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Introduction

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The cytochrome *bc* complexes catalyze electron transfer from a reduced quinol to cytochrome *c*, or an alternative electron acceptor, in the respiratory chains of mitochondria and bacteria, as well as in the photosynthetic electron transfer chains of plants and bacteria. These bc_1 complexes, or *bf* complexes as they are called in plants, catalyze the oxidation of ubiquinol (or plastoquinol) with the transfer of electrons to cytochrome *c* (or plastocyanine or cytochrome c_2) with the concomitant generation of an electrogenic proton gradient across the membrane. The electrochemical gradient is then subsequently used for the generation of ATP through the F_0/F_1 ATPase located in the membranes of all these energy-transducing organelles. These multi-protein *bc* enzyme complexes contain three different redox proteins including cytochrome *b*, a single polypeptide containing two *b* hemes with different properties, cytochrome c_1 (or cytochrome *f* in chloroplasts), and an iron–sulfur protein with a 2Fe–2S cluster.

Several articles in this review series are focused on understanding the central role of cytochrome *b* (or cytochrome b_6) in electron transport and proton translocation in the *bc* complexes from various organisms. Cytochrome *b* is a hydrophobic protein containing eight membrane-spanning alpha helices (four in the *bf* complex) and thus appears to be the central anchor for the other protein subunits which constitute the complex. In addition, several articles address structural studies of the iron–sulfur protein which along with cytochrome *b* is involved in the initial step of quinol oxidation in all of the *bc* complexes. Chemical modifications of the bc_1 complex with inhibitors of proton translocation as well as photoaffinity labels of quinone derivatives have also provided useful information about the structure of the proteins constituting the *bc* complexes and their interactions.

The function of the *bc* complexes in electron transfer and concomitant proton translocation across the membrane can best be described by the modified Q cycle, originally introduced by Mitchell (1976) and reviewed by Trumpower (1990). The Q cycle proposes that the reduced quinol is oxidized at a site near the positive side of the membrane, the Q_o site, with the transfer of one electron to the iron–sulfur protein (Fig. 1). Two protons are released from the membrane coupled with the formation of an unstable semiquinone. The reduced iron–sulfur cluster subsequently transfers an electron to cytochrome c_1, while the semiquinone is rapidly oxidized by the low-potential cytochrome *b* heme (b_L) which with the iron–sulfur cluster constitutes the Q_o site. The electron present on b_L is then transferred to the high-potential cytochrome *b* heme (b_H) localized on the opposite side of the membrane, thus contributing to the formation of the membrane potential. The cytochrome b_L is then oxidized by a quinone at the Q_i site to form a stable semiquinone which is reduced to a quinol by a second electron coming from an additional turnover of the cycle.

The central prediction of the Q cycle is the presence in the *bc* complexes of two distinct quinone binding sites: the Q_o site, where oxidation of quinol occurs, and the Q_i site where reduction of quinone occurs (Fig. 1). Moreover, the Q cycle predicts that these two quinone sites should be localized on opposite sides of the membrane, the positive (P) and the negative (N) sides, respectively. The topographical orientation of cytochrome *b* in the membrane as depicted on the cover of this journal indicates the presence of four conserved histidines in cytochrome *b* isolated from every species. The two pairs of histidines which bind the *b* hemes are localized on opposite

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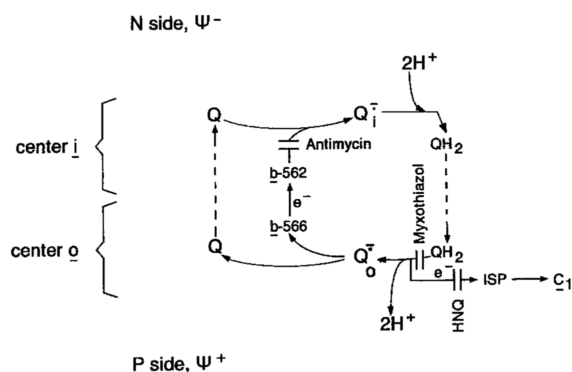

Fig. 1. A model of the Q cycle for electron transfer through the bc_1 complex. Q_o is the quinol oxidizing site and Q_i is the quinone reducing site. The sites of inhibitor action are depicted.

sides of the membrane, thus lending support to the suggestion of two distinct active sites in the complex. Moreover, two classes of inhibitors which interact with cytochrome *b* have provided evidence for the Q cycle mechanism. One set of inhibitors, exemplified by myxothiazol, binds to cytochrome b_L and inhibits the oxidation of quinol at the Q_o site, while the second set of inhibitors, exemplified by antimycin, binds to cytochrome b_H and blocks the reduction of quinone at the Q_i site (Fig. 1).

The first article in this issue by Gennis *et al.* provides a review of studies on the bc_1 complexes of *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. The bc_1 complexes in these organisms have a much simpler polypeptide composition than the mitochondrial bc_1 complexes, which facilitates structural studies of the complex. Moreover, these bacteria can be easily modified by site-directed mutagenesis. A systematic approach using site-directed mutagenesis of amino acids conserved in all species coupled with antibiotic-resistant mutants obtained spontaneously is currently providing important information about the amino acids which constitute both the Q_o and Q_i sites on cytochrome *b*. Certain residues appear not to be critical for function of the Q_o, quinol oxidizing site, but are important for maximum efficiencies of electron transfer. In similar studies with yeast, Colson's article provides an important historical background to the initial isolation of antibiotic-resistant mutants, the mapping of these mutants to the split cytochrome *b* gene, and finally the sequencing of these mutations such that the amino acid substitutions could be determined. These studies provided the initial critical data necessitating the revision of the topographical model for cytochrome *b* to an eight-helix model from the originally proposed nine-helix model. The eight-helix model placed all of the mutations involving the Q_o site inhibitors on one side of the membrane and the mutations affecting the Q_i site on the opposite side of the membrane as depicted on the cover of this journal. Current studies in Colson's laboratory involve the isolation and characterization of revertants which correct respiratory-deficient mutants. Many of these revertants arise from changes in amino acids which sometimes map in distant localizations on cytochrome *b*. This genetic approach has been very fruitful in the further elucidation of the Q cycle mechanisms and has provided additional information about the amino acids involved in both the Q_o and Q_i sites.

The structure of the quinol oxidizing site is also the focus of the article by Link *et al.* These workers have employed inhibitor-binding studies using both fluorescence and kinetic measurements to learn more about the structure of the Q_o site. Kinetic studies coupled with the binding parameters for the various inhibitors on both cytochrome *b* and the iron–sulfur protein have led to a detailed model for the quinol oxidizing site which is consistent with the changes in amino acid residues observed in the inhibitor-resistant mutants. The structure of the quinol oxidizing site and its relationship to the concomitant movement of protons is further explored in the article by Beattie in which the carboxyl-modifying reagent, DCCD, has been used to study the bc_1 and *bf* complexes. DCCD blocks proton translocation in both these complexes without affecting electron transfer by binding to an aspartate acid residue present in a hydrophobic, but extra-membranous, alpha helix of cytochrome *b* and cytochrome b_6.

The article by Graham *et al.* explores the iron–sulfur protein of the bc_1 complex and especially the mechanism by which this protein is inserted into the Q_o site of the complex. Structural studies of various mutants in this protein have provided evidence for the cysteine and histidine residues involved in binding the 2Fe–2S cluster to the protein and established the presence of flexible domains in the protein. These flexible domains form the boundaries for three domains which provide a hydrophobic shield around the iron–sulfur cluster. Further analyses of the mutants in the regions of the protein surrounding the iron–sulfur cluster will help in our understanding of the role the iron–sulfur protein plays in forming the quinol oxidizing site, Q_o, in conjunction with the heme of cytochrome *b*.

The site of quinol oxidation has also been explored by Yu and Yu in their elegant studies with photoaffinity labeled derivatives of quinone. Many of the necessary structural features of these molecules have been determined in these studies. Of great interest are the crystals of the bovine heart mitochondrial bc_1 complex displayed in this article. The crystalline complex contains the ten subunits of this complex and retains enzymatic activity if reconstituted with phospholipids and ubiquinone. The possibilities of obtaining X-ray analyses of the bc_1 complex are exciting for a resolution of this enzyme.

In conclusion, the six articles in this issue each provide a different approach to the study of the cytochrome *bc* complexes. It is clear, however, that the results obtained are all consistent with the models for the complex including the Q cycle to describe the pathway of electron transport from ubiquinol to cytochrome *c*. Continued studies of mutants of cytochrome *b* as well as the other subunits including cytochrome c_1, the iron-sulfur protein, and the proteins lacking a redox centre, will provide additional information about the structure and function of the enzyme complex. Only cursory mention has been made in these articles of the experimental approaches to the study of the associations between different subunits making up the complex or how these are assembled during biogenesis of the complex. As new experimental approaches are developed, additional information about the structure, assembly, and enzymatic activity, including proton translocation of the *bc* complex, will be provided and, undoubtedly, new questions will arise.

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