Cytochrome $b_{6}f$: bridging the divide

Pollen aperture evolution

GABA in plants: just a metabolite?

Mitochondrial carriers in *Arabidopsis*
Cytochrome $b_6f$: structure for signalling and vectorial metabolism

John F. Allen

Following decades of detailed kinetic and spectroscopic evidence, two new, independent X-ray structures for the cytochrome $b_6f$ complex of photosynthesis now reveal the arrangement of its key electron carriers relative to each other, and to their protein ligands. But these are not predictable additions to the structural collection. The complex is dimeric, and encloses a central chamber in which plastoquinone and its redox intermediates couple proton translocation with cytochrome oxidation and reduction. The structures also announce a fourth, wholly unexpected haem, that could be the long-sought, missing link of photosystem I cyclic photophosphorylation. One chlorophyll molecule and one carotenoid molecule add to the enigma of this dark, downhill electron transfer complex, linking the real photosystems I and II. Conserved structural features offer clues to the evolution of photosynthesis, and to the initiation of redox signals required for genome function.

There can be few proteins that compare with the cytochrome $b_6f$ complex of oxygenic photosynthesis for the precision and detail of the structural predictions that must follow from its biochemical and biophysical properties – provided, that is, our current understanding of biological energy transduction is correct. Photosynthesis researchers have been waiting, almost with bated breath, for a structural determination of cytochrome $b_6f$. It is said the longer you wait for a bus, the more likely it is that two will come along. To similar delight and surprise, 2003 saw the independent determination, by X-ray crystallography, of the structure of $b_6f$ from the unicellular green alga Chlamydomonas reinhardtii and from the thermophilic cyanobacterium Mastigocladus laminosus. The Chlamydomonas structure was determined by a team in Paris, France, headed by Daniel Picot and Jean-Luc Popot at the Institut de Biologie Physico-Chimique [1]. The determination of the Mastigocladus structure was a collaboration between the laboratories of William Cramer and Janet Smith in Purdue University, Indiana, USA [2].

The biologist’s ‘standard model’, indispensable for our current understanding of the functions of cells and their constituents, is the chemiosmotic theory. The two new structures are in agreement with its predictions, and with a special, chemical mechanism for the proton translocation it requires. Even across the evolutionary divide separating prokaryotes from eukaryotes, the differences between the two complexes are less than one Ångström unit for distances between key iron atoms. However, the structures are also unanimous on some completely unexpected features, absent from the homologous complex of mitochondria. Among these is an extra, unexpected haem, belonging to a kind of c-type cytochrome seen previously only in Gram-positive bacteria. And why does a light-independent, downhill electron transport complex contain embedded chlorophyll and carotenoid molecules?

**Downhill cytochrome chain of photosynthesis**

The Z-scheme of Robin Hill and Fay Bendall [3] provided solutions to many problems, one of which was the relationship between chloroplast ATP synthesis (photophosphorylation) and the comparable process (oxidative phosphorylation) in mitochondria. The Z-scheme (Figure 1) explicitly proposed that a chain of cytochromes and other carriers transports electrons thermodynamically downhill, through a site at which the energy released is used, as work, for ATP synthesis, just as in the respiration [4,5].

**Chemiosmosis and the Q-cycle**

At about the same time as the Z-scheme, a common mechanism for photosynthetic and oxidative phosphorylation was proposed. Peter Mitchell’s chemiosmotic theory [6] made several radical assumptions. One was that electron and hydrogen transfers are arranged vectorially across bioenergetic membranes, thus moving protons (hydrogen ions) across the membrane to establish an electrochemical gradient. Another cornerstone was the idea that this gradient, or ‘proton motive force’, supplies energy for ATP synthesis, because this, too, is coupled to vectorial movement of protons between aqueous phases on each side of the membrane [7,8].

The Q-cycle was a later addition [9,10] designed initially to explain higher observed proton-to-electron ratios than the original chemiosmotic theory seemed to predict [11]. Simple inspection of quinone redox chemistry suggested two electrons move only two protons (Equation 1).

$$Q + 2e^- + 2H^+ → QH_2 → Q + 2H^+ + 2e^- \quad (\text{Eqn 1})$$

An essential assumption of the Q-cycle is that the single species of quinine (plastoquinone in chloroplasts; ubiquinone in most mitochondria) drives translocation of twice as many protons across the membrane as this simple scheme

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*Supplementary data associated with this article can be found at doi:10.1016/j.tplants.2004.01.009.

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suggested. What makes this possible is a re-cycling of electrons through two cytochrome b haems, and two quinone-binding sites: one (the Qo site – 'o' for 'outside') donating two electrons to two different acceptors; the other (the Qi – 'i' for 'inside') accepting recycled electrons from one of the b-haems (Equations 2–5).

At the Qi site:

\[
\text{Q} + 2\text{H}_\text{in}^+ \rightarrow \text{QH}_2
\]  

(Eqn 2)

\[
\text{Q}^- + 2\text{H}_\text{in}^+ + 2\text{e}^- \rightarrow \text{QH}_2
\]  

(Eqn 3)

At the Qo site:

\[
\text{QH}_2 \rightarrow \text{Q}^- + 1\text{e}^- + 2\text{H}_\text{out}^+
\]  

(Eqn 4)

\[
\text{Q}^- + 1\text{e}^- \rightarrow \text{Q}
\]  

(Eqn 5)

Sum, for one electron transferred (Equation 6):

\[
2\text{H}_\text{in}^+ \rightarrow 2\text{H}_\text{out}^+
\]  

(Eqn 6)

The effect is to re-cycle one of the electrons from plastoquinol (PQH₂, also called ‘plastohydroquinone’). This electron (underlined in Equations 2 and 5) is supplied by the plastosemiquinone anion intermediate (PQ⁻) at the Qi site, to a short chain of two b-haems, and given back to plastosemiquinone at the Qo site. The whole process gives two protons translocated for each single electron transferred through the quinone pool, that is, a total of four protons, not two, for each pair of electrons passing through the quinone pool and the cytochrome complex (Equation 7).

\[
\text{Q} + 2\text{e}^- + 4\text{H}_\text{in}^+ \rightarrow \text{QH}_2 + [2\text{H}_\text{out}^+] \rightarrow \text{Q} + 4\text{H}_\text{out}^+ + 2\text{e}^-
\]  

(Eqn 7)

In the chloroplast, the Q-cycle is a chemical mechanism for proton translocation across the chloroplast thylakoid membrane from the chloroplast stroma into the lumen.

The Q-cycle uses vectorial electron transport to establish the proton motive force that is also coupled to ATP synthesis [12,13]. Figure 2 shows an outline of the Q-cycle and its context, as currently envisaged for the chloroplast thylakoid membrane.

**Expected structural features: unexpected precision and conservation**

The cytochrome b₆f structures from *Chlamydomonas reinhardtii* [1] (Figure 3) and *Mastigocladus laminosus* [2] (Figure 4) agree that the two b-haems span the membrane as the Q-cycle requires: one adjacent to the Qo site; the other adjacent to the Qi site. There is precise agreement, within experimental error, on distances between the iron atoms of the two haems in cytb₆f (20.8 Å) and in mitochondrial cytbc₁ (20.9 Å). This is also the case for the distance between the iron atom of the b-haem adjacent to the Qo site and the iron–sulfur centre (the ‘Rieske’ 2Fe–2S centre) that accepts the electron on its way out to the rest of the chain: this estimated value is 26.1 Å for b₆f and 26.2 Å for cytbc₁. Sequence comparisons suggest that this extraordinary conservation of inter-atomic distance is likely to span even the most fundamental biological divide on Earth, that between archaeabacteria (*Archaea*) and eubacteria (*Bacteria*) [14,15]. Thus, the cytochrome b structure might have arisen before the emergence of true cells [16]. Perhaps the conserved iron atoms participated in the pre-biotic, vectorial, hydrogen metabolism and phosphate transfer that was powered initially by geothermal convection, according to a new theory for the origin of life on Earth [17].

In both photosynthesis and respiration, the final electron transfer step from the complex is from the Rieske Fe–S centre to a c-type cytochrome. This cytochrome is called ‘f’ for ‘frons’ – Latin for ‘leaf’ – in chloroplasts, but is

Figure 1. The context of cytochrome b₆f The Z-scheme for photosynthetic electron transfer as envisaged by Hill and Bendall [3], drawn here to depict the components of cytochrome b₆f in a linear sequence corresponding to the ‘coupling site’ of ATP synthesis in both non-cyclic and cyclic photophosphorylation [4,5]. The two ‘light-reactions’, photosystems I and II, were originally described as a ‘working hypothesis’ [3], and their reaction centres are also now described in structural terms, at near-atomic resolution [46–48].
Figure 2. Cytochrome \(b_6f\): function, orientation and terminology. The figure presents a diagram of the components of the Q-cycle, with the stoichiometries of protons and electrons, and using the orientation and terminology described for Chlamydomonas by Stroebel et al. [1]. The alternative terms used by Kurisu et al. [2] with Mastigocladus are omitted, but a guide to translation is given in Box 1.

(a) The orientation of cytochrome \(b_6f\) in the chloroplast thylakoid membrane and its function as a proton motive, plastoquinone–plastocyanin (PC) oxidoreductase.

(b) Reactions at the Qo site.

(c) Reactions at the Qi site.

(d) The overall structure, function and orientation of cytochrome \(b_6f\) as a link between photosystems I and II (compare with Figure 1). The grey arrow in (d) indicates that one electron is carried, on the lumen side, from the Qo site to cytochrome \(f\) by physical relocation of the Rieske iron–sulfur [Fe–S] cluster (depicted in (b)) arising from a conformational change in its apoprotein. In (b–d), haem \(c_i\) of
a c-type cytochrome, with covalently bound haem, nonetheless. This last electron transfer occurs over a distance for which estimated values are identical, 31.9 Å, in photosynthetic and mitochondrial complexes. However, the position of this c-haem relative to the axes of symmetry of the two complexes differs considerably, by 16.3 Å. In the cytb6f complex, cytochrome f appears to form a bowl around what is presumed to be the rocking head of the Rieske Fe–S protein. The motion of the Fe–S centre was first suggested by the two positions seen in mitochondrial cytochrome bc1 complexes [see supplementary material (http://archive.bmn.com/supp/plants/Qo.MOV)] [18]. The significance of this motion is that the Fe–S centre accepts only single electrons, therefore the potentially lethal semiquinone (Q−) is produced by quinol (QH2) oxidation at the Q, site only if the first b-haem is oxidized and ready to accept the electron of the semiquinone to give the relatively safe quinone (Q). In addition, movement of the Fe–S centre away from the Q, site introduces a kinetic barrier to the thermodynamically favourable reduction of Fe–S by the second electron, the one from the semiquinone (Figure 2b).

This two-electron gate directs the semiquinone radical to reduce haem b1, not Fe–S. However, its occasional failure can cause a single electron to pass to molecular oxygen, which has had profound metabolic significance of this motion is that the Fe–S centre accepts only single electrons, therefore the potentially lethal semiquinone (Q−) is produced by quinol (QH2) oxidation at the Q, site only if the first b-haem is oxidized and ready to accept the electron of the semiquinone to give the relatively safe quinone (Q). In addition, movement of the Fe–S centre away from the Q, site introduces a kinetic barrier to the thermodynamically favourable reduction of Fe–S by the second electron, the one from the semiquinone (Figure 2b).

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and evolutionary consequences [19,20]. There is even a recent proposal that the semiquinone is not an intermediate at all, and that quinol oxidation at the Q\textsubscript{o} site proceeds all the way to quinone in one step, by a kinetically concerted two-electron transfer [21]. If this is the case, it is still true that univalent reduction of oxygen to superoxide is a constant danger of Q-cycle action under aerobic conditions [22–24], and the single-electron donor to oxygen might then be either one of the co-factors, or a semiquinone produced ‘by mistake’ when the perfectly tuned mechanism fails for some reason.

Whether or not Q\textsubscript{o} is a true, if transient, intermediate, it is likely that the superoxide anion radical and its products will eventually exacerbate the problem, increasing its own frequency of production. There might be multiple reasons, and pathways, for this ‘vicious circle’, one of which stretches back to free-radical-induced mutation within the mitochondrial or chloroplast genome [20,25].

**Unexpected features**

Biochemical evidence has been available for some time to suggest that b\textsubscript{6f} complexes, unlike b\textsubscript{c1} complexes, contain a chlorophyll molecule [26,27]. The structures give precise locations for the chlorophyll and for a familiar carotenoid, \(\beta\)-carotene [28]. The tip of the phytyl tail of chlorophyll protrudes into the Q\textsubscript{o} quinone-binding site. The distance between the chlorophyll and carotenoid molecules precludes the kind of functional interaction seen in photosynthetic reaction centres and light-harvesting complexes in quenching excited states generated by light absorption. Therefore, if cytochrome b\textsubscript{6f} works occasionally at energy transfer or photochemistry, it cannot do so alone. But the expected and well-known photochemical properties of the two pigment molecules are intriguing, particularly considering the arguments from sequence comparisons that...
cytochrome $b$ and photosynthetic reaction centres had a common evolutionary precursor [29].

A total surprise in the $b_f$ structures was the fourth haem of cytochrome $b_6$ itself, conclusively identified as an iron-containing electron density by Stroebel et al., and accounting for a peroxidase activity of $b_f$ complexes [30] that is absent from mitochondrial $b_c$. This electron density lies near the $Q_s$ site and close to the conventional, high-potential $b$-haem. Both Stroebel et al. and Kurisu et al. agree that this haem $c_1$ (‘i’ for inside) or ‘heme $x$’ (Box 1) is likely to provide a link to plastoquinone from ferredoxin on the acceptor side of photosystem I [31], thereby completing the photosystem I cyclic electron transport pathway [32], the first mode of ATP synthesis to be discovered in photosynthesis [4] (Figure 1). Stroebel et al. point out [1] that the unusual ligation of haem $c_1$, through a thioether linkage to cysteine 35 of the cytochrome $b_6$ apoprotein, is also seen in $firmicutes$ with positive bacteria such as the non-photosynthetic Bacillus subtilis and the photosynthetic heliobacteria. The photosynthetic heliobacteria group contains only one kind of subtilis and the photosynthetic heliobacteria. The photosynthetic heliobacteria group contains only one kind of subtilis and the photosynthetic heliobacteria. The photosynthetic heliobacteria group contains only one kind of subtilis and the photosynthetic heliobacteria.

Box 1. Guide to translation

David Stroebel et al. [1] report the structure of Chlamydomonas cytochrome $b_f$ (plastohydroquinone:plastocyanin oxidoreductase) using a particular terminology, and present the complex in an orientation familiar to most biochemists and plant scientists. In this orientation, the chloroplast thylakoid membrane separates two aqueous phases: conventionally, the chloroplast stroma is depicted above the membrane, and the thylakoid lumen below it (Figure 2). The quinone-binding sites are designated ’Qo’, for ‘outer’ (lumen side) and ’Qi’, for ‘inner’ (stromal, cytoplasmic side); the cytochrome $b_6$ haems are designated $b_6$ (for low potential) and $b_1$ (for high potential). The novel haem is given the name ‘haem $c_1$’ (for c-type cytochrome, inner). By contrast, Genji Kurisu et al. [2] in their report of the structure of Mastigocladus present the complex the other way up, and quinone and haems are designated according to whether they are closer to the $p$'-phase or $n$'-phase (Figure 2a,d). The key is that the cyano bacterial cytochrome (equivalent to the chloroplast stroma; ‘inside’ is the $n$’ (for negative)-phase; the lumen (‘outside’) is the $p$’ (for positive)-phase. Thus, the $Q_s$ site is synonymous with the $Q_s$ site and the nearby $b_6$ haem is ‘heme $b_6$’.

Everything else follows: the $Q_s$ site is synonymous with the $Q_s$ site, and the nearby $b_1$ is ‘heme $b_1$’. In other literature, $Q_s$ becomes ‘$Q_0$’ and stands for ‘quinol oxidase’, and $Q_i$ becomes ‘$Q_1$’ for ‘quinone reductase’. In addition, $p'$ and $n'$ are often given in uppercase lettering. To add a final terminological snare for the unwary, the novel, atypical haem that Stroebel et al. describe as ‘haem $c_1$’ becomes ‘heme $x$’ in the Kurisu et al. structure. Apart from ‘heme $x$’, the use of $p'$ and $n'$ for vertical orientation is self-consistent and recognizes the relative electrochemical polarity of the two aqueous phases and their adjoining membrane surfaces [2]. It also fits with the custom that the outside of a cell is up; the inside, down. However, the Stroebel et al. orientation (Figure 2) presents the pathway of electron transfer in such a way that the ‘Z-scheme’ (Figure 1) can almost be superimposed, and decreasing electrochemical potential is ‘up’, so that one can imagine electrons rolling gently downhill towards thermodynamic equilibrium (Figure 2d). The Rieske iron–sulfur centre is described by Stroebel et al. as ‘FeS$_2$’ [1] and by Kurisu et al. as ISP (for ‘iron–sulfur protein’) [2]. For students, the differing orientations and terminologies must seem perverse.

The protein at the interface between energetics and genome function

Apart from its obligatory role in the Q-cycle, plastoquinone is known to signal the relative rates of electron transport through photosystem I and photosystem II. The first indication of this signalling function arose when it was shown that reduction of plastoquinone activates a protein kinase that catalyses phosphorylation of light-harvesting complex II, thus redistributing absorbed light energy to photosystem I at the expense of photosystem II [35]. Subsequent work in several laboratories implicates cytochrome $b_f$ in this redox control [36,37]. It is suggested that a parallel plastoquinone redox control of reaction centre gene transcription underlies the self-adjustment of the stoichiometry of photosystems I and II [38]. The predicted pattern of up- and down-regulation of reaction centre gene transcription in chloroplasts supports this idea [38].

One view, currently gaining support, is that redox control of gene expression is so important that it alone justifies the cost of maintaining genomes in chloroplasts and mitochondria, in order that they might encode the proteins whose synthesis must be so tightly coupled to their function in electron transfer [19]. Chloroplast cytochrome $b_6$, like mitochondrial cytochrome $b$, is a case in point: in eukaryotes these proteins are always the products of organellar genes. Redox-activated chloroplast protein kinases [39,40] might therefore be implicated in both post-translational and transcriptional modes of redox signalling, and are likely to have had continuing and decisive influence in eukaryotic cell evolution.

The new structures of cytochrome $b_f$ are essentially identical in spite of the great evolutionary distance between the prokaryote (Mastigocladus) and the eukaryotic (Chlamydomonas) organelle. In addition, by comparison with recent structures for bacterial [41] and mitochondrial [42,43] cytochrome cyt$bc$ complexes, cytochrome $b_6$ [1,2] confirms the true homology of proton-motive, quinol-oxidizing electron transport in photosynthesis and in respiration, the first glimpse of which was the formulation of photosynthetic electron transport to include features in common with the respiratory chain [3].

Given the importance of transmitting redox information from this Q-cycle machine to many destinations, and to different levels of gene expression [19,44], one pointer for future research is clear. All the responses result, eventually, from interactions on the stromal or cytoplasmic ($Q_s$) sides of the bioenergetic membrane, where lie the genetic system and protein kinase activities.
What, then, is the primary quinone redox signal, and how is it exported? Does the mystery chlorophyll molecule of cytochrome b/f probe occupancy of the Q$_o$ site, and transmit that information across the membrane by some means, as Stroebe1 et al. [1] suggest? What are the interactions and the structural events delivering redox information to gene expression, and how are the signals processed? In short, where, in these structures, is the crucial redox sensor?

**Supplementary information**

The supplementary material (http://archive.bmn.com/supp/plants/Qo.MOV) is a Quicktime file Qo.mov (adapted from a movie by Antony R. Crofts) of an animation of the reactions occurring at the Q$_o$ site of cytochrome bc complexes generally, illustrating the predicted motion of the head of the Rieske Fe–S protein, and the point of bifurcation of electron transport from QH$_2$ to b$_1$, and to the iron–sulfur centre of the Rieske protein illustrated in Figure 2b.

**Acknowledgements**

I thank Jean-Luc Papon for discussions; Daniel Picot for correspondence on the manuscript; and Antony R. Crofts, S. Bendall, Antony R. Crofts, Fevzi Daldal and Sabeeha Merchant for the structures and for the file 1q90.pdb in advance of public release; Derek I thank Jean-Luc Popot for discussions; Daniel Picot for correspondence on the manuscript; and Antony R. Crofts, S. Bendall, Antony R. Crofts, Fevzi Daldal and Sabeeha Merchant for the structures and for the file 1q90.pdb in advance of public release; Derek I thank Jean-Luc Popot for discussions; Daniel Picot for correspondence on the manuscript; and Antony R. Crofts, S. Bendall, Antony R. Crofts, Fevzi Daldal and Sabeeha Merchant for the structures and for the file 1q90.pdb in advance of public release.

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