

Published in final edited form as:

FEBS Lett. 2012 March 9; 586(5): 617–621. doi:10.1016/j.febslet.2011.08.032.

Recent Advances in Cytochrome *bc*₁: Inter monomer Electronic Communication?

Bahia Khalfaoui-Hassani¹, Pascal Lanciano¹, Dong-Woo Lee², Elisabeth Darrouzet³, and Fevzi Daldal^{1,*}

¹University of Pennsylvania, Department of Biology, Philadelphia, PA 19104 USA

²Industrial Biotechnology and Bioenergy Research Center, Korea Research Institute of Bioscience & Biotechnology, Daejeon 305-806, Korea

³CEA, iBEB, SBTN, TIRO, Centre de Marcoule, Bât 170, BP17171, 30207 Bagnols sur Cèze, France

Abstract

The ubihydroquinone: cytochrome *c* oxidoreductase, or cytochrome *bc*₁, is a central component of photosynthetic and respiratory energy transduction pathways in many organisms. It contributes to the generation of membrane potential and proton gradient used for cellular energy production (ATP). The three-dimensional structures of cytochrome *bc*₁ indicate that its two monomers are intertwined to form a symmetrical homodimer. This unusual architecture raises the issue of whether the monomers operate independently, or function cooperatively during the catalytic cycle of the enzyme. In this review, recent progress achieved in our understanding of the mechanism of function of dimeric cytochrome *bc*₁ is presented. New genetic approaches producing heterodimeric enzymes, and emerging insights related to the inter monomer electron transfer between the heme *b* cofactors of cytochrome *bc*₁ are described.

Keywords

cytochrome *bc*₁; heterodimer; inter monomer electron transfer; Q cycle mechanism; *Rhodobacter capsulatus*

Introduction

The ubihydroquinone: cytochrome *c* oxidoreductase (cytochrome *bc*₁ or complex III) is a multi-subunit membrane bound enzyme central to photosynthetic and respiratory electron transport chains of many organisms, including bacteria, archaea, eukaryotic mitochondria and chloroplasts [1–3]. This enzyme catalyzes oxidation of hydroquinone (QH₂) to quinone (Q) and transfers the resulting electrons, usually via *c*-type cytochrome, to the reaction centers in photosynthetic and cytochrome *c* oxidases in aerobic respiratory growth conditions. It couples the free energy released by these reactions to the translocation of

© 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

*To whom correspondence should be addressed: Tel: (215)898-4394., Fax: (215)898-8780., fdaldal@sas.upenn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

electrons and protons across membrane to contribute to the formation of proton motive force used for ATP synthesis [4].

The static three dimensional (3D) structures of cytochrome bc_1 indicate that it is a symmetrical homodimer [5–9] with at least three catalytic subunits: the Rieske Fe/S protein with a high potential [2Fe2S] cluster, the cytochrome b with two b -type (one low potential (b_L) and one high potential (b_H)) hemes, and the cytochrome c_1 with a c -type heme [10] (Fig. 1). Additional subunits in variable numbers are also present in some species [6–8,11]. The Q-cycle model is generally used to describe the mechanism of function of cytochrome bc_1 [4,12,13]. Accordingly, two QH_2 molecules are oxidized at a QH_2 (Q_o) site on the positive side, and one Q molecule is reduced at a Q reduction (Q_i) site on the negative side, of the membrane. Each QH_2 oxidation is initiated by the oxidized Fe/S protein, which accepts one electron and transfers it to cytochrome c_1 via a large-scale movement of its cluster bearing extrinsic domain [14,15]. This electron is then transferred from cytochrome c_1 to other downstream electron carriers and terminal acceptors. The second electron from QH_2 oxidation is conveyed to the low potential hemes b_L and b_H , and then to a Q forming a stable semiquinone (SQ) at the (Q_i) site (Fig. 1). Following a second turnover of a Q_o site the SQ at the Q_i site is converted to QH_2 and released from the enzyme, completing the catalytic cycle [12]. This model accounts well for the electron transfer pathways between the cofactors of a monomeric cytochrome bc_1 . However, it does not describe whether the two consecutive QH_2 oxidations, which are required to complete a full catalytic cycle, take place at the Q_o site of a given monomer or at the two distinct Q_o sites of the dimeric enzyme. The 3D structures of cytochrome bc_1 revealed that its cofactors, except the mobile [2Fe2S] clusters, are distributed symmetrically within the dimeric enzyme. Moreover, the distances separating the hemes b_L – b_H in a given monomer, and the hemes b_L – b_L between the two monomers are quasi-identical (Fig. 1). These findings raised a number of intriguing questions (*e.g.*, see [16,17]), including the independent or co-ordinated functions of cytochrome bc_1 monomers. The architecture of cytochrome bc_1 might play a functional role in addition to the structural stability of the enzyme if the two consecutive QH_2 oxidations alternate between the two Q_o sites of the dimeric enzyme, or if inter monomer electronic communication occurs between the heme cofactors. A number of hypothetical models, including “activated Q cycle” [18], “alternating Q cycle” [19] or “heterodimeric Q cycle” [20], invoking different structural and functional interactions between the Q_o and Q_i sites within one monomer, or between both monomers, of cytochrome bc_1 have been described. Initially, peculiar transient kinetics data were interpreted as indication of a dimeric Q cycle mechanism for QH_2 oxidation [18] despite other possible explanations. In recent years, more convincing experimental approaches [21–23] and theoretical calculations [24] were reported. In this review, we survey recent studies on inter monomer electronic communication within cytochrome bc_1 . We describe the different genetic systems used to produce asymmetric cytochrome bc_1 heterodimers, and critically assess the outcomes of these efforts in understanding the mechanism of function of dimeric cytochrome bc_1 .

Recent approaches to probe inter monomer electronic communication in cytochrome bc_1

Half of the sites reactivity

Early on, Trumpower and colleagues described that one molecule of stigmatellin (a Q_o site inhibitor) per dimer was sufficient to completely inhibit cytochrome bc_1 [25], and that when yeast cytochrome bc_1 is inhibited by antimycin A (a Q_i site inhibitor) the amount of cytochrome c_1 reduced corresponded to half of that present in cytochrome bc_1 [21]. These studies assumed that equilibrium between the two hemes b_H is attained via electron transfer between the hemes b_L to support the “half of the sites reactivity” model for yeast [26] and

bacterial [27] cytochrome bc_1 . More recently, a *Paracoccus denitrificans* strain that produces a quasi-wild type and a Q_o site defective mutant variants of cytochrome bc_1 , tagged with His- and Strep-epitopes, respectively, (see below “two-plasmids system” for details) was described [28]. The quasi-wild type form of cytochrome bc_1 used in this study lacked the amino terminal acidic part of cytochrome c_1 and produced a dimeric enzyme, which otherwise forms a tetramer in its native state. From this strain, homodimeric (with two wild type Q_o , or two mutant Q_o sites) and heterodimeric (with a wild type Q_o site in one monomer, and a mutant Q_o site in the other monomer) cytochrome bc_1 were purified by affinity chromatography with appropriate epitope-tags. Purified homodimeric wild type and heterodimeric mutant cytochromes bc_1 exhibited comparable extents of cytochrome c_1 and cytochrome b_H reductions and enzyme activities, but antimycin-mediated stimulation was observed only with the homodimeric wild type enzyme. These findings suggested that only one of the two Q_o sites of the wild type homodimer, or the mutant heterodimer is active, and that inter monomer electron transfer occurs in cytochrome bc_1 [28]. These interesting findings are consistent with the alternating Q cycle model [19], although they do not prove directly the occurrence of either an inactive Q_o site in wild type, or inter monomer electron transfer between the hemes b_L in the heterodimeric mutant cytochrome bc_1 . In addition, this *P. denitrificans* mutant provides no information about the physiological ability of a heterodimeric cytochrome bc_1 to support cellular growth.

One-plasmid system

More recently, a different genetic system to probe inter monomer electron transfer in dimeric cytochrome bc_1 was reported by Osyczka and collaborators [29]. They adapted a previously described *Rhodobacter capsulatus* genetic system [30] to carry an ingeniously modified *petABC* (structural genes of cytochrome bc_1) operon. Two copies of *petB* (encoding cytochrome b) were connected to each other via a designed linker sequence to form a fused *petB-B* gene on a single plasmid, to produce a ‘cytochrome $b-b$ ’ subunit, which is tagged with Strep-epitope at its carboxyl end [29] (Fig. 2A). This “one-plasmid” system was considered to produce a non-native but fully active dimeric “cytochrome $b-bc_1$ ” in which the two monomers are covalently linked to each other via a linker between two cytochromes b (Fig. 1A). The system was used to generate single and double mutations on either or both cytochrome b copies to yield homodimeric and heterodimeric cytochrome $b-bc_1$ variants with active or inactive Q_o and Q_i sites. The cytochrome b G158W substitution that inhibits QH_2 oxidation at the Q_o site without affecting the other cofactors [30,31], and the H212N substitution that eliminates heme b_H [16,22] were used to inactivate one or both monomers to produce heterodimers (Figs. 1A and 2A). Using appropriate cytochrome $b-bc_1$ mutants and measuring their activities, the authors concluded that electrons move freely between the hemes b_L and distribute without regulation among the hemes b_H using an H-shaped electron transfer path (a “bus bar”) within a dimeric cytochrome $b-bc_1$ [29].

Our ongoing experiments indicate that the *R. capsulatus* one-plasmid system with cytochrome $b-b$ duplications, constructed as reported in [29], exhibit extreme genetic instability ($\sim >10^{-2}$) and does not confer normal photosynthetic growth to *R. capsulatus* cytochrome bc_1 -minus mutant. Even under respiratory conditions, where growth can be maintained via a cytochrome bc_1 independent branch (*i.e.*, via an alternate hydroquinone oxidase), selective pressure against intra-molecular direct cytochrome b repeats appears to be strong. Plasmids seem to rearrange immediately to discard one of the cytochrome b copies to yield wild type-like homodimeric cytochrome bc_1 . We also observe that high frequency intra-molecular rearrangements similar to those described above (*petAB-BC*) (Fig. 2A) occur when the entire *petABC* operon is duplicated as either direct (*petABC-ABC*) or inverted (*petABC-CBA*) repeats on a single plasmid. These strains also exhibit poor photosynthetic growth and high frequency of intra-molecular rearrangements, to quickly

generate uncontrollable mixtures of homo and heterodimeric cytochromes bc_1 (manuscript *in preparation*). Until we understand and control the occurrence of frequent intra-molecular rearrangements, these undesirable features restrict severely the use of the one-plasmid system for studies of heterodimeric cytochromes bc_1 .

Two plasmids system

A genetic system, similar to that used in [28], for studying inter monomer electron transfer in dimeric cytochromes bc_1 was initiated about a decade ago by our group, and reported recently [32]. This system also derives from earlier described *R. capsulatus* genetic system [30] and harbors two different plasmids (marked with different antibiotic resistance, Kan^R and Tet^R). Each of the plasmids has a single copy of *petABC* operon where each *petB* is carboxyl-terminally tagged with a different (Strep and Flag) epitope [32] (Fig. 2B). The cytochrome bc_1 subunits being produced and assorted independently in cells, the “two-plasmids” system yields two sets of homodimeric cytochrome bc_1 with the same epitope at their monomers (Strep-Strep or Flag-Flag) together with a set of heterodimeric cytochrome bc_1 with a different tag at each monomer (Flag-Strep) (Fig. 1B). Unlike the one-plasmid system, the two-plasmids system carrying wild type *petABC* operons confers quasi-wild type like photosynthetic growth to *R. capsulatus* cytochrome bc_1 -minus mutants. Inter-molecular genetic rearrangements among the two plasmids appear to be less frequent ($\sim < 10^{-4}$) than intra-molecular events seen with the one-plasmid system and intra-molecular duplications. With two-plasmids system, use of different replicons of different plasmid copy numbers changes steady-state amounts of homodimeric and heterodimeric cytochrome bc_1 variants in cells. In addition, the two-plasmid system is not restricted to heteromerization of cytochrome b only as it can yield heterodimers with different combinations of all cytochrome bc_1 subunits. However, extensive purification is required to isolate the desired heterodimeric cytochrome bc_1 variant among its multiple combinations produced.

This two-plasmids genetic system was used to probe inter monomer electronic communication between the hemes b_L of cytochrome bc_1 , by selective inactivation of one Q_o site of one monomer and one Q_i site of the other monomer in a heterodimeric construct (Fig. 1B and 2B). Based on trials and errors, the previously characterized cytochrome b Y147A and H212N substitutions for inactivating the Q_o and Q_i sites, respectively were chosen. The former mutation affects drastically the rate of electron transfer from QH_2 oxidation to heme b_L at the Q_o site [33], whereas the latter causes loss of heme b_H [16,22], eliminating SQ formation at the Q_i site. Strains that produced only homodimeric mutant cytochromes bc_1 (*i. e.*, carrying cytochrome b Y147A or H212N mutations on both plasmids) were unable to grow in photosynthesis and produced inactive enzymes. In contrast, strains producing heterodimeric cytochrome bc_1 (*i.e.*, carrying cytochrome b Y147A and H212N mutations on two different plasmids) exhibited slow photosynthetic growth compared with a wild type strain and had cytochrome bc_1 activity [32]. Thus, electron transfer between the hemes b_L can interconnect across the enzyme the active Q_o site on one monomer to the active Q_i site on the other monomer of the heterodimeric cytochrome bc_1 to generate enough enzyme activity to support photosynthetic growth. Detailed, molecular genetic, biochemical and biophysical studies of cells with two-plasmids revealed semi-quantitatively that inter molecular heme b_L - b_L - b_H electron transfer occurs more slowly than intra-monomer heme b_L - b_H electron transfer, but is sufficient to sustain slow photosynthetic growth. Precise determination of inter molecular hemes b_L - b_L electron transfer rates is complicated due to electronic coupling between the photosynthetic reaction centers and cytochromes bc_1 in membranes. Vigorous photosynthetic growth was observed when higher amounts of heterodimeric cytochromes bc_1 were produced [32] (*manuscript in preparation*), documenting the physiological relevance of the two-plasmids system with respect to the efficiency and amount of a heterodimeric cytochrome bc_1 .

Summary and future studies

Genetic approaches that were initiated in recent years are now probing functional roles of the dimeric architecture of cytochrome bc_1 [28,29,32]. Experiments are revealing, with varying degrees of rigor and reliability, the occurrence of slow but appreciable inter monomer electron transfer between the hemes b_L , at least in mutant heterodimeric cytochrome bc_1 enzymes that are forced to support photosynthetic growth [32]. Further studies of electronic communication between the hemes b_L , especially in a wild type cytochrome bc_1 , is of paramount importance to our understanding of the mechanism of function of a dimeric enzyme, but this requires additional work. First, currently available heterodimeric cytochrome bc_1 production systems need improvements. Choosing appropriate mutations and respiration-deficient backgrounds might assess the physiological capability of *P. denitrificans* heterodimeric mutant cytochrome bc_1 enzyme reported in [28]. Inactivating RecA enzyme, which is known to reduce the frequency of homologous recombination in many organisms [34], might reduce molecular rearrangements to increase genetic stability of the one-plasmid system. Using different kinds of epitope tag or different Q_o and Q_i site mutations might improve the stability of purified active heterodimeric cytochromes bc_1 produced by the two-plasmids *R. capsulatus* [32] (*manuscript in preparation*).

Second, inter monomer electronic communication *per se* deserves further studies. Direct determinations of inter monomer hemes b electron transfer rates might be achieved using photo-activatable ruthenium cytochrome c derivatives [35]. The role of aromatic residues, like F195 and Y199 of cytochrome b , located between the hemes b_L at the interface of the monomers in the dimeric cytochrome bc_1 with respect to inter monomer electron transfer [36,37] need reexamination. A two-plasmids system producing appropriate heterodimeric enzymes with an inactive Q_o site in one monomer and an inactive Q_i site in the other monomer, together with selected cytochrome b F195 or Y199 substitutions, might probe better the role of these residues on inter monomer electronic communication in dimeric cytochrome bc_1 . Moreover, availability of a reliable heterodimeric cytochrome bc_1 with one fully active and one fully inactive monomer generated via the two-plasmids system might probe if intra-monomer hemes b_L - b_H electron transfer alone can support photosynthetic growth without any hemes b_L - b_L electronic coupling. Comparing the amounts of non-productive bypass reactions (*i.e.*, ROS production) generated by an appropriate heterodimeric cytochrome bc_1 having only one active monomer with those produced by a wild type homodimeric enzyme with two active monomers, might be eye opening in respect to physiological regulation of cytochrome bc_1 . Could steady state inter monomer electronic communications within a dimeric cytochrome bc_1 minimize unproductive by-pass reactions? Could it play a protective role to minimize ROS mediated oxidative damages under specific physiological conditions, like high membrane potential or limited oxygen availability, rendering hemes b_L partially reduced [24]?

Third, time is ripe to start using heterodimeric cytochromes bc_1 to probe intra and inter monomer Q_o - Q_i sites interactions in particular for initiation and regulation of the first *versus* the second QH_2 oxidation during cytochrome bc_1 catalytic cycle. Recent structural rationalization [38] of the heterodimeric Q cycle model [20] begs experimental testing. Inspection of cytochrome b structure indicates that its “rotating” E helix (providing all necessary ligand interactions at the Q_i site) and its *ef* loop extension on the Q_o site might be tethered to the more “static” D helix upon SQ formation at the Q_i site. Faster electron transfer rates between hemes b_L - b_H as compared to those between hemes b_L - b_L - b_H [32] might favor intra, instead of inter, monomer SQ formation at the cognate Q_i sites of cytochrome bc_1 monomers. Consequently, following the first QH_2 oxidation conformation of the *ef* loop could change upon SQ production on the other side of the membrane, and

inhibit access of oxidized Fe-S protein to the cognate Q_o site on the same monomer [20,38]. After the second QH_2 oxidation, only dismutation of the SQ molecules via inter monomer electronic communication would then complete the catalytic cycle to produce Q and QH_2 , and relax the SQ-tethered Q_i sites, to render the cognate Q_o sites active for the next turnover of the enzyme. Production of appropriate heterodimeric cytochrome bc_1 variants should be invaluable to assess the validity, if any, of various aspects of speculative models [20].

In summary, the establishment of a dimeric architecture for cytochrome bc_1 raised in recent years interesting questions aimed at complementing the Q-cycle scheme generally used to describe the mechanism of function of a monomeric enzyme. In particular, do the monomers operate independently or function cooperatively? Do the two consecutive QH_2 oxidations required to complete the catalytic cycle occur in the same or different monomers? Does electronic communication occur between the hemes b cofactors of cytochrome bc_1 ? If so, is there any selective or beneficial advantage? These and other related outstanding questions awaiting answers enticed the development of new genetic approaches to produce novel heterodimeric cytochromes bc_1 . Ongoing studies of these variant enzymes are progressing on our understanding of the structure-function relationship of dimeric cytochrome bc_1 . Hopefully, future studies will better define whether or not the mechanism of function of a dimeric cytochrome bc_1 invokes intra and inter monomer cooperation and electronic communications.

Acknowledgments

This work was supported by grants from NIH (GM 38237) and DOE (91ER 20052) to F. D.

Abbreviations

Q	quinone
SQ	semiquinone
QH_2	hydroquinone

References

1. Darrouzet E, Moser CC, Dutton PL, Daldal F. Large scale domain movement in cytochrome bc_1 : a new device for electron transfer in proteins. *Trends Biochem Sci.* 2001; 26:445–51. [PubMed: 11440857]
2. Osyczka A, Moser CC, Dutton PL. Fixing the Q cycle. *Trends Biochem Sci.* 2005; 30:176–82. [PubMed: 15817393]
3. Cape JL, Bowman MK, Kramer DM. Understanding the cytochrome bc complexes by what they don't do. The Q-cycle at 30. *Trends Plant Sci.* 2006; 11:46–55. [PubMed: 16352458]
4. Mitchell P. Possible molecular mechanisms of the protonmotive function of cytochrome systems. *J Theor Biol.* 1976; 62:327–67. [PubMed: 186667]
5. Xia D, Yu CA, Kim H, Xia JZ, Kachurin AM, Zhang L, Yu L, Deisenhofer J. Crystal structure of the cytochrome bc_1 complex from bovine heart mitochondria. *Science.* 1997; 277:60–6. [PubMed: 9204897]
6. Iwata S, et al. Complete structure of the 11-subunit bovine mitochondrial cytochrome bc_1 complex. *Science.* 1998; 281:64–71. [PubMed: 9651245]
7. Zhang Z, et al. Electron transfer by domain movement in cytochrome bc_1 . *Nature.* 1998; 392:677–84. [PubMed: 9565029]
8. Hunte C, Koepke J, Lange C, Rossmannith T, Michel H. Structure at 2.3 Å resolution of the cytochrome bc_1 complex from the yeast *Saccharomyces cerevisiae* co-crystallized with an antibody Fv fragment. *Structure.* 2000; 8:669–84. [PubMed: 10873857]

9. Berry EA, Huang LS, Saechao LK, Pon NG, Valkova-Valchanova M, Daldal F. X-Ray Structure of *Rhodobacter capsulatus* Cytochrome *bc*₁: Comparison with its Mitochondrial and Chloroplast Counterparts. *Photosynth Res.* 2004; 81:251–75. [PubMed: 16034531]
10. Berry, EALD-W.; Huang, L-S.; Daldal, F. *The Purple Phototrophic Bacteria*. Springer; Berlin: 2009. Structural and mutational studies of the cytochrome *bc*₁ complex.
11. Esser L, Elberry M, Zhou F, Yu CA, Yu L, Xia D. Inhibitor-complexed structures of the cytochrome *bc*₁ from the photosynthetic bacterium *Rhodobacter sphaeroides*. *J Biol Chem.* 2008; 283:2846–57. [PubMed: 18039651]
12. Crofts AR, Meinhardt SW, Jones KR, Snozzi M. The role of the quinone pool in the cyclic electron-transfer chain of *Rhodospseudomonas sphaeroides*: a modified Q-cycle mechanism. *Biochim Biophys Acta.* 1983; 723:202–218. [PubMed: 21494412]
13. Trumpower BL. The protonmotive Q cycle. Energy transduction by coupling of proton translocation to electron transfer by the cytochrome *bc*₁ complex. *J Biol Chem.* 1990; 265:11409–12. [PubMed: 2164001]
14. Darrouzet E, Valkova-Valchanova M, Moser CC, Dutton PL, Daldal F. Uncovering the [2Fe2S] domain movement in cytochrome *bc*₁ and its implications for energy conversion. *Proc Natl Acad Sci U S A.* 2000; 97:4567–72. [PubMed: 10781061]
15. Darrouzet E, Daldal F. Movement of the iron-sulfur subunit beyond the ef loop of cytochrome *b* is required for multiple turnovers of the *bc*₁ complex but not for single turnover Q_o site catalysis. *J Biol Chem.* 2002; 277:3471–6. [PubMed: 11707449]
16. Osyczka A, Moser CC, Daldal F, Dutton PL. Reversible redox energy coupling in electron transfer chains. *Nature.* 2004; 427:607–12. [PubMed: 14961113]
17. Cramer WA, Hasan SS, Yamashita E. The Q cycle of cytochrome *bc* complexes: a structure perspective. *Biochim Biophys Acta.* 2011; 1807:788–802. [PubMed: 21352799]
18. Gupta OA, Feniouk BA, Junge W, Mulkidjanian AY. The cytochrome *bc*₁ complex of *Rhodobacter capsulatus*: ubiquinol oxidation in a dimeric Q-cycle? *FEBS Lett.* 1998; 431:291–6. [PubMed: 9708922]
19. Trumpower BL. A concerted, alternating sites mechanism of ubiquinol oxidation by the dimeric cytochrome *bc*₁ complex. *Biochim Biophys Acta.* 2002; 1555:166–73. [PubMed: 12206910]
20. Cooley JW, Lee DW, Daldal F. Across membrane communication between the Q_o and Q_i active sites of cytochrome *bc*₁. *Biochemistry.* 2009; 48:1888–99. [PubMed: 19254042]
21. Covian R, Gutierrez-Cirlos EB, Trumpower BL. Anti-cooperative oxidation of ubiquinol by the yeast cytochrome *bc*₁ complex. *J Biol Chem.* 2004; 279:15040–9. [PubMed: 14761953]
22. Cooley JW, Ohnishi T, Daldal F. Binding dynamics at the quinone reduction (Q_i) site influence the equilibrium interactions of the iron sulfur protein and hydroquinone oxidation (Q_o) site of the cytochrome *bc*₁ complex. *Biochemistry.* 2005; 44:10520–32. [PubMed: 16060661]
23. Covian R, Trumpower BL. Rapid electron transfer between monomers when the cytochrome *bc*₁ complex dimer is reduced through center N. *J Biol Chem.* 2005; 280:22732–40. [PubMed: 15833742]
24. Shinkarev VP, Wraight CA. Intermonomer electron transfer in the *bc*₁ complex dimer is controlled by the energized state and by impaired electron transfer between low and high potential hemes. *FEBS Lett.* 2007; 581:1535–41. [PubMed: 17399709]
25. Gutierrez-Cirlos EB, Trumpower BL. Inhibitory analogs of ubiquinol act anti-cooperatively on the Yeast cytochrome *bc*₁ complex. Evidence for an alternating, half-of-the-sites mechanism of ubiquinol oxidation. *J Biol Chem.* 2002; 277:1195–202. [PubMed: 11700316]
26. Covian R, Trumpower BL. Regulatory interactions between ubiquinol oxidation and ubiquinone reduction sites in the dimeric cytochrome *bc*₁ complex. *J Biol Chem.* 2006; 281:30925–32. [PubMed: 16908520]
27. Covian R, Kleinschroth T, Ludwig B, Trumpower BL. Asymmetric binding of stigmatellin to the dimeric *Paracoccus denitrificans* *bc*₁ complex: evidence for anti-cooperative ubiquinol oxidation and communication between center P ubiquinol oxidation sites. *J Biol Chem.* 2007; 282:22289–97. [PubMed: 17561507]
28. Castellani M, Covian R, Kleinschroth T, Anderka O, Ludwig B, Trumpower BL. Direct demonstration of half-of-the-sites reactivity in the dimeric cytochrome *bc*₁ complex: enzyme with

- one inactive monomer is fully active but unable to activate the second ubiquinol oxidation site in response to ligand binding at the ubiquinone reduction site. *J Biol Chem.* 2010; 285:502–10. [PubMed: 19892700]
29. Swierczek M, Cieluch E, Sarewicz M, Borek A, Moser CC, Dutton PL, Osyczka A. An electronic bus bar lies in the core of cytochrome *bc*₁. *Science.* 2010; 329:451–4. [PubMed: 20651150]
 30. Atta-Asafo-Adjei E, Daldal F. Size of the amino acid side chain at position 158 of cytochrome *b* is critical for an active cytochrome *bc*₁ complex and for photosynthetic growth of *Rhodobacter capsulatus*. *Proc Natl Acad Sci U S A.* 1991; 88:492–6. [PubMed: 1846443]
 31. Ding H, Moser CC, Robertson DE, Tokito MK, Daldal F, Dutton PL. Ubiquinone pair in the Q_o site central to the primary energy conversion reactions of cytochrome *bc*₁ complex. *Biochemistry.* 1995; 34:15979–96. [PubMed: 8519754]
 32. Lanciano P, Lee DW, Yang H, Darrouzet E, Daldal F. Intermonomer electron transfer between the low-potential *b* hemes of cytochrome *bc*₁. *Biochemistry.* 2011; 50:1651–63. [PubMed: 21261281]
 33. Saribas AS, Ding H, Dutton PL, Daldal F. Tyrosine 147 of cytochrome *b* is required for efficient electron transfer at the ubihydroquinone oxidase site (Q_o) of the cytochrome *bc*₁ complex. *Biochemistry.* 1995; 34:16004–12. [PubMed: 8519756]
 34. Cox MM. The bacterial RecA protein as a motor protein. *Annu Rev Microbiol.* 2003; 57:551–77. [PubMed: 14527291]
 35. Millett F, Durham B. Use of ruthenium photooxidation techniques to study electron transfer in the cytochrome *bc*₁ complex. *Methods Enzymol.* 2009; 456:95–109. [PubMed: 19348884]
 36. Gong X, Yu L, Xia D, Yu CA. Evidence for electron equilibrium between the two hemes *b*_L in the dimeric cytochrome *bc*₁ complex. *J Biol Chem.* 2005; 280:9251–7. [PubMed: 15615714]
 37. Crofts AR, et al. The Q-cycle reviewed: How well does a monomeric mechanism of the *bc*₁ complex account for the function of a dimeric complex? *Biochim Biophys Acta.* 2008; 1777:1001–19. [PubMed: 18501698]
 38. Cooley JW. A structural model for across membrane coupling between the Q_o and Q_i active sites of cytochrome *bc*₁. *Biochim Biophys Acta.* 2010; 1797:1842–8. [PubMed: 20513347]

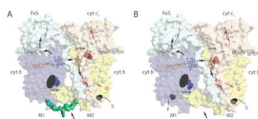


Fig. 1. Structure and function of heterodimeric cytochromes bc_1

A. A hypothetical structural model for a non-native heterodimeric cytochrome $b-bc_1$. The 3D structure of cytochrome bc_1 (PDB entry 1ZRT) was modified to include the Q_i site H212N mutation (blue) on one monomer (M1) and the Q_o site G158W mutation (red) on the other (M2) to depict a heterodimeric enzyme produced by the one-plasmid system [29]. The carboxyl end of one cytochrome b (blue) is linked to the amino end of the other (yellow) with a peptide (green) indicated by an arrow to create cytochrome $b-b$ that is not naturally present in cytochrome bc_1 . S refers to the Strep-tag on the carboxyl terminal end of cytochrome $b-b$. The Fe/S and cytochrome c_1 subunits are shown in light blue and light brown, with the cofactors ([2Fe2S] and hemes b_H , b_L and c_1) in monomer M1 in blue and those in M2 in red. The black arrows indicate the electron transfer pathways across the monomers, Q and QH_2 correspond to quinone and hydroquinone, respectively. The black ellipsoid indicates the absence of heme b_H on monomer M1. **B.** A hypothetical structural model for a native-like heterodimeric cytochrome bc_1 . The 3D structure of cytochrome bc_1 (PDB entry 1ZRT) was modified to include the Q_i site H212N mutation (blue) on one monomer (M1) and the Q_o site Y147A mutation (red) on the other (M2) to depict a heterodimeric enzyme produced by the two-plasmids system [32]. The labels are as in A, and note the absence (indicated by an arrow) of the peptide linking together the cytochromes b , and the presence of Flag- (F) and Strep (S)-tags on the carboxyl terminals of two cytochrome b on monomers M1 and M2, respectively.

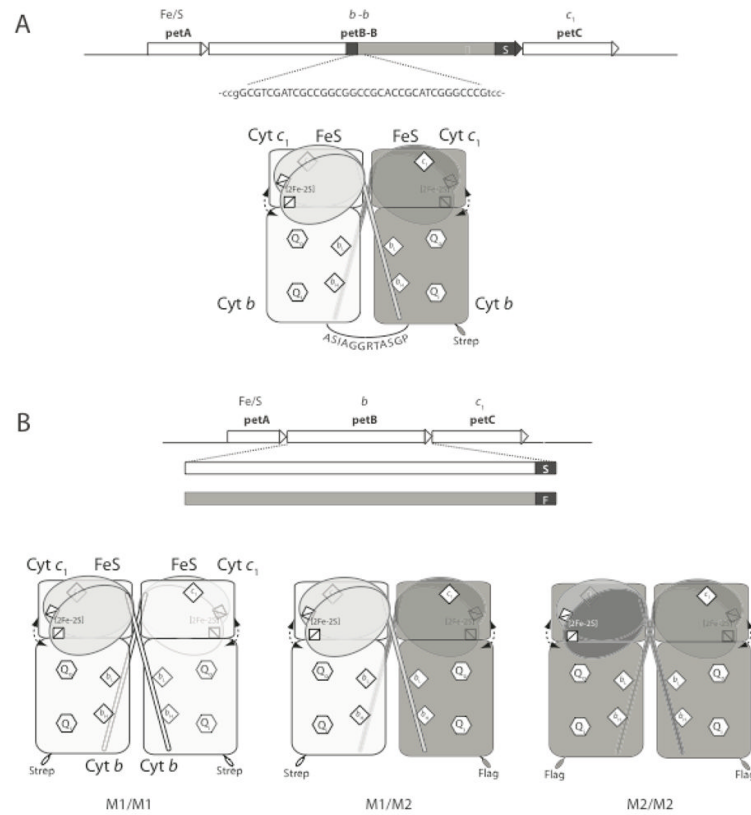


Fig. 2. Genetic systems producing heterodimeric cytochromes bc_1

A. One-plasmid system is genetically less stable possibly due to high frequency intra-molecular rearrangements between *petB* genes (encoding cytochrome *b*) duplications carried by the plasmid, but produces only heterodimeric cytochromes bc_1 [29]. *petB-B* encodes cytochrome *b-b*, formed by linking two cytochrome *b* copies by a dodecameric peptide. *petA* and *petC* refer to the structural genes of Fe/S protein and cytochrome c_1 , respectively. S indicates the Strep-tag epitope added to the carboxyl end of the second copy of cytochrome *b*. **B.** Two-plasmids system is genetically more stable, as only one copy of *petABC* (structural genes encoding cytochrome bc_1) is carried by each plasmid. Various subunits of cytochrome bc_1 are produced and re-assorted independently, leading the cells harboring this system to produce a combination of homodimeric (M1/M1 and M2/M2) and heterodimeric (M1/M2) cytochrome bc_1 that are differentially tagged by the Flag (F) and Strep (S) epitopes, [32], as shown. Apparently no high frequency inter-molecular rearrangements are observed in this system, but the heterodimeric cytochromes bc_1 thus produced need to be separated from homodimeric variants by affinity chromatography via appropriate epitope tags.