Review

Activated Q-cycle as a common mechanism for cytochrome bc_1 and cytochrome b_6f complexes

Armen Y. Mulkidjanian[a,b,*]

[a] School of Physics, University of Osnabrück, D-49069 Osnabrück, Germany, [b]A.N.Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119992, Moscow, Russia.

* *Correspondence t*o: Armen Y. Mulkidjanian, School of Physics, University of Osnabrück, D-49069 Osnabrück, Germany.

Phone +49-(0541)-9692698, FAX +49-(0541)-9692656.

E-mail: amulkid@uos.de

Key words: Photosynthesis; Electron transfer; Proton transfer; ubiquinol:cytochrome *c* oxidoreductase; ubiquinone; plastoquinone; cytochrome *b; Rhodobacter capsulatus; Rhodobacter sphaeroides*.

Abstract

Cytochrome bc_1-complexes of animals and bacteria (hereafter bc_1), as well as related cytochrome b_6f complexes of plants and cyanobacteria (hereafter *bf*) are dimeric quinol:cytochrome *c/*plastocyanin oxidoreductases capable of translocating protons across energy-converting membranes. The commonly accepted Q-cycle mechanism suggests that these enzymes oxidize two quinol molecules in their catalytic centers *P* to yield one quinol molecule in another catalytic center *N*. Earlier, based upon data on flash-induced redox changes of cytochromes *b* and c_1, voltage generation, and proton transfer in membrane vesicles of *Rhodobacter capsulatus*, we have put forward a scheme of an "activated Q-cycle" for the bc_1. The scheme suggests that the bc_1 dimers, being "activated" by injection of electrons from the membrane ubiquinol pool via centers *N*, steadily contain two electrons in their cytochrome *b* moieties under physiological conditions, most likely, as a bound semiquinone in center *N* of one monomer and a reduced high-potential heme *b* in the other monomer. Then the oxidation of each ubiquinol molecule in centers *P* of an activated bc_1 should result in a complete catalytic cycle leading to the formation of a ubiquinole molecule in the one of enzyme's centers *N* and to voltage generation. Here it is argued that a similar pre-loading by two electrons can explain the available data on flash-induced reactions in cytochrome b_6f- complexes of green plants and cyanobacteria.

1. Introduction

Cytochrome bc_1-complexes of animals and bacteria (hereafter bc_1), as well as related cytochrome b_6f complexes of plants and cyanobacteria (hereafter *bf*) are oligomeric quinol:cytochrome *c/*plastocyanin oxidoreductases capable of translocating protons across energy-converting membranes (see [1-7] for reviews). Thereby one of the membrane-adjoining water phases becomes *p*ositively charged (*p*-side), whereas the other one charges *n*egatively (*n*-side). The resulting transmembrane difference in electrochemical potential of proton ($\Delta\tilde{\mu}_{H^+}$) is contributed by chemical (ΔpH) and electrical (Δψ) components and reaches approx. 200–250 mV under physiological conditions [8]. Thus the proton translocation by these enzymes encounters a remarkable proton backpressure *in vivo*.

Triggering by flashes of light has been widely used for resolving the elementary catalytic steps in the bc_1 complexes of phototrophic bacteria and the *bf* complexes of green plants (see e.g. [9-17]). Earlier, based on this kind of data, I have suggested that the operation of cytochrome bc_1 complexes of phototrophic bacteria can be described by an "activated Q-cycle" model [18]. Here it is argued that the wealth of kinetic data on the operation of cytochrome *bf* complexes might be described by this scheme as well.

2. Mitchell's Q-cycle and its modifications

As revealed by X-ray crystallography [19-24], the bc_1 complexes are intertwined dimers (see Fig. 1A). The catalytic core of each bc_1-monomer is formed by three subunits: the membrane-embedded cytochrome *b,* the iron-sulfur Rieske protein, and the cytochrome c_1. The catalytic, hydrophilic domains of the two latter subunits are anchored in the membrane by solo hydrophobic α-helices (Fig.1A). Each cytochrome *b* is a bundle of 8 α-helices that accommodates two hemes, namely the *l*ow- and *h*igh-potential protohemes (b_l and b_h, respectively, see Table 1 for the (midpoint) redox potentials of the components involved).

The number of subunits in bc_1 complexes varies between 11 subunits in the mitochondrial bc_1 and 3 catalytic subunits in some bacteria. It is noteworthy that the X-ray structure of the simplest cytochrome bc_1-complexes of *Rb. capsulatus*, which contains only 3 subunits, matches the structure of the three catalytic subunits of mitochondrial bc_1 [25].

The operation of the cytochrome bc_1 complex is routinely described by the Mitchell's Q-cycle mechanism [26-28]. Its original scheme is shown in Fig. 1B, whereas the current, structure-based scheme of the Q-cycle is plotted over the structure of the bc_1 in Fig. 1A. As it could be seen, the interface between cytochrome *b* and the $[Fe_2S_2]$ cluster-carrying domain of the Rieske protein (hereafter the *FeS domain*) forms the catalytic center *P* of the enzyme where quinol molecules are oxidized. Upon the unique, "bifurcated" reaction (originally suggested by Wikström and Berden [29]), the FeS domain accepts the first electron from the quinol molecule to pass it further, via cytochrome c_1 and the water-soluble *c*-type cytochrome, to external electron carriers. The FeS domain, however, can reduce cytochrome c_1 only after undocking from cytochrome *b* and rotating by ca. 60° [1]. The semiquinone molecule left in center *P* reduces the nearest heme b_l of the cytochrome *b*. From heme b_l, the electron moves across the membrane to heme b_h and then to the quinone-binding center *N* where another quinone molecule could be reduced first to a semiquinone and then to a quinol. Oxidation of each quinol molecule in center *P* should be followed by release of two protons, while the formation of a quinol molecule in center *N* should be accompanied by binding of two protons from the other side of the membrane (see Fig. 1B). Because the ubiquinol molecule that is formed in center *N* can be then oxidized in center *P*, two protons should be ultimately transferred across the membrane per each electron that passes through the bc_1 complex. Therefore the Q-cycle scheme, besides accounting for a bizarre oxidant-induced reduction of cytochrome *b* [29, 30], explained the H^+/e^- stoichiometry of 2 as measured with the mitochondrial bc_1 complex [31].

It is noteworthy that Mitchell has initially suggested that the semiquinone molecule in center *N* can be reduced to ubiquinol by a some other enzyme shown as "*d*" in Fig. 1B (e.g. the succinate dehydrogenase was suggested to perform this function in mitochondria [27, 28]). However, the requirement of a special enzyme as a second electron donor for center *N* came in contradiction with the apparent ability of the liposome-incorporated bc_1 complexes to pump - on their own - two protons per each processed electron [32]. Accordingly, the idea that the bc_1 can work solo by turning over twice [33] gradually gained acceptance and was incorporated into the scheme of the "modified Q cycle" where a $Q_N^{\bullet -}$ anion was suggested to form in center *N* after the first turnover of center *P*, whereas the formation of a Q_NH_2 ubiquinol required oxidation of the next ubiquinol molecule in center *P* [13, 34].

Initially the operation of the cytochrome bc_1 complexes was studied with preparations either of animal mitochondria, or submitochondrial particles, or isolated cytochrome bc_1 complexes (see [35-38] for earlier reviews). These experimental models, however, were of little help in studying the mechanisms of $\Delta\tilde{\mu}_{H^+}$ generation. These mechanisms could be better studied with chromatophores (inside-out vesicles of inner cellular membrane), which can be obtained by disruption of cells of phototrophic α-proteobacteria *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* (see [12, 39] for reviews). By using a spectrophotometric setup, it is possible to trace simultaneously the flash-induced redox reactions in the bc_1 (by following the spectral changes of the hemes [12, 40]), the voltage generation (electrogenesis) at the chromatophore membrane (via the electrochromic spectral shift of intramembrane carotenoid pigments [9, 12, 39, 41]), and the binding or release of protons (by using pH indicators [42, 43]). Hence, the elementary steps of electron transfer (ET) can be correlated with the kinetics of voltage generation and of proton transfer (PT) in the same samples.

Chromatophore vesicles can be considered as bacterial energosomes since each of them contains a set of major energy-converting enzymes in their natural membrane environment. A

flash of light on a solution of chromatophores leads to the charge separation in the photochemical reaction centers (RC) and to the generation both of a reducing and oxidizing agents for the bc_1, namely mobile molecules of ubiquinol and oxidized cytochrome c_2, respectively. Accordingly, the reactions in bc_1 can be flash-triggered in two different ways. Under oxidizing conditions, the bc_1 is trigerred by the interaction with the ubiquinol molecules that are produced by the RC. The respective kinetic data, however, are ambigous since molecules of ubiquinol can bind not only in center *P* but also in center *N*. As the redox potentials of the ubiqunol/ubiquinone pair and of heme b_h are compatible (see Table 1), the molecules of ubiquinol can reduce heme b_h via center *N*, especially under alkaline conditions [1, 44, 45]. This kind of ambiguity is avoided in a more physiological set-up, when the membrane ubiquinone pool (ca. 100 molecules per one bc_1 dimer) is pre-reduced yielding so-called *reducing* conditions. In this case, a flash-induced generation of a ubiquinol molecule in the RC can only marginally shift the redox state of the membrane ubiquinone pool, so that only cytochrome c_2, after being oxidized by the RC, can initiate the turnover of bc_1 by oxidizing cytochrome c_1. The latter, in turn, can oxidize the FeS domain of the Rieske protein and provide thus an oxidant for ubiquinol in center *P*. On contrast, the fast reactions in the mitochondrial bc_1 are routinely studied by prompt mixing of oxidized enzyme with reduced quinol as substrate (see e.g. [46-49]). In this setup, the quinol molecules can interact with centers *N* as well as with centers *P*, so that aforementioned ambiguity in the interpretation of kinetic data can be hardly avoided.

Since α-proteobacteria are evolutionarily related to the ancestors of mitochondria [50], the bc_1 of *Rb. capsulatus* is sensitive to the typical inhibitors of mitochondrial bc_1 (see [1] and references cited therein). Accordingly, the reactions that are caused by ubiquinol oxidation in the bc_1 are routinely discriminated by comparing the kinetic traces as measured in the absence of specific inhibitors of bc_1 with the traces measured in their presence; routinely myxothiazol (a blocker of center *P*) and/or antimycin (that blocks center *N*) are used. In chromatophore

membranes, there are 2÷3 RCs per one bc_1 monomer, so that each bc_1 complex can turn over several times in response to a saturating flash of light. To establish genuine single-turnover conditions one has to use weak flashes of light, in response to which not more than one ubiquinol molecule can be oxidized in some bc_1 complexes [17]. In sum, the chromatophores of *Rhodobacter* can be considered as a model of choice for studying the mechanisms of energy conversion by the bc_1.

The original Q-cycle scheme did not focus on the detailed molecular mechanisms of $\Delta\tilde{\mu}_{H^+}$ generation in the bc_1 [26-28]. In the earlier works, the generation of $\Delta\tilde{\mu}_{H^+}$ in mitochondrial bc_1 was related to the oxidation of heme b_h since the backpressure of the membrane potential was shown to retard this oxidation [36, 37, 40]. After the position of the cytochrome *b* hemes in the membrane (see Fig. 1) was resolved [19-24, 51-53], it became customary to attribute the "working" step of the catalytic cycle to the ET from heme b_l to heme b_h, towards the negatively charged side of the membrane. However, if so, the membrane voltage should have prevented not the oxidation, but the reduction of heme b_h, in contrast to experimental observations [35-37].

In chromatophore vesicles, simultaneous monitoring of redox-reactions and voltage generation showed routinely that the onset of flash-induced voltage generation was notably slower than the reduction of cytochrome *b* [17, 40, 41, 43, 54-57]; instead, the kinetics of voltage generation correlated with the oxidation of heme b_h in some experiments. Based on these observations, it has been suggested that the transmembrane ET to heme b_h is electrically silent [17, 43, 57-59]. Accordingly, the voltage generation by the bc_1 was attributed predominantly to the electrogenic release of those protons that remained within the bc_1 after the oxidation of ubiquinol and that should become "excessive" after the negative charges of electrons were neutralized by formation of ubiquinol in center *N* [17, 43, 57-61]. The kinetic correlation between the proton ejection by bc_1 and the voltage generation [43, 60, 62, 63] can

be considered as a supportive evidence for this scheme. The electrical silence of the transmembrane ET towards heme b_h under coupled conditions is, most likely, related to the small driving force of this reaction. The equilibrium constant of the $b_l \leftrightarrow b_h$ reaction has been shown to be 10-15 at pH 7 [64] in the *absence* of $\Delta\psi$. In the *presence* of $\Delta\psi$ of > 100 mV (as after a single saturating flash [65]), the respective ΔG value should approach zero, so that ET against the transmembrane electric field, from heme b_l to heme b_h, could proceed only on condition of electric compensation. The compensation, as discussed elsewhere [18, 57-59], might be achieved by displacements of protons in the same direction and/or by dielectric relaxation of water molecules. Generally, the electrical silencing of the heme b_h reduction can be considered as a case of a protein control over biological ET, which, as argued elsewhere [66], should be a widespread phenomenon. For example, an electrically compensating, non-productive proton shuttling across the membrane, between the two cytochrome *b* hemes has been recently demonstrated for the membrane subunit of the fumarate reductase of *Wolinella succinogenes* [67].

The catalytic cycle of the bc_1 could be further scrutinized by studying the Zn-treated chromatophores of *Rb. capsulatus* [17, 63]. The Zn^{2+} ions are well-established inhibitors of mitochondrial bc_1 [68, 69] where they were suggested to block the proton release from center *P* [70] and were shown to bind close to this center in the crystal structure [71]. Venturoli and co-workers have recently characterized the Zn-binidng sites in the bc_1 of mitochondria and *Rb. capsulatus* by K-edge X-ray absorption fine-structure spectroscopy [72]. The spectra were interpreted as showing only one high-affinity Zn-binding site in the enzymes studied (at $[Zn^{2+}] < 1$ mM, G. Venturoli, personal communication). It was concluded that Zn binds in the bc_1 of *Rb. capsulatus* in the same position as in the crystal structure of mitochondrial bc_1, i.e. next to center *P* [72].

The investigation of flash-induced reactions in chromatophore vesicles of *Rb. capsulatus* under physiological conditions (with a half-reduced membrane ubiquinone pool) has shown that in the presence of Zn^{2+} ions, added at < 100 μM, the flash-induced voltage generation in the bc_1 became slower, but retained its full extent [17, 63]. Hence, the bc_1 remained fully functional. Concurrently, the oxidation of heme b_h and the re-reduction of the cytochrome c_1 by the Rieske protein slowed down. Due to the slower oxidation of heme b_h, the onset of its flash-induced reduction, otherwise elusive under these conditions, became resolvable. These data helped to identify two distinct steps of the catalytic cycle (see Fig. 2). During the first, Zn-insensitive step (Fig. 2A), the flash-induced oxidation of an ubiquinol molecule in center *P* led to the reduction of the FeS domain and to the fast, electrically silent (see above) reduction of approx. half of the heme b_h content. The re-reduction of cytochrome c_1 by the FeS domain was, however, delayed, most likely because the reduced FeS domain remained docked to cytochrome *b*. The re-reduction of the latter happened during the second, Zn-sensitive step of the catalytic cycle (Fig. 2B). This step was coupled with the oxidation of heme b_h, voltage generation, and proton release into the chromatophore lumen [17, 63].

The concurrent action of Zn^{2+} ions on the re-reduction of cytochrome c_1 by the Rieske protein, voltage generation, and proton release from the bc_1 [17, 63] might be related to the functioning of the Zn^{2+}-binding cluster of amino acid residues, as identified first in the structure of the mitochondrial bc_1 [71] and then in the bc_1 of *Rb. capsulatus* [72], as the proton outlet from center *P* [18, 58, 59, 70, 72, 73]. The comparative structural analysis shows that the Zn^{2+}-binding patch faces the water phase only when the FeS domain is undocked from cytochrome *b* (*cf.* Fig. 2A and 2B). When the FeS domain is docked to cytochrome *b*, it apparently "plugs" this tentative proton outlet (see Fig. 2A). Apparently, the proton(s) which are released upon the oxidation of ubiquinol in center *P* can escape into the water phase only after the FeS domain undocks and moves towards cytochrome c_1 (see Fig. 2B). As argued elsewhere [58, 59], the binding of Zn^{2+} ions can slow down both the proton

release from center *P* and the undocking of the FeS domain (e.g. by constraining the yellow-colored *ef* loop in Fig. 2 that have to be "pushed aside" to permit the movement of the FeS domain [74, 75]).

The observed kinetic correlation between the oxidation of heme b_h (via center *N*) and the re-reduction of cytochrome c_1 by the mobile FeS domain might reflect a coupling between the two quinone binding centers as suggested elsewhere [17, 58, 60, 61]. The molecular mechanism of this coupling in the bc_1 of *Rb. capsulatus* was addressed by Cooley and co-workers [76, 77], while Covian and co-workers studied it in the bc_1 of yeast and *Paracoccus denitrificans* [78-80]. In principle, the release of electrostatically uncompensated proton(s) from center *P* should be thermodynamically favorable and could help to drive the ubiquinol formation in center *N* [59].

It is noteworthy that a mechanistic impediment of ET from the FeS domain to the cytochrome c_1 was envisioned by Mitchell as early as in 1981 [34]. Such an impediment should contribute to the prevention of short-circuiting in the bc_1. A short-circuiting might occur not only during the fast turnover of center *P* (see [2, 58, 81-86] for tentative mechanisms that might prevent the undesired escape of both electrons to the FeS cluster upon ubiquinol oxidation), but also later, provided that electron(s) at cytochromes *b* could find a way to escape via the FeS domain. Such an escape might be especially dangerous under coupled conditions since $\Delta\psi$ steadily pushes electrons from center *N* and heme b_h towards center *P* [64, 87]. Hence, as argued elsewhere [58, 59], it might be useful to keep the reduced FeS domain docked to cytochrome *b* until the formation of ubiquinol molecule in center *N* - the ''no return'' point of the catalytic cycle.

Another set of modifications of the original Mitchell's scheme has addressed the dimeric nature of the bc_1. De Vries et al. envisioned the possibility of a functionally relevant electron exchange between the two monomers [38, 88, 89]. The X-ray structures of the bc_1 showed

the edge-to-edge distance between two b_l hemes in the bc_1 dimer of less than 11 Å [19-24], so that electrons, according to the empirical rate/distance dependence for biological ET [90], should equilibrate within the bc_1 dimer on the time scale of its turnover. This possibility was noted by Yu and co-workers [19] and was also incorporated into some structure-based schemes of dimeric Q-cycles [18, 61, 91-94].

3. Activated Q-cycle in the cytochrome bc_1 complex of phototrophic bacteria

In spite of all these modification of the original Q-cycle scheme, two sets of experimental observations, as obtained with chromatophores of *Rhodobacter*, have remained puzzling. First, in the absence of antimycin A, not more than a half of total heme b_h content could be reduced in response to a flash – in a broad range of experimental conditions [17, 40, 41, 43, 54-57]. Second, the relative extent and the rate of voltage generation by bc_1 did not decrease under the conditions of weak flashing - when only one ubiquinol molecule could be oxidized per flash in some bc_1 complexes [17]. It seemed that the catalytic cycle proceeded to completion even under single-turnover conditions, which were expected to yield only $Q_N^{\bullet -}$ semiquinone molecules in some centers *N* (see Fig. 1A).

These observations could be explicated by the scheme of an *activated Q-cycle* [18, 95]. The scheme (see Fig. 3) suggests that the bc_1 dimers, owing to a continuous electron injection from the membrane ubiquinol pool via centers *N*, are "activated" and contain two electrons in their cytochrome *b* moieties under physiological conditions. Most likely the two electrons asre stored as a bound semiquinone in center *N* of one monomer and a reduced high-potential heme *b* in the other monomer [18]. Indeed, an oxidation of a ubiquinol molecule via center *N* brings the bc_1 dimer into the state $Q_N^{\bullet -} b_h^{red} \,\|\, b_h^{ox}$ (see [1] and references therein). In this state, however, the two negative charges in a bc_1 monomer should repulse each other, prompting the escape of one electron into the other monomer (transition F → A in Fig. 3).

While electron exchange between the monomers during the flash-induced bc_1 turnover, as suggested in refs. [61, 91], is still debatable [1, 87], there is no thinkable way to prevent the "dark" electron equilibration between the monomers and, hence, the activation of the bc_1 complexes (provided that the membrane ubiquinone pool is, at least, half-reduced). The $Q_N^{\bullet-}$ semiquinone in the resulting $Q_N^{\bullet-} b_h^{ox} \parallel b_h^{red}$ state should be EPR-silent due to a antiferromagnetic interaction with the oxidized heme b_h [96-98]. This EPR-silence might be the reason why the presence of one $Q_N^{\bullet-}$ semiquinone per bc_1 dimer under physiological conditions was not taken into account until it has been suggested first in refs. [18, 95] and after that in ref. [99].

If the bc_1 dimer is in an activated $Q_N^{\bullet-} b_h^{ox} \parallel b_h^{red}$ state, already the first flash-triggered ubiquinol oxidation in center *P* should lead to the formation of an ubiquinol molecule in the respective center *N* and, accordingly, to the electrogenic reactions of proton binding and release (as shown in Fig. 3). Hence, the kinetics of voltage generation by the bc_1 complexes should not depend on the intensity of exciting flashes, and not more than half of the heme b_h content could be reduced by a flash of light, in accordance with experimental observations.

Otherwise, if the bc_1 is not activated (that could happen under non-physiological, oxidizing conditions), the formation of the first ubiquinol molecule in center *N* would require not one, but two enzyme turnovers, at least, as suggested by the modified Q-cycle scheme of Crofts and co-workers [13]. Generally, the bc_1 turns over much slower under oxidizing conditions than under reducing ones, when its activation is possible [17, 18, 56, 61].

The Mitchell's Q-cycle starts from the oxidation of a quinol molecule in center *P* (see Fig. 1). The activated Q-cycle, in fact, starts from the oxidation of a ubiquinol molecule in center *N* followed by the activation of the bc_1 (F → A transition in Fig. 3). This rather bizarre view on the Q-cycle matches the earlier observations that center *N* – which is commonly believed to be the quinone-reducing one – binds the molecules of ubiquinol by order of magnitude more tightly than molecules of ubiquinone [100]. Under oxidizing conditions, the ubiquinol

molecules, which were produced in the RC in response to flashes, were shown to interact with center *N* much faster than with center *P* [44, 101, 102]. The crystal structures show that centers *N* of the bc_1 dimer are easily accessible from the lipid bilayer in contrast to centers *P* [19].

It looks like that the bc_1 first makes one activating turn backwards to oxidize one ubiquinol molecule via center *N* and to "load" its cytochrome *b* moiety by two electrons. Only then the bc_1 makes two productive forward turns, each leading to an oxidation of one ubiquinol molecule in center *P* and formation of one ubiquinol molecule in center *N*. The total balance of the activated Q-cycle is the same as in the earlier Q cycle models [13, 33], namely two ubiquinol molecules are oxidized in centers *P* and one is formed in centers *N*. The pre-activation of the bc_1 appears to preclude the situations when an eventual impediment in oxidation of a second ubiquinol molecule in center *P* could prevent the ubiquinol formation in center *N*. In addition, the pre-activation might be kinetically advantageous, particularly under coupled conditions; the estimates from refs. [64, 87], indicate that the electron injection into the bc_1 dimer via center *N* (the "pre-loading" of the bc_1) should be driven by $\Delta\psi$ (see [18] for details).

4. Activated Q-cycle in the cytochrome b_6f complex?

The cytochrome *bf* complexes of green plants and cyanobacteria (see Table 2 for some of their properties) differ structurally from the bc_1 complexes [3, 23, 103, 104]. For example, the cytochrome *b* of the bc_1 (formed by 8 transmembrane helices) corresponds to the two subunits of the *bf*: the N-terminal part of the cytochrome *b* is homologous to the cytochrome b_6 (4 transmembrane helices), whereas the C-terminal part resembles the subunit IV of the *bf* complex (3 transmembrane helices). The cytochrome b_6 subunit, besides accommodating two *b*-type hemes, as in the bc_1, carries an additional *c*-type heme (heme c_n or heme c_i) that does

not have a counterpart in the bc_1 [3, 103-106]. The iron atom of this heme is connected to the propionate of heme b_h by a water bridge. The subunit IV binds single molecules of chlorophyll *a* and β-carotene, which are likely to be involved in photoprotection [7, 107-109]. In addition, the cytochrome *f,* which accepts electrons from the mobile FeS domain of the Rieske protein, although carries a *c*-type heme, is structurally unrelated to the cytochrome c_1 of the bc_1 [110]. In fact, only the positions of the *b*-type hemes overlap in the structures of bc_1 and *bf* complexes [103].

In spite of these differences, the data on flash-induced reactions in the *bf* complexes show similarity with the respective reactions in the bc_1 of phototrophic bacteria. In chloroplast thylakoids, where the plastoquinol oxidation in center *P* was triggered by flashes of light, the reduction of cytochrome b_6 was usually faster than (i) re-reduction of cytochrome *f,* (ii) voltage generation in the *bf,* (iii) proton release into the thylakoid lumen, and (iv) proton binding by the *bf* [11, 15, 16, 111-113]. Similar to the situation in the bc_1, the latter four reactions showed a rough kinetic correspondence with the oxidation of cytochrome b_6 (see e.g. [16, 113]).

Cramer and co-workers have shown that the flash-induced reduction of cytochrome b_6 can be kinetically decoupled from the re-reduction of cytochrome *f* and voltage generation. Diverse mutations of amino-acid residues in cytochrome *f* retarded both the re-reduction of cytochrome *f* and the generation of the membrane voltage without affecting the cytochrome b_6 reduction rate. Thereby the latter reaction was distinctly faster than the former two [114]. Furthermore, the kinetic discrepancy between the reduction of cytochrome b_6 and the re-reduction of cytochrome *f* increased with the increase in the lumenal viscosity [115]. These results indicate that the undocking of a reduced FeS domain from cytochrome b_6 – a precondition of the cytochrome *f* re-reduction - might be coupled to an electrogenic reaction, as it seems to happen in the bc_1.

Rappaport and Deniau have shown that the the initial slope of voltage generation by the *bf* was slowed four-fold by a H_2O/D_2O substitution, in contrast with the redox-reactions of cytochromes *f* and b_6 that were only little affected [116]. These authors concluded that a proton pump was triggered by the oxidation of plastoquinol in center *P*. In principle, these data are compatible with the aforementioned scheme where the voltage generation in the bc_1 resulted from the ultimate, electrostatically compensating re-distribution of protons.

Furthermore, two inhibitors of the *bf* complex, namely 2-*n*-nonyl-4-hydroxyquinoline *N*-oxide (NQNO) [117-119] and E-β-methoxyacrylate-stilbene (MOA-stilbene) [15], were shown to retard concurrently the flash-induced re-reduction of cytochrome *f*, the oxidation of cytochrome b_6 and the onset of voltage generation, thereby hardly affecting the rate of the flash-induced cytochrome b_6 reduction and the steady turnover of the *bf*. Apparently, this kind of weak inhibition resembles the aforementioned action of Zn^{2+} ions on the bc_1. NQNO and MOA-stilbene were suggested to interact with center *N* of the *bf*. The X-ray structure of the *bf* that was crystallized in the presence of NQNO has shown that, indeed, this quinone analogue binds as an axial ligand to heme c_n and apparently imitates the binding of a plastoquinone molecule [120]. Hence, the slowing of the cytochrome *f* re-reduction and voltage generation in response to the binding of NQNO in center *N* might imply a connection between centers *N* and *P*, similar to the coupling between the two quinone-binding centers in the bc_1 (*vide supra*).

It was repeatedly shown that, in the absence of inhibitors, only about 0.5 heme *b* per *bf* monomer could be reduced in response to a flash, with this fraction increasing two-fold in the presence of NQNO [11, 15, 16, 111-113]. Later it was found that NQNO decreases the midpoint potential of heme c_n by > 200 mV [121]. Hence, in the absence of NQNO the reduction of cytochrome b_6 by the first electron leads apparently to an even spreading of this electron over the c_n/b_h heme pair [106]. Appearance of a second electron at the c_n/b_h heme

pair should prompt a two-electron reduction of a plastoquinone molecule to plastoquinol in center *N* [3, 106], surpassing a stable semiquinone as a reaction intermediate (in contrast to the bc_1). Not surprisingly, a stable semiquinone has never been observed in the *bf* complexes. The flash-induced reactions in the *bf* were shown to be much faster in pre-illuminated samples than after their dark adaptation under oxidizing conditions (see [15, 122] and references therein). To explain this feature, Rich and co-workers put forward a scheme where the initial turnover(s) of an oxidized *bf* yielded a metastable, slowly relaxing enzyme intermediate, *I*, with one electron being spread over the ($b_l b_h Q_N$) system. It was proposed that if the subsequent turnover of the enzyme proceeded before the state *I* decayed to the initial oxidized state, the rate of this turnover was rapid. In the view of new data on functional dimerization of the *bf* [103, 104, 123] and on redox properties of its constituents [7, 106, 121], the hypothetical state *I* might be tentatively attributed to a dimeric *bf* where one electron is shared by the c_n/b_h heme couple in each monomer. This state, however, formally corresponds to the activated state $Q_N^{\bullet -} b_h^{ox} \parallel b_h^{red}$ in the bc_1 complex (state A in Fig. 3) where one electron is "stored" in each cytochrome *b* moiety.

Based on this analysis, a scheme of an activated Q-cycle in the cytochrome *bf* complex can be suggested (Fig. 4). Here the activated *bf* is pre-loaded by two electrons that reside at the two c_n/b_h heme pairs. Then the oxidation of the each plastoquinol molecule in center *P* - in response to a flash of light - will yield a plastoquinol molecule in center *N*. By analogy with the bc_1, it is tempting to suggest that the reduction of the FeS cluster and the c_n/b_h heme pair after the oxidation of a plastoquinol molecule in center *P* (transition A → B in Fig. 4) might proceed faster than the subsequent steps coupled with formation of plastoquinol in center *N* (transition B → C in Fig. 4). The electrical neutralization of the system upon plastoquinol formation in center *N* might be coupled with electrogenic proton displacements and explain the observed kinetic coupling between the oxidation of cytochrome b_6, re-reduction of cytochrome *f*, voltage generation and proton transfer reactions [11, 15, 16, 111-113].

The lack of a semiquinone stabilizing capacity should hinder the dark activation of the *bf* via plastoquinol oxidation in center *N*, as well as the eventual decay of this state via plastoquinol formation in center *N*. The *bf* complex, however, can be dark-activated via reduction of heme c_n by ferredoxin as depicted in Fig. 4. Although this reaction remains highly debated, evidence in its favor were obtained both *in vitro* [124] and *in vivo* (see [125, 126] and references therein).

The ubiquinone pool is half-reduced both in cells of *Rhodobacter* [127] and in respiring mitochondria [128]. Therefore the activated state of the bc_1 seems to be in equilibrium with the membrane ubiquinol pool *in vivo* and represent the physiological state of the enzyme [18]. To what extent the activated state of the *bf* is populated in living organisms is yet to be established. On the one hand, the plastoquinol pool can become oxidized *in vivo* (see [129, 130] and references therein). On the other hand, a continuous reduction by ferredoxin might help to keep the *bf* in a metastable, "pre-loaded" state. In addition, the aforementioned absence of a stable semiquinone in center *N* might account for the long life time of the activated state in the *bf*, this life time can reach minutes in the absence of externally added redox mediators [15, 122]. Hence, owing to the possibility of heme c_n reduction by ferredoxin and the longevity of the activated state, the *bf* complexes might remain activated even when the membrane plastoquinone pool gets sporadically oxidized. It is tempting to speculate that the well-recognized redox regulation of electron flows between the two photosystems of green plants (see [129-131] and references therein) might help to keep the cytochrome *bf* complex in an activated state.

5. Conclusions and outlook

The Q-cycle schemes of Peter Mitchell [27, 28] have invoked the delivery of the second electron to center *N* by some other enzyme complex (shown as "*d*" in Fig. 1B). Although Mitchell has noted that center (i) (center *N*) of mitochondrial bc_1 may be, in principle, a self-contained ubiquinone reductase [28], the Q-cycle schemes that he suggested for the bc_1 complexes of phototrophic bacteria and the *bf* complexes of plants all invoked tentative second electron donors [28]. By invoking additional electron donors, Mitchell may have tried to avoid the incompleteness of the Q-cycle: while the substrate quinol is irreversibly utilized in center *P*, the formation of the product quinol in center *N* is incomplete in the absence of a second electron donor and depends on the availability of a second quinol molecule for center *P* (see e.g. Fig. 1A). The activated Q-cycle schemes (see Fig. 3 and 4) suggest an alternative way to avoid unfinished catalytic cycles: here the cytochrome *b* moieties can be pre-reduced by mobile quinol and/or ferredoxin molecules, that should enable one-shot quinol formation in centers *N*.

Which enzyme complexes provide electrons for the molecules of quinol and ferredoxin? In chromatophores of *Rhodobacter* the major supplier of reduced ubiquinol molecules is the RC - that was suggested to donate the second electron for ubiquinone reduction in center *N* (center (i)) by Mitchell [28]. In mitochondria, the molecules of ubiquinol come from various dehydrogenases, including the succinate dehydrogenase that was envisioned by Mitchell as the donor of the second electron. In the green plants and cyanobacteria, Mitchell suggested the photosystem II as the second electron donor for the *bf* [28]. Here it is yet to be established yet, whether the *bf* is activated by plastoquinol molecules that come from the Photosystem II or by ferredoxin molecules that are reduced by Photosystem I, or by both. In this framework, the concept of the activated Q-cycle (Fig. 3 and 4) might be considered as an updated version of the original Mitchell's scheme (Fig. 1B).

6. Acknowledgements

Stimulating discussions with Drs. E.A. Berry, D.A. Cherepanov, J.W. Cooley, W.A. Cramer, A.R. Crofts, F. Daldal, L.A. Drachev, L. Esser, M.Y. Galperin, O.A. Gopta, C. Hunte, W. Junge, S.S. Klishin, A.A. Konstantinov, C.R.D. Lancaster, M.D. Mamedov, P. Mitchell, D. Picot, F. Rappaport, P. Rich, V.P. Shinkarev, V.P. Skulachev, M.V. Verkhovsky, D. Xia, and C.-A. Yu are gratefully appreciated. The research of the author was supported by the Volkswagen Foundation, and by grants from the *Deutsche Forschungsgemeinschaft* (Mu-1285/1, SFB 431-P15, 436-RUS-113/ 210).

References

[1] E.A. Berry, M. Guergova-Kuras, L.S. Huang, A.R. Crofts, Structure and function of cytochrome *bc* complexes, Ann. Rev. Biochem., 69 (2000) 1005-1075.

[2] P.R. Rich, The quinone chemistry of *bc* complexes, Biochim Biophys Acta, 1658 (2004) 165-171.

[3] W.A. Cramer, H. Zhang, J. Yan, G. Kurisu, J.L. Smith, Transmembrane traffic in the cytochrome b_6f complex, Annu Rev Biochem, 75 (2006) 769-790.

[4] D.M. Kramer, W. Nitschke, J.W. Cooley, The cytochrome bc_1 and related *bc* complexes: The Rieske/Cytochrome *b* complex as the functional core of a central electron/proton transfer complex in: N. Hunter, F. Daldal, M. Thurnauer, J.T. Beatty (Eds.) The Purple Phototrophic Bacteria, Springer, Dordrecht, 2008, pp. 451-473.

[5] C. Hunte, S. Solmaz, H. Palsdottir, T. Wenz, A structural perspective on mechanism and function of the cytochrome bc (1) complex, Results Probl Cell Differ, 45 (2008) 253-278.

[6] C. de Vitry, R. Kuras, The cytochrome b_6f complex, in: D.B. Stern (Ed.) The Chlamydomonas sourcebook. Second Edition: Organellar and Metabolic Processes, Volume 2 Elsevier, Oxford, 2008, pp. 603-637.

[7] D. Baniulis, E. Yamashita, H. Zhang, S.S. Hasan, W.A. Cramer, Structure-function of the cytochrome b_6f complex, Photochem Photobiol, 84 (2008) 1349-1358.

[8] W.A. Cramer, D.B. Knaff, Energy Transduction in Biological Membranes: A Textbook of Bioenergetics, Springer-Verlag, 1990.

[9] J.B. Jackson, A.R. Crofts, The kinetics of light induced carotenoid changes in *Rhodopseudomonas spheroides* and their relation to electrical field generation across the chromatophore membrane, Eur J Biochem, 18 (1971) 120-130.

[10] R.J. Cogdell, J.B. Jackson, A.R. Crofts, The effect of redox potential on the coupling between rapid hydrogen-ion binding and electron transport in chromatophores from *Rhodopseudomonas spheroides*, J Bioenerg, 4 (1973) 211-227.

[11] B.R. Velthuys, Electron flow through plastoquinone and cytochromes b_6 and *f* in chloroplasts, Proc Natl Acad Sci U S A, 76 (1979) 2765-2769.

[12] A.R. Crofts, C.A. Wraight, The electrochemical domain of photosynthesis, Biochim. Biophys. Acta, 726 (1983) 149-185.

[13] A.R. Crofts, S.W. Meinhardt, K.R. Jones, M. Snozzi, The role of the quinone pool in the cyclic electron transfer chain of *Rhodopseudomonas sphaeroides* Biochim. Biophys. Acta, 723 (1983) 202-218.

[14] P. Joliot, A. Joliot, Proton pumping and electron transfer in the cytochrome b/f complex of algae, Biochim. Biophys. Acta, 849 (1986) 211-222.

[15] P.R. Rich, S.A. Madgwick, S. Brown, G. vonJagow, U. Brandt, MOA-stilbene: A new tool for investigation of the reactions of the chloroplast cytochrome bf complex, Photosynth. Res., 34 (1992) 465-477.

[16] A.B. Hope, R.R. Huilgol, M. Panizza, M. Thompson, D.B. Matthews, The flash-induced turnover of cytochrome b-563, cytochrome f and plastocyanin in chloroplasts. Models and estimation of kinetic-parameters, Biochim. Biophys. Acta, 1100 (1992) 15-26.

[17] S.S. Klishin, W. Junge, A.Y. Mulkidjanian, Flash-induced turnover of the cytochrome bc_1 complex in chromatophores of *Rhodobacter capsulatus*: binding of Zn^{2+} decelerates likewise the oxidation of cytochrome *b*, the reduction of cytochrome c_1 and the voltage generation, Biochim Biophys Acta, 1553 (2002) 177-182.

[18] A.Y. Mulkidjanian, Proton translocation by the cytochrome bc_1 complexes of phototrophic bacteria: introducing the activated Q-cycle, Photochem Photobiol Sci, 6 (2007) 19-34.

[19] D. Xia, C.A. Yu, H. Kim, J.Z. Xia, A.M. Kachurin, L. Zhang, L. Yu, J. Deisenhofer, Crystal structure of the cytochrome bc_1 complex from bovine heart mitochondria, Science, 277 (1997) 60-66.

[20] Z. Zhang, L. Huang, V.M. Shulmeister, Y.I. Chi, K.K. Kim, L.W. Hung, A.R. Crofts, E.A. Berry, S.H. Kim, Electron transfer by domain movement in cytochrome bc_1, Nature, 392 (1998) 677-684.

[21] S. Iwata, J.W. Lee, K. Okada, J.K. Lee, M. Iwata, B. Rasmussen, T.A. Link, S. Ramaswamy, B.K. Jap, Complete structure of the 11-subunit bovine mitochondrial cytochrome bc_1 complex, Science, 281 (1998) 64-71.

[22] C. Lange, C. Hunte, Crystal structure of the yeast cytochrome bc_1 complex with its bound substrate cytochrome *c*, Proc Natl Acad Sci U S A, 99 (2002) 2800-2805.

[23] E.A. Berry, L.S. Huang, L.K. Saechao, N.G. Pon, M. Valkova-Valchanova, F. Daldal, X-ray structure of *Rhodobacter capsulatus* cytochrome bc_1: Comparison with its mitochondrial and chloroplast counterparts, Photosynth Res, 81 (2004) 251-275.

[24] L. Esser, M. Elberry, F. Zhou, C.A. Yu, L. Yu, D. Xia, Inhibitor-complexed structures of the cytochrome bc1 from the photosynthetic bacterium Rhodobacter sphaeroides, J Biol Chem, 283 (2008) 2846-2857.

[25] E.A. Berry, L.S. Huang, Observations concerning the quinol oxidation site of the cytochrome bc1 complex, FEBS Lett, 555 (2003) 13-20.

[26] P. Mitchell, Protonmotive redox mechanism of the cytochrome b-c1 complex in the respiratory chain: protonmotive ubiquinone cycle, FEBS Lett, 56 (1975) 1-6.

[27] P. Mitchell, The protonmotive Q cycle: a general formulation, FEBS Lett, 59 (1975) 137-139.

[28] P. Mitchell, Possible molecular mechanisms of the protonmotive function of cytochrom systems, J. Theor. Biol., 62 (1976) 327-367.

[29] M.K. Wikstrom, J.A. Berden, Oxidoreduction of cytochrome *b* in the presence of antimycin, Biochim. Biophys. Acta, 283 (1972) 403-420.

[30] B. Chance, D.F. Wilson, P.L. Dutton, M. Erecinska, Energy-coupling mechanisms in mitochondria: kinetic, spectroscopic, and thermodynamic properties of an energy-transducing form of cytochrome b, Proc Natl Acad Sci U S A, 66 (1970) 1175-1182.

[31] H.G. Lawford, P.B. Garland, Proton translocation coupled to quinol oxidation in ox heart mitochondria, Biochem. J., 136 (1973) 711-720.

[32] E.C. Hurt, N. Gabellini, Y. Shahak, W. Lockau, G. Hauska, Extra proton translocation and membrane potential generation--universal properties of cytochrome bc_1/b_6f complexes reconstituted into liposomes, Arch. Biochem. Biophys., 225 (1983) 879-885.

[33] P.B. Garland, R.A. Clegg, D. Boxer, J.A. Downie, B.A. Haddock, Proton-translocating nitrate reductase of *Escherichia coli*., in: E. Quagliariello, S. Papa, F. Palmieri, E.C. Slater, N.

Siliprandi (Eds.) Electron Transfer Chains and Oxidative Phosphorilation, North-Holland Publ. Co., Amsterdam, 1975, pp. 351-358.

[34] A.R. Crofts, The Q-cycle - a personal perspective, Photosynth Res, 80 (2004) 223-243.

[35] E.C. Slater, The mechanism of action of the respiratory inhibitor, antimycin, Biochim.Biophys. Acta, 301 (1973) 129-154.

[36] M.K. Wikstrom, The different cytochrome *b* components in the respiratory chain of animal mitochondria and their role in electron transport and energy conservation, Biochim. Biophys. Acta, 301 (1973) 155-193.

[37] M. Wikstrom, K. Krab, M. Saraste, Proton-translocating cytochrome complexes, Ann. Rev. Biochem., 50 (1981) 623-655.

[38] S. de Vries, The pathway of electron transfer in the dimeric QH2: cytochrome c oxidoreductase, J Bioenerg Biomembr, 18 (1986) 195-224.

[39] J.B. Jackson, Bacterial photosynthesis, in: C. Anthony (Ed.) Bacterial energy transduction, Academic Press, London, 1988, pp. 317–376.

[40] P.L. Dutton, R.C. Prince, Reaction center driven cytochrome interactions in electron and proton translocation and energy conservation, in: R.K. Clayton, W.R. Sistrom (Eds.) The Photosynthetic Bacteria, Academic Press, New York, 1978, pp. 525–570.

[41] L.A. Drachev, M.D. Mamedov, A.Y. Mulkidjanian, A.Y. Semenov, V.P. Shinkarev, M.I. Verkhovsky, B.S. Kaurov, V.P. Skulachev, Flash-induced electrogenic events in the photosynthetic reaction center and cytochrome bc_1 -complex of *Rhodobacter sphaeroides* chromatophores, Biochim. Biophys. Acta, 973 (1989) 189-197.

[42] K. Petty, J.B. Jackson, P.L. Dutton, Factors controlling the binding of two protons per electron transferred through the ubiquinone and cytochrome b/c2 segment of Rhodopseudomonas sphaeroides chromatophores, Biochim. Biophys. Acta, 546 (1979) 17-42.

[43] A.Y. Mulkidjanian, W. Junge, Calibration and time resolution of lumenal pH-transients in chromatophores of *Rhodobacter capsulatus* following a single turnover flash of light: proton release by the cytochrome bc_1-complex is strongly electrogenic, FEBS Letters, 353 (1994) 189-193.

[44] E.G. Glaser, S.W. Meinhardt, A.R. Crofts, Reduction of cytochrome b-561 through the antimycin-sensitive site of the ubiquinol-cytochrome c2 oxidoreductase complex of Rhodopseudomonas sphaeroides, FEBS Letters, 178 (1984) 336-342.

[45] D.E. Robertson, K.M. Giangiacomo, S. de Vries, C.C. Moser, P.L. Dutton, Two distinct quinone-modulated modes of antimycin-sensitive cytochrome b reduction in the cytochrome bc1 complex, FEBS Letters, 178 (1984) 343-350.

[46] B.E. Schultz, K.C. Hansen, C.C. Lin, S.I. Chan, Rapid photochemical generation of ubiquinol through a radical pathway: an avenue for probing submillisecond enzyme kinetics, J Org Chem, 65 (2000) 3244-3247.

[47] B.E. Schultz, S.I. Chan, Structures and proton-pumping strategies of mitochondrial respiratory enzymes, Annu.Rev.Biophys.Biomol.Struct., 30:23-65. (2001) 23-65.

[48] J. Zhu, T. Egawa, S.R. Yeh, L. Yu, C.A. Yu, Simultaneous reduction of iron-sulfur protein and cytochrome b(L) during ubiquinol oxidation in cytochrome bc(1) complex, Proc Natl Acad Sci U S A, 104 (2007) 4864-4869.

[49] R. Covian, B.L. Trumpower, The dimeric structure of the cytochrome bc(1) complex prevents center P inhibition by reverse reactions at center N, Biochim Biophys Acta, 1777 (2008) 1044-1052.

[50] S.G.E. Andersson, A. Zomorodipour, J.O. Andersson, T. Sicheritz-Ponten, U.C.M. Alsmark, R.M. Podowski, A.K. Naslund, A.S. Eriksson, H.H. Winkler, C.G. Kurland, The genome sequence of Rickettsia prowazekii and the origin of mitochondria, Nature, 396 (1998) 133-140.

[51] M. Saraste, Location of haem-binding sites in the mitochondrial cytochrome b FEBS Letters, 166 (1984) 367-372.

[52] W.R. Widger, W.A. Cramer, R.G. Herman, A. Trebst, Sequence homology and structural similarity between cytochrome b of mitochondrial complex III and the chloroplast b6-f complex: position of the cytochrome b hemes in the membrane, Proc. Natl. Acad. Sci USA, 81 (1984) 674-678.

[53] C.A. Yu, J.Z. Xia, A.M. Kachurin, L. Yu, D. Xia, H. Kim, J. Deisenhofer, Crystallization and preliminary structure of beef heart mitochondrial cytochrome-bc_1 complex, Biochim. Biophys. Acta, 1275 (1996) 47-53.

[54] D.P. O'Keefe, P.L. Dutton, Cytochrome *b* oxidation and reduction reactions in the ubiquinone- cytochrome b/c_2 oxidoreductase from *Rhodopseudomonas sphaeroides*, Biochim. Biophys. Acta, 635 (1981) 149-166.

[55] J.R. Bowyer, A.R. Crofts, On the mechanism of photosynthetic electron transfer in *Rhodopseudomonas capsulata* and *Rhodopseudomonas sphaeroides*, Biochim. Biophys. Acta, 636 (1981) 218-233.

[56] K. Matsuura, D.P. O'Keefe, P.L. Dutton, A reevaluation of the events leading to the electrogenic reaction and proton translocation in the ubiquinol-cytochrome *c* oxidoreductase of *Rhodopseudomonas sphaeroides*, Biochim. Biophys. Acta, 722 (1983) 12-22.

[57] A. Mulkidjanian, M.D. Mamedov, L.A. Drachev, Slow electrogenic events in the cytochrome bc_1-complex of *Rhodobacter sphaeroides*. The electron transfer between cytochrome *b* hemes can be non-electrogenic, FEBS Lett, 284 (1991) 227-231.

[58] A.Y. Mulkidjanian, Ubiquinol oxidation in the cytochrome bc1 complex: reaction mechanism and prevention of short-circuiting, Biochim Biophys Acta, 1709 (2005) 5-34.

[59] A.Y. Mulkidjanian, Proton in the well and through the desolvation barrier, Biochim Biophys Acta, 1757 (2006) 415-427.

[60] A.Y. Mulkidjanian,W. Junge, Electrogenic proton displacements in the cytochrome bc_1 complex of *Rhodobacter capsulatus*, in: P. Mathis (Ed.) Photosynthesis: from Light to Biosphere: Proceedings of the Xth International Photosynthesis Congress, Vol. II, Kluwer Academic Publishers, Dordrecht, 1995, pp. 547–550.

[61] O.A. Gopta, B.A. Feniouk, W. Junge, A.Y. Mulkidjanian, The cytochrome bc_1 complex of *Rhodobacter capsulatus* : ubiquinol oxidation in a dimeric Q-cycle?, FEBS Letters, 431 (1998) 291-296.

[62] M.R. Jones, J.B. Jackson, Proton efflux from right-side-out membrane vesicles of *Rhodobacter sphaeroides* after short flashes, Biochim. Biophys. Acta, 1019 (1990) 51-58.

[63] .S. Klishin, A.Y.Mulkidjanian, Proton transfer paths at the quinol oxidizing site of the *Rb. capsulatus* cytochrome *bc* complex, in: A. van der Est, D. Bruce (Eds.) Photosynthesis: Fundamental aspects to global perspectives, Vol. 1, Montreal, 2005, pp. 260–262.

[64] V.P. Shinkarev, A.R. Crofts, C.A. Wraight, The electric field generated by photosynthetic reaction center induces rapid reversed electron transfer in the bc1 complex, Biochemistry, 40 (2001) 12584-12590.

[65] B.A. Feniouk, M.A. Kozlova, D.A. Knorre, D.A. Cherepanov, A.Y. Mulkidjanian, W. Junge, The proton driven rotor of ATP synthase: Ohmic conductance (10 fS), and absence of voltage gating, Biophys. J., 86 (2004) 665-680.

[66] D.A. Cherepanov, L.I. Krishtalik, A.Y. Mulkidjanian, Photosynthetic electron transfer controlled by protein relaxation: analysis by Langevin stochastic approach, Biophys J, 80 (2001) 1033-1049.

[67] M.G. Madej, H.R. Nasiri, N.S. Hilgendorff, H. Schwalbe, C.R. Lancaster, Evidence for transmembrane proton transfer in a dihaem-containing membrane protein complex, EMBO J, 25 (2006) 4963-4970.

[68] V.P. Skulachev, V.V. Chistyakov, A.A. Jasaitis, E.G. Smirnova, Inhibition of the respiratory chain by zinc ions, Biochem.Biophys.Res.Comm., 26 (1967) 1-6.

[69] M. Lorusso, T. Cocco, A.M. Sardanelli, M. Minuto, F. Bonomi, S. Papa, Interaction of Zn^{2+} with the bovine-heart mitochondrial bc_1 complex, Eur J Biochem, 197 (1991) 555-561.

[70] T.A. Link, G. von Jagow, Zinc ions inhibit the Q_P center of bovine heart mitochondrial bc_1 complex by blocking a protonatable group, J.Biol Chem., 270 (1995) 25001-25006.

[71] E.A. Berry, Z. Zhang, H.D. Bellamy, L. Huang, Crystallographic location of two Zn^{2+}-binding sites in the avian cytochrome bc_1 complex, Biochim Biophys Acta, 1459 (2000) 440-448.

[72] L. Giachini, F. Francia, G. Veronesi, D.W. Lee, F. Daldal, L.S. Huang, E.A. Berry, T. Cocco, S. Papa, F. Boscherini, G. Venturoli, X-Ray absorption studies of Zn^{2+} binding sites in bacterial, avian, and bovine cytochrome bc_1 complexes, Biophys J, 93 (2007) 2934-2951.

[73] T. Wenz, P. Hellwig, F. MacMillan, B. Meunier, C. Hunte, Probing the role of E272 in quinol oxidation of mitochondrial complex III, Biochemistry, 45 (2006) 9042-9052.

[74] S. Izrailev, A.R. Crofts, E.A. Berry, K. Schulten, Steered molecular dynamics simulation of the Rieske subunit motion in the cytochrome bc(1) complex, Biophys J, 77 (1999) 1753-1768.

[75] E. Darrouzet, F. Daldal, Movement of the iron-sulfur subunit beyond the ef loop of cytochrome b is required for multiple turnovers of the bc1 complex but not for single turnover Qo site catalysis, J Biol Chem, 277 (2002) 3471-3476.

[76] J.W. Cooley, T. Ohnishi, F. Daldal, Binding dynamics at the quinone reduction (Qi) site influence the equilibrium interactions of the iron sulfur protein and hydroquinone oxidation (Qo) site of the cytochrome bc1 complex, Biochemistry, 44 (2005) 10520-10532.

[77] J.W. Cooley, D.W. Lee, F. Daldal, Across membrane communication between the Q(o) and Q(i) active sites of cytochrome bc(1), Biochemistry, 48 (2009) 1888-1899.

[78] R. Covian, B.L. Trumpower, Regulatory interactions between ubiquinol oxidation and ubiquinone reduction sites in the dimeric cytochrome bc1 complex, J Biol Chem, 281 (2006) 30925-30932.

[79] R. Covian, T. Kleinschroth, B. Ludwig, B.L. Trumpower, Asymmetric binding of stigmatellin to the dimeric Paracoccus denitrificans bc1 complex: evidence for anti-cooperative ubiquinol oxidation and communication between center P ubiquinol oxidation sites, J Biol Chem, 282 (2007) 22289-22297.

[80] R. Covian, B.L. Trumpower, Regulatory interactions in the dimeric cytochrome bc(1) complex: the advantages of being a twin, Biochim Biophys Acta, 1777 (2008) 1079-1091.

[81] U. Brandt, G. von Jagow, Analysis of inhibitor binding to the mitochondrial cytochrome c reductase by fluorescence quench titration. Evidence for a 'catalytic switch' at the Qo center, Eur J Biochem, 195 (1991) 163-170.

[82] T.A. Link, The role of the 'Rieske' iron sulfur protein in the hydroquinone oxidation (Q(P)) site of the cytochrome bc1 complex. The 'proton-gated affinity change' mechanism, FEBS Letters, 412 (1997) 257-264.

[83] A. Osyczka, C.C. Moser, P.L. Dutton, Fixing the Q cycle, Trends Biochem.Sci, 30 (2005) 176-182.

[84] J.L. Cape, M.K. Bowman, D.M. Kramer, Understanding the cytochrome bc complexes by what they don't do. The Q-cycle at 30, Trends Plant Sci, 11 (2006) 46-55.

[85] D. Xia, L. Esser, L. Yu, C.A. Yu, Structural basis for the mechanism of electron bifurcation at the quinol oxidation site of the cytochrome bc1 complex, Photosynth Res, 92 (2007) 17-34.

[86] S.E. Chobot, H. Zhang, C.C. Moser, P.L. Dutton, Breaking the Q-cycle: finding new ways to study Qo through thermodynamic manipulations, J Bioenerg Biomembr, 40 (2008) 501-507.

[87] V.P. Shinkarev, C.A. Wraight, Intermonomer electron transfer in the bc1 complex dimer is controlled by the energized state and by impaired electron transfer between low and high potential hemes, FEBS Lett, 581 (2007) 1535-1541.

[88] S. De Vries, S.P. Albracht, J.A. Berden, E.C. Slater, The pathway of electrons through OH2:cytochrome c oxidoreductase studied by pre-steady -state kinetics, Biochim Biophys Acta, 681 (1982) 41-53.

[89] S. De Vries, S.P. Albracht, J.A. Berden, C.A. Marres, E.C. Slater, The effect of pH, ubiquinone depletion and myxothiazol on the reduction kinetics of the prosthetic groups of ubiquinol:cytochrome c oxidoreductase, Biochim Biophys Acta, 723 (1983) 91-103.

[90] C.C. Moser, J.M. Keske, K. Warncke, R.S. Farid, P.L. Dutton, Nature of biological electron transfer, Nature, 355 (1992) 796-802.

[91] A. Osyczka, C.C. Moser, F. Daldal, P.L. Dutton, Reversible redox energy coupling in electron transfer chains, Nature, 427 (2004) 607-612.

[92] R. Covian, E.B. Gutierrez-Cirlos, B.L. Trumpower, Anti-cooperative oxidation of ubiquinol by the yeast cytochrome bc1 complex, J Biol Chem, 279 (2004) 15040-15049.

[93] R. Covian, B.L. Trumpower, Rapid electron transfer between monomers when the cytochrome bc1 complex dimer is reduced through center N, J Biol Chem, 280 (2005) 22732-22740.

[94] M. Castellani, R. Covian, T. Kleinschroth, O. Anderka, B. Ludwig, B.L. Trumpower, Direct demonstration of half-of-the-sites reactivity in the dimeric cytochrome bc1 complex: enzyme with one inactive monomer is fully active but unable to activate the second ubiquinol oxidation site in response to ligand binding at the ubiquinone reduction site, J Biol Chem, 285 (2010) 502-510.

[95] A.Y. Mulkidjanian, Proton transfer by membrane proteins: Whether there are rules?, Biochim. Biophys. Acta, 1757, Supplement 1 (2006) 92.

[96] J.N. Siedow, S. Power, F.F. de la Rosa, G. Palmer, The preparation and characterization of highly purified, enzymically active complex III from baker's yeast, J.Biol Chem., 253 (1978) 2392-2399.

[97] F.F. de la Rosa, G. Palmer, Reductive titration of CoQ-depleted Complex III from Baker's yeast. Evidence for an exchange-coupled complex between QH . and low-spin ferricytochrome b, FEBS Letters, 163 (1983) 140-143.

[98] P.R. Rich, A.E. Jeal, S.A. Madgwick, A.J. Moody, Inhibitor effects on redox-linked protonations of the *b* haems of the mitochondrial bc_1 complex, Biochim. Biophys. Acta, 1018 (1990) 29-40.

[99] R. Covian, K. Zwicker, F.A. Rotsaert, B.L. Trumpower, Asymmetric and redox-specific binding of quinone and quinol at center N of the dimeric yeast cytochrome bc1 complex. Consequences for semiquinone stabilization, J Biol Chem, 282 (2007) 24198-24208.

[100] D.E. Robertson, R.C. Prince, J.R. Bowyer, K. Matsuura, P.L. Dutton, T. Ohnishi, Thermodynamic properties of the semiquinone and its binding site in the ubiquinol-cytochrome *c* (c_2) oxidoreductase of respiratory and photosynthetic systems, J.Biol Chem., 259 (1984) 1758-1763.

[101] E.G. Glaser, A.R. Crofts, A new electrogenic step in the ubiquinol:cytochrome c_2 oxidoreductase complex of *Rhodopseudomonas sphaeroides*, Biochim. Biophys. Acta, 766 (1984) 322-333.

[102] A.Y. Mulkidjanian, M.D. Mamedov, A.Y. Semenov, V.P. Shinkarev, M.I. Verkhovsky, L.A. Drachev, Partial reversion of electrogenic reaction in ubiquinol: cytochrome c_2 reductase of *Rhodobacter sphaeroides* under the neutral and alkaline conditions FEBS Letters, 277 (1990) 127-130.

[103] D. Stroebel, Y. Choquet, J.L. Popot, D. Picot, An atypical haem in the cytochrome b(6)f complex, Nature, 426 (2003) 413-418.

[104] G. Kurisu, H. Zhang, J.L. Smith, W.A. Cramer, Structure of the cytochrome b6f complex of oxygenic photosynthesis: tuning the cavity, Science, 302 (2003) 1009-1014.

[105] H. Zhang, A. Primak, J. Cape, M.K. Bowman, D.M. Kramer, W.A. Cramer, Characterization of the high-spin heme x in the cytochrome b6f complex of oxygenic photosynthesis, Biochemistry, 43 (2004) 16329-16336.

[106] F. Baymann, F. Giusti, D. Picot, W. Nitschke, The ci/bH moiety in the b6f complex studied by EPR: a pair of strongly interacting hemes, Proc Natl Acad Sci U S A, 104 (2007) 519-524.

[107] Y. Pierre, C. Breyton, Y. Lemoine, B. Robert, C. Vernotte, J.L. Popot, On the presence and role of a molecule of chlorophyll a in the cytochrome b6 f complex, J.Biol Chem., 272 (1997) 21901-21908.

[108] H. Zhang, D. Huang, W.A. Cramer, Stoichiometrically bound beta-carotene in the cytochrome b6f complex of oxygenic photosynthesis protects against oxygen damage, J.Biol Chem., 274 (1999) 1581-1587.

[109] N. Dashdorj, H. Zhang, H. Kim, J. Yan, W.A. Cramer, S. Savikhin, The single chlorophyll a molecule in the cytochrome b6f complex: unusual optical properties protect the complex against singlet oxygen, Biophys J, 88 (2005) 4178-4187.

[110] S.E. Martinez, D. Huang, A. Szczepaniak, W.A. Cramer, J.L. Smith, Crystal structure of chloroplast cytochrome f reveals a novel cytochrome fold and unexpected heme ligation, Structure, 2 (1994) 95-105.

[111] M.A. Selak, J. Whitmarsh, Kinetics of the electrogenic step and cytochrome b_6 and cytochrome *f* redox changes in chloroplasts - evidence for a Q-Cycle, FEBS Letters, 150 (1982) 286-292.

[112] D.P. O'Keefe, Structure and function of the chloroplast cytochrome bf complex, Photosynth.Res., 17 (1988) 189-216.

[113] A.B. Hope, P.R. Rich, Proton uptake by the chloroplast cytochrome *bf* complex, Biochim. et Biophys. Acta, 975 (1989) 96-103.

[114] M.V. Ponamarev, W.A. Cramer, Perturbation of the internal water chain in cytochrome f of oxygenic photosynthesis: loss of the concerted reduction of cytochromes f and b6, Biochemistry, 37 (1998) 17199-17208.

[115] S. Heimann, M.V. Ponamarev, W.A. Cramer, Movement of the Rieske iron-sulfur protein in the p-side bulk aqueous phase: effect of lumenal viscosity on redox reactions of the cytochrome b6f complex, Biochemistry, 39 (2000) 2692-2699.

[116] C. Deniau, F. Rappaport, New insights on the proton pump associated with cytochrome b6f turnovers from the study of H/D substitution effects on the electrogenicity and electron transfer reactions, Biochemistry, 39 (2000) 3304-3310.

[117] R.W. Jones, J. Whitmarsh, Origin of the electrogenic reaction in the chloroplast cytochrome *b/f* complex, Photobiochem. Photobiophys., 9 (1985) 119-127.

[118] R.W. Jones, J. Whitmarsh, Inhibition of electron transfer and the electrogenic reaction in the cytochrome *b/f* complex by 2-n-nonyl-4-hydroxyquinoline N- oxide(NQNO) and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), Biochim. Biophys. Acta, 933 (1988) 258-268.

[119] P.R. Rich, S.A. Madgwick, D.A. Moss, The interactions of duroquinol, DBMIB and NQNO with the chloroplast cytochrome bf complex, Biochim. Biophys. Acta, 1058 (1991) 312-328.

[120] E. Yamashita, H. Zhang, W.A. Cramer, Structure of the cytochrome b6f complex: quinone analogue inhibitors as ligands of heme cn, J Mol Biol, 370 (2007) 39-52.

[121] J. Alric, Y. Pierre, D. Picot, J. Lavergne, F. Rappaport, Spectral and redox characterization of the heme c_i of the cytochrome b_6f complex, Proc Natl Acad Sci U S A, 102 (2005) 15860-15865.

[122] S. Heimann, C. Klughammer, U. Schreiber, Two distinct states of the thylakoid bf complex, FEBS Lett, 426 (1998) 126-130.

[123] D. Huang, R.M. Everly, R.H. Cheng, J.B. Heymann, H. Schagger, V. Sled, T. Ohnishi, T.S. Baker, W.A. Cramer, Characterization of the chloroplast cytochrome b6f complex as a structural and functional dimer, Biochemistry, 33 (1994) 4401-4409.

[124] P.N. Furbacher, M.E. Girvin, W.A. Cramer, On the question of interheme electron transfer in the chloroplast cytochrome b6 in situ, Biochemistry, 28 (1989) 8990-8998.

[125] P. Joliot, A. Joliot, Cyclic electron flow in C3 plants, Biochim Biophys Acta, 1757 (2006) 362-368.

[126] C. Breyton, B. Nandha, G.N. Johnson, P. Joliot, G. Finazzi, Redox modulation of cyclic electron flow around photosystem I in C3 plants, Biochemistry, 45 (2006) 13465-13475.

[127] K.I. Takamiya, P.L. Dutton, Ubiquinone in Rhodopseudomonas sphaeroides. Some thermodynamic properties, Biochim. Biophys. Acta, 546 (1979) 1-16.

[128] P.F. Urban, M. Klingenberg, On the redox potentials of ubiquinone and cytochrome *b* in the respiratory chain, Eur. J. Biochem., 9 (1969) 519-525.

[129] J.F. Allen, Botany. State transitions--a question of balance, Science, 299 (2003) 1530-1532.

[130] S. Eberhard, G. Finazzi, F.A. Wollman, The dynamics of photosynthesis, Annu Rev Genet, 42 (2008) 463-515.

[131] A.V. Vener, P.J. van Kan, P.R. Rich, I.I. Ohad, B. Andersson, Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: Thylakoid protein kinase deactivation by a single-turnover flash, Proc.Natl.Acad.Sci.U.S.A, 94 (1997) 1585-1590.

[132] W. Humphrey, A. Dalke, K. Schulten, VMD: Visual molecular dynamics, Journal of Molecular Graphics, 14 (1996) 33-38.

[133] C. Hunte, J. Koepke, C. Lange, T. Rossmanith, H. Michel, Structure at 2.3 A resolution of the cytochrome bc(1) complex from the yeast Saccharomyces cerevisiae co-crystallized with an antibody Fv fragment, Structure Fold.Des., 8 (2000) 669-684.

[134] D.E. Robertson, E. Davidson, R.C. Prince, W.H. van den Berg, B.L. Marrs, P.L. Dutton, Discrete catalytic sites for quinone in the ubiquinol-cytochrome c_2 oxidoreductase of *Rhodopseudomonas capsulata*. Evidence from a mutant defective in ubiquinol oxidation, J. Biol Chem, 261 (1986) 584-591.

[135] C.H. Yun, A.R. Crofts, R.B. Gennis, Assignment of histidine axial ligands to the cytochrome b_h and cytochrome b_l components of the bc_1 complex from *Rhodobacter sphaeroides* site directed mutagenesis, Biochemistry, 30 (1991) 6747-6754.

[136] S. Meinhardt, A.R. Crofts, Kinetic and thermodynamic resolution of cytochrome c_1 and cytochrome c_2 from *Rhodopseudomonas sphaeroides* FEBS Letters, 149 (1982) 223-227.

[137] D.E. Robertson, H. Ding, P.R. Chelminski, C. Slaughter, J. Hsu, C. Moomaw, M. Tokito, F. Daldal, P.L. Dutton, Hydroubiquinone-cytochrome c_2 oxidoreductase from *Rhodobacter capsulatus*: definition of a minimal, functional isolated preparation, Biochemistry, 32 (1993) 1310-1317.

[138] V.P. Shinkarev, D.R.J. Kolling, T.J. Miller, A.R. Crofts, Modulation of the midpoint potential of the [2Fe-2S] Rieske iron sulfur center by Q(o) occupants in the bc_1 complex, Biochemistry, 41 (2002) 14372-14382.

[139] E. Darrouzet, M. Valkova-Valchanova, F. Daldal, The [2Fe-2S] cluster E_m as an indicator of the iron-sulfur subunit position in the ubihydroquinone oxidation site of the cytochrome bc_1 complex, J Biol Chem, 277 (2002) 3464-3470.

[140] P.R. Rich, P. Heathcote, D.A. Moss, Kinetic studies of electron transfer in a hybrid system constructed from the cytochrome *bf* complex and photosystem I, Biochim. Biophys. Acta, 892 (1987) 138-151.

Captions to Figures

Fig. 1. Q-cycle in the cytochrome bc_1 complex.

A, the conventional Q-cycle scheme as plotted over the X-ray structure of the three catalytic subunits of a dimeric bc_1 of phototrophic bacteria (the X-ray structure of the bc_1 of *Rhodobacter sphaeroides* (PDB entry 2QJY [24]) was used). Yellow arrows, ET steps; green arrows, ubiquinone/ubiquinol exchange events. The figure was produced with the VMD software package [132].

B, the original Q-cycle scheme of Mitchell. The figure is based on Fig. 1 from ref. [27] and Fig. 3 from ref. [28]. The symbol (o) corresponds to center *P*, the symbol (i) marks center *N*. The additional *d*onor of the second electron to center (i) is denoted "*d*". Otherwise the original Mitchell's notation was changed to the currently used one (see the text for details).

Fig. 2. Electron and proton transfer events during the turnover of the bc_1 (the figure is taken from ref. [59]). Black arrows, electron transfer events; red arrows, proton transfer events; dotted dark red arrows, dielectric relaxation of protein/water. The redox centers are colored as in Fig. 1. The segment of the *ef* loop of cytochrome *b* that interferes with the movement of the FeS domain is shown as a thick yellow tube.

(A) The faster step of ubiquinol oxidation in center *P*. The picture is based on the high resolution structure of the yeast bc_1 (PDB entry 1EZV [133]). The bound stigmatellin in center *P* was replaced by ubiquinone. Water molecules, which are found in the vicinity of center *P* are shown as red balls. The four amino acid residues which bind a Zn^{2+} ion in chicken bc_1 are depicted in violet.

(B) The slower, Zn-dependent step of ubiquinol reduction in center *N*. The picture is based on the structure of the chicken bc_1 (PDB entry 1BCC [20]). Ubiquinol in center *N*

is shown in the same position as it is found in the yeast bc_1 [133]. The four amino acid residues, which bind a Zn^{2+} ion in the bc_1 of chicken [71] and, most likely, of *Rb. capsulatus* [72], are colored as follows: Asp-253, pink, Glu-255, violet, His-268, green (all three are residues of cytochrome *b*), His-121 of cytochrome c_1, light green.

Fig. 3. Activated Q-cycle in the cytochrome bc_1 complex. Thin black arrows, electron transfer steps; thick red arrows, proton transfer steps; thick gray arrows, quinone/quinol exchange reactions. Blue "lightning signs" indicate those steps that can be triggered by flashes of light in the bc_1 of phototrophic bacteria. Protons are depicted as red circled crosses. The cartoon in the middle shows a tentative structure of an activated bc_1 with a $Q_N^{\bullet -}$ semiquinone and a reduced heme b_h present in different monomers (colored in magenta and red, respectively, the PDB entry 1EZV [133] was used). For other explanations, see the text and ref. [18].

Fig. 4. Activated Q-cycle in the cytochrome *bf* complex (tentative scheme). Electrons at the c_n/b_h heme pair are shown as circled minus signs (magenta). Because of space limitations, plastoquinone is denoted as Q and not PQ. Otherwise the notation is similar to that in Fig. 3. A, the activated state of the *bf*: the FeS clusters and cytochromes *f* are pre-reduced and one electron is shared by each c_n/b_h heme pair. Transition A → B, fast oxidation of a plastoquinol molecule in center *P*: an electron vacancy migrates from the photosystem I to the *bf* complex after the flash; the oxidized FeS domain docks to cytochrome b_6 and binds a plastoquinol molecule in center *P*; oxidation of this plastoquinol molecule is accompanied by a delivery of an electron and a proton to the FeS cluster leaving a semiquinone in center *P*; the second electron goes from the semiquinone, via heme b_l, to the pre-reduced c_n/b_h heme pair; the FeS domain remains docked to cytochrome b_6 and plugs the proton outlet from center *P*. Transition B→ C , slow formation of a plastoquinol molecule in center *N* and voltage generation: a two-electron oxidation of the c_n/b_h heme pair generates a plastoquinol molecule

in center *N* and causes electrogenic binding of two protons from the *n*-side of the membrane; the formation of this plastoquinol molecule (or its release from center *N*) enables undocking of a reduced FeS domain and its movement towards cytochrome *f*; the oxidation of the reduced and protonated FeS domain by cytochrome *f* is accompanied by proton release into the water phase; concomitantly protons leave center *P* via the outlet that is now open; displacement of all these protons across the membrane dielectric, as well as the re-orientation of the intra-membrane dipoles, account for a large electrogenic reaction. Transition C → D and D → E describe the oxidation of the second plastoquinol molecule in the other center *P* and are essentially similar to the transitions A→ B and B → C, respectively. Blue dashed arrows indicate that the *bf* can be activated (transition F → A) either via the reduction of heme c_n by ferredoxin, or via the light-induced oxidation of two plastoquinol molecules in center *P*, or through the dark oxidation of a plastoquinol molecule via center *N*.