FEBS 27213

FEBS Letters 545 (2003) 39–46

Minireview

Protonmotive pathways and mechanisms in the cytochrome bc_1 complex

Carola Hunte[a,*], Hildur Palsdottir[a], Bernard L. Trumpower[b,*]

[a] *Department Molecular Membrane Biology, Max-Planck-Institute Biophysics, D-60439 Frankfurt am Main, Germany*
[b] *Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, USA*

Received 18 March 2003; revised 11 April 2003; accepted 14 April 2003

First published online 6 May 2003

Edited by Bernard Trumpower

Abstract The cytochrome *bc*$_1$ complex catalyzes electron transfer from ubiquinol to cytochrome *c* by a protonmotive Q cycle mechanism in which electron transfer is linked to proton translocation across the inner mitochondrial membrane. In the Q cycle mechanism proton translocation is the net result of topographically segregated reduction of quinone and reoxidation of quinol on opposite sides of the membrane, with protons being carried across the membrane as hydrogens on the quinol. The linkage of proton chemistry to electron transfer during quinol oxidation and quinone reduction requires pathways for moving protons to and from the aqueous phase and the hydrophobic environment in which the quinol and quinone redox reactions occur. Crystal structures of the mitochondrial cytochrome *bc*$_1$ complexes in various conformations allow insight into possible proton conduction pathways. In this review we discuss pathways for proton conduction linked to ubiquinone redox reactions with particular reference to recently determined structures of the yeast *bc*$_1$ complex.

Key words: bc_1 complex; Protonmotive; Q cycle; Crystal structure; Cytochrome *c* reductase; Cytochrome *b*

1. Introduction

The cytochrome bc_1 (ubiquinol:cytochrome *c* oxidoreductase complex) is an energy-transducing enzyme located in the inner mitochondrial membrane of oxygen utilizing eukaryotic cells, where it participates in cell respiration. A functionally similar but structurally simpler version of the bc_1 complex is located in the plasma membrane of a wide variety of bacteria, where it takes part in respiration, denitrification, nitrogen fixation, and cyclic photosynthetic electron transfer, depending on the species. In all of these organisms the bc_1 complex oxidizes a membrane-localized quinol and reduces a water-soluble, *c*-type cytochrome and links this redox reaction to translocation of protons across the membrane in which the bc_1 complex resides.

*Corresponding authors.
E-mail addresses: hunte@mpibp-frankfurt.mpg.de (C. Hunte), trumpower@dartmouth.edu (B.L. Trumpower).

Abbreviations: UQ6, ubiquinone-6; UHDBT, 3-undecyl-2-hydroxy-1,4-dioxobenzoxythiazol; HHDBT, 3-heptyl-2-hydroxy-1,4-dioxobenzoxythiazol; CL, cardiolipin

The mechanism by which the bc_1 complex carries out this energy-transducing electron transfer is known as the protonmotive Q cycle [1]. The electron transfer pathway intrinsic to the Q cycle mechanism has been supported by an extensive body of experimental evidence as summarized elsewhere [2]. In the Q cycle mechanism proton translocation is the net result of topographically segregated reduction of quinone and reoxidation of quinol on opposite sides of the membrane, with protons being carried across the membrane as hydroxy hydrogen atoms on the quinol. The sites where quinone is reduced and quinol is oxidized are referred to as center N and center P, respectively, since they are located toward the electronegative and electropositive sides of the membrane. Protons are thus taken up at center N, carried across the membrane by the quinol, and released at center P.

Ubiquinone and ubiquinol partition exclusively into the hydrophobic phase of the mitochondrial membrane, owing to the extreme hydrophobicity of the 30–50 carbon isoprenoid side-chain. In addition, since the quinone and quinol redox reactions involve a semiquinone intermediate, it is advantageous to have these reactions localized to a non-aqueous environment in order to provide a thermodynamic and mechanistic barrier to formation of deleterious oxygen radicals by aberrant reactivities of the semiquinone [3]. Consequently, the linkage of proton chemistry to electron transfer during quinol oxidation at center P and quinone reduction at center N requires mechanisms for moving protons to and from the aqueous phase and the hydrophobic environment in which the quinol and quinone reside.

Cytochrome bc_1 complexes from bovine [4,5], chicken [6] and yeast mitochondria [7] have been crystallized and the structures determined by X-ray crystallography. The structure of the yeast complex is especially useful since it provides the basis for a combined approach by site-directed mutagenesis and X-ray structure analysis to investigate structure/function relationships within the enzyme [8,9]. In addition, the yeast bc_1 complex has recently been crystallized with an inhibitor that is structurally related to an intermediate of ubiquinol oxidation bound at center P, and this structure provides new insight into the possible pathways of proton conduction in that region of the enzyme [10].

2. Structure of the cytochrome *bc*$_1$ complex

The cytochrome bc_1 complex from eukaryotic organisms is a homodimeric, multi-subunit membrane protein complex (Fig. 1a). Each monomer contains three catalytic subunits

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00391-0

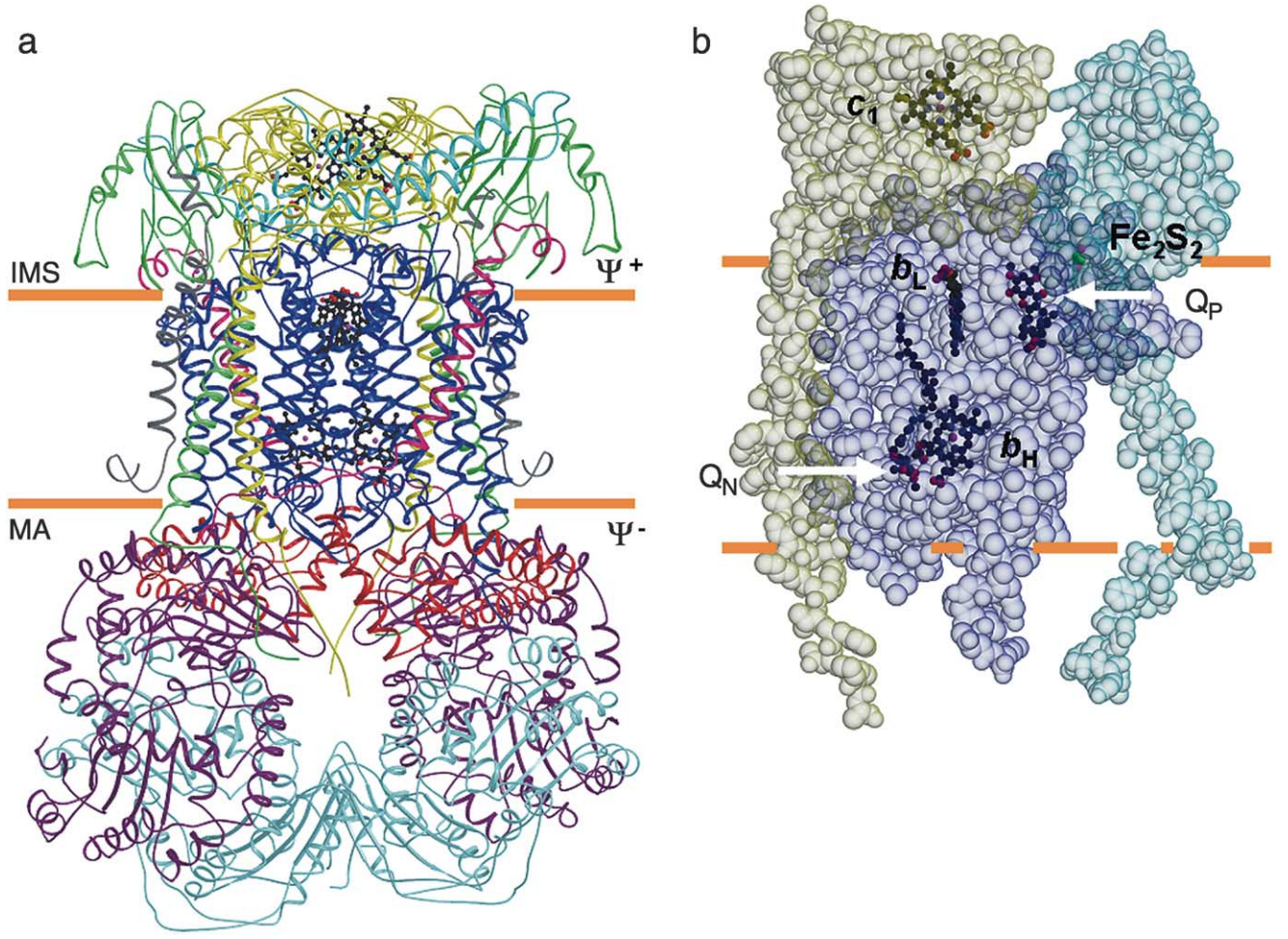

Fig. 1. Structure of the yeast cytochrome bc_1 complex [7]. Panel (a) shows the homodimeric complex comprised of the catalytic subunits cytochrome *b* (blue), Rieske protein (green), and cytochrome c_1 (yellow) with their respective co-factors (shown in ball-and-stick representation) and six additional subunits. Cor1p (blue-violet) and Qcr2p (blue-green) extrude into the matrix (MA), the smaller subunits Qcr6p (cyan), Qcr7p (red), Qcr8p (magenta), Qcr9p (gray) are laterally attached to the complex. Panel (b) shows the catalytic subunits of one functional unit in the same orientation as transparent surface presentation. The center P (Q_P)-specific inhibitor stigmatellin and UQ6 bound at center N (Q_N) visualize the relative position of the centers in respect to the phospholipid bilayer (orange bars). The arrows mark the positions of the primary sites of proton chemistry within the membrane, indicating the necessity of pathways for proton exchange with the aqueous environment.

carrying prosthetic groups, namely cytochrome *b* with two *b*-type hemes, cytochrome c_1 with a *c*-type heme, and the Rieske protein (Rip1p) containing a [2Fe–2S] cluster. Up to eight additional subunits are present in mitochondrial complexes [11]. The 2.3 Å resolution structure of the yeast bc_1 complex, which was crystallized with the help of antibody fragments, is the atomic structure of highest resolution available so far [7]. It provided a detailed description of substrate- and inhibitor-binding sites elucidating parts of the enzyme mechanism and suggesting pathways for proton transfer.

The central domain of the complex is formed by eight transmembrane helices of cytochrome *b* per monomer. Cytochrome c_1 and the Rieske protein are anchored to this core via single transmembrane helices; their catalytic domains are located in the intermembrane space. Two or three single transmembrane helices of the small subunits are attached to the periphery of the catalytic core in the yeast or the bovine complex, respectively. The so-called hinge protein, a small acidic subunit probably involved in binding of cytochrome *c*, is part of the intermembrane portion of the complex. The large domain of the complex extruding in the matrix space is formed by core proteins 1 and 2, and one additional subunit, Qcr7p, in yeast. Functions for most of the subunits that lack redox groups are not clear.

The structures clearly show that the bc_1 complex is a functional dimer (Fig. 1b). One functional unit is comprised of cytochrome *b* and cytochrome c_1 of one monomer, whereas the Rieske protein is connected with its transmembrane anchor in the second monomer. In principal, both functional units are fully active, but a regulatory interplay is discussed (see below). An important feature of the Rieske protein is the mobility of its catalytic domain, which was first shown in the avian bc_1 complex structure, in which the Rieske protein was either mobile and not visible in the X-ray structure or fixed and thereby resolved in a defined conformation by the use of an inhibitor [6]. It was proposed that the domain acts as a mobile electron shuttle, taking up electrons at center P when bound in the *b*-position and releasing them to cytochrome c_1 after swinging over to the *c*-position. Several mutagenesis studies confirmed that mobility of the catalytic Rieske domain is crucial for bc_1 complex activity. Mutations that limit the flexibility of the linker connecting the catalytic Rieske domain

and its transmembrane anchor result in lower activity of the complex [8,12–14]. However, it is an open question whether there is any control or trigger for the movement. Molecular dynamics simulation predicted a steered but stochastic movement [15]. In contrast, electron paramagnetic resonance studies of oriented membranes suggest a redox dependent movement of the domain [16]. The true nature of the movement remains to be elucidated.

The relative orientation of the complex in respect to the phospholipid bilayer can be judged from several parameters, such as the percentage of solvent-exposed carbon atoms as a measure for apolarity of the surface, or a layer of exposed tryptophans. In the structure of the yeast bc_1 complex phospholipid molecules have been identified, which are bound to the surface of the complex. They not only indicate the position of the complex in the phospholipid bilayer, but their tight and specific binding suggests specific roles for some of them for structural and functional integrity of the complex as well as for its assembly [9].

The sites of ubiquinol oxidation and ubiquinone reduction, center P and center N, have been structurally described with site-specific inhibitors [5,7,10,17]. In addition, the structure of the yeast bc_1 complex contained the natural substrate coenzyme Q_6 (UQ6) bound to center N [7]. Both substrate-binding sites are located at the border of the hydrophobic core of the enzyme, therefore, short proton transfer pathways are required for proton uptake and release upon ubiquinone/ubiquinol redox reactions (Fig. 1b). Details of these binding sites important for the mechanism will be discussed below.

3. The protonmotive Q cycle

The protonmotive Q cycle, shown in Fig. 2, involves divergent oxidation of two molecules of ubiquinol and recycling of one electron from each oxidation through the bc_1 complex, while the second electron from each oxidation is passed through the Rieske iron–sulfur cluster and cytochrome c_1 en route to cytochrome c. The two electrons that are recycled through the enzyme bring about re-reduction of one molecule of ubiquinone via a stable semiquinone intermediate. The oxidation of two ubiquinol molecules releases four protons to the intermembrane space, while re-reduction of ubiquinone results in uptake of two protons from the matrix. As noted above, these redox reactions and associated proton chemistry take place at two topographically segregated centers, center N and center P, which are disposed toward the electronegative and electropositive surfaces of the bc_1 complex (Fig. 1b).

In the first step of the Q cycle, ubiquinol is oxidized at center P in a concerted reaction that divergently transfers the two electrons from ubiquinol to the Rieske iron–sulfur cluster and the cytochrome b_L heme (reactions 1a–1c in Fig. 2). In reaction 2 the reduced Rieske cluster oscillates to within electron transfer distance of cytochrome c_1 as discussed above, allowing electron transfer from the iron–sulfur cluster to the c_1 heme. Two protons are released from center P coincident with ubiquinol oxidation. In reaction 3 an electron is transferred from the b_L to b_H heme, which in turn reduces ubiquinone to ubisemiquinone (reaction 4). Following oxidation of a second ubiquinol at center P and reduction of the b cytochromes the b_H heme reduces ubisemiquinone to ubiquinol (reaction 5), accompanied by uptake of two protons at center N.

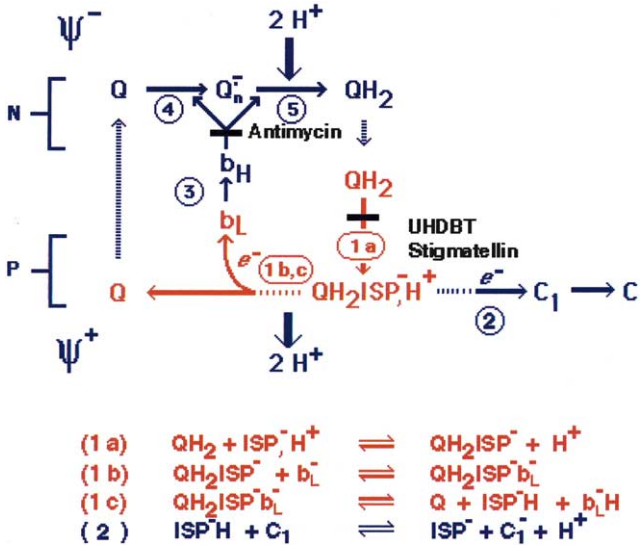

Fig. 2. The protonmotive Q cycle. Electron transfer reactions are numbered and circled. Dashed arrows designate movement of ubiquinol or ubiquinone between centers N and P and movement of the iron–sulfur protein between cytochrome b and cytochrome c_1. Solid black bars indicate sites of inhibition by antimycin, UHDBT and stigmatellin. Oxidation of ubiquinol at center P is depicted as a concerted reaction, consisting of three component reactions, 1a–c, shown in red. In reactions 1a–c oxidation of ubiquinol delivers two electrons divergently to the Rieske cluster and the b_L heme and the resulting ubiquinone leaves center P. In reaction 1a ubiquinol replaces a proton of the imidazole nitrogen of His181 on the Rieske iron–sulfur protein to form a transient quinol–imidazolate complex. In reaction 1b the quinol–imidazolate complex forms a hydrogen bond to Glu272 of cytochrome b to form the electron donor complex. In reaction 1c electrons are transferred simultaneously from the electron donor complex to the Rieske cluster and the b_L heme, resulting in dissociation of the complex and release of ubiquinone. In reaction 2 the reduced Rieske cluster oscillates to within electron transfer distance of cytochrome c_1, resulting in electron transfer from the iron–sulfur cluster to the c_1 heme. One proton is released to the aqueous phase from center P when the Rieske cluster is oxidized by cytochrome c_1. The other proton is transferred in parallel with the electron to heme b_L when the protonated Glu272 of cytochrome b dissociates from the electron donor complex and moves toward the heme propionate of heme b_L, from which the proton is conducted to the aqueous phase via Arg79 of cytochrome b. In reaction 3 an electron is transferred from the b_L to b_H heme, which in turn reduces ubiquinone to ubisemiquinone (reaction 4). Following oxidation of a second ubiquinol at center P and reduction of the b cytochromes the b_H heme reduces ubisemiquinone to ubiquinol (reaction 5), accompanied by uptake of two protons at center N.

The first step of the Q cycle, oxidation of ubiquinol at center P, has been proposed to be a concerted reaction, alternating in a controlled manner between the two monomers of the dimeric enzyme, and the evidence for such has been discussed elsewhere [18]. The concerted aspect of the reaction derives from the necessity of coupling the thermodynamically unfavorable reduction of the Rieske iron–sulfur cluster by an unstable ubiquinol/ubisemiquinone to the thermodynamically favorable reduction of the b_L heme, following the proposal of Rich and coworkers [19]. Consequently, the concentration of semiquinone is so low as to be non-existent as a distinct reaction intermediate during ubiquinol oxidation at center P.

The concerted reaction involves replacement of the ionizable proton of the imidazole nitrogen of His181 on the Rieske protein by a hydroxyl group from ubiquinol to form a quinol–imidazolate complex (reaction 1a in Fig. 2), which simulta-

neously forms a second hydrogen bond to Glu272 of cytochrome *b* (reaction 1b in Fig. 2). This results in an electron donor complex with two hydrogen bonds from the quinol, conferring partial negative charge on the quinol oxygens, and allowing essentially simultaneous electron transfer to the Rieske cluster and the b_L heme (reaction 1c in Fig. 2). It should be noted that the recent X-ray structure of the yeast complex with a 3-undecyl-2-hydroxy-1,4-dioxobenzoxythiazol (UHDBT) analog bound showed the stabilization of the negative charge of the hydroxyquinone at center P, suggesting that an anti-ferromagnetically coupled ubisemiquinone might be formed under some conditions [10].

When ubiquinol is oxidized the hydrogen bonds to His181 of the Rieske protein and Glu272 of cytochrome *b* are broken and the electron donor complex dissipates. The ubiquinone that is formed as a result of the oxidation leaves center P and generally has been assumed to enter the ubiquinone pool. However, it is possible that ubiquinone remains in the bc_1 complex and that the quinone ring rotates out of center P and into center N in the opposite half of the dimer. Dissipation of the electron donor complex also allows the Rieske protein to oscillate toward cytochrome c_1 and Glu272 of cytochrome *b* to rotate toward the propionate of heme b_L, as discussed below.

A model of the proposed electron donor complex was constructed and is shown in Fig. 3a. To generate this model ubiquinol was docked into the position occupied by stigmatellin in the crystal structure of the yeast enzyme (1EZV) and then submitted to an energy minimization. Evidence that the binding pockets for stigmatellin and ubiquinol are the same has been outlined elsewhere [7,20]. After energy minimization the quinol hydroxyl groups are $\sim$2.05 and 2.40 Å from the imidazole nitrogen of His181 and carboxyl oxygen of Glu272, respectively. This demonstrates the feasibility of the two hydrogen bonds requisite to the electron donor complex.

Electron transfer is proposed to occur through the hydrogen bond and the imidazole ring to the iron–sulfur cluster and by tunneling to the porphyrin from the quinol hydroxyl group that is hydrogen-bonded to Glu272. The closest distance to the heme porphyrin ring, estimated at 10.6 Å, will be one of the determinants of the electron transfer rate for the concerted reaction. The second determinant of the electron transfer rate is the driving force, ΔG_{net}, which is the ΔG for electron transfer from ubiquinol to the iron–sulfur cluster plus the ΔG for electron transfer to the b_L heme. These two electron transfer reactions generate a ΔG_{net} that varies from -0.05 to -0.3 eV, depending on the redox poise of the ubiquinol pool and cytochrome *c*. With a rate determining distance of 10.6 Å one can calculate from the Moser–Dutton equation [21] that these potential increments should result in electron transfer rates between 1×10^6 and 2×10^7 s^{-1}. Since these rates are three to four orders of magnitude greater than the measured rates of the bc_1 complex, one can conclude that the usual application of Marcus theory that typically assumes a value for the reorganization energy (λ) = 1 is not applicable to ubiquinol oxidation at center P. This discrepancy is to be expected if ubiquinol oxidation is a concerted reaction as proposed.

4. Proton conduction from ubiquinol oxidation at center P

From the crystal structure of the yeast bc_1 complex with stigmatellin bound it can be seen that cytochrome *b* and the Rieske protein surround stigmatellin and block direct access to the aqueous phase [7]. We assume that ubiquinol is similarly sequestered from the aqueous phase. Consequently, there must be some mechanism to conduct protons from the electron donor complex to the aqueous phase when ubiquinol is oxidized. Based on the crystal structure of the bc_1 complex with stigmatellin bound [7] and the structure when 3-heptyl-2-hydroxy-1,4-dioxobenzoxythiazol (HHDBT) is bound at center P [10], it appears that the two protons are conducted by two separate mechanisms. One of these depends on movement of the Rieske protein, the other depends on movement of Glu272 of cytochrome *b*. A notable feature of these proton-conducting mechanisms is that both protons released from ubiquinol oxidation move to the aqueous surface with minimal charge separation. One moves with the reduced Rieske iron–sulfur cluster while the other moves in parallel with an electron to heme b_L.

Ubiquinol cytochrome *c* reductase activity of the bc_1 complex is pH dependent and described by a bell-shaped curve that can be fit by assuming three contributing protonatable groups with pK_a = 5.2, 7.5, and 9.2 [22]. The midpoint potential of the Rieske iron–sulfur protein exhibits a pH dependence that indicates the oxidized protein has pK_a = 7.6 and 9.1 [23]. The crystal structures of the bc_1 complex with stigmatellin bound show that it forms hydrogen bonds to His181 of the Rieske protein and Glu272 of cytochrome *b*. Taken together these results suggest that the three protonatable groups contributing to the pH dependent activity are the carboxyl group of Glu272 on cytochrome *b*, the imidazole nitrogen on His181 of the Rieske protein, and the imidazole nitrogen on His161 of the Rieske protein, respectively. Formation of the electron donor complex as discussed above would be enhanced by deprotonation of both Glu272 and His181, while deprotonation of His161 would lower the midpoint potential of the Rieske cluster and thus slow the rate of ubiquinol oxidation.

When the Rieske iron–sulfur cluster is reduced the imidazole nitrogen on His181 of the Rieske protein is protonated. Thus, the redox reaction is formally a hydrogen transfer from the quinol, and the Rieske protein acts as both an electron and proton carrier. As noted above, when the hydrogen bond between the imidazole nitrogen of His181 and ubiquinol is broken by oxidation of the quinol, the electron donor complex dissipates and the Rieske protein is able to move proximal to cytochrome c_1 [6,24], where it is oxidized. When the iron–sulfur cluster is oxidized the imidazole nitrogen on His181 becomes a weak base (pK_a = 7.6) and the proton dissociates.

The second proton from ubiquinol oxidation is transferred to Glu272 when the quinol–carboxylate hydrogen bond is broken as a result of electron transfer to the b_L heme. When the electron donor complex dissipates Glu272 rotates 170° to form a hydrogen bond to a water molecule that is hydrogen-bonded to a propionate on the porphyrin ring of heme b_L as can be seen in the yeast complex with a UHDBT analog bound (shown in Fig. 3b). The subsequent proton release to the aqueous surface is mediated by a hydrogen-bonded water chain stabilized by cytochrome *b* residues Arg79, Asn256, Glu66 and Arg70 [10]. In this manner a proton is carried more than 16 Å from ubiquinol to the aqueous surface through a proton conduction pathway that involves as central elements the conserved amino acid, Glu272, a bound water, and the porphyrin ring of heme b_L. Glu272 is com-

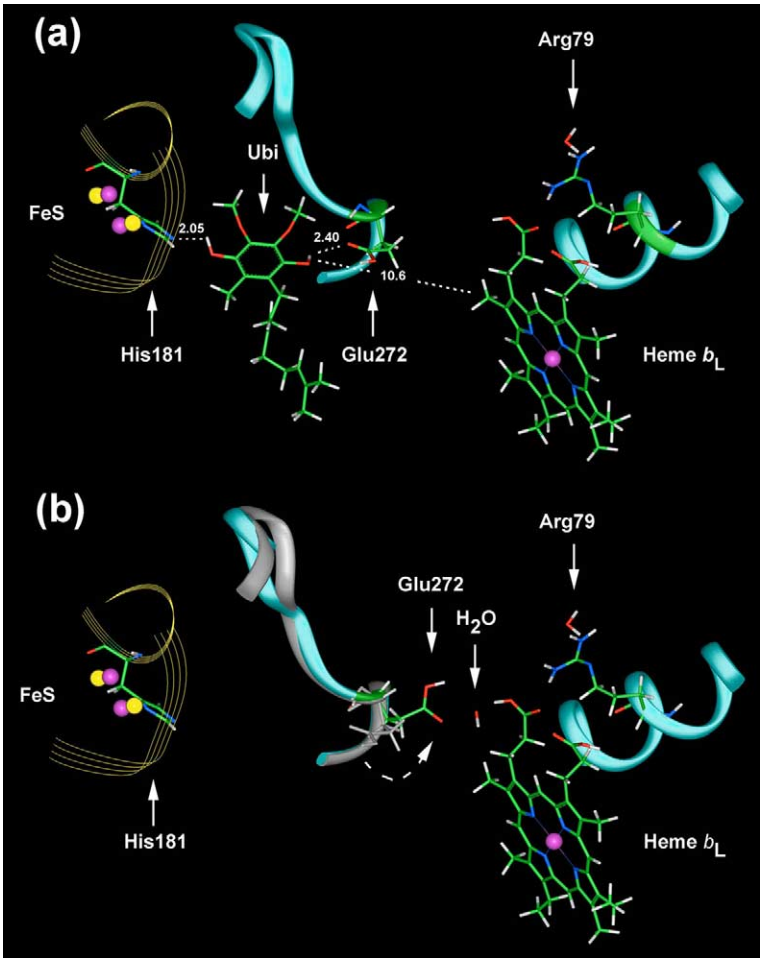

Fig. 3. Structural basis for electron transfer and proton conduction at center P. Panel (a) shows ubiquinol hydrogen-bonded to His181 of the Rieske iron–sulfur protein and Glu272 of cytochrome *b* in the yeast cytochrome bc_1 complex. The view is from the membrane interior, looking toward center P. Portions of the Rieske protein between residues 160–165 and 178–182 are represented as yellow strands. Portions of cytochrome *b* that include residues 75–85 and 265–275 are shown as cyan ribbons. The iron–sulfur cluster is at the upper left, with iron and sulfur atoms colored magenta and yellow. The b_L heme is at the lower right, with the iron atom colored magenta. Ubiquinol, the b_L heme, His181 of the Rieske protein, and Arg79 and Glu272 of cytochrome *b* are shown as stick models, with carbon atoms colored green, oxygen atoms colored red, and nitrogen atoms colored blue. Dashed white lines indicate hydrogen bonds between the ubiquinol hydroxyl groups and the imidazole nitrogen of His181 of the Rieske protein and the carboxyl oxygen of Glu272 of cytochrome *b*, and the distances are indicated in Angstroms. The distance between the ubiquinol oxygen and the b_L heme is similarly indicated. Ubiquinol containing two isoprenyl groups in the side-chain was docked into the Q_p site by replacing stigmatellin in the coordinates for the stigmatellin-liganded yeast enzyme [7]. An energy-minimized structure for the docked quinol was calculated with the Discover® program in Insight II®, using molecular dynamics and the CFF91 force-field. Panel (b) shows the structure of the ubiquinol-binding pocket after Glu272 of cytochrome *b* moves proximal to the propionate side-chain of the b_L heme as it is in the yeast bc_1 complex with bound HHDBT [10]. The dashed white arrow depicts the presumed movement of Glu272 during catalysis to the position proximal to the propionate of the b_L heme. A gray ribbon and a gray stick model of Glu272 indicate the position of residues 265–275 when Glu272 is hydrogen-bonded to ubiquinol. The position of residues 265–275 after Glu272 has moved proximal to the heme propionate is indicated by a cyan ribbon and a colored stick model of Glu272. A water molecule is hydrogen-bonded between Glu272 and the heme propionate allowing a direct proton transfer from the primary proton acceptor Glu272 to the propionate.

pletely conserved in mitochondrial cytochrome *b* [25] and the importance of the residue for proton transfer is supported by mutagenesis studies as replacement by glutamine abolishes ubiquinol oxidation in *Rhodobacter sphaeroides* [26]. Also, kinetic studies recently showed that protonation of a group with pK_a of 5.7 blocked catalysis and this effect was assigned to Glu272 [27].

The movement of Glu272 which is central to this proton conduction pathway is inferred from the location of Glu272 proximal to stigmatellin in the stigmatellin-liganded yeast bc_1 complex [7] and proximal to the heme propionate in the non-liganded bovine bc_1 complex [5] and has been proposed earlier as an initial step for the release of the second proton [7,28,29]. Experimental support is provided by a recently determined structure of the yeast bc_1 complex with an analog of UHDBT bound [10], where the 2-OH group on the benzoquinone ring is hydrogen-bonded to His181 of the Rieske protein, while the carbonyl group of the benzoquinone ring is facing Glu272. The latter, devoid of any stabilizing interaction, is rotated proximal to the heme propionate. This suggests that the absence of a second OH group on the UHDBT analog, as would be present on ubiquinol, allows the Glu272 to swing away from the position it occupies in the electron donor complex. Furthermore, in this structure a single, hydrogen-bonded water molecule is present between Glu272 and the heme propionate A, demonstrating a feasible delivery route of the proton from the primary acceptor to the heme. The short array of hydrogen-bonded water molecules stabilized by cytochrome *b*

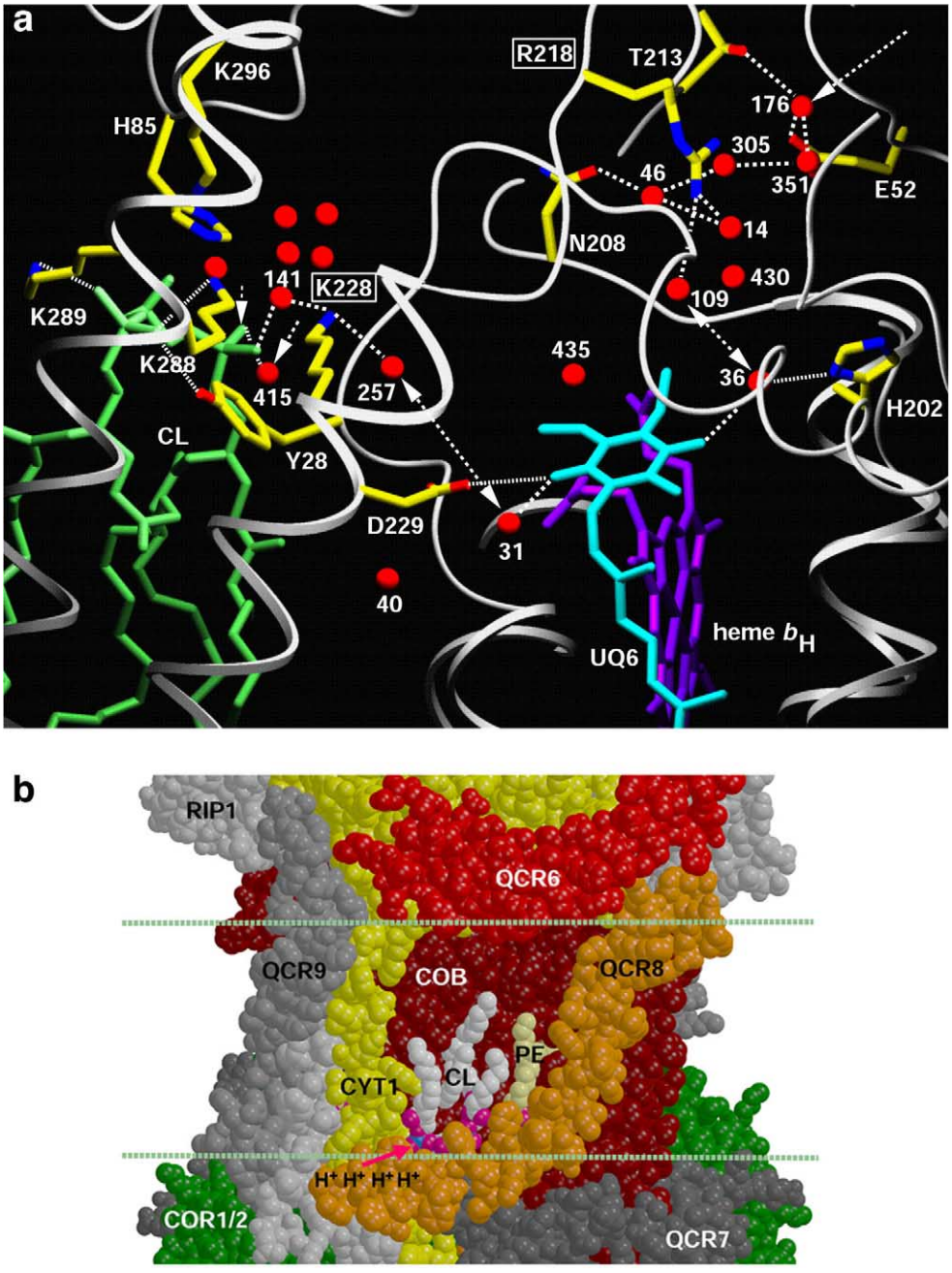

Fig. 4. Putative proton uptake pathways for ubiquinone reduction at center N. Panel (a) shows two arrays of hydrogen-bonded water molecules that connect the bulk solvent at the matrix side with the binding pocket. The entrance to the E/R-pathway is formed by Glu52 of Qcr7p and a water molecule. The gating residue towards the quinone-binding pocket is Arg218 of cytochrome *b*. CL is positioned at the entrance to the CL/K-pathway, for which Lys228 of cytochrome *b* is the gating residue. Arrows indicate the access sites from the bulk solvent; double-headed arrows indicate proton transfer between the key residues Arg218 or Lys228 of cytochrome *b* and UQ6. Side-chains of amino acid residues that are involved in hydrogen bond interactions or ion pair formation are shown (standard colors). Dashed lines indicate hydrogen bond interactions. Dotted lines are used for hydrogen bond interactions of UQ_6 and CL (taken from [9]). Panel (b) shows a surface close-up view of the cardiolipin-binding site. The dianionic CL (white) and a phosphatidylethanolamine molecule (yellow) are bound in a depression of the surface of the yeast bc_1 complex close to the Q_N site. These boundary phospholipids contribute to position the complex in the phospholipid bilayer (indicated with dashed lines). The suggested proton uptake from the membrane surface via cardiolipin is schematically indicated.

residues Arg79, Asn256, Glu66 and Arg70 has been noted in both crystal structures of the yeast enzyme [7,10] and has been suggested as a proton exit pathway from the heme propionate to the aqueous environment [7,10]. The parallel route of electron and proton transfer from ubiquinol to the *b* heme might be of advantage for electrostatic reasons and may speed up proton transfer.

5. A proton conduction pathway to ubiquinone reduction at center N

Binding of the natural substrate UQ6 to the Q_N site has been described in the structure of the yeast bc_1 complex [7]. The ubiquinone head-group is oriented towards the matrix and the isoprenoid tail extends along the hydrophobic cleft

reaching into the cavity of the Q_P site of the second monomer. The carbonyl oxygen atoms of UQ6 are in hydrogen-bonding distance to a water molecule as well as Asp229 of cytochrome *b* on one side and to a water molecule that is stabilized by His202 of cytochrome *b* on the other side. These highly conserved residues were proposed to stabilize the protein substrate complex [7,29]. For the bovine bc_1 complex direct protonation of quinone by histidine and arginine was proposed [29]. However, the resolution of the available structure did not allow determination of the positions of water molecules. Furthermore, it was suggested that the histidine side-chain is mobile and may change orientation upon quinone binding and release, thereby opening the binding pocket to the bulk solvent. In contrast, based on the high-resolution structure of the yeast complex it was suggested that not His202 but a water molecule is the primary proton donor on one side, and the second proton is either donated by a water molecule or Asp229 [7,9,20]. The donors have to be re-protonated from the matrix. As the binding pocket is located at the border of the transmembrane hydrophobic core and as it lacks a direct opening to the bulk solvent the uptake requires a short proton 'wire' or a hydrogen-bonded 'network' from the protein surface [7,9].

Two distinct proton uptake pathways have been suggested from the study of the yeast bc_1 complex, namely the E/R-pathway and the cardiolipin (CL)/K-pathway (Fig. 4). As they are located on either side of the substrate and can directly provide protons to the two carbonyl groups in the described orientation, reduction of quinone by heme b_H most likely takes place without major reorientation of the quinone head-group. Interestingly, CL is positioned at the entrance to one of them (Fig. 4). Well-defined water molecules are present in the spacious ubiquinone-binding pocket and each of the carbonyl groups of the quinone ring is in hydrogen-bonding distance to a water molecule (Wat31 and Wat36). Further disordered water molecules are likely to be present in the two lobes of the binding pocket, which extend to the matrix side.

The E/R-pathway is located on the His202 oriented side of the ubiquinone head-group. Here, Arg218 mediates between the water molecules of the binding pocket with an array of several water molecules that are connected by hydrogen bonds and stabilized by interactions with neighboring residues (Fig. 4). Glu52 of Qcr7p is positioned at the exit of this array to a hydrophilic cavity, which is formed by cytochrome *b*, subunit Qcr7p and subunit Qcr8p and is open to the aqueous environment. Upon reduction of ubiquinone a proton is abstracted from Arg218, and proton transfer occurs directly via the hydrogen-bonded water molecules, thereby allowing replenishment of this proton from the matrix side. The presumed gating role of Arg218 is supported by the fact that it is highly conserved and only replaced by lysine in mitochondrial cytochrome *b*. Further support derives from mutations of Asn223 of cytochrome *b*, the prokaryotic homolog to Asn208. The latter is part of the suggested proton-conducting array. An exchange of this residue to valine in a prokaryotic organism led to disturbance of the Q_N kinetics [26].

On the Asp229 oriented side of ubiquinone, Lys228, which is highly conserved in mitochondrial cytochrome *b*, is the key residue for the CL/K-pathway (Fig. 4). Lys228 connects the water molecules of the binding pocket with the aqueous solvent via a single water molecule, which is present in hydrogen bond distance between Lys228 and a CL molecule, which is tightly bound to the protein surface. It was proposed that during reduction of ubiquinone a proton is abstracted from Lys228. This will consequently be replenished from the matrix side either directly by reversible deprotonation of the CL phosphodiester group B or proton exchange occurs via Wat415, which is located between phosphodiester group B of CL and the head-group of the neighboring phospholipid. Consequently, the boundary phospholipid CL not only ensures structural integrity of the Q_N site, but also provides the entry point for proton uptake. This allows the direct coupling of the ubiquinone reduction with the proton source at the membrane surface of the matrix side. It was proposed that the high surface charge density of bilayer anionic phospholipids acts as a trap or buffer that concentrates protons and passes them directly to a proton-translocating pore [30].

Double and triple mutants of the main CL ligands in yeast, namely K289L/K296L and K288/K289L/K296L, exhibited a slow growth phenotype on non-fermentable carbon source and lower bc_1 complex content, indicating that the specific CL-binding site is important for stability and integrity of the complex [9]. Further evidence derives from the observation that CL can restore binding of the Q_N site-specific inhibitor antimycin in delipidated yeast bc_1 complex [31]. Further studies are required to prove the functional role of CL in gating proton uptake for quinone reduction in addition to its importance for the structural integrity of the Q_N site architecture.

Acknowledgements: C.H. acknowledges support by the Deutsche Forschungsgemeinschaft (SFB 472) and B.L.T. acknowledges support by NIH Grant GM 20379.

References

[1] Mitchell, P. (1976) J. Theor. Biol. 62, 327–367.
[2] Trumpower, B.L. (1990) J. Biol. Chem. 265, 11409–11412.
[3] Muller, F. (2000) J. Am. Aging Assoc. 23, 227–253.
[4] Xia, D., Yu, C.A., Kim, H., Xia, J.Z., Kachurin, A.M., Zhang, L., Yu, L. and Deisenhofer, J. (1997) Science 277, 60–66.
[5] Iwata, S., Lee, J.W., Okada, K., Lee, J.K., Iwata, M., Rasmussen, B., Link, T.A., Ramaswamy, S. and Jap, B.K. (1998) Science 281, 64–71.
[6] Zhang, Z., Huang, L., Schulmeister, V.M., Chi, Y.I., Kim, K.K., Hung, L.W., Crofts, A.R., Berry, E.A. and Kim, S.H. (1998) Nature 392, 677–684.
[7] Hunte, C., Koepke, J., Lange, C., Rossmanith, T. and Michel, H. (2000) Structure 8, 669–684.
[8] Nett, J.H., Hunte, C. and Trumpower, B.L. (2000) Eur. J. Biochem. 267, 5777–5782.
[9] Lange, C., Nett, J.H., Trumpower, B.L. and Hunte, C. (2001) EMBO J. 20, 6591–6600.
[10] Palsdottir, H., Gomez-Lojero, Trumpower, B.L. and Hunte, C. (2003) J. Biol. Chem., in press.
[11] Link, T.A., Schägger, H. and von Jagow, G. (1986) FEBS Lett. 204, 9–15.
[12] Tian, H., White, S., Yu, L. and Yu, C.A. (1999) J. Biol. Chem. 274, 7146–7152.
[13] Darrouzet, E., Valkova Valchanova, M. and Daldal, F. (2000) Biochemistry 39, 15475–15483.
[14] Xiao, K., Yu, L. and Yu, C.A. (2000) J. Biol. Chem. 275, 38597–38604.
[15] Izrailev, S., Crofts, A.R., Berry, E.A. and Schulten, K. (1999) Biophys. J. 77, 1753–1768.
[16] Brugna, M., Rodgers, S., Schricker, A., Montoya, G., Kazmeier, M., Nitschke, W. and Sinning, I. (2000) Proc. Natl. Acad. Sci. USA 97, 2069–2074.
[17] Kim, H., Xia, D., Yu, C.A., Xia, J.Z., Kachurin, A.M., Zhang,

L., Yu, L. and Deisenhofer, J. (1998) Proc. Natl. Acad. Sci. USA 95, 8026–8033.
[18] Trumpower, B.L. (2002) Biochim. Biophys. Acta 1555, 166–173.
[19] Jünemann, S., Heathcote, P. and Rich, P.R. (1998) J. Biol. Chem. 273, 21603–21607.
[20] Hunte, C. (2001) FEBS Lett. 504, 126–132.
[21] Moser, C.C., Page, C.C., Farid, R. and Dutton, P.L. (1996) J. Bioenerg. Biomembr. 27, 263–274.
[22] Covian, R. and Moreno-Sanchez, R. (2001) Eur. J. Biochem. 268, 5783–5790.
[23] Link, T.A., Hagen, W.R., Pierik, A.J., Assman, C. and von Jagow, G. (1992) Eur. J. Biochem. 208, 685–691.
[24] Crofts, A.R., Hong, S., Ugulava, N., Barquera, B., Gennis, R., Guerrgova-Kuras, M. and Berry, E. (1999) Proc. Natl. Acad. Sci. USA 96, 10021–10026.
[25] Esposti, M.D., De, V., Crimi, M., Ghelli, A., Patarnello, T. and Meyer, A. (1993) Biochim. Biophys. Acta 1143, 243–271.
[26] Brasseur, G., Saribas, A.S. and Daldal, F. (1996) Biochim. Biophys. Acta 1275, 61–69.
[27] Covian, R. and Moreno, S. (2001) Eur. J. Biochem. 268, 5783–5790.
[28] Crofts, A.R., Barquera, B., Gennis, R.B., Kuras, R., Guergova, K. and Berry, E.A. (1999) Biochemistry 38, 15807–15826.
[29] Berry, E.A., Zhang, Z., Huang, L.S. and Kim, S.H. (1999) Biochem. Soc. Trans. 27, 565–572.
[30] Haines, T.H. and Dencher, N.A. (2002) FEBS Lett. 528, 35–39.
[31] Tsai, A.L. and Palmer, G. (1986) Biochim. Biophys. Acta 852, 100–105.