Review

Ubiquinol oxidation in the cytochrome \(/\text{bc}_{1}\) complex:
Reaction mechanism and prevention of short-circuiting

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Abstract

This review is focused on the mechanism of ubiquinol oxidation by the cytochrome \(/\text{bc}_{1}\) complex \((/\text{bc}_{1})\). This integral membrane complex serves as a "hub" in the vast majority of electron transfer chains. The \(/\text{bc}_{1}\) oxidizes a ubiquinol molecule to ubiquinone by a unique "bifurcated" reaction where the two released electrons go to different acceptors: one is accepted by the mobile redox active domain of the \([-2\text{Fe}–2\text{S}]\) iron–sulfur Rieske protein (FeS protein) and the other goes to cytochrome \(/\text{b}\). The nature of intermediates in this reaction remains unclear. It is also debatable how the enzyme prevents short-circuiting that could happen if both electrons escape to the FeS protein. Here, I consider a reaction mechanism that (i) agrees with the available experimental data, (ii) entails three traits preventing the short-circuiting in \(/\text{bc}_{1}\), and (iii) exploits the evident structural similarity of the ubiquinone binding sites in the \(/\text{bc}_{1}\) and the bacterial photosynthetic reaction center (RC). Based on the latter congruence, it is suggested that the reaction route of ubiquinol oxidation by \(/\text{bc}_{1}\) is a reversal of that leading to the ubiquinol formation in the RC. The rate-limiting step of ubiquinol oxidation is then the re-location of a ubiquinol molecule from its standby site within cytochrome \(/\text{b}\) into a catalytic site, which is formed only transiently, after docking of the mobile redox domain of the FeS protein to cytochrome \(/\text{b}\). In the catalytic site, the quinone ring is stabilized by Glu-272 of cytochrome \(/\text{b}\) and His-161 of the FeS protein. The short circuiting is prevented as long as: (i) the formed semiquinone anion remains bound to the reduced FeS domain and impedes its undocking, so that the second electron is forced to go to cytochrome \(/\text{b}\); (ii) even after ubiquinol is fully oxidized, the reduced FeS domain remains docked to cytochrome \(/\text{b}\) until electron(s) pass through cytochrome \(/\text{b}\); (iii) if cytochrome \(/\text{b}\) becomes (over)reduced, the binding and oxidation of further ubiquinol molecules is hampered; the reason is that the Glu-272 residue is turned towards the reduced hemes of cytochrome \(/\text{b}\) and is protonated to stabilize the surplus negative charge; in this state, this residue cannot participate in the binding/stabilization of a ubiquinol molecule.

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1. Introduction

Cytochrome \(/\text{bc}_{1}\)-complexes of animals and bacteria (hereafter \(/\text{bc}_{1}\)), as well as closely related cytochrome \(/\text{b}_{6}f\)-complexes of plants and cyanobacteria (hereafter \(/\text{b}_{6}f\)), are oligomeric membrane enzymes that function as electrogenic quinol:cytochrome \(/\text{c}\) oxidoreductases (see [1,2] for comprehensive reviews on \(/\text{bc}_{1}\) and \(/\text{b}_{6}f\), respectively). These membrane complexes catalyze the oxidation of diverse quinols by high potential redox-carriers \((c\text{-type cytochromes in mitochondria and bacteria or plastocyanin in cyanobacteria and higher plants})\). The free energy of the redox reaction is used to transfer protons across the energy-transducing membrane. Thereby, one of the membrane-adjoining water phases becomes positively charged \((p\text{-side})\) whereas the other one charges negatively \((n\text{-side})\). Proton transfer leads to the formation of the difference in electrochemical activity of

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protons across the membrane (ΔµH⁺). The latter can be considered as a sum of chemical (ΔpH) and electrical (Δφ) components and reaches approximately 200–250 mV under physiological conditions [3]. Thus, bc₁ has to pump protons against remarkable backpressure at steady state.

The cytochrome bc₁ complex (see Fig. 1) is an intertwined dimer both in crystals [4–11] and in solution [12]. The catalytic core of each bc₁ monomer is formed by three subunits: the membrane-embedded cytochrome b, the [2Fe–2S] cluster-carrying Rieske protein, and cytochrome c₁ (see Fig. 1). Each cytochrome b carries one low- and one high-potential protoheme (b₁ and b₇, respectively, the redox and spectroscopic properties of the cofactors are given in Table 1). The number of subunits in cytochrome bc₁-complexes varies from only 3 catalytic ones in some bacteria up to 11 in the mitochondrial bc₁.

With bc₁-containing proteoliposomes, it was shown that two protons were transferred across the membrane per each oxidized quinol [13,14]. Mitchell has explained this stoichiometry by a Q-cycle mechanism [15,16]. The mechanism invoked the bifurcation of electron flow at the site of quinol oxidation, as originally suggested by Wikström and Berden [17], with one electron reducing cytochromes c and another one going to cytochrome b. In addition, Q-cycle implied that electrons, after passing through cytochrome b, reduce a quinone molecule from the other side of the membrane as shown in Fig. 1. According to the current, structure-based Q-cycle models (see e.g., [1,6,8,18–20]), quinol molecules are oxidized at the interface between cytochrome b and the mobile [2Fe–2S] cluster-carrying domain of the Rieske protein (hereafter the FeS domain, see Fig. 1). This interface forms the catalytic center P of the enzyme (which corresponds to center o in the original Mitchell’s notation). The FeS domain takes the first electron from a ubiquinol to pass it further, via cytochrome c₁ and the further c-type cytochromes (cytochrome c in mitochondria and cytochromes c₂ and c₂ in phototrophic bacteria [21]), to external electron carriers. The semiquinone remaining in center P reduces the heme b₁. From this heme, the electron moves across the membrane to heme b₇ and then to the further quinone-binding center N (center i in the Mitchell’s notation). In center N, a ubiquinone molecule is reduced first to a semiquinone anion Qₜ⁻ and then, after the oxidation of the next ubiquinol in center P, to a QₙH₂ ubiquinol. This ubiquinol can be oxidized by bc₁ as well, so that two charges are “pumped” across the membrane per each ubiquinol processed by bc₁. It is convenient to distinguish two electron transfer (ET) branches in bc₁, namely the high-potential one, which is formed by the FeS domain and cytochrome c₁ (c-chain according to [20]), and the low-potential one formed by two hemes of cytochrome b and the Qₙ quinone (b-chain).

The Mitchell’s idea of doubling the enzyme efficiency by the internal electron cycling is generally accepted (see, however, [22]). Further details on the enzyme reaction remain still controversial. It is debatable whether ubiquinol is oxidized sequentially, in two steps, or this reaction is concerted. There are diverse hypotheses explaining why both electrons, which are released upon quinol oxidation, do not escape via the FeS domain towards cytochromes c.

In this review, the ubiquinol oxidation in center P of bc₁ is compared with the reduction of the secondary quinone Q₀ in the photosynthetic reaction center (RC) of purple photo-

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Fig. 1. Overview of structure and function of the cytochrome bc₁ complex. The scheme shows the Q-cycle scheme superimposed on the X-ray structure of the three catalytic subunits of the dimeric yeast bc₁. The X-ray structure of the yeast bc₁ co-crystallized with cytochrome c (PDB entry 1KYO [10]) was used for the presentation. Color code: grey/black, cytochrome b subunits; blue, thick line, the Rieske subunits; green, cytochromes c₁; red, cytochrome c; yellow, hemes. Figure was produced with the help of the VMD software package [216].
synthetic bacteria. The mechanism of the latter reaction is well understood because it is possible to feed electrons into the RC one by one by firing short flashes of light, so that intermediate states can be trapped and studied (see e.g., [23–29]). So far, the parallels were drawn between the mechanisms of sequential ubiquinone reduction in the RC and in the center N of bc$_1$ [9,19,30–32]. As argued below, the quinone-processing machinery in the RC can serve as useful paradigm for understanding the mechanism of the bifurcated ubiquinol oxidation by bc$_1$ as well.

2. Mechanism of ubiquinol oxidation by the cytochrome bc$_1$ complex

2.1. Electron cycling in chromatophore vesicles from purple phototrophic bacteria

The data on the operation of bc$_1$ in a flash-triggered mode, as discussed in the following sections, were mostly obtained with preparations of chromatophores (inside-out vesicles of inner cellular membrane) from purple phototrophic bacteria Rhodobacter sphaeroides and Rhodobacter capsulatus (see Fig. 2 and [33–35] for reviews). Absorption of light quantum by photosynthetic pigments leads to the electrogenic transmembrane charge separation in the photosynthetic reaction center (RC, see Fig. 3) and to generation of a reducing agent (Q$_{BH_2}$ ubiquinol) and of an oxidant (oxidized cytochrome c$_2$) for bc$_1$. The cytochrome bc$_1$ complex catalyzes the ET from ubiquinol back to cytochrome c$_2$ (see Fig. 2); this reaction is coupled with $\Delta\psi$ generation (see [3,33,34] for reviews). The flash-induced redox changes of cytochromes can be monitored optically [36,37], whereas the generation of $\Delta\psi$ can be followed both optically, via the electrochromic shifts of intramembrane carotenoids [38,39], and by capacitive electrometry [39,40]. The reactions of proton binding and release could be traced by appropriate pH-indicators [41–43]. The reactions in bc$_1$ are routinely discriminated by application of inhibitors that selectively block quinol

| Cytatochrome bc$_1$ complex of Rh. capsulatus: electrochemical and spectral properties of the redox cofactors |
|-----------------|------------------|------------------|--------|
| Redox cofactor  | $E_m$ (mV)       | $\lambda_{max}$ (nm) | $g_x$ (EPR) |
| Heme $b_1$      | $-90^{..}-115$   | 558/565$^{ab}$     |        |
| Heme $b_h$      | approximately 0 mV | 560$^{ab}$       |        |
| cytochrome $c_1$| +50/+60 mV$^{ab}$ | 552$^c$          |        |
| [2Fe–2S] cluster| +270 (FeS)$_{\delta}/\gamma$ 460 mV (FeS)$_h$ | – | 1.800 (ubiquinone present)$^l$ |

References: a, [213]; b, [33]; c, [214]; d, [64]; e, [56]; f, [215]; g, [106]; h, [109]; i, [45].

![Fig. 2. Schematic presentation of a chromatophore membrane based on a picture by Boris Feniouk. White arrows depict electron cycling between the RC and the bc$_1$. The red color of the interfacial water layer indicates its higher acidity at steady state, which can increase the protonic backpressure on the cytochrome bc$_1$ complex [217,218].](image)
oxidation (e.g., of myxothiazol) and by comparison of the kinetic traces in the presence and in the absence of an inhibitor.

Besides providing the possibility to study the bc$_1$ in a pulsed mode, chromatophores have several other advantages: (i) the bc$_1$ complexes of purple bacteria are simple and are made of only 3 or 4 subunits [11,35,44,45]; (ii) chromatophore vesicles are easy to isolate; they remain stable at room temperature for many hours; (iii) mutants with site-specific amino acid substitutions in the subunits of the bc$_1$ are available (see [46] for their survey); (iv) evolutionarily, purple bacteria are close to mitochondria [47]; not surprisingly, the X-ray structure of the simplest three-subunit bc$_1$ from Rh. capsulatus, which has been recently resolved up to 3.8 Å [11], overlaps with the three core subunits of the mitochondrial bc$_1$, and the same specific inhibitors are effective in both systems.

When the membrane quinones are oxidized in the dark, the flash-induced turnover of bc$_1$ is triggered by the arrival of a ubiquinol molecule formed in the RC. At neutral pH, the oxidation of this ubiquinol by bc$_1$ leads to a partial reduction of heme $b_h$ at approximately 3 ms followed by slower (i) re-reduction of the flash-oxidized cytochrome c$_1$ by electrons coming from ubiquinol, (ii) $\Delta \psi$ generation, and (iii) proton release to the $p$-side of the membrane. These three reactions take approximately 10–20 ms [18,36,39,43,48–51]. Usually, there are 2–3 RCs per one bc$_1$ monomer in the membranes of Rhodobacter, so that each bc$_1$ dimer turns over several times after a saturating flash of light. Therefore, the kinetic mismatch between the faster heme $b_h$ reduction and slower cytochrome c$_1$ re-reduction and electrogenesis was usually explained by the contribution of the multiple turnovers of bc$_1$ to the latter reactions (see e.g., [52]). The cytochrome bc$_1$ complex can be, however, compelled to turn over only once (i) if the substrate ubiquinol is in shortage [18,53] or (ii) if the exciting flash is weak [51,54]. Under such conditions, the heme $b_h$ was still reduced at 3 ms, whereas the flash-induced $\Delta \psi$ generation and cytochrome c re-reduction proceeded at approximately 30–40 ms, by order of magnitude slower [18,51,53].

Under reducing conditions, when ubiquinol is abundant in the membrane (that corresponds to the native situation [55]), the oxidation of ubiquinol by bc$_1$ is triggered by the migration of an electron vacancy, via cytochromes c, to the FeS domain ([56], see Fig. 1). In flash experiments, the ubiquinol oxidation by bc$_1$ manifests itself in the re-reduction of cytochromes c at 2–4 ms. The kinetics of this re-reduction corresponds to the kinetics of $\Delta \psi$ generation by bc$_1$. No flash-induced redox changes of cytochrome b can be resolved; apparently, under these conditions, the latter is oxidized faster than is reduced (for reviews, see [33,34,36]).

Recently, it has been found that the implementation of Zn$^{2+}$, a well-established inhibitor of the mitochondrial bc$_1$ [57,58], can help to resolve the partial reactions in an intact bc$_1$ even under reducing conditions [54,59]. The Zn$^{2+}$ ions retarded the oxidation of heme $b_h$ and made the kinetics of its reduction visible. In the presence of $\leq$100 μM Zn$^{2+}$, the electrogenic reaction in bc$_1$ slowed down but retained its full extent. The latter observation indicated that the bc$_1$ complexes remained fully functional. The flash-induced oxidation of ubiquinol led to the reduction of heme $b_h$ at 1.5–2 ms. The re-reduction of cytochrome c$_1$ by ubiquinol, the $\Delta \psi$ generation, and the proton release into the chromatophore interior took about 10 ms. The retardation of the latter reactions relative to the heme $b_h$ reduction was apparent even after a weak flash of light, which triggered only one turnover in some bc$_1$ monomers [54,59].

One conclusion from these data was that the turnover of bc$_1$ proceeds in two steps, at least, as depicted in Fig. 4 (see also [54,59]). During the first, faster step (see Fig. 4A), a ubiquinol molecule is oxidized in center P, and two electrons arrive at the FeS cluster and at heme $b_h$, respectively. During the second, slower step protons are released from center P, $\Delta \psi$ is generated, and cytochrome c$_1$
is re-reduced by the FeS cluster, as depicted in Fig. 4B. These slower reactions seem to correlate with the oxidation of heme $b_h$ in center $N$. In this review, we focus on the first, faster step of ubiquinol oxidation. The slower events are considered only in relation to the prevention of short circuits in $bc_1$ (see Sections 2.5.1 and 3.2).

Another conclusion from the data was that the flash-induced reduction of heme $b_h$ was not accompanied by a compatibly fast $\Delta\psi$ generation in an intact $bc_1$ [54,59]. Hence, the transmembrane ET from center $P$ to heme $b_h$ over a distance of $>20$ Å (see Fig. 4) was electrically compensated. This situation differs from that in the RC where the transmembrane charge separation is accompanied by pronounced electrogenesis [38]. Seemingly paradoxical, the electrical silence of the transmembrane ET towards heme $b_h$ could be due to the small driving force of the reaction. As it has been argued elsewhere, the low-exothermic ET reactions in proteins can be controlled/directed by the medium relaxation [60,61]. In other words, if the reaction driving force $\Delta G$ is small, the initial electron exchange between reactants brings the system into an intermediate, unrelaxed state, the energy level of which might lie above that of the initial state (see Fig. 5 and its legend). In this case, the observable ET is driven by the relaxation of this intermediate state into the final one (red arrow in Fig. 5A and the transition between the solid and dashed blue energy terms in Fig. 5B). In other words, the observable ET follows the protein relaxation around the redox center(s). As shown by Dutton et al., the rate constant of ET in proteins depends on the edge-to-edge distance between reactants $R$ as $\log_{10} k = 15 - 0.6R - 3.1(\Delta G + \lambda^2/\lambda)$, where $\lambda$ is the medium reorganization energy [62]. If the ET rate is by orders of magnitude slower than expected from this empirical relation, a relaxation control over ET might be the reason. As it is argued in refs. [60,61], the observable rate of biological ET is likely to be determined by the rate of protein reorganization if $\Delta G|l < 100$ meV.

While the transmembrane charge separation in the RC is a highly exothermic reaction with $\Delta G$ of $< -400$ meV (see e.g., [36]), the driving force for heme $b_h$ reduction by heme $b_1$ is much smaller. The equilibrium constant of the $b_1 \leftrightarrow b_h$ reaction has been shown to be $10^{-15}$ at pH 7 [63], which corresponds to a $\Delta G$ value of electron transfer between $b_1$ and $b_h$ of about $-60/-100$ meV at equilibrium and in the absence of $\Delta\psi$. In the presence of $\Delta\psi$, this counteracts the ET from heme $b_1$ to heme $b_h$, the respective $\Delta G$ value is likely to approach zero, so that electrons can
flow from heme b\(_l\) to heme b\(_h\) only on condition of electrical compensation. In this case, however, the ET from b\(_l\) to b\(_h\) would not be accompanied by notable DC generation, in agreement with experimental observations [18,43,50,54,59,64]. Quite recently, by using ultra-fast microfluidic mixer and freeze-quenching device, coupled with EPR, Zhu et al. succeeded to determine the pre-steady state kinetic of ubiquinol oxidation by bc\(_1\) [65]. The heme b\(_l\) was reduced at 250 μs, simultaneously with the FeS cluster. The heme b\(_h\) was reduced slower, at 2.5 ms (cf. with the above considered Zn\(^{2+}\)-treated chromatophores of Rb. capsulatus [54,59]). The Dutton’s ruler [62] predicts, however, that the ET from heme b\(_l\) to heme b\(_h\), across the edge-to-edge distance of ~10 Å, has to proceed at <1 μs, i.e., by three orders of magnitude faster than was actually observed [65]. Apparently, the reduction of heme b\(_h\) cannot proceed to completion unless compensated by protein.

The electrical compensation of the transmembrane ET could be due to the displacement of protons and/or charged protein groups. Notably, the \(E_m\) value of heme b\(_l\) in Rb. sphaeroides is pH dependent in the whole physiological pH range [66,67], so that at neutral pH the reduction of heme b\(_l\) is likely to be accompanied by protonation event(s). A water chain connecting the propionates of heme b\(_l\) with the water phase from the \(p\)-side of the membrane was predicted from molecular dynamics simulations [68] and found in the high-resolution X-ray structure of the yeast bc\(_1\) [9]. This chain has been already suggested to participate in the electrostatic compensation of heme b\(_l\) reduction [9]. Proton transfer towards the propionate groups of heme b\(_l\) in response to its reduction, as shown in Fig. 4A, would contribute to the electrical silencing of the ET from heme b\(_l\) to heme b\(_h\) because the rate-limiting step of ubiquinol oxidation is coupled with the preceding events in center P, not with the ET from heme b\(_l\) to heme b\(_h\) proper (see [69] and Section 2.5.2). Another thinkable possibility is that the docking of the mobile FeS domain to cytochrome b, kinetically coupled with the reduction of heme b\(_h\) (see Section 2.5 below), might play a part in the electrical compensation of the ET across cytochrome b.

The dielectric relaxation can be also accomplished by dipole reorientation of water molecules, as shown schematically by dotted arrow in Fig. 4A. In the case of Rps. viridis RC, it has been shown that water molecules occupy the Q\(_B\)-binding cavity in the absence of the quinone molecule [70]. The same must be true for bc\(_1\): water molecules are expected to penetrate the enzyme upon the...
quinone retreat/exchange reactions in the four catalytic centers. Although mostly unresolvable in crystal structures, these water molecules still manifest themselves indirectly (see e.g., [71]).

Importantly, a reversion of those reactions that “silence” the electron on its way across the membrane would cause $\Delta \psi$ generation. Such a reversion is expected after the neutralization of the negative charge(s) at cytochrome $b$ upon the reduction and protonation of a ubiquinone molecule in center $N$ [43]. In the Zn$^{2+}$-treated $bc_1$ of $Rb$. capsulatus, the $\Delta \psi$ generation indeed correlated with the oxidation of heme $b_1$ [54,59]. The slowing of $\Delta \psi$ generation and of proton release by Zn$^{2+}$ ions can be explained by a suggestion that the Histidine-rich Zn$^{2+}$-binding patch, as identified in the crystals of mitochondrial $bc_1$ [71], serves as a proton outlet from center $P$ (H-pathway in Fig. 4B). Structurally, the patch resembles the proton inlet of the $Rb$. sphaeroides RC, which can be blocked by Zn$^{2+}$ (see [28], Fig. 3, and Section 2.4). The involvement of the water chain leading to heme $b_1$ in proton release, as suggested by Crofts et al. [72], is less likely. This chain (R-pathway in Fig. 4A) is flanked by two strictly conserved arginine residues, which are expected to hamper the proton release via this way. More likely is the involvement of this water chain in the electrostatic compensation of negative charge at heme $b_1$, as shown in Fig. 4A and discussed above (see also Sections 2.5.1 and 3.2 and refs. [54,59] for further details).

Because the flash-induced redox reactions of cytochrome $b$ are hardly resolvable, many studies of $bc_1$ were done in the presence of antimycin A (hereafter antimycin, see [1,73] for reviews). Antimycin binds close to center $N$ [8,31], blocks the oxidation of heme $b_1$, and allows only a “half-turnover” of the enzyme. In chromatophores from $Rb$. sphaeroides and $Rb$. capsulatus, the $\Delta \psi$ generation and proton release accelerate after the addition of antimycin, as suggested by Crofts et al. [72], is less likely. This chain (R-pathway in Fig. 4A) is flanked by two strictly conserved arginine residues, which are expected to hamper the proton release via this way. More likely is the involvement of this water chain in the electrostatic compensation of negative charge at heme $b_1$, as shown in Fig. 4A and discussed above (see also Sections 2.5.1 and 3.2 and refs. [54,59] for further details).

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2.2. Inhibitor-binding sites in the center $P$ of the cytochrome $bc_1$ complex

Brandt, von Jagow, and their co-workers have identified two different inhibitor-binding sites in center $P$, one for stigmatellin and 3-n-undecyl-2-hydroxynaphthoquinone (UHDBT), and another one for the methoxy-acrylate-stylobene (MOA) and myxothiazol [78,79]. The X-ray structures of mitochondrial $bc_1$ confirmed the existence of two distinct, although partly overlapping inhibitor-binding sites at the junction of the cd1, cd2, and ef helical loops connecting the transmembrane helices of cytochrome $b$ from the p-side of the membrane [5–9,31,80–83]. One of these sites is proximal to heme $b_1$ and binds myxothiazol, MOA-type inhibitors, and a non-oxidizable ubiquinol analogue 2,3,4-trimethoxy-5-decyl-6-methyl-phenol (TMDMP) (see e.g., [81,83]). In the recent study of Esser et al. [83], three highly conserved residues from the cd1 helix, Gly142, Val145, and Ile146 (in bovine nomenclature) were identified as the critical ones for the inhibitor binding in this site. Another inhibitor binding site (the distal one relative to heme $b_1$) is on the interface of cytochrome $b$ and the FeS domain; it binds stigmatellin, UHDBT-type inhibitors, and famaxadone [6,8,9,81–84]. Esser et al. identified a strong involvement of the conserved PEWY motif of the ef loop as the main feature of inhibitor binding in this position [83].

Depending on the presence of inhibitors and crystallization conditions, the FeS domain was found in different positions, indicating its rotation by approximately 60° [5–9,31,82,83]. Stigmatellin, UHDBT, 2-nonyl-4-hydroxyquinoline N-oxide (NQNO), and famaxadon trapped the FeS domain closer to cytochrome $b$, as shown in Fig. 4A, whereas in the absence of these inhibitors or in the presence of myxothiazol the domain was found closer to cytochrome $c_1$, as depicted in Fig. 4B (see [1,32,83] for surveys). It was concluded that the FeS domain moves, upon shuttling an electron from center $P$ to cytochrome $c_1$, from a position where the FeS cluster is close to heme $b_1$ (FeS$_b$ state) into the “cytochrome $c_1$” position, where the cluster interacts with the cytochrome $c_1$ heme (FeS$_c$ state) [5–9]. In addition, Iwata et al. have identified an intermediate, “loose” state of the FeS domain, halfway between these two positions [7]. Esser et al. have exploit the correlation between the position of the FeS domain and nature of inhibitor bound to classify the inhibitors of center $P$ into two groups [83]. According to this classification, the subgroup Pm (mobile FeS domain) includes myxothiazol, MOA-type inhibitors, and azoxy-strobulin, while stigmatellin, famaxadon, and UHDBT-type inhibitors form to the subgroup Pf (for fixed FeS domain).
2.3. Divergence of electron flows in the cytochrome bc₁ complex

The divergent pattern of inhibitor binding in center P and the mobility of the FeS domain are related to the divergence of electron fluxes in the bc₁. As noted in Introduction, it remains debatable why both electrons released at ubiquinol oxidation do not escape via the FeS cluster. The latter, just by turning over twice, could carry both electrons from ubiquinol to cytochromes c. The FeS cluster with its midpoint potential at pH 7.0 (Eₘᵇ) of ≥300 mV seems to be a more attractive oxidant for the semiquinone in center P than the hemes of cytochrome b (see Table 1 for the Eₘ values). The electron bifurcation in center P is rather rigorous: when, in the presence of antimycin, both hemes of cytochrome b become reduced, the turnover rate of bc₁ drops to about 2% of its usual value although both the FeS cluster and cytochrome c₁, fully oxidized under these conditions, are ready to accept electrons from ubiquinol [73]. And the other way around, both hemes of cytochrome b, after being pre-reduced by light flashes in chromatophore vesicles from Rhodobacter capsulatus treated by antimycin, stay reduced on a time scale of seconds [85], although a two-electron reduction of a quinone in center P seems to be thermodynamically favorable. The latter phenomenon is especially striking in the view of general reversibility of bc₁: a quinone in center P can be reduced by electrons coming via the FeS cluster in the presence of external membrane potential (see [86] and references cited therein). A potential danger of short-circuiting in bc₁ has been recently addressed by several authors [20,87,88].

Based on the dependence of inhibitor binding in center P on the redox state of bc₁, Brandt and von Jagow have advanced the general principle of a redox-dependent conformational switch to rationalize the obligatory electron bifurcation in bc₁ [78,79]. The tentative mechanisms of such a switch, as suggested so far, can be subdivided into two major groups. In one set of models, the escape of the second electron into the high-potential branch is prevented by the prompt dissociation of the reactants. It has been suggested that the FeS domain, initially docked to cytochrome b, promptly moves into the FeS₁, position close to cytochrome c₁ after being reduced [89,90]. It has been hypothesized that the oxidized FeS domain cannot dock back unless the heme b₁ is oxidized [89]. In addition to the fast dissociation of the FeS domain from cytochrome b, the possibility of a semiquinone relocation into the alternative quinone-binding site close to heme b₁ has been invoked to explain how the oxidation of semiquinone by the returning oxidized FeS domain is prevented [8,32,69]. This set of models, however, was essentially weakened by the finding that one ubiquinol molecule could be still oxidized in a bifurcated mode in chromatophores of Rh. capsulatus where the FeSₚ → FeSₗ motion was blocked by a mutation [91].

Alternatively, the first electron can get transiently trapped at the FeS cluster. Then, the cluster cannot be reduced anymore, and the second electron is compelled to reduce heme b₁ [8,18,32,51,54,59,92,93]. Initially, this type of mechanism was invoked to explain the faster reduction of heme b₉ as compared to the re-reduction of cytochrome c₁ [51]. Thereby, it was hypothesized that the formation of a semiquinone in center P is coupled with the transition of the center into a “closed” conformation with the Eₘ value of the FeS cluster becoming (transiently) higher than the Eₘ value of cytochrome c₁ [51]. In this state, the FeS domain cannot promptly reduce cytochrome c₁. The hypothesis was based on the observation that the tightly binding stigmatellin, believed to be a semiquinone analogue, increased the Eₘ value of the FeS cluster up to 540 mV in the mitochondrial bc₁ [94]. The “opening” of center P, leading to the decrease in the Eₘ value of FeS and to the transfer of the “trapped” electron to cytochrome c₁, was suggested to happen only after the oxidation of heme b₉ in center N [51]. A semiquinone, which is bound to the FeS-cluster with an elevated Eₘ, was the key element also in the hypothetical model of Link, who, however, assumed that the high-potential state of the FeS cluster persists only until heme b₁ is reduced by the semiquinone [92]. The mobility of the FeS-cluster could be incorporated in the tentative mechanisms of this type by a suggestion that the oxidation of the FeS cluster by cytochrome c₁ is prevented by the transient “locking” of the reduced FeS cluster in the cytochrome b position, as shown in Fig. 4A, too far away from cytochrome c₁ [8,18,32,54]. Kim et al. considered the possibility that the undocking of the FeS domain happens after the oxidation of heme b₁ by heme b₉ [8]. The slowness of the re-reduction of cytochrome c₁ as compared to the reduction of heme b₉ [18,51,54,95–97] indicates, however, that the undocking of the FeS domain happens even later, it seems to correlate with the oxidation of heme b₉, as shown in Fig. 4B (see Sections 2.5.1 and 3.2 for the further consideration of this point).

Elaborated studies in several labs yielded a wealth of functional data on the mobility of the FeS domain and its interaction with cytochrome b and cytochrome c₁, respectively [77,90,91,98–108]. Particularly, Dalda et al. have shown that the Eₘ value of the FeS cluster was elevated up to 460 mV in several site-specific mutants of Rh. capsulatus even in the absence of stigmatellin-type inhibitors. Correspondingly, it was concluded that the elevated Eₘ is the intrinsic property of the FeS cluster in the cytochrome b position [109], in support of the earlier guesses [51,92]. EPR studies showed that, unlike the reduced FeS domain that stays docked to cytochrome b, the oxidized FeS domain resides in an alternative, supposedly cytochrome c₁ position [103,105].
2.4. Ubiquinone processing in the photosynthetic reaction center of Rb. sphaeroides

As described above, the quinol-oxidizing center P of bc$_1$ provides two binding sites for inhibitors, namely a nonpolar one proximally to heme b$_1$, and a more polar distal one, on the interface between cytochrome b and FeS$_b$ (see [1,31,78–81,83] and references cited therein). As noted in the previous section, several authors have considered the possibility that the oxidation of quinol takes place in the distal site, after which the formed neutral semiquinone relocates closer to heme b$_1$ to reduce the latter. It was suggested that the relocation might prevent the escape of the second electron to the FeS cluster (see e.g., [6,8,32,80,110]). A detailed version of such a mechanism has been formulated by Crofts et al. [32,80,110,111].

On presenting their tentative model, Crofts et al. [80] used the analogy with the RC of phototrophic bacteria where the secondary quinone acceptor Q$_B$ is reduced to a quinol Q$_B$H$_2$ via a semi-quinone intermediate Q$_B^\cdot$ (see Fig. 3 for the general scheme and [25,27–29] for reviews). The low-temperature X-ray crystallography of the Rb. sphaeroides RC has indeed revealed two quinone-binding sites in the Q$_B$-binding pocket, at the interface of the H- and L-subunits of the RC [24]. To emphasize the functional difference between them, Stowell et al. have denoted them as active and inactive one, respectively [24]). These two quinone-binding sites also differ in their polarity: the active site that interacts with a hydrogen-bonded water cluster penetrating from the outside is apparently more polar. However, the model of quinol oxidation in bc$_1$ that was suggested by Crofts et al. is not consistent with the situation in the Q$_B$ site of the RC. The semiquinone anion Q$_B^\cdot$ is seen in the polar site, close to the non-heme iron atom [24], opposite to what has been suggested for bc$_1$. Further on, the lifetime of the Q$_B^\cdot$ semiquinone anion reaches minutes at appropriate conditions [112], which indicates its tight binding by the protein and rules out its movement in the site.

Several mutually compatible schemes, where the initial steps of electron and proton transfer to Q$_B$ were arranged with the displacements of quinoline ring, were suggested for the RC of Rb. sphaeroides [24–27,61,113] and of Rps. viridis [23], respectively. It has been even possible to clarify the sequence of events coupled with the last steps of Q$_B$ turnover, when the second electron is transferred to Q$_B^\cdot$ together with two protons and the formed ubiquinol leaves the binding pocket [26,27,61,114,115]. The available data allow to describe the Q$_B$ turnover in the RC as follows (see Fig. 6):

1. The high-resolution low-temperature X-ray structure [24] indicates that the molecules of Q$_B$ ubiquinone are distributed between two binding sites in the ground state. To reach a coherency with the further presentation, the distal, nonpolar “inactive” site (see Panel A of Fig. 6) is hereafter denoted as a stand-by site, whereas the proximal, polar “active” site is denoted as a catalytic site (see Panel B in Fig. 6). The relative occupation of the two sites by ubiquinone seems to differ depending on the preparation studied, conditions, and the length of the ubiquinone tail.

In the case of the isolated RC of Rb. sphaeroides and at room temperature, ubiquinone is predominantly in the catalytic site [116], although functional evidence of heterogeneity of Q$_B$ can be also found in the literature [117–120]. On temperature decrease, quinone relocates into the stand-by site, as it follows from the earlier functional studies [121] and from the low-temperature crystal structure of the RC [24]. In the case of crystal structures of the Rps. viridis RC, the short-tail ubiquinone Q-2 was seen predominantly in the catalytic site, while the native long-tail Q-7 occupied the stand-by position [23].

Apparently, the affinity of the tail to the hydrophobic environment affects the ubiquinone distribution between the two binding sites. When the RCs of Rb. sphaeroides were studied in native chromatophore membranes, ubiquinone seemed to be distributed between the two binding sites [27,113,122]. Apparently, the two ubiquinone positions are almost isoenergetic [25]. Correspondingly, there is some evidence that a partial relocation of Q$_B$ molecules into the catalytic site takes place already in response to the reduction of Q$_A$ [123,124].

2. When Q$_B$ is in the stand-by site, its C4 carbonyl forms a hydrogen bond with the backbone nitrogen of the Ile-224 of the L subunit (Ile-L224, see panel A in Fig. 6).

3. In the catalytic site, as shown in panel B of Fig. 6, the quinone ring makes a 180° twist and comes ~5 Å closer to the conserved Glu-L212 and His-L190 [24]. The latter residue ligates the non-heme iron atom. In this conformation, the C1-carbonyl of Q$_B$ forms a hydrogen bond with Ile-L224 whereas the C4 carbonyl is hydrogen bonded to His-L190. Based on the structural [125,126] and functional evidence [119,120], an additional (weak) hydrogen bond between Glu-L212 and the C3-methoxy oxygen of Q$_B$ was suggested [25,113]; such an interaction could account for the high apparent pH of Glu-L212 (pK$_{a212}$) of ~10.0 [127]. In support of this guess, MD simulations [128,129] and electrostatic calculations [130] indicated that the binding of Q$_B$ in the catalytic position correlated with a protonated state of the Glu-L212 residue [130].

4. The ET from Q$_B^\cdot$ to Q$_B$ results in a formation of a tightly bound semiquinone anion Q$_B^\cdot$. The respective X-ray structure (see Panel C of Fig. 6) shows that the whole Q$_B^\cdot$ population is now in the catalytic site [24]. MD simulations indicated that the Q$_B^\cdot$ semiquinone is essentially stabilized by its interaction with iron [131]. The X-ray structure of the RC with a light-trapped Q$_B^\cdot$ shows that he latter is also...
stabilized by hydrogen bonds of the C1-carbonyl with Ser-L223, Ile-L224, and Gly-L225, of the C4-carbonyl with His-L190, and of the C2-methoxy group with Gly-L225 and, perhaps, Thr-L226. The negative charge of Q_{B^-} shifts \( pK_{212} \) to >12.0 [132].

5. The transfers of the second electron and of the first proton to Q_{B^-} are tightly coupled. As depicted in Fig. 3, protons enter the RC via a histidine-rich patch that can be blocked by Zn\(^{2+}\) ions (see [28] for a review). The fast protonation of Q_{B^-} to Q_{BH}\(^{+}\) is followed by a rate-limiting ET reaction [28,114]. The formed ubiquinone-anion Q_{BH}\(^{-}\) remains bound by the RC [133]. As it is depicted in Panel D of Fig. 6, Q_{B^-} is believed to bind in the catalytic site similarly to stigmatellin, which is assumed to be an analogue...
of a ubiquinol–anion (see [23] and Fig. 9 in Section 3.1 below).

(6) The second proton is likely to be donated to Q$_{b}$H$^{-}$ by Glu-L212 [127,134]. The transfer of the second proton proceeds with a high activation energy ($E_a$) of approximately 60 kJ/mol [115]. According to the kinetic analysis [26], this reaction step is governed by the detachment of the quinol ring from His-L190 and by its rotational movement towards the stand-by site, where the ubiquinol Q$_{b}$H$_2$ is seen in the respective crystal structure (see panel E of Fig. 6 and [135]). Upon this ubiquinol retreat, the water molecules enter the Q$_b$ pocket and form a bridge between Glu-L212 and His-L190. As long as Glu-L212 is involved in this bridge as a hydrogen bond acceptor, its $pK_{212}$ dramatically decreases, so that the protonation of Q$_{b}$H$^{-}$ to Q$_{b}$H$_2$ by Glu-L212 becomes irreversible [25,26]. The MD simulations essentially confirmed this scenario [129].

Hence, the proximal Q$_b$-binding site of the RC, which is formed by His-L190 connected with an iron atom, binds the charged species, namely Q$_{b}^{-}$ and Q$_{b}$H$^{-}$, and serves as the catalytic center where the reactions of reduction and protonation take place. The alternative site binds the uncharged species Q$_{b}$ and Q$_{b}$H$_2$ and serves as a stand-by position for a quinone/quinol molecule. The highest activation barriers on ubiquinone reduction seem to be coupled with the journeys of the quinone ring between the stand-by and catalytic sites [26,27,61,115,122]. It is likely that a prompt relocation of the freshly formed ubiquinol into the stand-by site prevents the potentially detrimental reversion of the reaction.

2.5. The catalytic cycle of ubiquinol oxidation by the cytochrome bc$_1$ complex as a reversal of the ubiquinone reduction cycle in the RC

In the previous section, the catalytic cycle of Q$_b$ was described in such detail because just the reversal of this cycle and its application to the bc$_1$ can yield a coherent ubiquinol oxidation picture that is in agreement with available data. The similarity between the Q$_b$ site of the RC and the center $P$ of bc$_1$ is not limited to the presence of two quinone-binding loci and of an iron atom in the vicinity. The high-resolution X-ray structure of the yeast bc$_1$ [9] shows stigmatellin, a supposed analogue of a ubiquinol-anion [23], bound between His-181 of the Rieske protein and Glu-272 of cytochrome $b$. This arrangement resembles the situation in the RC where His-L190 and Glu-L212 are involved both in Q$_b$ binding and stabilization of the Q$_b^{-}$ semiquinone (see Fig. 6 in Section 2.4 and also Fig. 9 in Section 3.1 below). The list of analogies can be continued, but instead, it seems worthy to consider what happens if the cycle of ubiquinone reduction in the RC, as depicted in Fig. 6 and as described above, is “turned around” in the center $P$ of bc$_1$, as depicted in Fig. 7 and described below.

2.5.1. Mechanistic scheme of ubiquinol oxidation

Fig. 7 shows a hypothetical scheme of ubiquinol oxidation in bc$_1$. Its relation to other tentative schemes of center $P$ operation is discussed in Section 4. For coherency with previous models, the avian numeration of the key residues is used. The Glu-272 of the avian cytochrome $b$ corresponds to Glu-271 of beef bc$_1$, Glu-272 of yeast bc$_1$ and Glu-295 of Rb. capsulatus bc$_1$. The counterparts of His-161 in avian bc$_1$ are His-161 in beef bc$_1$, His-181 in yeast bc$_1$, and His-156 in the bc$_1$ of Rb. capsulatus.

The key feature of the scheme is the involvement of two binding sites for the neutral ubiquinone/ubiquinol molecules but of only one binding locus for the anionic forms of ubiquinol and of ubisemiquinone.

(1) The cycle starts from a ubiquinol Q$_{b}$H$_2$ residing within cytochrome $b$ (Panel A of Fig. 7). Most likely, Q$_{b}$H$_2$ resides proximally to heme $b_1$, in the position of Pm-type inhibitors (see Section 2.2 above). It is noteworthy that a non-oxidizable ubiquinol(!) derivative TMDBP has been reported to bind close to the myxothiazol-binding pad in the respective crystal structure [81]. As well, the photoaffinity labeling of the bovine bc$_1$ by ubiquinone analogue yielded crosslinks with the amino acid residues of the cd1 helix that is crucial for binding of the myxothiazol-type inhibitors (see [83] and Section 2.3 above), but not with the residues of the ef loop, which is crucial for binding of stigmatellin or UHDBT. The ubiquinol proper, however, is not seen in this position in the crystal structures. Therefore, it cannot be completely ruled out that the only stable quinol/quinone binding site is in the center N, which is occupied in the X-ray structures, and that the binding site of Pm-type inhibitors is only transiently “visited” by a ubiquinol molecule upon its re-location from center N into the catalytic site in center $P$.

(2) The catalytic site is formed at the interface of cytochrome $b$ after docking of the FeS domain and corresponds to the binding site of the Pf-type inhibitors (see panel B of Fig. 7, Section 2.2 above, and refs. [9,31,83]). The EPR data indicate that the affinity of the oxidized FeS domain to the docking interface of cytochrome $b$ is low [103]. Still ubiquinol does bind to the oxidized FeS domain; the stigmatellin- and UHDBT-containing crystal structures of bc$_1$ allowed several authors to model how ubiquinol forms a hydrogen bond with the His-161 residue of the Rieske protein [9,72,82,136], as it is depicted in panel B of Fig. 7. To form this bond, His-161 has to be deprotonated. The available estimates give values in the range of 6.0–8.0 for the apparent $pK$ of this histidine residue [82,92,137–140]. It was argued [9,72,82], based on the inhibitor-containing crystal structures of bc$_1$, that the second hydroxyl group of ubiquinol forms a hydrogen bond with Glu-272 of cytochrome $b$ (as it is shown in panel B of Fig. 7).

(3) In almost all current models, the oxidation of Q$_{b}$H$_2$ to ubisemiquinone by the FeS cluster is coupled to its deprotonation to a ubiquinol anion (see panel C of Fig. 7). As argued by Rich, the redox potential of the Q$_{b}$H$_2$/QH$_2^-$ couple is >850 mV, therefore the ubiquinone-anion Q$_{b}$H$^{-}$, with estimated $E_m$ of ~190 mV, is the form that can be
oxidized in biological ET [141]. The pK of the QH$_2$/QH$^-$ couple, deduced from model systems [141] and from the properties of Q$_b$H$_2$ in the RC [26,142], is expected in the range of 8.5–11.0. At least two tentative reaction routes could lead to a ubisemiquinone in center $P$:

(i) In one mechanism, the relocation of the first proton from Q$_b$H$_2$ to His-161 of the FeS domain is followed by the electron transfer from Q$_b$H$_2$ to the FeS cluster yielding a neutral ubisemiquinone (see e.g., [1,19,32]). In this mechanism, which can be named “FeS-first”, no genuine Q$_b$H$_2$
anion is formed; the first proton does not go away, but just shifts along the hydrogen bond between QbH2 and His-161 by approximately 1 Å. Therefore it remains unclear whether this minor proton shift is sufficient to decrease the oxidizing potential of QbH2 from the estimated >850 mV to an acceptably low value. The formed neutral semiquinone QbH+ is likely to give away the proton (as long as the pH of the QbH+/Qb− couple is expected to be on the order of 5.0, see the considerations below, in Section 4.1), leaving a ubisemiquinone anion radical Qb− in the catalytic center. The most probable acceptor of the second proton is then the Glu-272 residue of cytochrome b [19].

(ii) Alternatively, it is imaginable that Glu-272 of cytochrome b might serve as an acceptor of the first proton (“Glu-272-first” mechanism). Indeed, Covian and Moreno-Sanchez have shown that quinol binding to bc1 requires a de-protonated state of a group with pH of 5.2–5.7 and attributed this group to Glu-272 [139]. The latter residue can attain two different configurations (see Fig. 7 and also Fig. 4 in Section 2.2): while in the stigmatellin-containing bc1 structure Glu-272 forms a hydrogen bond with the inhibitor [9], in those X-ray structures of bc1 where center P is empty [7,72], the side chain of Glu-272 points towards the heme b1 (see Fig. 9 in Section 3.2. below). Thus, Glu-272 shuttles between two conformational sub-states, turning either towards the FeS cluster on ubiquinol binding (Glu-272FeS) or towards heme b1 on the retreat of the quinone ring (Glu-272bl) (see refs. [19,72]). Because the Glu-272FeS → Glu-272bl equilibrium is shifted to the right when center P is empty [7,72], the estimate of the functional pH of Glu-272FeS as obtained by Covian and Moreno-Sanchez [139], relates apparently to Glu-272bl. The yet unknown functional pH of Glu-272FeS in the presence of quinol/semiquinone is expected to be higher than that of Glu-272bl because the polarity of the pocket is likely to decrease with the intrusion of the ubiquinol ring and the water retreat. The example of the RC shows that the functional pH value of Glu-L212 residue interacting with the Qb ring is about 9.0–10.0 [127,143]. The pH value of the proton-transporting Glu-286 in the cytochrome c oxidase has been estimated as high as 9.4 (see [144] and references therein). If the functional pH of Glu-272FeS is compatible high, this residue can outdo His-161 in accepting the first proton from Qh2 to yield a Qh2− anion (see panel C of Fig. 7). In the “Glu-272-first” mechanism, a subsequent coupled transfer of an electron and a proton from Qh2− to the FeS domain would yield a bound Qh2− semiquinone anion in the catalytic site, so that at this point of the catalytic cycle, the two tentative mechanisms converge (panel D of Fig. 7). In fact, the Glu-272 residue, which is halfway between two quinol-binding sites, is likely to encounter the ubiquinol before His-161. By analogy with the Qb turnover, it is expected that the iron-containing catalytic site has high (electrostatic) affinity for negatively charged Qh2−/Qh− species. Then, by accepting the first proton, Glu-272 might facilitate the formation of a histidine-mediated complex between the ubiquinol-anion and the oxidized FeS cluster. It is noteworthy that in this case, which is depicted in Fig. 7, the catalytic cycle of ubiquinol oxidation represents an exact reversion of the ubiquinone reduction cycle in the RC (cf. with Fig. 6).

(iii) Finally, it is also imaginable that proton attraction by both Glu-272 and His-161 is needed to promote a prompt oxidation of ubiquinol by the FeS cluster (see e.g., [136]). Resolving this question would require further experimental studies.

By analogy with Qb− in the RC, a tight binding of the Qb− semiquinone to the reduced FeS domain is expected. Such a binding, on one hand, would keep the reduced FeS domain in the cytochrome b position and prevent the escape of the second electron to the FeS cluster (see also Section 3.1 below) and, on the other hand, would make the semiquinone EPR invisible due to its antiferromagnetic interaction with the iron (see refs. [92,145] and the discussion in Section 4.4).

(4) The redox potential of the semiquinone in center P is likely to resemble those of Qb− or Qc− in the RC, that is, to be in the range of −200/−50 mV [146–150]. Indeed, the polarity of the environment resembles that in the quinone-binding sites of the RC, not to mention the similarity between the putative ligands of Qb− and the intimate neighbors of Qb−. Because of the ability of the Qb− semiquinone to reduce oxygen at steady state [151], the most realistic estimate range is −200/−150 mV (The equilibrium Q− + O2 ↔ Q + Qb− is shifted to the right already at Enm(Q/Qb−) < −150 mV [152]). This value is low enough to enable the reduction of heme b1. The Qb− → Q transition is most probably coupled with the rotation of the side chain of the protonated Glu-272 towards heme b1, as depicted on panel E of Fig. 7. The latter suggestion agrees with the observation that Glu-272 does not form a bond with a deprotonated oxygen atom of a quinone analogue, 5-n-heptyl-6-hydroxy-4,7-dioxobenzothiazole [82]. After turning towards heme b1, the protonated side chain of Glu-272 contributes to the stabilization of the negative charge at cytochrome b (see also Section 3.3). The negative charge might be also compensated by proton redistribution along the water chain, which connects the heme b1 with the p-surface of the membrane (see Fig. 4A in Section 2.1 and Panel E of Fig. 7). On the next step, electron is transferred towards heme bh at approximately 2 ms [65,85]. The relatively slow time constant indicates that the reaction proceeds on condition of electrostatic compensation (see Section 2.1).

After the reduction of cytochrome b, a neutral quinone is left in the catalytic site (panel E of Fig. 7). The reduced and protonated FeS domain can now, in principle, undock and move to cytochrome c1. This seems to happen, however, only in the presence of antimycin (see Sections 2.1 and 3.2). In the intact bc1, the re-reduction of cytochrome c1 by electrons coming from ubiquinol is delayed as compared to the reduction of heme bh both in chromatophores of Rh. capsulatus [18,36,48–50,54,59] and in the preparations of isolated mitochondrial bc1 [65,96,97]. The “trapped”
reduced FeS cluster prevents a futile electron escape from the 
b-chain into the c-chain (see Section 3.2 for a detailed 
consideration of this point).

(5) The undocking of the FeS domain and its relocation 
towards cytochrome $c_1$ (panel F of Fig. 7) seems to be coupled 
with the oxidation of heme $b_h$ and ubiquinone reduction in 
center $N$ [51,54,59]. This suggestion was based on the 
kinetic correlation between the re-reduction of cytochrome 
$c_1$ and the oxidation of heme $b_h$ [36,48–50,54,59,95–97], 
especially under single-turnover conditions [54] (see a more 
detailed discussion in Section 3.2). As depicted on panel F of 
Fig. 7 and in Fig. 4B above, (i) the reduction of a quinone/ 
semiquinone in center $N$ by heme $b_h$ is accompanied by 
proton binding from the n-side of the membrane; (ii) the pK 
of the Glu-272 residue decreases to the initial acidic value 
because no surplus negative charge is left at cytochrome $b$ 
(see Section 3.3), so that Glu-272 releases its proton, 
operating as a pK switch (see [25] for definition of a pK- 
switch); (iii) other protons, which, supposedly, were com- 
penating the negative charge on cytochrome $b$, are released 
from the p-side as well; (iv) the reduced and protonated FeS 
domain moves towards cytochrome $c_1$, reduces the latter, 
and deprotonates. The transfer of all these charges across the 
membrane dielectric, together with the re-orientation of the 
intramembrane water dipoles (see Section 2.1), account for 
a large electrogenic reaction, the rate of which correlates 
both with the rate of the cytochrome $c_1$ re-reduction and the 
rate of proton release into the chromatophore interior 
[18,54,59]. The (i) kinetic correlation between proton 
release/voltage generation and cytochrome $c_1$ re-reduction and the 
(ii) concurrent slowing of these reactions by Zn$^{2+}$ 
ions (see Section 2.1 and [54,59]) can be explained as 
follows. The histidine-rich patch of cytochrome $b$, which 
binds Zn$^{2+}$ in the mitochondrial $bc_1$ [71], can serve, on 
one hand, as a proton outlet from center $P$ (as discussed 
in Section 2.1, see Fig. 4) and, on the other hand, can 
interfere, via the ef loop of cytochrome $b$, with the 
movement of the FeS domain to cytochrome $c_1$ (as 
discussed in more detail in Section 3.2). The putative 
molecular mechanisms, which might kinetically couple the 
electrogenic proton release from center $P$ with the re- 
duction of cytochrome $c_1$ by the FeS domain, are 
considered in more detail elsewhere in relation to the 
experimental data on electrogenic proton transfer [54,59].

2.5.2. Rate-limiting step of ubiquinol oxidation

The scheme in Fig. 7 provides a new insight on the 
possible nature of the rate-determining step in ubiquinol 
oxidation. In model systems, it has been shown that the rate 
of quinone oxidation is limited by the formation of a complex 
between a quinol-anion $QH^-$ and the electron acceptor [153– 
155]. In the case of $bc_1$, Crofts et al. have suggested, however, 
that the partial reaction with the limiting rate and the highest 
activation energy was the oxidation of the bound ubiquinol 
to a semiquinone (C → D transition in Fig. 7, see [69,110,156]). 
Turning to the analogy with the RC, it is worth noting that the 
respective reverse $QH^+ \rightarrow QH_2^-$ reaction (see the C → D 
transition in Fig. 6) seems to proceed without relocation of 
quinone ring [23,26,133] and with an activation energy of 
only about 10 kJ/mol [115]. There are no evident structural 
or chemical reasons to expect high activation barrier for the 
congruent reaction step in $bc_1$.

Crofts et al. have ruled out the formation of the 
substrate-enzyme complex as the main activation step 
because the measured $E_a$ of ubiquinol oxidation was 
independent of the redox state of the quinone pool and, 
hence, of the quinol presence in the binding site before the 
flash [69,110,156]. Thereby, it was presumed that there is a 
single quinol binding site in center $P$, on the interface 
between cytochrome $b$ and the oxidized FeS domain. 
Alternatively to this view, the scheme in Fig. 7 implies two 
quenol-binding sites in center $P$, namely the permanent 
stand-by site within cytochrome $b$ and a catalytic site, 
which forms only transiently after the docking of the 
oxidized FeS domain. A stand-by site for ubiquinol is 
dispensable because the quinol molecule has to reside 
somewhere when the FeS domain is not docked to 
cytochrome $b$. Correspondingly, the main activation step 
of ubiquinol oxidation could be coupled with the move- 
ment of ubiquinol from this stand-by site into the 
transiently formed catalytic one and with the formation of a 
complex between the oxidized FeS domain and the 
ubiquinol ring. The activation barrier for this reaction 
would be independent of redox potential because the latter 
correlates with the incidence of quinol in the stand-by site, 
but not in the catalytic one, which, under any conditions, 
forms only transiently. The quinol binding to the FeS 
domain is coupled with the counter-movements (i) of the 
quenol ring into the catalytic site and (ii) of the oxidized 
FeS domain towards cytochrome $b$. The data on site- 
specific mutants [109] indicate that the hinge region of the 
Rieske protein needs to be strained to enable the docking 
of the FeS domain to cytochrome $b$ (“spring loaded mechanism” [111]). To explain the high $E_a$ value of 
ubiquinol oxidation, Krshatalik has invoked the necessity 
to overcome the mutual repulsion of amino acid side 
chains upon docking of the FeS domain to cytochrome $b$ 
[157]. Moreover, the side chain of Glu-272 has to turn 
towards the FeS cluster upon quinol binding. All these 
reactions can account for a relatively high $E_a$ value. The 
rate of the quinol oxidation, if limited by the docking of the 
FeS domain, would depend on viscosity, in agreement 
with recent observations of Cramer et al. made with the 
chloroplast $bf$ complex [158]. The suggestion is also 
corroborated by the observation of Yu et al. who have 
found that the activation energy of the steady $bc_1$ turnover 
correlates with the rigidity of the hinge region of the 
Rieske protein [98]. In the $Rb. sphaeroides$ RC, exactly the 
considered reaction, although going in the opposite direc- 
tion, is characterized by the highest activation energy of 60 
kJ/mol and is the rate-limiting step of the whole $Qb$ turnover, 
at least at $t < 20^\circ$C [26,115]. Although the $QbH^- \rightarrow QbH_2$
reaction in the RC is coupled with a proton transfer event, its activation energy was shown to be independent of pH at 6.0 < pH < 8.1 [115], which indicates that proton transfer per se was not limiting. This situation again resembles the situation in bc₁, where the $E_a$ value of quinol oxidation in response to a single flash was shown to be pH-independent in the range of 5.5 < pH < 9 [69].

Assuming that stigmatellin, in the X-ray structures of bc₁, occupies the site where the Q₉⁻ semiquinone is bound, the edge-to-edge distance between Q₉⁻ in the catalytic site and heme $b_1$ can be expected to be about 11 Å. From the empirical rate/distance dependence for ET in proteins, as put forward by Dutton et al. [62,159] and as given in Section 2.1, the time constant of semiquinone Q₉⁻ oxidation by heme $b_1$ can be predicted to be $\leq 10^{-3}$ s under the assumptions that (i) the $\Delta G$ value of the reaction is about $-100/0$ meV [69], and (ii) the value of $\lambda$ is about 1.0 eV, which is typical for proteins [29,114,157,159,160]. This estimate is compatible with the time constant of 3 μs, as found for the ET between Q₉⁻ and Qₐ in the edge-to-edge distance of 13 Å in the RC of Rb. sphaeroides [161]. Hence, the oxidation of the Q₉⁻ semiquinone in the catalytic site by heme $b_1$ is expected to be fast enough and not to hamper the steady enzyme turnover by semiquinone accumulation in center $P$. Hence, there is no need to invoke a relocation of a semiquinone closer to heme $b_1$ as suggested in [32].

The actual rate of heme $b_1$ reduction is slower than estimated above, ~250 μs, but it is apparently limited by the preceding reaction steps [65].

2.5.3. Energy profile of ubiquinol oxidation

The corresponding energy diagram is depicted in Fig. 8. Here, the first reaction step can be written as

$$
Q_PH_2 + FeS^{ox} + b_1^{ox}b_h^{ox} \rightarrow Q_PH^- + H^+ + FeS^{ox}_b + b_1^{ox}b_h^{ox}
$$

where the first proton (H⁺) is accepted either by His-161 residue of the FeS domain or by Glu-272 residue of cytochrome $b$ (see the considerations in Section 2.5.1). Although the electrostatic attraction between QH⁻ and the iron atom(s) might contribute to the “trapping” of the FeS domain, the available structural and functional data indicate that the affinity of the oxidized FeS domain to the docking site at cytochrome $b$ is low [103,109,162]. Therefore the Q₉⁻H⁻ + H⁺FeS₉²⁺b₁₂⁻b₉²⁻ state, an unstable intermediate, is placed high on the energy diagram (see Fig. 8). The top of the activation barrier corresponds to the Q₉H₂FeS₉²⁻b₁²⁻b₉²⁻ state, which is supposed to be extremely unstable (this state is shadowed in Fig. 8). Correspondingly, the reaction (1) would be endothermic and would account for the major part of the activation barrier of the overall transition ($E_a^{conf}$).
The next step is the reduction and protonation of the FeS domain:

$$Q_b^\bullet H^+ \cdot FeS^{ox}_{b} h^ox_h \rightarrow Q_b^\bullet H^+ \cdot FeS^{red}_{b} h^ox_h$$

(2)

This reaction contributes to the stabilization of the FeS domain in the cytochrome $b$ position as long as the reduced FeS domain, unlike the oxidized one, shows affinity to the docking site at cytochrome $b$ [103, 106, 162]. As well, the $\sim 150$ mV increase in the $E_m$ value of the FeS cluster upon its docking to cytochrome $b$, as shown for the Rb. sphaeroides $bc_1$ ([107, 109], see also Section 3.2), would additionally stabilize the $Q_b^\bullet H^+ FeS^{red}_{b} h^ox_h$ state. In this case one can speak about a kinetic trapping of the FeS domain by the reduction of its FeS cluster, similarly to the kinetic trapping of oxygen in the cytochrome $c$ oxidase [163]. This reaction step (2) is exothermic and drives the quinol oxidation. This ET reaction is characterized by its characteristic activation energy, $E_a^{ET}$, which, together with $E_a^{conf}$, account for the overall activation barrier of the quinol oxidation (see Fig. 8). The $Q_b^\bullet H^+ FeS^{red}_{b} h^ox_h$ state can be considered as a metastable intermediate as long as it can manifest itself via the interaction with oxygen and superoxide generation [87, 151, 164, 165].

The following electron transfer steps, first from $Q_b^\bullet$ to heme $b_1$ (reaction (3)), and then from heme $b_1$ to heme $b_h$ (reaction (4)) contribute to the further stabilization of the system:

$$Q_b^\bullet H^+ FeS^{red}_{b} h^ox_h \rightarrow Q_b^\bullet H^+ FeS^{red}_{b} h^ox_h$$

(3)

$$Q_b^\bullet H^+ FeS^{red}_{b} h^ox_h \rightarrow Q_b^\bullet H^+ FeS^{red}_{b} h^ox_h$$

(4)

The rate of ubiquinol oxidation can be then written as:

$$v \approx A \cdot e^{-E_a^{ET}/RT} \cdot [Q_bH_2]^* \cdot [FeS^{ox}_{b} h^ox_h]$$

(5)

where $A \cdot e^{-E_a^{ET}/RT}$ is a pH-independent activation term (rate constant), $[Q_bH_2]^*$ is a redox-dependent term reflecting the amount of available ubiquinol, and $[FeS^{ox}_{b} h^ox_h]$ is a pH- and redox-dependent term that reflects the availability of $bc_1$ complexes with oxidized and de-protonated electron/proton acceptors in the appropriate conformational states. In other terms, ubiquinol binding and oxidation could proceed if the FeS cluster and heme $b_h$ are oxidized, the immediate acceptors of protons from $Q_bH_2$ (His-161 of FeS and Glu-272 of cytochrome $b$ in our scheme) are de-protonated, the FeS domain is in the FeS$_{S}$ state, and the Glu-272 residue is in the Glu-272$^{\text{Glu}}$ conformation. It is noteworthy that the requirement of de-protonated proton acceptors implies that the rate of the quinol oxidation, contrary to $E_a$, would decrease with acidification, in agreement with experimental observations [166].

With site-specific mutants, it has been shown that the rate of the heme $b_h$ reduction slowed down with the decrease in the midpoint potential of the FeS cluster both in chromatophores of Rb. sphaeroides in the presence of antimycin [166] and in the isolated preparations of the yeast $bc_1$, which were studied in a pre-steady state mode [167]. The slowing, however, was weak as the $E_m$ value of the FeS cluster was decreased from approximately 300 mV to approximately 240 mV, but became more pronounced with the further decrease in $E_m$ [166, 167]. The energy diagram in Fig. 8 shows how the relation between $E_a^{conf}$ and $E_a^{ET}$ can determine whether the reaction rate depends on the $E_m$ value of the FeS cluster or not. No notable dependence on $DG^{ET}$ is expected as long as the activation barrier of the ET reaction (2), $E_a^{ET}$ in Fig. 8, stays lower than the activation barrier of the reaction (1) (this situation would correspond to a moderate decrease in the $E_m$ value of the FeS cluster). Upon the further decrease in the $E_m$ value of the FeS cluster, the activation barrier of the ET reaction (2) grows (see $E_a^{ET}$ (mut) in Fig. 8) and can become higher that that of the reaction (1). Then the reaction (2) would contribute to the activation barrier of ubiquinol oxidation, and a Marcus-type dependence on $DG^{ET}$, with reaction rate changing by a factor of 10 per 120 mV change in $DG^{ET}$ [168], could be expected, in agreement with experimental observations [166, 167].

### 3. Protection from short-circuits in the cytochrome $bc_1$ complex

As it has been already discussed in the literature (see e.g., [104]), the major task of the $bc_1$ machinery is to avoid an “energy collapse” that would happen if both electrons slip from $Q_bH_2$ into the high potential $c$-chain (the possible short-circuit routes are considered in Refs. [20, 165]). The mechanistic scheme of quinol oxidation in Fig. 7 implies three traits that can protect the $bc_1$ from short-circuiting.

#### 3.1. Binding of $Q_b^\bullet$ to the FeS domain

By analogy with the $Q_b^\bullet$ binding in the RC, the $Q_b^\bullet$ semiquinone is expected to be tightly bound/stabilized by its interaction with the iron of the FeS cluster [51, 92, 137], Fig. 9 compares the relative arrangements of iron and stigmatellin in the $Q_b$-binding site of the Rps. viridis RC [169] and in the center $P$ of the yeast $bc_1$ [9] (because no structures of $bc_1$ with a semiquinone in center $P$ are available, the stigmatellin is considered as an analogue of an anionic semiquinone/ ubiquinol [169]). The structural similarity is evident: in both cases the interaction with iron is mediated by a histidine ring, with the same distance of 6.67 Å between the iron atom and the nearest oxygen of stigmatellin. Molecular dynamics simulations of the RC showed a strong interaction between the negatively charged $Q_b$ species and the iron atom [131], which, apparently, accounts for the high stabilization of the $Q_b^\bullet$ semiquinone. This stabilization manifests itself in the long lifetime of $Q_b^\bullet$ that can reach minutes under appropriate conditions [112]. The structural congruency, as seen in Fig. 9, implies a comparably tight binding of the semiquinone to the FeS domain in $bc_1$. The bound $Q_b^\bullet$ is likely to “hold” the reduced FeS domain and prevent its relocation towards
cytochrome $c_1$. Then, the second electron is compelled to reduce heme $b_1$.

### 3.2. FeS-lock mechanism

As noted in Sections 2.1 and 2.5.1, the re-reduction of cytochrome $c_1$ by electrons coming from ubiquinol is delayed as compared to the reduction of heme $b_1$. Hence, the FeS domain seems to stay transiently locked in the cytochrome $b$ position even after the Q$_P^-$ semiquinone is oxidized to Q$_P$. This “FeS-lock” might be important under the coupled conditions, where the oxidation of ubiquinol is impeded by the backpressure from the membrane potential. Indeed, the small edge-to-edge distances of <11 Å between heme $b_1$, heme $b_h$ and Q$_N$ as well as minor differences in their midpoint potentials imply that an electron, after entering the $b$-chain, remains essentially equilibrated between the low-potential redox components of $bc_1$. Moreover, edge-to-edge distance between two $b_1$ hemes in the $bc_1$ dimer is also <11 Å, so that electrons, according to the rate/distance dependence for biological ET [159], are likely to equilibrate between two low-potential ET branches of the $bc_1$ dimer on the time scale of the turnover. This possibility of electron exchange between the monomers was considered by several authors (see e.g., [5,170–173]) and was explicitly incorporated in some structure-based schemes of the dimeric Q-cycle [18,20]. Then, if the FeS domain is not constrained in its shuttling between cytochrome $c_1$ and cytochrome $b$, the electron can be driven back into center $P$ by $\Delta \psi$ and eventually can even escape via an oxidized FeS domain (e.g., using a quinone molecule in center $P$ as a ET bridge). Therefore, it seems advantageous to prevent the oxidation of the FeS domain until the turnover of $bc_1$ reaches some point of “no return”. As argued elsewhere [51,54,59], such a point of “no return” might be the oxidation of heme $b_h$ leading to the ubiquinol formation in center $N$.

The retardation of the cytochrome $c_1/f$ re-reduction as compared to the reduction of cytochrome $b$ seems to be a phenomenon that is common both to $bc_1$ and to $bf$ complexes. Besides being observed in the flash experiments with photo-trophic bacteria [18,36,48–51,54], this phenomenon was also seen in pulsed experiments with the isolated intact mitochondrial $bc_1$ as studied by stop-flow [95,96] and by using “caged” ubiquinol [97]. Similar observations were done with the cytochrome $bf$ complexes of green plants [174–177]. In many of these cases, the cytochrome $c_1/cytochrome\ f$ re-reduction correlated with the oxidation of heme $b_h$.

In the case of the cytochrome $bf$ complexes, the kinetic mismatch became accepted (see e.g., [1]) after Cramer et al. have provided a key evidence for the absence of a tight coupling between the reduction of cytochrome $b$, from the one side, and the reduction of cytochrome $f$ and electrogenesis, from the other side. These authors have found out that mutations of amino-acid residues in cytochrome $f$ retarded both the reduction of cytochrome $f$ and the generation of the transmembrane voltage without affecting the rate of cytochrome $b$ reduction. Thereby, the latter reaction was distinctly faster than the former two [178]. Further on, the kinetic discrepancy between cytochrome $b$ reduction and the re-reduction of cytochrome $f$ increased with the increase in the luminal viscosity [158].

In the case of $bc_1$, the cause of the kinetic disparity is still under debate (see e.g., [179]). The studies of intact $bc_1$ under single-turnover conditions, which could be created, even in the absence of antimycin, by substrate shortage [18,96,97,180] or by weak light flash (see Section 2.1 and [51,54,59]), seem to be instrumental in solving this controversy. As well, the addition of Zn$^{2+}$ ions to the intact, membrane-embedded $bc_1$ of Rh. capsulatus increased the kinetic mismatch between the faster reduction of heme $b_h$ and the slower cytochrome $c_1$ re-reduction and voltage generation up to a factor of ~10 [54,59], making the discrepancy unambiguous (the resultant scheme of a two-step operation of $bc_1$ is depicted in Fig. 4 and briefly discussed in Sections 2.1 and 2.5.1).

A direct evidence of electron trapping by the FeS domain has been recently obtained by Zhu and co-workers who used ultrafast microfluidic mixer and freeze-quenching device, coupled with EPR (see [65] and Section 2.1). These authors succeeded to determine the pre-steady state kinetic of ubiquinol oxidation. The FeS cluster was reduced, after a 100µs lag, with half time of 250µs. A similar reduction kinetic was also observed for cytochrome $b_1$ indicating simultaneous reduction of both the FeS cluster and heme $b_1$. The time constants of ~2.5 ms and ~6 ms were observed for the reduction of heme $b_h$ and cytochrome $c_1$, respectively, in good

![Fig. 9. Binding of stigmatellin in the Q$_h$ site of the RC (A) and in the center $P$ of $bc_1$ (B). Color code as in Fig. 3. (A) Stigmatellin binding in the RC of Rps. viridis as seen in the respective crystal structure (PDB entry 1PRC [23]). (B) Stigmatellin binding in center $P$ of yeast $bc_1$, as seen in the respective crystal structure (PDB entry 1KYO [10]).](image-url)
correspondence with the respective time constants, as found in the \( \text{Zn}^{2+} \)-treated chromatophores of \( \text{Rh. capsulatus} \) [54,59].

Mechanistically, the \( \text{FeS}_b \rightarrow \text{FeS}_c \) transition can be retarded in two ways, namely: (i) if the \( E_m \) of \( \text{FeS}_b \) is much higher than that of \( \text{FeS}_c \); in this case, the \( \text{FeS}_b \rightarrow \text{FeS}_c \) transition goes energetically uphill and would be retarded even if the intrinsic constant of the \( \text{FeS}_b \rightarrow \text{FeS}_c \) movement is fast; (ii) if the return of the \( \text{FeS} \) domain into the \( \text{FeS}_c \) position is conformationally constrained. These two possibilities are not mutually exclusive and there is experimental evidence in favor of both of them, so that most probably some combination of (i) and (ii) is involved. Particularly, Daldal et al. have shown that the \( E_m \) value of the \( \text{FeS} \) cluster was increased, up to 460 mV, in the site-specific mutants of \( \text{Rh. capsulatus} \) with hinge domain of the Rieske protein elongated by the addition of alanine residues [109]. Correspondingly, it was concluded that a tight inhibitor binding is not a precondition for an increase in \( E_m \), and that the elevated \( E_m \) is the intrinsic property of the \( \text{FeS} \) cluster in the cytochrome \( b \) position [109]. In support of this view, it has been recently shown that even after the extraction of ubiquinone the \( E_m \) value of the \( \text{FeS} \) cluster remained elevated by \( \sim 50 \text{ mV} \) in the alanine mutant [107]. The reason for the \( E_m \) elevation might be straightforward. It is well established that the midpoint potentials of the \([2\text{Fe}−2\text{S}]\) clusters in the Rieske-type iron-sulphur proteins increase with the number of hydrogen bonds that stabilize the reduced cluster [140,181]. The docking of the \( \text{FeS} \) domain to cytochrome \( b \) implies that the \([2\text{Fe}−2\text{S}]\) cluster, which is only partially covered by protein, comes into the medium with lower dielectric permittivity (\( \epsilon_{\text{eff}} \)). At lower \( \epsilon_{\text{eff}} \) the hydrogen bonds are expected to strengthen, which would lead to the increase in the \( E_m \) value of the cluster.

Darrouzet and Daldal have shown that the \( \epsilon f \) loop of cytochrome \( b \), which is shown as a yellow tube in Fig. 4, acts as a barrier that needs to be crossed by the \( \text{FeS} \) domain on its way to cytochrome \( c_1 \) [91], in confirmation of the earlier MD simulations [68]. In the site-specific mutant with an additional alanine residue inserted into the hinge region of the Rieske protein, the oxidation of a RC-generated ubiquinol proceeded normally, but no electron was delivered to cytochrome \( c_1 \). The function was, however, partially restored in the double mutants, where the leucine at position 286 in the \( \epsilon f \) loop of cytochrome \( b \) was replaced with a phenylalanine. Apparently, the \( \epsilon f \) loop prevented the \( \text{FeS}_b \rightarrow \text{FeS}_c \) motion in the single mutant [91]. The picture that emerges from these elaborated studies offers a wide range of possibilities to control the \( \text{FeS}_b \rightarrow \text{FeS}_c \) transition, from the one side, via docking interactions in center \( P \) and, from the other side, via conformational impact on the hinge region of Rieske protein and/or on the \( \epsilon f \) loop of cytochrome \( b \).

The apparent “release” of the \( \text{FeS}-\text{lock} \) in response to a redox reaction in center \( N \), and the deactivation of the lock in the presence of antimycin (see Section 2.1, 2.5.1 and [18,51, 54,59,96,97,182]) can be considered as one more evidence of a conformational coupling between centers \( N \) and \( P \). After being put forward in [51], the possibility of such a coupling was tackled in several publications [18,101,108,136]. Daldal et al. have shown that the cleavage of the Rieske protein at the hinge region by thermolysin depended on the state of center \( N \) [101]. While in the untreated \( bc_1 \), only 50% of the \( \text{FeS} \) domains were cleavable by thermolysin, in the presence of stigmatellin the cleavage was blocked, and in the presence of antimycin the cleavage was about 80%. Because, as it is known from X-ray data, stigmatellin fixes the \( \text{FeS} \) domain in the cytochrome \( b \) position, it could be straightforwardly suggested that the cleavability depended on the position of the \( \text{FeS} \) domain, so that the protein was accessible to thermolysin only when the \( \text{FeS} \) domain was close to cytochrome \( c_1 \) or stayed in some intermediate position. In this case, the data might indicate that antimycin shifted the \( \text{FeS}_b \rightarrow \text{FeS}_c \) equilibrium to the right. The situation, however, was not so simple: the antimycin-treated \( bc_1 \) showed a typical \( g_x = 1.800 \) EPR signal indicating an interaction of the \( Q_p \) ubiquinone with a reduced \( \text{FeS} \) domain in the cytochrome \( b \) position. Therefore, the authors attributed the enhanced cleavability of the Rieske protein to the antimycin-induced conformational changes in the hinge region proper, which was the target of the protease. It is noteworthy, however, that the cleavage experiments were carried out in the absence of added electron donors, so that one can expect that the \( \text{FeS} \) cluster was oxidized during the assay. The EPR measurements, from the other side, are routinely conducted with a pre-reduced \( \text{FeS} \) cluster because the oxidized one is EPR-silent. Unless \( \text{Qh}_2 \) is present, an oxidized \( \text{FeS} \) domain seems to reside in the cytochrome \( c_1 \) position unlike the reduced one, which shows weak affinity to the docking site at cytochrome \( b \) in the presence of ubiquinone [103,106]. Thus, the data of Daldal et al. might indicate that the position of the oxidized \( \text{FeS} \) domain depends on the presence of antimycin in center \( N \). In support of the earlier suggestions of a functionally relevant conformational coupling between centers \( N \) and \( P \) [18,51,54,59,96,97,180], Cooley and Daldal have recently shown, by using EPR spectroscopy, that either inhibitor binding or single residue mutation events at the \( Q_N \) site altered the interaction of the \( \text{FeS} \) domain with ubiquinol at the \( Q_p \) site in a manner that was dependent upon the nature of interactions at the \( Q_N \) pocket [77]. These results of Daldal et al. might be related to the earlier observations (i) on the easier dissociability of the Rieske protein from the isolated \( bc_1 \) in the presence of antimycin (see [183] and references cited therein), and (ii) on the propagation of the antimycin-induced conformational change towards heme \( b_1 \) [184]. An extensive further discussion of how center \( N \) can be mechanistically coupled to the Rieske protein can be found in [101].

It is noteworthy that even when the \( \text{FeS}-\text{lock} \) is deactivated, the electron divergence in center \( P \) can be still ensured by the tight semiquinone binding (\textit{vide supra} Section 3.1) and by the involvement of the Glu-272 switch (\textit{vide infra} Section 3.3). It remains unclear, however, whether a \( bc_1 \) complex with a deactivated \( \text{FeS}-\text{lock} \) is able to pump protons against the membrane potential backpressure: both the antimycin-treated and the heme \( b_1 \)-depleted enzymes, in which the FeS-
lock, apparently, does not operate, cannot perform a complete electrogenic turnover.

3.3. “Glu-272” switch

As noted in Section 2.5.1 and depicted in Figs. 4 and 7, the comparative analysis of X-ray structures has shown that the side chain of Glu-272 can turn towards heme \( h_1 \) and the water-filled channel that connects the heme with the external water phase \([9,72,82]\). Correspondingly, it has been speculated that the rotation of Glu-272 is required for proton release into this channel (see \([72,82]\) and comment in Section 2.1). The mobility of Glu-272 might play an even more important role in controlling the events in center \( P \). The FTIR spectroscopy of the \( Rb. capsulatus \) bc1 has shown that the reduction of heme \( h_1 \) was coupled with the protonation of a carboxy group peaking at 1720 cm

\(^{-1}\) \([67]\) (in an independent study of \( Rb. capsulatus \) bc1 by the ATR-FTIR spectroscopy this peak has been recently confirmed at 1723 cm

\(^{-1}\) \([185]\)). Because the signal was twice as large at pH 6.5 than at pH 8.7, the pK of this carboxyl was estimated as \( \sim 8.7 \) \([67]\). From these data, the extent of pK shift at this group in response to heme \( h_1 \) reduction can be estimated as >2.5. Taking a value of 8–12 for \( \varepsilon_{\text{eff}} \) (see \([186,187]\) for estimates of \( \varepsilon_{\text{eff}} \) values inside membrane proteins), the distance between the heme iron and the carboxyl can be estimated from the Coulomb’s equation as <8–12 Å. The structure of the \( Rb. capsulatus \) bc1 \([11]\) implies that the only carboxyl that is close enough to heme \( h_1 \) is the Glu-272 residue when it is turned towards the heme (Glu-272\(_{\text{hb}}\)). Other carboxyls are >20 Å away from heme \( h_1 \). It is thinkable that when heme \( h_1 \) is pre-reduced at equilibrium, Glu-272 stabilizes the negative charge at the heme by taking a proton from the medium, perhaps, via the water chain (see Fig. 4A). Further on, the same signal at 1720 cm

\(^{-1}\) has been observed in response to the reduction of heme \( h_3 \) as well \([67]\). The heme \( h_3 \) is 22 Å away from Glu-272 in the crystal structure. Assuming a \( \varepsilon_{\text{eff}} \) value of 6–8 for the inner, hydrophobic part of cytochrome \( b \), the energy of electrostatic interaction between heme \( h_3 \) and Glu-272 can be estimated as about 90 mV, implying a pK shift of 1.5 units at Glu-272 in response to the heme \( h_3 \) reduction. The smaller pK shift correlates with the observation that when only heme \( h_3 \) was reduced, the signal at 1720 cm

\(^{-1}\) was seen at pH 6.5 but not at pH 8.7 \([67]\).

These electrostatic considerations point to a following tentative scenario, which is schematically depicted in Fig. 10. When center \( P \) is empty and the hemes of cytochrome \( b \) are oxidized, Glu-272 is turned towards heme \( h_1 \) in the respective crystal structures \([7,72]\). This indicates that the intrinsic Glu-272\(_{\text{hb}} \rightleftharpoons \text{Glu-272}_{\text{FeS}} \) equilibrium (\( A \rightleftharpoons B \) equilibrium in Fig. 10) is shifted to the left, i.e., towards the state \( A \) in Fig. 10. With the reduction of the cytochrome \( b \) hemes and the expected protonation of Glu-272 the system would get into a state \( \text{H}^{+}\)-Glu-272\(_{\text{hb}} \) (state \( C \) in Fig. 10); thereby, the negative charge at the hemes would further stabilize the \( \text{H}^{+}\)-Glu-272\(_{\text{hb}} \) state. The ubiquinol binding requires, however, a deprotonated Glu-272\(_{\text{FeS}} \) residue (state \( B \) in Fig. 10). Hence, the binding of a ubiquinol in the catalytic site under the conditions where the hemes of cytochrome \( b \) are pre-reduced would require a “diagonal” \( C \rightarrow B \) alteration of the Glu-272 residue (deprotonation + flip, see Fig. 10). This requirement would strongly hamper the binding and oxidation of ubiquinol when cytochrome \( b \) is reduced. Although the flips of the Glu-272 side chain proper are expected to happen at pico/nanoseconds (see e.g., \([188]\) for molecular dynamics (MD) simulations of carboxyl side chain mobility), a steady population of the deprotonated form of Glu-272\(_{\text{FeS}} \), which determines the ubiquinol oxidation rate (see Eq. (5)), would be low.

The here suggested redox-controlled swinging of the Glu-272 side chain resembles two well-studied cases of carboxy side chain mobility in redox enzymes. The first case is the one of the strongly conserved Glu-286 in the cytochrome \( c \) oxidase. Here, the comparative analysis of various crystal structures showed that the protonated side chain of Glu-286 might move in response to redox changes in the cytochrome \( c \) oxidase and connect thereby the input and output proton channels \([144]\). The other relevant case is the one of the Asp-15 in ferredoxin I from \( Azotobacter vinelandii \). This residue was shown to transfer protons, by a swinging movement, from the water phase towards the \( \mu_2S \) sulphur atom of the \([3\text{Fe}–4\text{S}] \) cluster \([189]\). The MD simulations showed that a reaction mechanism based on a side-chain flipping is effective only as long as two configurations of the side chain are isoenergetic. The enzyme function is effectively blocked if one of the conformations is selectively stabilized and the mobile residue becomes “fixed” \([188]\). This seems to happen with the protonated Glu-272\(_{\text{hb}} \) after the complete reduction of cytochrome \( b \).

It is noteworthy to emphasize that the heme \( h_1 \)-due electrostatic influence, which was discussed above in relation to Glu-272, is not a prerogative of this specific residue, but of any potential proton acceptor located between the quinol ring and heme \( h_1 \). This notion might be related to the ubiquinol oxidation by mutants lacking Glu-272 \([72]\) and to the plastoquinol oxidation by the cytochrome \( bh \) complex.

3.4. Conformational gating

Hitherto it has been assumed that, besides the considered twists of the side chain of Glu-272, there is no other allosteric impact on the stabilization/binding of the quinol/semiquinone ring in center \( P \) either (i) from the reduction of cytochrome \( b \) hemes or (ii) from the binding of inhibitors in
Both assumptions might be wrong. The reduction of cytochrome $b$ is coupled with the relative angular displacement of the hemes [190], which implies a major conformational change. If, due to a conformational change in cytochrome $b$, the Glu-272 would lose the ability to reach the ubiquinol ring and to form a hydrogen bond with the latter, the ubiquinol oxidation in center $P$ would be efficiently impeded. The same is true for other residues involved in the stabilization of the quinol ring (see e.g., [82]). As mentioned above, the data of Daldal et al. on the higher cleavability of the Rieske protein in the presence of antimycin [101] might indicate that the FeS$_b$ \(-\) \(\rightarrow\) FeS$_c$ equilibrium for the oxidized FeS domain is shifted to the right in the presence of this inhibitor. Such a shift would impede the stabilization of quinol/semiquinone in the catalytic site as well. The binding/stabilization of ubiquinol can be also affected by the antimycin-induced long-range conformational interactions in cytochrome $b$ [184]. Cooley and Daldal have concluded in their already cited study of the coupling between centers $N$ and $P$ by means of EPR spectroscopy ([77], see also Section 3.2 above), that “the structure of cytochrome $b$ submit itself changes as a function of the dynamic events at one side or the other”.

4. Comparison of different models of ubiquinol oxidation by the cytochrome $bc_1$ complex

4.1. Mobility of ubisemiquinone

Concerning the mobility of the ubisemiquinone in center $P$, the suggested mechanism of ubiquinol oxidation is at odds with models that imply a neutral ubisemiquinone that moves between the two binding sites (see Section 2.3 and [80,110,111]).

Other tentative models of ubiquinol oxidation, however, imply a single binding site for the semiquinone. The earlier insightful model of Link [92] implies a single binding site for an immobile ubisemiquinone. In this model, however, the nature of proton accepting groups was not specified. As well, the recent structure-based models of Hunte et al. [82] and of Berry and Huang [136] imply only one binding site for ubisemiquinone.

The mobility of a ubisemiquinone is expected to depend on its charge. While a relocation of neutral semiquinone between the two binding sites is imaginable, the negatively charged $Q_S^-$ would be tightly bound to the FeS domain by the electrostatic interaction with iron. The $pK$ value of a $Q^-/QH^+$ couple in an aqueous environment has been estimated to be about 5.0 [191]. In agreement with this estimate, the EPR-detectable ubisemiquinones in different membrane proteins, to the best knowledge of the author, showed characteristic EPR and optical spectra of anion-radicals at neutral pH values (see e.g., [192] and references cited therein). The anionic nature of these semiquinones is likely to be due (i) to the abundance of water molecules in the respective binding pockets [9,31,193] and (ii) to the electrostatic influence from positively charged iron atoms, which are usually present in vicinity. The high resolution X-ray structure of $bc_1$ [9] shows water molecules in center $P$. The expected, from the stigmatellin binding, position of the $Q_S^-$ semiquinone (cf. Figs. 7 and 9) is 7 \(\AA\) away from the nearest iron atom of the FeS cluster. The electrostatic influence from iron does shift the $pK$ values of the
histidine ligands of the FeS cluster from \( \sim 14.0 \) to \( \sim 6.0-9.0 \) [137,140,181]. A semiquinone in the catalytic site would feel this electrostatic impact of iron as well. Thus, there is no apparent reason to expect a neutral semiquinone in center \( P \) under physiological conditions.

4.2. Two binding sites for ubiquinol/ubiquinone

The tentative mechanisms of ubiquinol oxidation, as suggested so far, have considered either (i) a single binding site in center \( P \) for neutral ubiquinone/ubiquinol species (see e.g., [19,80,136]) or (ii) two ubiquinone/ubiquinone molecules simultaneously bound with different affinities to two binding sites in center \( P \), as suggested by the double occupancy model [194,195]. Concerning the latter model, the X-ray structures of \( bc_1 \) did not show any ubiquinone bound in center \( P \). Crofts et al. have suggested alternative explanation for the EPR data, on which the double occupancy model was based [1,32,80]. However, one has also to take into account the recent indications of the binding of two quinone-like molecules of DBMIB in \( bf \) [196] and of two \( Q_0 \) ubiquinone molecules in the mitochondrial \( bc_1 \) [197]. It is plausible that the center \( P \) is large enough to accommodate two quinone rings, especially if tail-less species are involved, as in the works cited above. Still it seems that the available structures of \( bc_1 \) do not offer enough space in center \( P \) for two single-tailed molecules of ubiquinone getting in and out through the same protein tunnel, not to mention menaquinone, which is even more bulky, but can be efficiently oxidized by the mitochondrial \( bc_1 \) [96]. The possibility that a second long-chain quinone can access the center \( P \) directly from the phospholipid bilayer, avoiding the protein tunnel, cannot be fully ruled out, especially in the case of the smaller bacterial \( bc_1 \). This option, which might be useful in rationalizing bypass ET reactions in \( bc_1 \) (see e.g., [164,165]), deserves further clarification.

To the best knowledge of the author, the possibility of a functionally relevant rocking of the same neutral ubiquinol/ubiquinone molecule between the two binding sites has not been explicitly addressed so far. It looks like that such a rocking is a distinct feature of the suggested mechanistic model of ubiquinol oxidation by \( bc_1 \).

4.3. Proton gating

Deprotonation of ubiquinol has been suggested to be the activation step in at least two models of ubiquinol oxidation [92,198]. The suggestion, on one hand, was based on the model experiments, which have shown that the deprotonation of \( QH_2 \) to \( QH^- \) is the most probable first step of ubiquinol oxidation [141]. On the other hand, the activation energy of ubiquinol oxidation, as determined for the steady turnover of the mitochondrial \( bc_1 \), was shown to decrease with pH [199]. The latter observation has prompted Brandt to hypothesize that the ubiquinol oxidation is proton-gated, with the FeS center acting as the acceptor of the first proton [198,199]. However, as already noted in Section 2.5.2, the \( E_a \) of ubiquinol oxidation, as determined for the \( Rb. \text{sphaeroides} \ bc_1 \) in single-flash experiments by Crofts et al., found to be independent of pH at \( 5.5 < \text{pH} < 9.0 \) [69].

This disparity between the data obtained at steady state with mitochondrial \( bc_1 \) [199] and in flash-experiments with the \( bc_1 \) of \( Rb. \text{sphaeroides} \) [69] deserves consideration. One possible solution follows from the scheme in Fig. 7. Here, the flash-induced reduction of heme \( b_h \), which was studied by Crofts et al. [69], corresponds to the \( D \rightarrow E \) transition. The steady turnover of \( bc_1 \), which was studied by Brandt et al., is, however, kinetically limited upon the later turnover step, which is coupled with the oxygenation of heme \( b_h \), the re-reduction of cytochrome \( c_1 \) and the \( \Delta \psi \) generation. This step is the rate limiting one both in coupled mitochondria [200,201] and in chromatophores of \( Rb. \text{sphaeroides} \) [36]. In flash experiments with \( bc_1 \) of \( Rhodobacter \), the oxidation of heme \( b_h \) and the re-reduction of cytochrome \( c_1 \) were concomitantly slowed down by the membrane potential [36] and by the ubiquinol shortage [18]. The scheme in Fig. 7 attributes this rate-limiting step to the \( E \rightarrow F \) transition. Upon this step protons are released from \( bc_1 \) into the surrounding water phase by Glu-272 of cytochrome \( b \) and by His-161 of the Rieske protein (as illustrated by Fig. 4B; see also Section 2.5.1). The activation energy of this proton release could decrease with pH elevation because of gradual deprotonation of proton relay(s) involved. The facilitation of proton ejection at higher pH could decrease the \( E_a \) of the overall turnover at steady state.

In earlier work [69], Crofts et al. ruled out the possibility of proton gating because the \( E_a \) of ubiquinol oxidation was independent of pH in flash experiments (see above). Thereby, the high \( E_a \) of ubiquinol oxidation was attributed to the rate limiting ET reaction (see refs. [69,110] and Section 2.5.2 above). This led, however, to a reorganization energy value of \( \sim 2 \) eV that was unrealistically high for a ET reaction in protein. To overcome this difficulty, Crofts has recently invoked the participation of protons to explain the high \( E_a \) of ubiquinol oxidation [156]. It has been suggested that the transfer of the first electron from \( Q_bH_2 \) to the FeS cluster proceeds by proton-coupled electron transfer (PCET) mechanism, so that the activation barrier is partly due to the “uphill” proton transfer from \( Q_bH_2 \) to the FeS domain (see Section 2.5.1 for the respective pK estimates). As an analogous case, Crofts considered the coupled transfer of the second electron and the first proton to \( Q_b \) in the \( Rb. \text{sphaeroides} \) RC (\( Q_b^- \rightarrow Q_bH^- \) reaction that corresponds to the \( C \rightarrow D \) transition in Fig. 6). The \( Q_b^- \rightarrow Q_bH^- \) reaction, however, proceeds not by PCET mechanism but sequentially: the ET takes place only after the \( Q_b^- \) semiquinone is protonated to \( Q_bH^- \) by proton coming from the surface histidine cluster (see Fig. 3 and [28]). Although this “uphill” proton transfer slows the reaction rate, it does not contribute to
the activation barrier, so that the $E_a$ value of this reaction is only about 10 kJ/mol [115]. Hence, the “uphill” proton transfer to $Q_b^-$ along a rather long chain of amino acid residues (see Fig. 3) does not elevate the activation barrier of the reaction. In the case of $bc_1$, the “uphill” proton transfer corresponds to a proton shift by only 1 Å along a hydrogen bond connecting the bound ubiquinol with the His-161 residue of the Rieske protein. It is unlikely that this reaction can limit the overall transition. The dependence of the ubiquinol oxidation rate on the $E_m$ of the FeS cluster but not on pH (see Section 2.5.3 and references cited therein) also speaks against a genuine PCET mechanism. As well, a rate limitation by PCET mechanism poorly corresponds with the strong dependence of the rate of cytochrome $b$ reduction on the viscosity in the cytochrome $hf$ complex [158]. The correlation between the activation energy of the steady $bc_1$ turnover and the rigidity of the hinge region of the Rieske protein [98] can be also hardly explained by a rate-limiting PCET mechanism. As well, one would expect a notable H/D isotope effect for a quinol oxidation that is limited by a genuine PCET mechanism [202,203]. The H/D effect, as measured by cytochrome $b$ reduction, was $<2.0$ in the case of the $hf$ complex of *Chlamydomonas reinhardtii* [204] and $\sim 1.5$ in the $bc_1$ of *Rh. capsulatus* (Klishin and Mulkidjanian, unpublished observations). All these findings are, however, compatible with the formation of the $Q_bH_2$–FeS complex as the rate-limiting step of ubiquinol oxidation (see Section 2.5.2).

4.4. Semiquinone stabilization

The extent of the $Q_b^-$ semiquinone stabilization is another matter of debate. It is worthwhile to discriminate between thermodynamic and kinetic modes of semiquinone stabilization. A thermodynamically stable semiquinone in center $P$ can be ruled out. Evidently, such a semiquinone with a stability constant $K_{>1}$ (see [3] for the definition of $K_s$) would have midpoint potential above that of the $Q_bH_2/Q_b$ redox pair ($\sim 100$ mV at pH 7.0 [48]) and would be unable to reduce the $b_1$ heme. The $Q_b^-$ semiquinone reduces heme $b_1$ even when the $b_1$ heme is pre-reduced and the $E_m$ of heme $b_1$ is approximately $\sim 100$ mV (see Table 1 and [64,85]). Hence, the functional redox potential of the $Q_b^-$ semiquinone can be estimated as $<-150$ mV at pH 7.0 (see also Section 2.5.1); this redox potential would formally correspond to a stability constant of $<10^{-3}$. The formal stability constant, however, is defined only for semiquinone molecules interacting in a homogenous solution. The stability of a semiquinone that is occluded in a protein is determined by the ability to exchange electrons with the surrounding. An impediment in electron exchange can lead to the formation of semiquinone, which is unstable thermodynamically, but stable kinetically. This happens in the RC where the $Q_b^-$ semiquinone, although thermodynamically unstable, can live for minutes at least [112,150].

As long as an oxidized heme $b_1$ is available, the $Q_b^-$ semiquinone can be considered to be unstable both thermodynamically and kinetically because the ET to heme $b_1$ is faster than the activation step of ubiquinol oxidation (see [65] and the discussion in Section 2.5.2). The reduction of heme $b_1$, as caused by antimycin treatment or by a membrane potential backpressure, seems to lead, however, to the partial kinetic stabilization of the $Q_b^-$ semiquinone. The (partially) stabilized semiquinone manifests itself by its ability to reduce oxygen to superoxide $Q_b^- \cdot \bullet$ in a stigmatellin-sensitive reaction (see [151,164,165] and references cited therein). This semiquinone is apparently EPR-silent; the EPR-silence has been explained by the antiferromagnetic coupling between the semiquinone and the reduced FeS cluster (see e.g., [92]). A direct evidences of such silencing were obtained upon the studies of the cytochrome $hf$ complex: first Malkin [205] and then Schoepp et al. [206] have shown by using 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), a quinone analogue which tightly binds to the FeS domain in the plant $hf$, that the generation of the semiquinone form of DBMIB by redox titration resulted in the loss of the EPR signal; the loss was apparently due to the antiferromagnetic coupling in the semiquinone–Fe$^{2+}$ pair.

4.5. Concerted ubiquinol oxidation

A concerted mechanism has been suggested by several authors for the divergent ubiquinol oxidation by $bc_1$ (see e.g., [20,85,136,207] and references cited therein). It is useful to discriminate between (i) an apparently concerted reaction, where two sequential ET reactions proceed without measurable delay because the overall reaction rate is determined either by the first ET step or even by some preceding event, and (ii) a genuinely concerted reaction where two electrons go to different acceptors at once. The above discussed data indicate that ubiquinol is oxidized by $bc_1$ in an apparently corrected way; the reaction rate is then determined either by the formation of a complex between $Q_bH_2$ ($Q_bH^-$) and the FeS domain (as argued in Section 2.5.2) or by the oxidation of $Q_bH_2$ to a semiquinone (as suggested by several authors, see [32,156,165,208] and references cited therein). The genuinely concerted mechanism, although very helpful in rationalizing the electron bifurcation phenomenon [20], seems to be, however, unlikely. The electrochemistry of quinones implies that the oxidation of a $Q_bH^-$ anion to a $Q_bH^+$ radical is followed by a deprotonation event because a QH$^+$ semiquinone can hardly be directly oxidized to a QH$^+$ cation (by analogy with the QH$_2$/QH$^+_2$ pair (see [141]), the QH$^+$ $\rightarrow$ QH$^+$ reaction would require an impractically high oxidizing potential). Then, a genuinely concerted reaction of ubiquinol oxidation has to invoke a simultaneous transfer of two electrons and of one proton to different acceptors. To the best knowledge of the author, such a triple concerted reaction does not have precedents in
chemistry. Moreover, from general considerations, a large kinetic isotope effect is expected for such a reaction because of proton involvement, in contrast to available data discussed in the previous section. A moderate H/D isotope effect is, however, compatible with the limitation of the reaction by a conformational change (see Section 2.5.2 above).

Turning to the chemical definition, “two or more primitive changes are said to be concerted if they occur within the same elementary reaction”. An elementary reaction, in turn, is definable as “a reaction for which no reaction intermediates have been detected or need to be postulated to describe a chemical reaction on a molecular scale” (both definitions are taken from [209]). Apparently, this definition can be hardly applied to the ubiquinol oxidation by bc1. Under conditions where the heme b1 is kept reduced (either by antimycin or by the backpressure from the membrane potential), the Qb− semiquinone, although EPR-silent, is detectable as a reaction intermediate via its ability to reduce oxygen to superoxide. The formation of the latter can be monitored by EPR probes (see [210] and references therein), by fluorescent dyes [151], as well as via H2O2 formation (see [165] and references therein).

Trumpower has argued that “semiquinone at center P only exists as an aberrant by-product” and that the reduction of oxygen to superoxide by semiquinone “reflects the “error rate“ at which the concerted reaction is disrupted” [207]. Kramer et al., however, have recently reported a similar pattern of thermal activation, including a unusual $E_{a}(H) > E_{a}(D)$ feature, for biomimetic systems, where the reaction of ubiquinol oxidation proceeds in two steps, and for the intact yeast bc1 [208]. Based on these data, it was suggested that even in a non-inhibited bc1 the oxidation of ubiquinol proceeds via two one-electron steps.

4.6. Logical gating/double gating

The idea of logical/double gating has been recently put forward by several authors to explain both the obligatory bifurcation of electron flows and the prevention of short circuits in bc1 [208]. According to this view, a “productive” binding of ubiquinol in center P is possible only when all the potential electron and proton acceptors are, respectively, oxidized and deprotonated. And other way around, a productive ubiquinone binding is only possible when these electron and proton acceptors are reduced and protonated. This scheme provides an elegant explanation of the emptiness of catalytic center P in the crystal structures of bc1; none of these “productive” states can be achieved at equilibrium. The here suggested scheme and the logical/double gating becomes clear upon consideration of experimental data. For example, two following experimental observations can be rationalized in the framework of both schemes, although the consideration of the conformational mobility of Glu-272 provides a more elaborated picture.

(1) At steady state, bc1 stops to oxidize ubiquinol in the presence of antimycin after both cytochrome b hemes become reduced, even if an oxidant, which keeps both the FeS cluster and cytochrome c1 oxidized, is available [73].

(2) At high membrane potential, the cytochrome b becomes over-reduced while cytochrome c1 stays oxidized. This situation, however, does not lead to the short-circuiting of bc1 by the transfer of both electrons from the ubiquinol to the high-potential ET branch [200,201].

Although both observations are widely cited in evidence of an obligatory electron bifurcation in center P, the underlying mechanisms has remained unclear. In the framework of the logical/double gating, the reduced state of heme b1 is expected to prevent a “productive” ubiquinol binding. On a more detailed level, the observations can be rationalized by a suggestion that the reduction of cytochrome b leads to the protonation of Glu-272 and to the trapping of its side chain in the “heme $b_1$” position (state C in Fig. 10) due to electrostatic and, perhaps, allosteric interactions, as discussed above. Then, as long as cytochrome b is reduced, Glu-272 is “turned away” and impeded in the formation of a hydrogen bond with the quinol ring. Thus, the ubiquinol oxidation is blocked already on the step of ubiquinol binding/first deprotonation. The turnover of bc1 under such conditions is expected to resemble one in the Glu-272 → Gln mutant. And indeed, the residual activity of about 2%, which seems to be typical for an antimycin-inhibited bc1 and which manifests itself both in the slow cytochrome c reduction [73] and in the generation of superoxide [164], is compatible to the residual activity of the corresponding Glu-295 → Gln mutant of Rh. sphaeroides [72].

The next experimental observation can be hardly explained by a logical/double gating alone, but requires the consideration of the conformational gating/Glu-272 switch:

(3) In the presence of antimycin, the flash-reduced hemes of cytochrome b remain reduced in the dark for seconds [85].

Because under these conditions, the FeS cluster is reduced by redox mediators at milliseconds, the logical/double gating mechanism would predict ubiquinone binding in center P followed by a thermodynamically favorable oxidation of cytochrome b. The absence of such oxidation can be rationalized by taking into account the role of Glu-272 in the semiquinone stabilization, which is crucial for reversed reactions in center P. The redox potential of ubisemiquinone is low in solutes with small $\epsilon_{eff}$ on the order of $-600$ mV [211]. Therefore, a quinone in center P can be reduced only if the redox potential of the Qb/Qb− redox couple is elevated, due to the relative stabilization of the semiquinone by the...
surrounding charged/polar groups, to acceptably high values. For example, as already noted in Section 2.4, the stabilization of the Q\(\text{b}^-\) semiquinone in the RC is accomplished, besides the electrostatic interaction with the iron atom [131], by a direct interaction with several amino acid residues [24]. It has been argued recently that specifically the formation of a hydrogen bond with Ser-L223 makes the reduction of Q\(\text{b}^-\) possible [212]. When both hemes of cytochrome \(b\) are pre-reduced by flashes in the presence of antimycin, the participation of the Glu-272 in semiquinone stabilization is impeded by its trapping in the Glu272\(bl\) state in response to the electrostatic and, perhaps, allosteric impact from the reduced heme \(b_1\) (i.e., the C \(\leftrightarrow\) D equilibrium in Fig. 10 is strongly shifted to the left). The poor semiquinone stabilization would prevent the oxidation of the cytochrome \(b\) hemes via center \(P\). The stability of the Q\(\text{b}^-\) semiquinone could be, in addition, allosterically affected by antimycin in center \(N\) (see above); the latter point deserves further experimental investigation.

A compatibly tight trapping of the protonated Glu-272\(bl\) state is not expected under conditions of the reverse electron transfer in \(bc_1\). In this case, antimycin is absent, heme \(b_1\) is oxidized, and only heme \(b_2\) is partly reduced [86]. Under these conditions, Glu-272 can contribute to the stabilization of a semiquinone and enable the ubiquinone reduction in center \(P\).

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