

Fixing the Q cycle

Artur Osyczka, Christopher C. Moser and P. Leslie Dutton

The Johnson Research Foundation, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, 19104, USA

Mitchell's key insight that all bioenergetic membranes run on the conversion of redox energy into transmembrane electrical and proton gradients took the form 30 years ago of a working model of the Q cycle of cytochrome *bc*₁, which operates reversibly on coupled electron and proton transfers of quinone at two binding sites on opposite membrane faces. His remarkable model still stands today, but he had no structural information to provide understanding into how dangerous short-circuit redox reactions were avoided. Now, it is clear that the Q cycle must be fixed with a special mechanism that avoids semiquinone-mediated short circuits. Either the redox states of the quinone electron-transfer partners double-gate the semiquinone-intermediate stability, or semiquinone is avoided altogether in concerted double-electron transfer.

Original insight into bioenergetic Q action

Thirty years ago, Peter Mitchell realized that Nature exploits the special redox properties of the quinone two-electron, two-proton redox couple to drive energy conversion between redox energy and the transmembrane electrical and proton gradient in nearly all bioenergetic membranes. His elegantly simple Q cycle is shown in Figure 1a as it applies to the cytochrome *bc* proteins of respiration and photosynthesis [1–3]. At a quinone site towards one side of the membrane, quinone (Q) is reversibly and doubly reduced, picking up two protons in the process to form hydroquinone (QH₂). The electrically neutral and non-polar QH₂ diffuses across the membrane to a second quinone site, at which QH₂ is reversibly and doubly oxidized to Q, releasing two protons. This creates or changes the transmembrane pH gradient, ΔpH. The next part of the cycle directs electrons through the protein across the membrane between the quinone sites. This creates or changes a transmembrane electrical gradient, ΔΨ. Electrically neutral Q diffuses back to the first site to complete the cycle. The combined ΔpH and ΔΨ gradients make up the proton motive force, ΔμH⁺, which is now recognized as the common bioenergetic membrane currency for a host of other processes, such as ion pumping and ATP synthesis (see Refs [4,5] for reviews of this central feature of membrane bioenergetics, and Refs [6–9] for recent reviews of cytochrome *bc* complexes operation in particular).

To drive transmembrane electron transfer and interconvert redox energy and ΔμH⁺, it is essential that the

two electrons exchanged during oxidation and reduction of quinone travel separate paths in at least one of the quinone sites. Otherwise, quinone oxidation with proton release at one site would be accompanied simply by transmembrane electron transfer of the two electrons and quinone re-reduction with proton uptake at the other site, with no net oxidation or reduction – a pointless interconversion of ΔpH and ΔΨ, with no productive change in ΔμH⁺. Without the benefit of clear structural information, Mitchell could not decide between his two proposals: (i) that the right-most quinone (now known as Q_i or Q_n) exchanged electrons with two different redox centers ('d' and 'b_R' in Figure 1a), or, as we now know to be the case, (ii) the right-most quinone exchanged electrons with just one center, now known as 'b_H'. Because Q_i exchanges exclusively with b_H, Mitchell's Q cycle requires that the other left-most quinone (now known as Q_o or Q_p) must stand at a redox divide between a high-potential redox chain and a low-potential redox chain (Figure 1b).

With the resolution of microbial, animal and plant cytochrome *bc* crystal structures [10–16], we now know the high-potential 'c chain' is usually composed of an iron–sulfur [2Fe2S] cluster (FeS), heme *c*₁ and diffusible substrate cytochrome *c*, whereas the low-potential 'b chain' includes heme *b*_L, heme *b*_H and the other quinone site Q_i. Redox centers within the chains need to be closely spaced, typically with an edge-to-edge distance of <14 Å [17,18], to ensure that electron tunneling within each chain is productive and faster than the millisecond catalytic turnover of the enzyme. In an unexpected twist, various crystal structures of cytochrome *bc*₁ revealed that FeS assumed at least two different positions [11,12,19] – one near the Q_o site and the other close enough for rapid tunneling with heme *c*₁; constrained diffusion between these positions is needed to carry out the electron-relay function of FeS. Despite its movement, FeS never approaches closer than 23 Å from heme *b*_L. This means that the danger of unproductive (short-circuiting) direct electron transfer from heme *b*_L to FeS is minimal; electrons would have to tunnel over a distance so long that it would take many seconds [17,18].

The exact position of the quinone within the 23-Å gap is unknown because quinone has never been resolved in the Q_o site. Indeed, it seems that there might even be more than one quinone near this site that influences the electron paramagnetic resonance (EPR) spectrum of FeS [20–23] and the rate of Q_o-site catalytic turnover [21]. In addition, it seems that more than one quinone is released when inhibitors are added to the Q_o site [24]. Crystal structures that include Q_o-site inhibitors (Figure 1c) show

Corresponding author: Dutton, P.L. (dutton@mail.med.upenn.edu).

Available online 8 March 2005

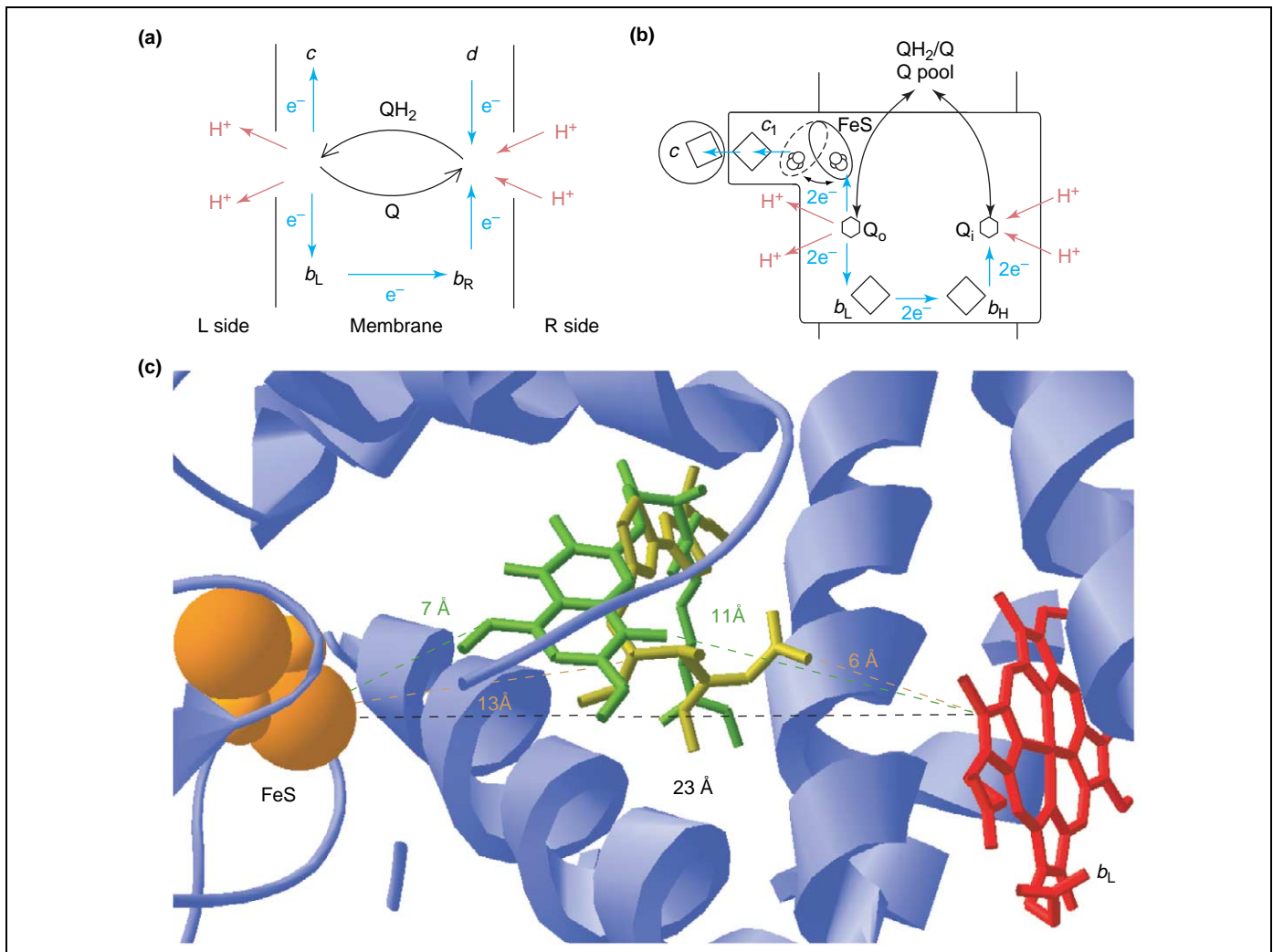


Figure 1. Q cycle and components in cytochrome *bc*₁. **(a)** Original flow diagram of proton-motive Q cycle. Arrows represent the formal forward direction of reversible transfers of electrons and protons and translocations of oxidized quinone (Q) and reduced hydroquinone (QH₂) that lead to bioenergetic production of transmembrane proton motive force ($\Delta\mu\text{H}^+$). Adapted from Ref. [2]. **(b)** Modern arrangement of components in cytochrome *bc*₁. Center *c* is now a moving FeS cluster, and the two centers *d* and *b_R* are now one center, heme *b_H*. **(c)** Close-up molecular view of the Q_o site showing a range of possible redox cofactor edge-to-edge tunneling distances based on inhibitor as proxies for quinone. Black, FeS → heme *b_L*; green, stigmatellin ring → iron-sulfur cluster (FeS) and heme *b_L*; tan, myxothiazol → FeS and heme *b_L*.

two binding loci at which quinone redox catalysis might occur: one inhibitor, stigmatellin, approaches within $\sim 7 \text{ \AA}$ of FeS [25], and the other, myxothiazol, approaches within $\sim 6 \text{ \AA}$ of heme *b_L* [26].

In Mitchell's view, when quinone is in the Q_o catalytic site between the high- and low-potential chains, its two electrons have widely different reducing power or redox-midpoint potentials (E_m). The first electron has a very positive or oxidizing potential, approximately comparable to the E_m of the high-potential chain components, whereas the second electron has a very reducing potential, which is comparable to the E_m of components of the low-potential chain; this means that the stability constant (K_{stab}) is small (Box 1). By contrast, in a Q cycle in which Q_i exchanges both electrons with the same heme *b*, it is an advantage for the SQ intermediate at Q_i to be relatively stable; depending on the pH, K_{stab} might be close to one [27]. Both midpoint potentials of Q_i are similar to each other and near, or slightly above, the average E_m of the Q pool. Regardless of the exact value of the stability constant, it is imperative that the Q_o does not allow both

electrons to move along the high- or low-potential chain, or to allow the electrons to move from the low-potential chain to the high-potential chain – this would be an energy-wasting short-circuit reaction, which would do nothing but produce heat.

Mitchell made it clear that the assumption that the Q-site reactions are readily reversible means that the Q_o site will turnover repeatedly until there is a redox balance between the high- and low-potential chains and the Q pool; in other words, the average of the redox potential (E_h) of the *c* chain and *b* chain equals the E_h of the Q pool. Early on, Chance and Hollunger demonstrated that the electron transfer in the reverse of the physiologically forward direction (i.e. electron transfers that lead to reduction of Q and oxidation of the high-potential redox chain) was fast enough to cause net reverse electron transfer via cytochrome *bc*₁ and complex I on a minutes timescale when ATP was added to mitochondria [28]. Recent experiments involving cofactor knockouts have proven that even without added ATP or a transmembrane electric field, reverse-electron-transfer rates are fast

Box 1. Semiquinone stability constant

It can be useful to break down the $n=2$ electron redox couple of species such as quinone (Q) and the doubly reduced hydroquinone (QH₂) into two separate $n=1$ redox couples, Q/semiquinone (SQ) and SQ/QH₂. The average redox midpoint potentials of these two couples are the same as the average $n=2$ redox midpoint of the Q/QH₂ couple (Figure 1):

$$(E_{m_{Q/SQ}} + E_{m_{SQ/QH_2}})/2 = E_{m_{aveQ/QH_2}}$$

On one hand, if $E_{m_{Q/SQ}}$ is more positive than $E_{m_{aveQ/QH_2}}$, (and the $E_{m_{SQ/QH_2}}$ equally more negative), then an equilibrium redox titration will reveal a stable SQ species; the SQ stability constant K_{stab} will have a value > 1 .

$$K_{stab} = \frac{[SQ]^2}{[Q][QH_2]} = 10^{(E_{m_{SQ/QH_2}} - E_{m_{Q/SQ}})/60}$$

On the other hand, if $E_{m_{Q/SQ}}$ is less than $E_{m_{aveQ/QH_2}}$, then $K_{stab} < 1$ and an equilibrium redox titration will reveal little SQ. SQ is unstable under these equilibrium redox conditions because the addition of the first electron to form the SQ is quickly followed by the favorable addition of the second electron to form QH₂. EPR titrations have revealed the presence of SQ at many quinone-binding sites, but not Q_o, suggesting that $E_{m_{SQ/QH_2}}$ is much more positive than the $E_{m_{FeS}}$, and that K_{stab} is much less than 10^{-7} [47,48].

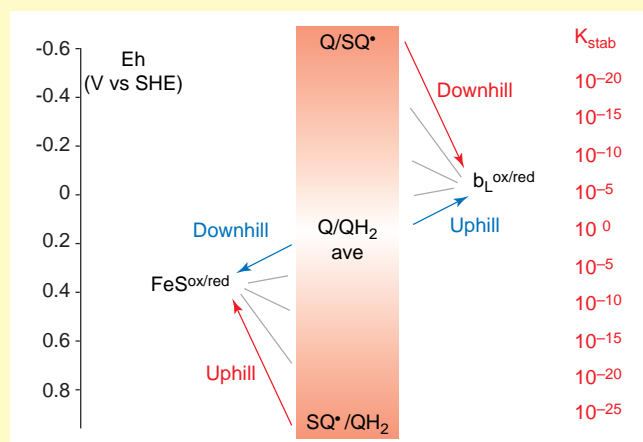


Figure 1. K_{stab} and the split in quinone redox couples. As K_{stab} becomes small, the two redox couples SQ-/QH₂ and Q/SQ- pass the FeS and heme b_L redox midpoints, so that the first and second electron transfers in QH₂ oxidation become uphill and downhill energetically, respectively.

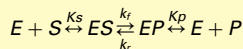
enough to limit the extent of QH₂ oxidation at Q_o on the catalytic millisecond timescale [29]. However, because researchers have focused on the molecular details of Q_o site catalysis and the means by which cytochrome bc_1 assures efficient conversion of redox energy to $\Delta\mu H^+$, the role of reverse electron transfers have often been suppressed or forgotten [30–33] (Box 2). Ignoring these reactions has dangerous consequences because, in a simple Q cycle model, these rapid reverse electron transfers repeatedly expose cytochrome bc_1 to several types of short-circuit failure, especially when the enzyme labors against a physiological transmembrane $\Delta\mu H^+$.

Q-cycle short circuits introduced by rapid reversibility

If semiquinone is an intermediate state in electron transfer at the Q_o site, placed somewhere between FeS and heme b_L so that the redox centers are within 14-Å

Box 2. Reversibility and 'one-way' enzymes

All enzymes are reversible, although large driving forces or subsequent reactions can make enzymes 'almost irreversible'. However, even when there is no net driving force, an enzyme can have a catalytic rate in the forward direction (k_f) that is faster than the catalytic rate in the reverse direction (k_r), provided the effective dissociation constants are different for the substrate (K_s) and the product (K_p) [49,50]. In a classical scheme



the equilibrium constant K_{eq}

$$K_{eq} = \frac{[P]}{[S]} = \frac{K_p k_f}{K_s k_r}$$

is a ratio of catalytic rates and dissociation constants (see Ref. [50] for a more extensive mathematical consideration). Even if there is no net driving force for the reaction ($K_{eq}=1$), it is still possible for the forward catalytic rate to be much faster than the reverse, giving the appearance of a 'one-way' enzyme, provided $K_p \ll K_s$, for example, if the product is bound quite tightly. Catalytic rates are usually measured under conditions of saturating substrate or product. However, at equilibrium with equal amounts of substrate and product, mass action in the forward direction is still balanced by mass action in the reverse direction. It is not possible to use this binding affinity adjustment to slow the catalytic rate in the reverse direction in cytochrome bc_1 in an attempt to prevent SQ-mediated short circuits at Q_o because the catalytic rates are both $\sim 10^{3\sigma-1}$, and the dissociation constants for Q and QH₂ are comparable.

tunneling distance of one another, what is to prevent the short circuits of both electrons from being exchanged with either the high- or low-potential chain, or for an electron to be transferred from the low-potential chain, via the SQ, to the high-potential chain? The revelation that FeS must undergo a constrained diffusion between the Q_o site and heme c_1 was seen by some as a design element to prevent short circuits [7,31,34,35]. After all, if QH₂ at the Q_o site were to give its first electron to FeS, and the reduced FeS remained nearby or took a relatively long time to move to heme c_1 and be re-oxidized, then the second electron of the Q would have little choice but to productively enter the low-potential b chain and a short circuit would be impossible. However, this simple viewpoint does not take into account that the cytochrome bc_1 is reversible, and at least some of the time the heme b next to the SQ_o will be reduced and unable to take an electron, while FeS is re-oxidized and returns to the Q_o site to pick up the second electron (Figure 2a). It also does not consider several short circuits that need no FeS motion, for example, when QH₂ reduces FeS to form SQ and the SQ is re-reduced by the low-potential b chain, which has the net effect of transferring one electron from the low-potential chain to the high-potential chain (Figure 2b). Similarly, Q at Q_o can be reduced to SQ by the low-potential chain, and then transfer its electron to oxidized FeS (Figure 2c). Finally, the low-potential chain can reduce Q at Q_o to SQ and then reduce it again to QH₂ (Figure 2d).

All these individual electron transfers operate in a fully reversible cytochrome bc_1 and in these inappropriate sequences would disastrously run down the bioenergetic energy gradients in respiration and photosynthesis. For

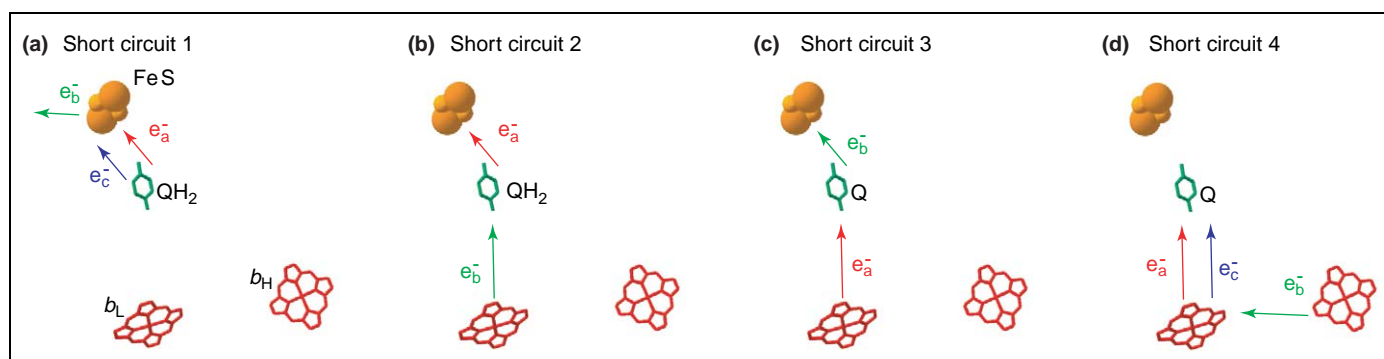


Figure 2. Energy-wasting short-circuit reactions mediated by semiquinone (SQ) at the Q_o site. Red, green and blue indicate first, second and third electron transfer in a sequence, respectively, involving FeS, Q_o , heme b_L and heme b_H . For each of the four types of short circuits the net result is unproductive loss of energy. **(a)** QH_2 donates both its electrons to the high-potential redox chain. **(b)** QH_2 first reduces FeS, and the SQ in turn is reduced by heme b_L , with net electron transfer from heme b_L to FeS. **(c)** Q is first reduced by heme b_L , and the SQ so formed goes on to reduce FeS. **(d)** Heme b_L reduces Q to SQ, and after heme b_L is reduced by heme b_H , SQ is reduced to QH_2 .

this reason, several gated models have been developed, all of which try to control the electron transfer at the Q_o site to prevent the first type of short circuit. In some models, the FeS does the gating [31,34–36], being in a position to accept or not accept electrons depending on the redox state of heme b_L , Q_o or Q_i . In other models, Q_o does the gating – in one model by moving near FeS or heme b_L depending on the redox state of Q_o [32,33], or, in another, transferring electrons exclusively with FeS or heme b_L depending on the redox state of FeS [30]. Still other Q_o -gated models forbid QH_2 binding if FeS or heme b_L is reduced [37] or enable SQ to form only if FeS is in the correct redox state to take a proton and if heme b_L is also oxidized [38]. However, none of these models prevent all types of short circuits and some even impede cytochrome bc_1 from operating reversibly.

To provide an illustration of how previously proposed models avoid some short circuits consider a Q-gated model in which the quinone moves about the Q_o site depending on its redox state [32,33]. Reduced quinone is postulated to be closer to, and engage in electron transfer exclusively with, FeS, whereas the SQ state moves closer to, and engages in exclusive electron transfer with, heme b_L . In this way, short-circuiting double-electron transfer between QH_2 and FeS is avoided (Figure 2a). This model also avoids the scenario in which FeS is reduced by b_L via SQ because SQ is assumed not to react with FeS (Figure 2c). However, the association of SQ with heme b_L only makes short circuits illustrated in Figure 2b,d more likely in this model. Furthermore, this model also makes reverse electron transfer difficult because SQ formed from reduction of Q by heme b_L is stated as unable to communicate with reduced FeS and cannot form the QH_2 needed for reversible electron transfer.

For any model using moving cofactors, it is important to remember that the tunneling electron is promiscuous; it will tunnel in any direction to any redox center providing there is a net favorable driving force with a rate that has an approximately exponential dependence on distance [17,18]. It is difficult to position the quinone anywhere in the Q_o site so that it is $>13 \text{ \AA}$ away from a potentially short-circuiting center. Using the inhibitors myxothiazol and stigmatellin to bracket the possible range of a quinone moving within the structure, the electron-tunneling distance between SQ and FeS will be at most $\sim 13 \text{ \AA}$.

This would lead to inherent tunneling rates of approximately microseconds, which is hardly slow enough to prevent short-circuiting electron transfer. Distance, by itself, will not sufficiently slow and prevent all semiquinone-mediated short circuits; redox-state-dependent energetic gating is also required. In fact, a single gating is not sufficient. In models using semiquinone, a minimum of two such gates are required [29]. Because no single value of a SQ stability constant will enable both productive reversible electron transfer and prevent unproductive short-circuit reactions, any SQ intermediate at Q_o must have its effective stability constant modulated by the redox state of both FeS and heme b_L .

Repairing the Q cycle

A double-gated model allows QH_2 oxidation when FeS and heme b_L are both oxidized, and permits Q reduction when FeS and heme b_L are both reduced, but forbids quinone electron transfer when FeS is oxidized and heme b_L is reduced [29]. It is not necessary to forbid electron transfer via SQ from reduced FeS to oxidized heme b_L – which, incidentally, is the redox state commonly observed by EPR – because this electron transfer is energy conserving and not short-circuiting.

The double-gated model has complex constraints that can be more severe than the previously proposed single-gated models. Figure 3 illustrates how this might be realized on an atomic scale. Clearly, the redox states of FeS and heme b_L would both have to contribute to the ability of the quinone to reach a semiquinone intermediate. This could be done by the FeS and heme b_L redox states controlling the conformation of the amino acids around the Q_o binding site to (i) enable the hydrogen bonding and binding of QH_2 when FeS and heme b_L are both oxidized [Figure 3a(i)]; (ii) enable a different pattern of hydrogen bonding and Q binding when FeS and heme b_L are both reduced [Figure 3b(i)]; but (iii) to forbid catalytically competent QH_2 or Q binding when FeS is oxidized and heme b_L is reduced [Figure 3a(ii) and 3b(ii)]. Because overall quinone oxidation and reduction involves two protons, it is just as likely that water molecules are involved in at least one of the protonation/deprotonation reactions as it is that the surrounding amino acids are. In this case, the redox state of FeS and heme b_L could control the pattern of water-molecule orientations or protonations

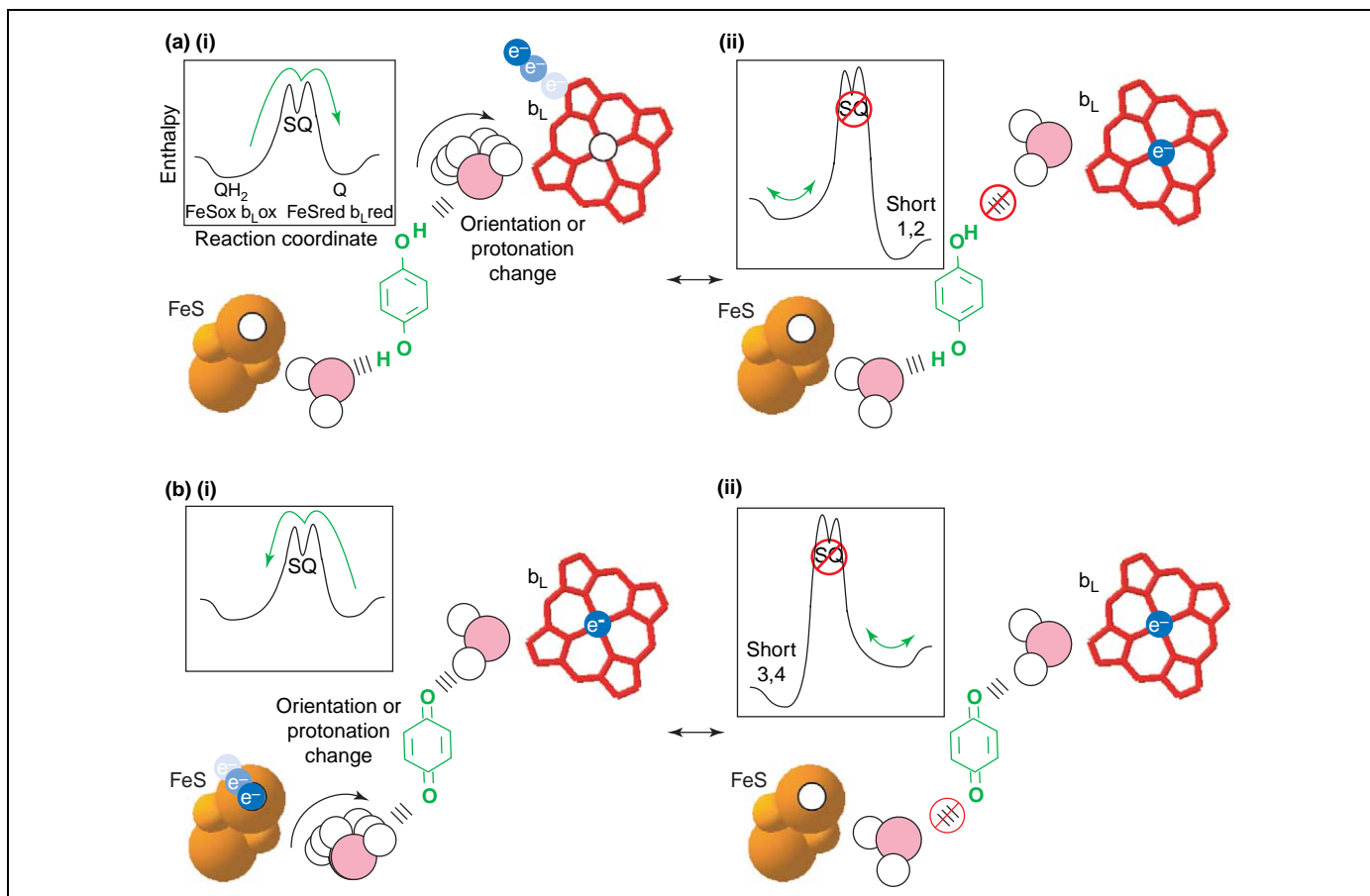


Figure 3. Avoiding short circuits by double gating. Double gating is required to prevent short circuits if SQ is an intermediate, for example, by reorientation or protonation/deprotonation of amino acid or water proton donors/acceptors as a function of the redox state of FeS and heme b_L . For simplicity, the gating with water molecules (pink) that can reorientate and change hydrogen bonding under redox control are shown. Inserts show the corresponding free energy profiles. (a) The redox state of heme b_L would raise or lower the barrier to form SQ from reduced QH_2 . (i) FeS is oxidized; upon heme b_L oxidation, a nearby water molecule reorients and can hydrogen bond to a QH_2 and favor SQ formation. (ii) FeS is still oxidized, but heme b_L is reduced and the water molecule cannot hydrogen bond to a QH_2 or stabilize SQ formation. (b) By contrast, the redox state of FeS gates SQ accessibility from oxidized Q. (i) Heme b_L is reduced; upon FeS reduction, a nearby water molecule reorients and can hydrogen bond to a Q and favor SQ formation. (ii) Heme b_L is still reduced, but FeS is oxidized and the water molecule cannot hydrogen bond to Q or stabilize SQ formation.

at the site that would lower or raise the barrier to forming a SQ intermediate state. This would be mildly analogous to the control of water chains by the redox state of hemes a and a_3 as proposed for cytochrome oxidase [39]. For example, the redox state of heme b_L could control the orientation or protonation state of a water molecule (or amino acid) to enable hydrogen bonding and deprotonation of QH_2 to form SQ only if heme b_L is oxidized (Figure 3a). At the same time, the redox state of FeS could control the orientation or protonation of a water molecule (or amino acid) to enable hydrogen-bonding of, and proton donation to, Q to form SQ only if FeS was reduced (Figure 3b). Only one quinone is illustrated in Figure 3, but it is entirely possible that two quinones are involved in catalysis at Q_o [20–24].

The histidines that ligand FeS are often mentioned as centers that could be involved in redox-linked protonations [25,32,33,36,38,40,41] and cannot be mutated without disastrous effects [42]. Although Glu295 – near heme b_L – hydrogen bonds to the inhibitor stigmatellin and is frequently referred to as one of the key mechanistic elements extracting protons from QH_2 [32,33,41,43], mutational work shows only modest kinetic effects and no phenotypic impairment of the operation of cytochrome bc_1 or b_6f under photosynthetic conditions [32,44]. This is

in contrast to cytochrome oxidase, in which proton channels can be effectively turned off with a single mutant [45,46], suggesting that the proton management at the Q_o site might be somewhat ‘soft’ and, at least partly, mediated by mobile water. Indeed, a ‘soft’ pattern of hydrogen bonding of mobile water and quinone might underlie the difficulty of resolving Q_o -site quinone structure in X-ray crystallography [10–14].

The double-gating requirement has been recently interpreted in terms of amino-acid hydrogen bonds that forbid QH_2 binding except when FeS and heme b_L are both oxidized, and forbid Q binding except when FeS and heme b_L are both reduced [40]. The gating effect is similar to our view, although more severe than needed. In our view, SQ can be discouraged even though quinone is bound, and quinone binding or SQ formation is harmless when FeS is reduced and heme b_L oxidized.

The double-gating logic diagram of control of access to the semiquinone state is sufficiently complex that an alternative, in some ways simpler, model deserves equal consideration. A truly concerted quinone-electron transfer has both electrons transferred within picoseconds of each other, with no relaxed semiquinone intermediate state available to participate in short-circuit reactions [29]. In this case, no gating is required because the quinone is

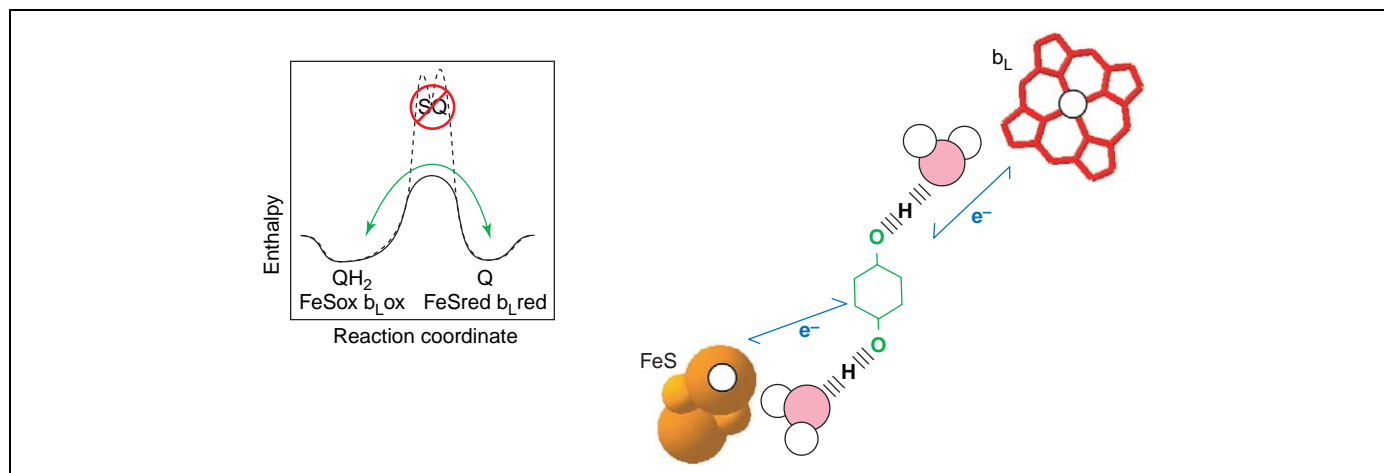


Figure 4. Avoiding short circuits by concerted electron transfer. Unlike sequential electron-transfer mechanisms, concerted double-electron transfer has no SQ state to mediate short circuits because both electrons are transferred in picoseconds or less. The chemical transition state is not a SQ but has covalent and hydrogen bond lengths intermediate between the Q and QH₂ states.

incapable of supporting the sequential single electron transfers of the short circuits. All that is required is that the site disfavors the formation of a semiquinone state (perhaps electrostatically) but favors the concerted transition state, which, presumably, has a geometry in which the carbonyl bond lengths are intermediate between the long C–O bond of QH₂ and the short C–O bond of oxidized Q, and at least one of the protons is at a distance intermediate between the short O–H bond of QH₂ and the long O–H hydrogen bond of the oxidized Q (Figure 4). In other words, the Q_o site needs to maintain the low stability constant that quinone naturally has when in the lipid membrane while bringing it close to the proton acceptors and donors of the Q_o site and of course the FeS and heme b_L electron acceptors and donors. The only short circuit remaining is the relatively long 23 Å direct distance between heme b_L and FeS, the same distance as that found in the photosynthetic reaction center between photo-reduced Q_B and the oxidized bacteriochlorophyll dimer; such a distance safely slows the electron tunneling short circuit to the seconds timescale.

Concluding remarks

As this year sees the 30-year anniversary of Peter Mitchell's visionary 'Q cycle', we can appreciate which of Mitchell's insights have, and have not, stood the test of decades of extensive experiment and the revelations of crystal structures that Mitchell could only imagine. As Mitchell's appealingly simple Q cycle entered textbooks and bioenergetics teaching, and as ever-increasing detailed experiments and models were designed, Mitchell's early emphasis on the reversibility of his cycle was lost. This had important consequences for the ability of the Q-cycle models to function, even in principle, as efficient bioenergetic machines. To some extent, this might have been encouraged by Mitchell only showing one direction of electron transfer in his Q-cycle model (Figure 1a), and leaving the reversibility to the figure legend, thus, avoiding portrayal of the problem of SQ intermediates and reverse electron transfers that might participate in unproductive short-circuit electron transfers. With improved structural information and an awareness of

electron-tunneling distances and rates, it is now clear that when the formally forward and reverse reactions are explicitly considered together, there must be several sequences of these electron-transfer steps that present a danger and, ultimately, short the Q cycle. Only by eliminating the SQ intermediate in a concerted reaction, or by introducing double gating of a SQ intermediate, can the reversible Q cycle spin smoothly again. Indeed, it seems that Nature must rely on this sort of control whenever her redox-transducing molecular machines have such modest driving forces that forward and reverse reactions are nearly balanced and unable to use large amounts of energy, such as that available to photosynthetic reaction centers and cytochrome oxidase, to slow the reverse reactions.

Acknowledgements

We acknowledge grant support from National Institutes of Health GM27309 to P.L.D. We thank C-A. Yu and D. Xia for providing coordinates of myxothiazol bound cytochrome b_c1 structure.

References

- Mitchell, P. (1975) Protonmotive redox mechanism of the cytochrome b-c₁ complex in the respiratory chain: protonmotive ubiquinone cycle. *FEBS Lett.* 56, 1–6
- Mitchell, P. (1975) The protonmotive Q cycle: a general formulation. *FEBS Lett.* 59, 137–139
- Mitchell, P. (1976) Possible molecular mechanisms of the protonmotive function of cytochrome systems. *J. Theor. Biol.* 62, 327–367
- Nicholls, D.G. and Ferguson, S.J. (2002) *Bioenergetics* (3rd edn), Academic Press
- Dutton, P.L. *et al.* (2000) Coenzyme Q oxidation reduction reactions in mitochondrial electron transport. In *Coenzyme Q: Molecular Mechanisms in Health and Disease* (Kagan, V.E. and Quinn, P.J., eds), pp. 65–82, CRC Press
- Berry, E.A. *et al.* (2000) Structure and function of cytochrome bc complexes. *Annu. Rev. Biochem.* 69, 1005–1075
- Darrrouzet, E. *et al.* (2001) Large scale domain movement in cytochrome bc₁: a new device for electron transfer in proteins. *Trends Biochem. Sci.* 26, 445–451
- Allen, F.J. (2004) Cytochrome b₆f: structure for signalling and vectorial metabolism. *Trends Plant Sci.* 9, 130–137
- Darrrouzet, E. *et al.* (2004) The cytochrome bc₁ complex and its homologue the b₆f complex: similarities and differences. *Photosynth. Res.* 79, 25–44

- 10 Xia, D. *et al.* (1997) Crystal structure of the cytochrome *bc*₁ complex from bovine heart mitochondria. *Science* 277, 60–66
- 11 Iwata, S. *et al.* (1998) Complete structure of the 11-subunit bovine mitochondrial cytochrome *bc*₁ complex. *Science* 281, 64–71
- 12 Zhang, Z. *et al.* (1998) Electron transfer by domain movement in cytochrome *bc*₁. *Nature* 392, 677–684
- 13 Hunte, C. *et al.* (2000) Structure at 2.3 Å resolution of the cytochrome *bc*₁ complex from the yeast *Saccharomyces cerevisiae* co-crystallized with an antibody F_v fragment. *Structure* 8, 669–684
- 14 Berry, E.A. *et al.* (2004) X-ray structure of *Rhodobacter capsulatus* cytochrome *bc*₁: comparison with its mitochondrial and chloroplast counterparts. *Photosynth. Res.* 81, 251–275
- 15 Stroebel, D. *et al.* (2003) An atypical haem in the cytochrome *b*_{6f} complex. *Nature* 426, 413–418
- 16 Kurisu, G. *et al.* (2003) Structure of the cytochrome *b*_{6f} complex of oxygenic photosynthesis: tuning the cavity. *Science* 302, 1009–1014
- 17 Moser, C.C. *et al.* (1992) Nature of biological electron transfer. *Nature* 355, 796–802
- 18 Page, C.C. *et al.* (1999) Natural engineering principles of electron tunneling in biological oxidation–reduction. *Nature* 402, 47–52
- 19 Kim, H. *et al.* (1998) Inhibitor binding changes domain mobility in the iron-sulfur protein of the mitochondrial *bc*₁ complex from bovine heart. *Proc. Natl. Acad. Sci. U. S. A.* 95, 8026–8033
- 20 Ding, H. *et al.* (1992) Cytochrome *bc*₁ complex [2Fe–2S] cluster and its interaction with ubiquinone and ubiquinol at the Q_o site: a double-occupancy Q_o site model. *Biochemistry* 31, 3144–3158
- 21 Ding, H. *et al.* (1995) Ubiquinone pair in the Q_o site central to the primary energy conversion reactions of cytochrome *bc*₁ complex. *Biochemistry* 34, 15979–15996
- 22 Sharp, R.E. *et al.* (1999) Ubiquinone binding capacity of the *Rhodobacter capsulatus* cytochrome *bc*₁ complex: effect of diphenylamine, a weak binding Q_o site inhibitor. *Biochemistry* 38, 3440–3446
- 23 Sharp, R.E. *et al.* (1999) Effect of inhibitors on the ubiquinone binding capacity of the primary energy conversion site in the *Rhodobacter capsulatus* cytochrome *bc*₁ complex. *Biochemistry* 38, 14973–14980
- 24 Bartoschek, S. *et al.* (2001) Three molecules of ubiquinone bind specifically to mitochondrial cytochrome *bc*₁ complex. *J. Biol. Chem.* 276, 35231–35234
- 25 Berry, E.A. and Huang, L.-S. (2003) Observations concerning the quinol oxidation site of the cytochrome *bc*₁ complex. *FEBS Lett.* 555, 13–20
- 26 Esser, L. *et al.* (2004) Crystallographic studies of quinol oxidation site inhibitors: a modified classification of inhibitors for the cytochrome *bc*₁ complex. *J. Mol. Biol.* 341, 281–302
- 27 Robertson, D.E. *et al.* (1984) 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, 1758–1763
- 28 Chance, B. and Hollunger, G. (1961) The interaction of energy and electron transfer reactions in mitochondria. *J. Biol. Chem.* 236, 1562–1568
- 29 Osyczka, A. *et al.* (2004) Reversible redox energy coupling in electron transfer chains. *Nature* 427, 607–612
- 30 Brandt, U. and von Jagow, G. (1991) Analysis of inhibitor binding to the mitochondrial cytochrome-*c* reductase by fluorescence quench titration – evidence for a ‘catalytic switch’ at the Q_o center. *Eur. J. Biochem.* 195, 163–170
- 31 Brandt, U. (1998) The chemistry and mechanics of ubiquinol oxidation at center P (Q_o) of the cytochrome *bc*₁ complex. *Biochim. Biophys. Acta* 1365, 261–268
- 32 Crofts, A.R. *et al.* (1999) Pathways for proton release during ubiquinol oxidation by the *bc*₁ complex. *Proc. Natl. Acad. Sci. U. S. A.* 96, 10021–10026
- 33 Crofts, A.R. *et al.* (1999) Mechanism of ubiquinol oxidation by the *bc*₁ complex: different domains of the quinol binding pocket and their role in the mechanism and binding of inhibitors. *Biochemistry* 38, 15807–15826
- 34 Yu, C.A. *et al.* (2002) Inter- and intra-molecular electron transfer in the cytochrome *bc*₁ complex. *Biochim. Biophys. Acta* 1555, 65–70
- 35 Klishin, S.S. *et al.* (2002) 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, 177–182
- 36 Link, T.A. (1997) The role of the ‘Rieske’ iron sulfur protein in the hydroquinone oxidation (Q_p) site of the cytochrome *bc*₁ complex. The ‘proton-gated affinity change’ mechanism. *FEBS Lett.* 412, 257–264
- 37 Snyder, C.H. *et al.* (2000) Evidence for a concerted mechanism of ubiquinol oxidation by the cytochrome *bc*₁ complex. *J. Biol. Chem.* 275, 13535–13541
- 38 Brandt, U. (1996) Bifurcated ubiquinol oxidation in the cytochrome *bc*₁ complex by proton-gated charge transfer. *FEBS Lett.* 387, 1–6
- 39 Wikstrom, M. *et al.* (2003) Water-gated mechanism of proton translocation by cytochrome *c* oxidase. *Biochim. Biophys. Acta* 1604, 61–65
- 40 Rich, P.R. (2004) The quinone chemistry of *bc* complexes. *Biochim. Biophys. Acta* 1658, 165–171
- 41 Trumpower, B.L. (2002) A concerted, alternating sites mechanism of ubiquinol oxidation by the dimeric cytochrome *bc*₁ complex. *Biochim. Biophys. Acta* 1555, 166–173
- 42 Davidson, E. *et al.* (1992) Potential ligands to the [2Fe–2S] Rieske cluster of the cytochrome *bc*₁ complex of *Rhodobacter capsulatus* probed by site-directed mutagenesis. *Biochemistry* 31, 3342–3351
- 43 Hunte, C. *et al.* (2003) Protonmotive pathways and mechanisms in the cytochrome *bc*₁ complex. *FEBS Lett.* 545, 39–46
- 44 Zito, F. *et al.* (1998) Glu78, from the conserved PEWY sequence of subunit IV, has a key function in cytochrome *b*_{6f} turnover. *Biochemistry* 37, 10395–10403
- 45 Adelroth, P. *et al.* (1997) Glutamate 286 in cytochrome *aa*₃ from *Rhodobacter sphaeroides* is involved in proton uptake during the reaction of the fully-reduced enzyme with dioxygen. *Biochemistry* 36, 13824–13829
- 46 Brzezinski, P. (2004) Redox-driven membrane-bound proton pumps. *Trends Biochem. Sci.* 29, 380–387
- 47 Takamiya, K.-I. and Dutton, P.L. (1979) Ubiquinone in *Rhodospirillum rubrum* some thermodynamic properties. *Biochim. Biophys. Acta* 546, 1–16
- 48 Junemann, S. *et al.* (1998) On the mechanism of quinol oxidation in the *bc*₁ complex. *J. Biol. Chem.* 273, 21603–21607
- 49 Jencks, W.P. (1975) Binding energy, specificity, and enzymatic catalysis: the Circe effect. *Adv. Enzymol.* 43, 219–410
- 50 Cornish-Bowen, A. (1995) The reversible Michaelis–Menten mechanism. In *Fundamentals of Enzyme Kinetics*, pp. 37–43, Portland Press