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Introduction: Study of the Cytochrome bc_1 Complex Entering a New Phase

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The cytochrome bc_1 complex (ubiquinol-cytochrome *c* reductase) is an essential segment of the energy-conserving electron transfer chains of mitochondria and many respiratory and photosynthetic bacteria. These enzymes catalyze oxidation of quinol and reduction of *c*-type cytochromes with concomitant generation of a proton gradient and membrane potential for ATP synthesis by the F_1F_0-ATP synthase complex. A structurally and functionally similar complex, the cytochrome b_6f complex, participates in the photosynthetic electron transfer chain of chloroplasts, algae, and certain photosynthetic bacteria. The cytochrome bc_1 complex contains three to eleven protein subunits, depending on the source. The essential redox components of the complex are: two *b*-type cytochromes (b_L and b_H), one *c*-type cytochrome (c_1), one high-potential iron–sulfur cluster (2Fe–2S), and a ubiquinone.

Study of the mitochondrial cytochrome bc_1 complex has been intensive since its discovery in the early 1960s. The presence of "colored" redox prosthetic groups and the availability of various inhibitors have facilitated spectrophotometric studies of the electron transfer sequence in this complex. Bacterial complexes with simpler subunit composition and well-developed molecular genetic systems provide good models for structure–function studies of the mitochondrial complex. The recent success in crystallization of mitochondrial bc_1 complex in various forms and the determination of the atomic structure at about 3 A resolution, took the structure–function studies of cytochrome bc_1 to yet another level (Yu *et al.*, 1996). The first three articles, by Iwata *et al.*, Berry *et. al.*, and my group, are devoted to structural analyses of various crystal forms of mitochondrial bc_1 complex in native or inhibitor-loaded complexes. The article of Cramer *et al.* extends structural work to the cytochrome b_6f complex.

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Although the structural data of mitochondrial bc_1 complex are generally in line with data obtained or predicted from biochemical or molecular genetic studies, there have been two surprises. First, is the mobile nature of the extramembrane domain of the iron–sulfur protein, as indicated by the varying position of the [2Fe–2S] cluster in different crystal forms and by observation that the position of [2Fe–2S] is affected by Q_o site inhibitors. This structural revelation has lead to a hypothesis that movement of the extramembranous domain of the iron–sulfur protein is involved in the catalytic cycle of the complex. Several articles in this special issue provide different points of view on this subject. Investigation of the role of iron–sulfur protein in assembly of the yeast complex, of Beattie and coworkers, has lead to the same conclusion. A second surprise is that the bc_1 complex exists as a structural and functional dimer. Structural evidences for a dimeric complex are: (i) the short distance between the two b_L in the two symmetric monomers; (ii) the intertwine of iron-sulfur protein between the two symmetric monomers; and (iii) the presence of two noncommunicating cavities in the dimeric complex, each connecting the Q_o pocket of one monomer to the Q_i pocket of the other.

Although the current atomic structure information for mitochondrial bc_1 complex is far from complete, it is certainly sufficient to guide the selection of amino acid residues for mutagenesis in bacterial or yeast bc_1 complexes in functional and mechanistic investigations. The article of Daldal *et al.* provides an excellent summary of mutants of bacterial bc_1 complexes. Current structural information accommodates well the Q-cycle mechanism (Trumpower, 1990) and the idea of iron–sulfur protein domain movement is compatible with a catalytic switch hypothesis (Brandt and von Jagow, 1991). The key features of the Q-cycle mechanism are the bifurcation of quinol oxidation at the Q_o site and quinone reduction at the Q_i site. Articles of

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Dutton *et al.*, Trumpower *et al.*, and Brandt deal with the reaction mechanism at the Q_o site in the context of the atomic structure of the complex. Although different models are proposed, each has its own merits and supporting data. A true reaction mechanism for bifurcation of quinol oxidation at the Q_o site must await the availability of high-resolution structural data and experimental evidence from approaches such as structure-guided site-directed mutagenesis and kinetic analyses. Also, one should bear in mind, that the bc_1 structure determined by X-ray crystallography represents a stable conformation of this complex under a specific condition; this conformation may or may not be the functional one. The functional significance of the structure in the area with less defined electron density should be considered with caution.

Among the eleven subunits of the mitochondrial bc_1 complex, only three bear redox prosthetic groups and are present in the bacterial complex. The remaining subunits, referred to as supernumerary subunits, are believed to play structural and regulatory roles without participating directly in catalysis. The article of Yu *et al.* provides evidence for the structural role of the only supernumerary subunit in *Rhodobacter sphaeroides* bc_1 complex. Recently, a mitochondrial-processing peptidase activity was found to be associated with the core subunits of the plant mitochondrial bc_1 complex. A similar peptidase activity was activated from the bovine heart mitochondrial complex under specific conditions. Glaser *et al.* brings us up to date in this interesting area. In addition to electron transfer, proton translocation, and peptidase activities, the mitochondrial bc_1 complex possesses a potential-regulated superoxide-generating activity. A schematic expression of the interrelationship between the Q cycle and superoxide cycle is given in the article from our laboratory.

As the study of bc_1 enters a mature phase, high-resolution structural data are urgently needed. The details of quinol binding at the Q_o site and of the proton translocation path are key areas that require better structure. Unfortunately, progress here has been slower than expected. It has become apparent that success in obtaining bc_1 crystals that diffract X-rays at high resolution requires more than just fine-tuning of reported conditions. A drastic change in enzyme preparation and/or the use of an uncommon source may be worth exploring.

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