc_1 complex biogenesis.

Interestingly, the catalytic mechanism described by the Q-cycle does not require that the bc_1 complex exist as a dimeric enzyme [78]. However, the Rip1 subunits of each monomer cross the homodimeric interface of the complex, resulting in its catalytic globular domain residing in one monomer and its transmembrane helix in the adjacent monomer. Furthermore, only the fully formed dimeric bc_1 complex can further associate into higher-order supercomplexes, containing complex IV in *S. cerevisiae* and both complex IV and NADH dehydrogenase (complex I) in other eukaryotes [48,102,103]. In *S. cerevisiae*, bc_1 is capable of forming three complexes: its dimeric form and supercomplexes with either one or two monomers of complex IV. Supercomplex formation is dependent on properly assembled bc_1 and complex IV, and the most recently proposed interfaces of the bovine bc_1/complex IV supercomplex include cytochrome *b*, the Rieske Fe/S protein, and SU11 (which are Cob, Rip1, and Qcr10 in yeast, respectively) of the bc_1 complex and Cox3, CoxVIa, and CoxVIIa (Cox3, Cox13, and Cox7 in yeast, respectively) of complex IV [97]. The interface subunits are predicted from modeling of cryoEM image reconstruction data. The recent single particle reconstruction differs from an earlier lower resolution study in the prediction of the bc_1/cytochrome oxidase interface [104]. The revised interface supports the dimeric structures observed for complex IV and has led to the proposal of even higher-order organization of respiratory supercomplexes into respiratory strings in higher eukaryotes [105,106].

3.4. Roles of non-subunit proteins

The formation and assembly of the respiratory chain enzymes in eukaryotes is a complicated process that requires the assistance of a multitude of additional (nuclear-encoded) proteins. A number of these non-subunit proteins have been identified as being important for the biogenesis of the individual respiratory complexes. Often, the precise function of such assembly factors is not obvious, but their roles affect the correct assembly of the subunits and/or the processing and incorporation of redox cofactors. These roles are exemplified in more than 30 assembly factors that have been identified for specific steps in the formation of complex IV (for review, see [107]). Conversely, relatively few assembly factors are currently known for bc_1 complex assembly, despite its similar level of structural complexity to complex IV, and additional factors may remain to be discovered.

Several of the known bc_1 assembly factors have already been discussed, including the Cob translational activators Cbs1 and Cbs2 and assembly factors Cbp3, Cbp4, and Cbp6 (Section 3.1.1), as well as the Cyc3 and Cyt2 heme lyases that mediate covalent attachment of heme to apo-Cyt1 (Section 3.1.3). The roles of the remaining assembly factors are discussed in this section, and all non-subunit proteins known to be involved in bc_1 complex biogenesis are summarized in Table 2.

3.4.1. Central core assembly factors

A recently identified metazoan protein with a putative role as a bc_1 assembly factor is *TTC19*, which encodes tetratricopeptide repeat (TPR) domain 19 [108]. The mature TTC19 is approximately 35 kDa and is located in the mitochondrial IM. TPR motifs are degenerate sequences of approximately 34 amino acids; proteins bearing this motif can act as scaffolds for assembly of multi-protein complexes. TTC19 has been shown to physically interact with the bc_1 complex by coimmunoprecipitation studies in wild-type mouse liver mitochondria. Furthermore, 2D-PAGE analysis of mitochondria from patients harboring a TTC19 mutation showed accumulation of a Cor1/2 subcomplex, despite low levels of the Cor1 protein, suggesting that bc_1 complex assembly may be impaired prior to formation of the

Table 2
Known bc_1 complex assembly factors.

Name	Function
Cbs1	Translational activator of *COB* mRNA
Cbs2	Translational activator of *COB* mRNA
Cbp1	Translational activator of *COB* mRNA
Cbp2	Splicing factor of *COB* pre-mRNA
Cbp3	Translational activator of *COB* mRNA; also interacts in a complex with newly synthesized Cob
Cbp4	Interacts in a complex with newly synthesized Cob
Cbp6	Translational activator of *COB* mRNA; also interacts in a complex with newly synthesized Cob
Cyt2	Heme lyase involved in maturation of cytochrome c_1
Cyc2	IMS protein involved in maturation of cytochromes *c* and c_1
Bca1	IM protein involved in early bc_1 complex biogenesis in fungi
TTC19	IM protein involved in bc_1 complex biogenesis in metazoans
Bcs1	AAA-ATPase required for Rieske Fe/S insertion into the bc_1 complex
Mzm1	Matrix protein involved in Rieske Fe/S protein insertion into the bc_1 complex

late core intermediate. Orthologs to TTC19 in other eukaryotes (plants or fungi) are not known.

Conversely, Bca1 is a recently identified bc_1 complex assembly factor found only in fungi, with no known orthologs in plants or metazoans [109]. In yeast, Bca1 is approximately 67 kDa and contains a single transmembrane domain with a large soluble C-terminal domain exposed to the IMS. This globular domain contains homology to the regulator of chromosome condensation (RCC1) family and beta-lactamase-inhibitor protein II (BLIPII) superfamily, which contain known structural motifs involved in protein-protein interactions [110]. Bca1 was reported to cause attenuated levels of Rip1 in its absence; furthermore, the additional deletion of Bca1 when bc_1 complex assembly is stalled at the late core subassembly complex (e.g., in *rip1*Δ) results in diminution of the subcomplex by approximately 60%. When Bca1 is present but bc_1 complex assembly is stalled at the late core intermediate (*rip1*Δ or *bcs1*Δ), Bca1 forms a 600-kDa complex with unknown protein partners. Therefore, Bca1 is postulated to act at an undefined step prior to Rip1 insertion. Interestingly, Bca1 was identified in a screen based on the observation that, in yeast, the expression of genes encoding translational activators and assembly factors of respiratory complex components precedes expression of the genes for the actual subunits; this approach may lead to the identification of additional bc_1 complex assembly factors.

3.4.2. Role of Bcs1 in maturation of the late core subcomplex

Bcs1 is an assembly factor that is essential for the insertion of the Rieske Fe/S protein into the bc_1 complex [100]. Bcs1 in yeast has 456 amino acid residues, which comprise three functional domains. The N-terminal domain consists of the first 126 amino acids and contains all of the sequence information for the mitochondrial targeting and stop-transfer mechanism of membrane insertion of Bcs1, although the protein lacks a typical targeting sequence at the extreme N-terminus. Rather, Bcs1 proteins are anchored in the IM by a single transmembrane segment, giving the protein an overall N_{out}–C_{in} topology. Stop-transferred proteins of this orientation contain a short N-terminal tail in the IMS with an internal single transmembrane region followed by a positively charged amphipathic helix that drives the rest of the protein into the mitochondrial matrix [111].

The Bcs1-specific domain is defined by residues 127–218 and does not contain homology to any other known protein domains. Bcs1 is functionally conserved in higher eukaryotes with no known prokaryotic ortholog. The human (BCS1L) and yeast proteins share 50% sequence identity, with most of the variability occurring in the Bcs1-specific region. Conversely, the C-terminal domain (amino acids 219–456) is highly homologous to the AAA+ (ATPases associated with various cellular activities) family, in which Bcs1-like proteins phylogenetically comprise their own AAA+ subfamily [112]. Mitochondria contain six AAA+ proteins of known function [113]: the m-AAA, i-AAA, and Lon proteases involved in mitochondrial protein quality control, Mcx1 and Hsp78, which are AAA+ proteins with classical chaperone-like functions in the mitochondrial matrix, and Bcs1. AAA+ ATPase family members bind ATP within a domain of 200–250 residues that contains conserved structural elements, including the Walker A, Walker B, and second region of homology (SRH) motifs. AAA ATPases typically form large oligomeric (often hexameric) structures with a central pore channel through which substrates are extruded in an ATP-dependent manner. Likewise, the human BCS1L forms oligomeric structures consistent with a hexameric ring of BCS1L monomers, which responds to ATP hydrolysis [114].

Bcs1 functions in the insertion of Rip1 into the bc_1 complex, which is permitted by the presence of functional Bcs1 with the late core subcomplex. Expression of wild-type Bcs1 under the control of a regulatable promoter in a *bcs1*Δ yeast strain was able to restore incorporation of Rip1 and thus bc_1 complex assembly [115]. An important implication of this experiment is that the late core subcomplex represents a true assembly intermediate, rather than a degradation product as a result of failed bc_1 biogenesis. Binding of Bcs1 to, and subsequent release from, the late core subcomplex is dependent on ATP hydrolysis [72]. Additionally, when Rip1 incorporation into the bc_1 complex is stalled, Bcs1 is observed as a component of the late core intermediate by 2D-PAGE [91].

The maturation of Rip1 involves the initial complete import into the mitochondrial matrix followed by the extrusion of the C-terminal folded domain to the IMS side of the inner membrane [51]. Rip1 is presumed to acquire its Fe/S cluster from the matrix ISC machinery prior to subsequent translocation. This translocation of the Rip1 C-terminus necessitates moving the folded, metallated domain across the inner membrane.

The translocation of a cofactor-containing Rieske Fe/S protein across an impermeable membrane has already been established in *Paracoccus denitrificans* [116]. In this species and other organisms possessing primitive bc_1 complexes, the Rieske protein is translocated from the cytoplasm to the periplasm and inserted into the plasma membrane by the Tat translocase machinery (for review see [117,118]). In contrast to the abundant Sec-type translocation machinery that only transports unfolded proteins, the Tat translocases can transport fully folded proteins across and into membranes. This system may be required because the typical bacterial Tat substrates are redox cofactor-containing proteins that belong in the periplasm but whose cofactors can only be inserted in the cytoplasm and require substantial or complete protein folding in order to accommodate the redox center.

This Tat machinery by which Fe/S-containing Rieske protein is moved amongst the prokaryotic compartments that became the matrix, IM, and IMS of eukaryotic mitochondria through its endosymbiotic origins is entirely absent in eukaryotes. Likewise, Bcs1 is absent in prokaryotes. Bcs1 performs the function of the Tat translocase in eukaryotes [119]. From the recent studies of Wagener et al. [119] Bcs1 transiently associates with Rip1 in an ATP-dependent manner during the translocation process. Rip1 attains a folded state within the matrix presumably by the formation of the 2Fe–2S center. The insertion of Rip1 into the bc_1 complex is dependent on a functional oligomeric Bcs1 ATPase, but Rip1 translocation is not dependent on the presence of the late core assembly intermediate. The C-terminal segment of Rip1 is critical for Bcs1 binding and translocation. Release of Rip1 from the Bcs1 complex is dependent on the N-terminal transmembrane segment and hydrolysis of Bcs1-bound ATP.

Details of the mechanism of Bcs1-mediated translocation of Rip1 remain to be elucidated. Structural alignments of Bcs1 with crystallographic structures of known AAA+ ATPases suggest that a series of residues corresponding to the channel pore formed by the hexameric ring are absent in the Bcs1 protein. An increase in the pore diameter may facilitate the passage of the folded, [2Fe–2S]-containing Rip1 through a Bcs1 oligomer.

3.4.3. Role of Mzm1 in maturation of the late core subcomplex

Mzm1 is a 14-kDa protein that is directed to the mitochondrial matrix but lacks a cleavable targeting sequence. The N-terminus of Mzm1 contains a "LYR" motif named for three conserved amino acid residues characteristic of a protein family that may be associated with Fe/S proteins. Isd11 and Sdh6 are two other proteins in the LYR protein family; the former is involved in Fe/S cluster biosynthesis, and the latter is proposed to be involved in maturation of Sdh2, a Fe/S-containing protein. Mutation of the conserved tyrosine residue in the LYR motif of Mzm1 renders the protein non-functional. Previously, Mzm1 was shown to be important for the maintenance of the bioavailable zinc pool known to exist in mitochondria [120]. However, mitochondrial zinc deficiency has been revealed to be a general phenotype in the absence of functional bc_1 complex, rather than a unique phenotype of *mzm1*Δ cells (our unpublished results).

Recent work has instead established Mzm1 as an assembly factor for the bc_1 complex in yeast, and conservation of Mzm1 in metazoans suggests that it may have a conserved role in higher eukaryotes [98]. Mzm1, like Bcs1, functions in the mitochondrial matrix during a late step in bc_1 complex assembly involving the insertion of Rip1. Cells lacking Mzm1 exhibit a modest bc_1 defect at 30 °C, and the defect is exacerbated at elevated temperatures. The defect is attributed to Rip1, as steady-state protein levels are decreased at 30 °C, and markedly attenuated at elevated temperatures in *mzm1*Δ cells. Respiratory growth at 37 °C is restored by overexpression of Rip1. Conversely, overexpression of Mzm1 in cells where bc_1 complex assembly is stalled at the late core subcomplex (e.g., *bcs1*Δ) is able to stabilize Rip1, where it would otherwise be susceptible to proteolytic degradation (our unpublished results). Therefore, Mzm1 may function to stabilize Rip1 prior to IM insertion. Alternatively, since Mzm1 interacts with Rip1 just prior to IM insertion, Mzm1 may aid in the presentation of Rip1 to Bcs1 for IM extrusion.

3.5. bc_1 complex deficiencies in human disease

3.5.1. Mutations in bc_1 complex subunits

Deficiencies in the bc_1 complex are relatively rare and are most commonly associated with mutations in the mitochondrial DNA encoding cytochrome *b*; upwards of 27 different mutations have been identified in the *COB* gene. Mutations in the mitochondrial genome are difficult to reproduce in model organisms, but a novel technique to introduce mutations into *COB* mitochondrial DNA in yeast has been recently established. This technique involves replacing the mitochondrial *COB* gene with the *ARG8* gene that is normally encoded by nuclear DNA and results in arginine auxotrophy. Then mutated versions of *COB* are re-introduced to the locus and identified by loss of auxotrophy [121]. This technique should allow for a thorough investigation of the specific phenotypes associated with the numerous disease-causing mutations in this protein. Clinically, the symptoms associated with *COB* mutations are variable and span a wide range of neuromuscular disorders and myopathies. Some patients have isolated complex III deficiencies, whereas other patients have combined complex I and III deficiencies, presumably due to the critical role of respirasome formation in the stability of complex I [122]. The only known patient mutations in nuclear genes encoding structural subunits of the bc_1 complex occur in the genes for SU7 (*UQCRB*, Qcr8 in yeast) [123] and SU6 (*UQCRQ*, Qcr7 in yeast) [124]. The patient in the former case exhibited isolated complex III deficiency and hepatic dysfunction with recurrent hypoglycemia and lactic acidosis. In the latter case, the patient displayed severe neurologic impairment.

3.5.2. Mutations in bc_1 complex assembly factors

Mutations in two human bc_1 assembly factors BCS1L (Bcs1 in yeast) and TTC19 (no yeast ortholog) have been reported in infants with variable clinical presentation and severity. However, the molecular defect in most bc_1-deficient individuals remains to be identified, suggesting that additional assembly factors with deleterious mutations remain to be discovered that may contribute to our understanding of bc_1 complex deficiencies.

Human mutations in the protein TTC19 were recently identified in patients with marked bc_1 deficiency, with no other known mutations in bc_1 complex subunits or BCS1L [108]. Interestingly, a delayed clinical presentation was observed associated with TTC19 mutations. A slowly progressive neurological disorder normally follows onset in late infancy, but can also manifest by rapid onset later in life, as was observed in one patient with no clinical symptoms until age 42. Studies using *Drosophila melanogaster* corroborated observations in human patients, where the homologous gene knockout is associated with severe neurological abnormalities.

More than 20 pathogenic mutations have been attributed to all three domains of *BCS1L* [114,125], and mutations to the different domains likely have variable effects. For example, mutations to the N-terminus may affect import and membrane topogenesis of BCS1L, while mutations to the C-terminal regions may impair the ATPase activity of BCS1L. Different *BCS1L* mutations are associated with variable clinical pathologies, tissue specificities, and progression of disease, which encompass three main clinical syndromes (reviewed in [126] and elsewhere). The first is simply referred to as Complex III deficiency, which is characterized by progressive encephalopathies resulting in hypotonia and developmental delays. Neurological phenotypes can present alone or with the involvement of other organ impairment (e.g., liver and kidney). Bjornstad syndrome is the least severe BCS1L-associated disease and is defined as an autosomal recessive disorder characterized by neurosensory hearing loss and *pili torti* (brittle hair). GRACILE syndrome is the most common and unfortunately, most severe BCS1L-associated disorder. GRACILE syndrome was first identified in patients of Finnish descent and is characterized by fetal growth restriction, aminoaciduria, cholestasis, iron overload in the liver, lactic acidosis, and early death. A homozygous $Ser^{78}Gly$ exchange is the single causative mutation of GRACILE syndrome [127]. In cases of Complex III deficiency and Bjornstad syndrome, the bc_1 complex defect can usually be directly attributed to an assembly defect of the Rieske Fe/S protein subunit. However, in patients with GRACILE syndrome, while total BCS1L protein and Rieske protein in the bc_1 complex are low, only liver and hepatic tissues show decreased bc_1 complex activity [128]. In addition, the cause of iron overload in GRACILE patients is unknown. BCS1L has also been reported to interact with the mitochondrial protein LETM1, which is an IM protein important for maintaining the tubular morphology of mitochondria and maintaining cell viability. Mutations in the LETM1 complex cause another physical and neurological developmental disorder, Wolf–Hirschhorn syndrome [129]. Together, these phenotypes may suggest that BCS1L impacts additional functions in mitochondria distinct from its role in bc_1 complex assembly, and it remains to be determined whether BCS1L has a role in iron homeostasis and/or mitochondrial morphology. Recently, a mouse model of the GRACILE mutation was developed, representing the first viable mouse model of bc_1 complex deficiency and the first nuclear transgenic model of a human mitochondrial disease, which will allow further investigation into the disease mechanism and explanation of unresolved phenotypes [125].

4. Concluding remarks and future outlook

The last several years have afforded new insights and techniques for studying biogenesis of the bc_1 complex. However, several important questions remain to be investigated. Cofactor insertion is assumed to occur prior to subunit incorporation into the bc_1 complex, but nothing is known about the mechanism by which Cob receives its heme cofactors or the involvement of any additional proteins in this maturation. Similarly, it is assumed that Rip1 receives its Fe/S

cluster in the matrix, but the location of this maturation has not been definitively shown, nor have Rip1-specific matrix chaperone proteins been identified for Fe/S cluster insertion. Furthermore, details of the mechanism by which Bcs1 mediates Rip1 translocation remain of great interest. The use of yeast as a model system to study bc_1 complex biogenesis has been extremely valuable to identify novel assembly factors and gain a better overall understanding of bc_1 complex assembly in mammalian orthologs. The emergence of mammalian models based on these studies is evidence of the continued progress in understanding bc_1 complex mitochondrial disease in humans.

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