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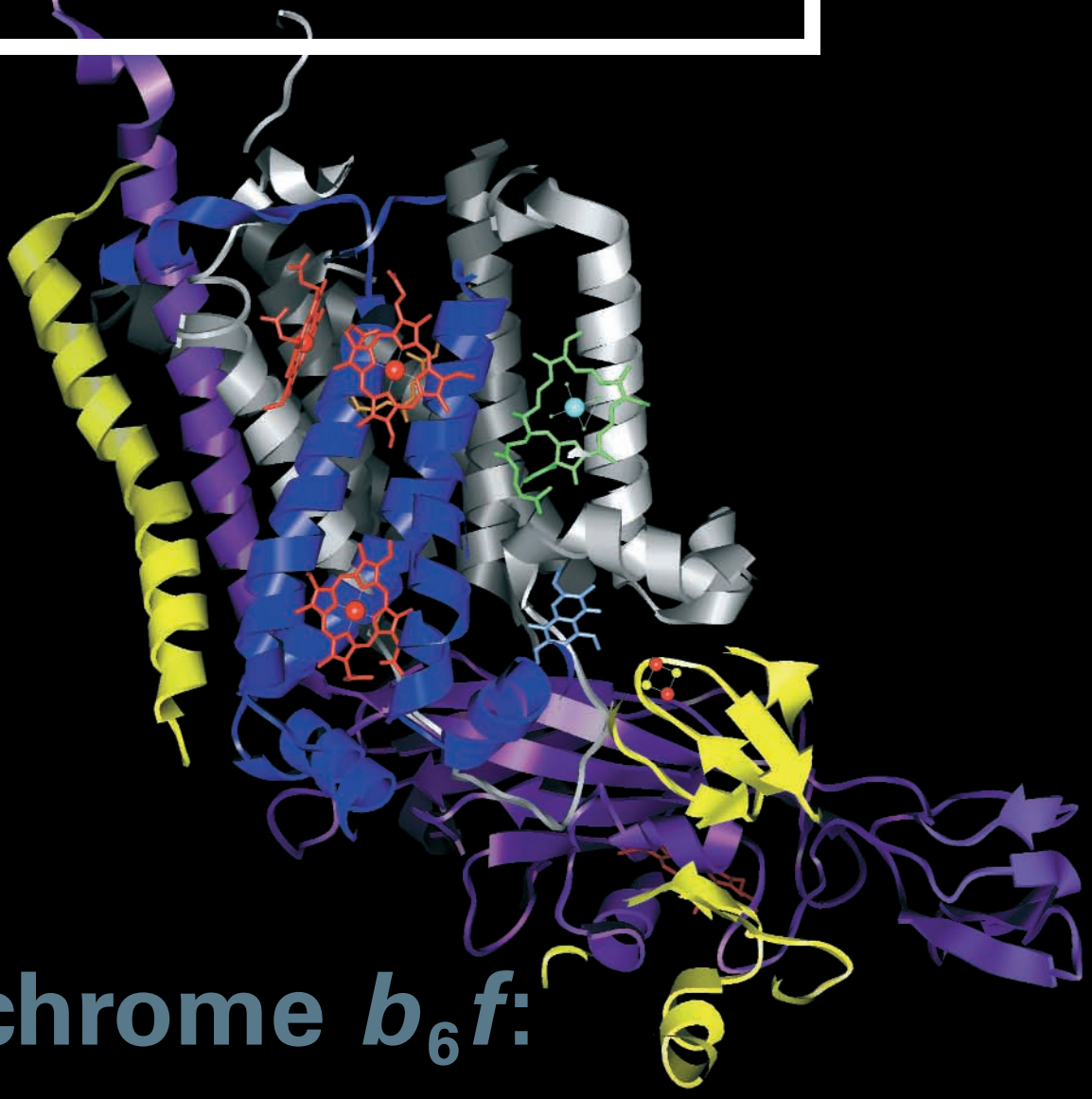

Cytochrome b_6f: bridging the divide

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Trends Plant Sci. March 2004 Vol. 9 No. 3, pp. xxx–xxx ISSN 1360-1385

Review TRENDS in Plant Science Vol.9 No.3 March 2004

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Cytochrome b_6f: structure for signalling and vectorial metabolism☆

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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).

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

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

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

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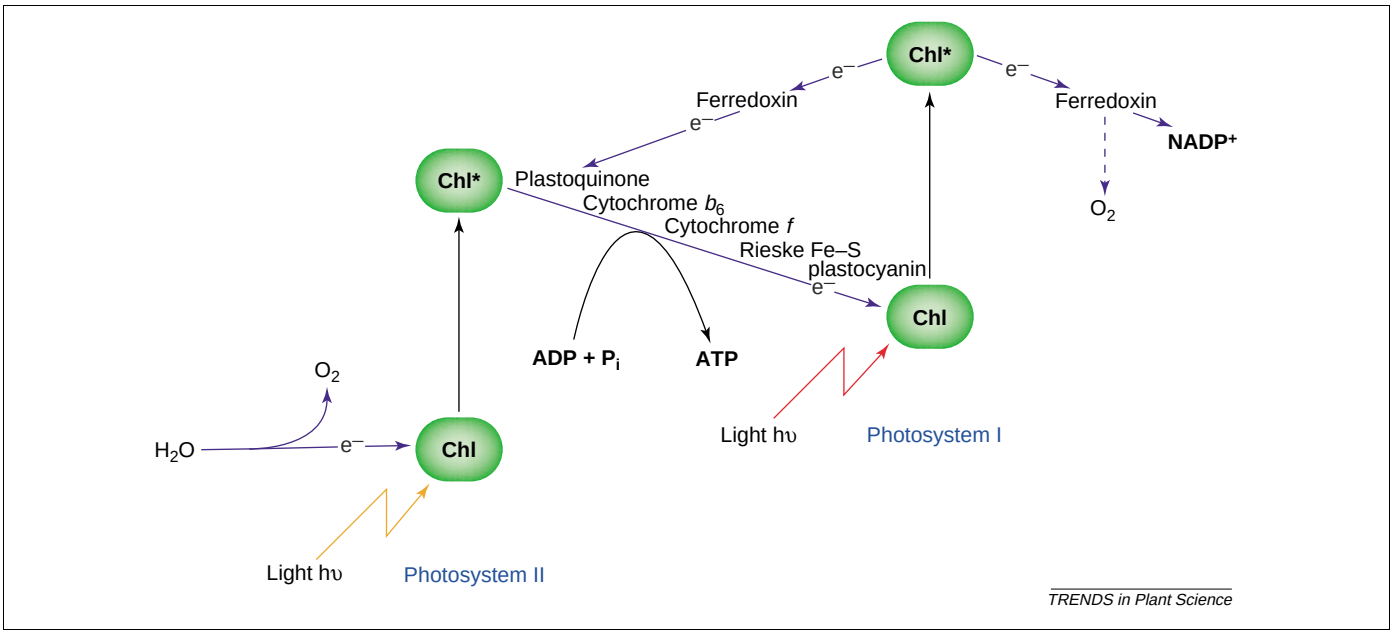

Figure 1. The context of cytochrome b_6f The Z-scheme for photosynthetic electron transfer as envisaged by Hill and Bendall [3], drawn here to depict the components of cytochrome b_6f in a linear sequence corresponding to the 'coupling site' of ATP synthesis in both non-cyclic and cyclic photophosphorylation [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 [45–48].

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

At the Q_i site:

$$Q + \underline{1e^-} \rightarrow Q\cdot^- \quad \text{(Eqn 2)}$$

$$Q\cdot^- + 2H^+_{[in]} + 1e^- \rightarrow QH_2 \quad \text{(Eqn 3)}$$

At the Q_o site:

$$QH_2 \rightarrow Q\cdot^- + 1e^- + 2H^+_{[out]} \quad \text{(Eqn 4)}$$

$$Q\cdot^- \rightarrow Q + \underline{1e^-} \quad \text{(Eqn 5)}$$

Sum, for one electron transferred (Equation 6):

$$2H^+_{[in]} \rightarrow 2H^+_{[out]} \quad \text{(Eqn 6)}$$

The effect is to re-cycle one of the electrons from plastoquinol (PQH_2, also called 'plastohydroquinone'). This electron (underlined in Equations 2 and 5) is supplied by the plastosemiquinone anion intermediate ($PQ\cdot^-$) at the Q_o site, to a short chain of two *b*-haems, and given back to plastosemiquinone at the Q_i 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).

$$Q + 2e^- + 4H^+_{[in]} \rightarrow QH_2 + [2H^+] \rightarrow Q + 4H^+_{[out]} + 2e^- \quad \text{(Eqn 7)}$$

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 b_6f 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 Q_o site; the other adjacent to the Q_i site. There is precise agreement, within experimental error, on distances between the iron atoms of the two haems in cytb_6f (20.8 Å) and in mitochondrial cytbc_1 (20.9 Å). This is also the case for the distance between the iron atom of the *b*-haem adjacent to the Q_o 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 b_6f and 26.2 Å for cytbc_1. 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 archaebacteria (*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

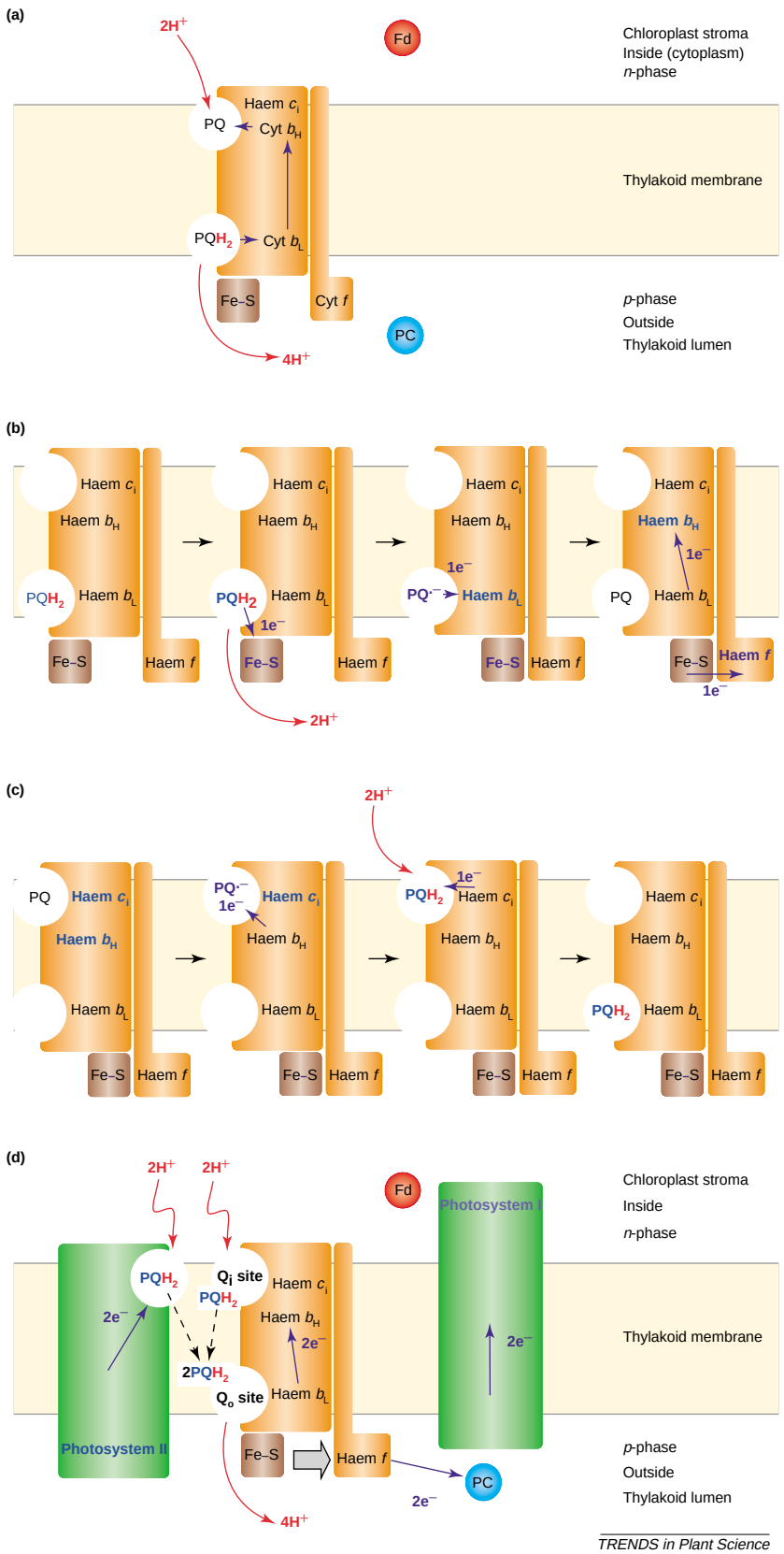

Figure 2. Cytochrome b_6f: function, orientation and terminology The figure presents a diagram of the components of the Q-cycle, with the stoichiometries of protons and electrons, and using the orientation and terminology described for *Chlamydomonas* by Stroebel *et al.* [1]. The alternative terms used 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 Q_o site. **(c)** Reactions at the Q_i 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 Q_o 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

Figure 3. The structure of cytochrome b_6f from *Chlamydomonas reinhardtii* derived from X-ray diffraction at 3.1 Å resolution, as determined by Stroebel *et al.* [1]. The view is parallel to the membrane plane, and 'up' is the stromal side, as in the schematic diagrams in Figure 2. In the present figure, only one monomer is shown for clarity. The view is of a cut through the Z-axis to expose the prosthetic groups or cofactors, therefore some polypeptides are missing, and helices, particularly of cytochrome b_6, are incomplete or missing, because they lie behind the observer's view. The line of sight is roughly normal to the porphyrin rings of the central cytochrome *b*-haems (red), and is taken as if from the centre of the native structure, which is dimeric. The native dimer encloses a central, inner cavity in which the redox reactions of the quinone species all take place. Polypeptides: purple, cytochrome *f* (PetA); blue, cytochrome b_6 (PetB); yellow, Rieske Fe–S protein (Pet C; incomplete soluble domain and complete, single, membrane helix); grey, subunit IV (PetD) and four smaller subunits (see later). Cofactors: red, haems with Fe atoms as spheres. The plane-perpendicular haems in the centre of the figure are b_L (lower) and b_H (upper; lying in front of the orange carotenoid). The plane-parallel haem to the left of b_H is haem c_i; to the left of haem c_i is the empty Q_i site. The nearly plane-parallel haem towards the bottom right of the figure is that of cytochrome *f*. Just above cytochrome *f* haem is the Fe_2S_2 cluster of the Rieske protein. The cornflower blue molecule just above the Fe_2S_2 cluster of the Rieske protein, and to the right of haem b_L, is tridecylstigmatellin, a quinone analogue that occupies the Q_o site. Q_o is situated between b_L and Fe_2S_2, exactly as predicted by its function (Figure 2b). Chlorophyll *a* is depicted in green with the Mg atom as a cyan sphere. β-carotene is depicted in orange (just visible behind b_H). The assignment of small protein subunits as given by Stroebel *et al.* [1] differs from that assigned by Kurisu *et al.* in their paper [2] and in their coordinate file 1um3.pdb. Translating from the Stroebel *et al.* structure to the Kurisu *et al.* structure: PetG is named Pet L; PetL, PetN; PetN, PetG. The two groups agree on PetM. In the figure, all these subunits, plus PetD (subunit IV) are grey. The two structures also differ in assignment of loops between cytochrome b_6 and the stroma, and in the position of the tridecyl tail of the inhibitor tridecylstigmatellin (the tail is located in the active site according to Kurisu *et al.*) The cytochrome b_6 loops of Kurisu *et al.* (Figure 4) favour the interpretation that haem c_i ('heme *x*') is available for reduction by ferredoxin. It is likely that these detailed differences between the two structures arise from differences in crystallographic interpretation rather than from true structural differences between cytochrome b_6f from *Chlamydomonas* and *Mastigocladus*. The graphic shown here was produced using UCSF Chimera [50], http://www.cgl.ucsf.edu/chimera, from the file of atomic coordinates 1q90.pdb.

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 cytb_6f 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 bc_1 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 ($Q^{\cdot -}$) is produced by quinol (QH_2) oxidation at the Q_o 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_o 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 b_L, not Fe–S. However, its occasional failure can cause a single electron to pass to molecular oxygen, which has had profound metabolic

cytochrome b_6 is proposed to hold one of the two electrons required for concerted reduction of PQ to PQH_2, and is therefore reduced, in parallel with b_H, by one of the two turnovers that take place at the Q_o site for each turnover at Q_i. This suggestion views the Q_i site as a mirror image of the Q_o site [21], but does not exclude the possibility that haem c_i can accept electrons from ferredoxin (Fd) (a,d), and thus from photosystem I. A more detailed description of the overall stoichiometries of protons, electrons and ATP was given previously [4] and suggests that complete photosynthesis is likely to require photosystem I-driven cyclic electron transport [31,32,49] as originally proposed (Figure 1; [3]). Proton and electron transport associated directly with reaction centres also contributes to the proton motive force [7].

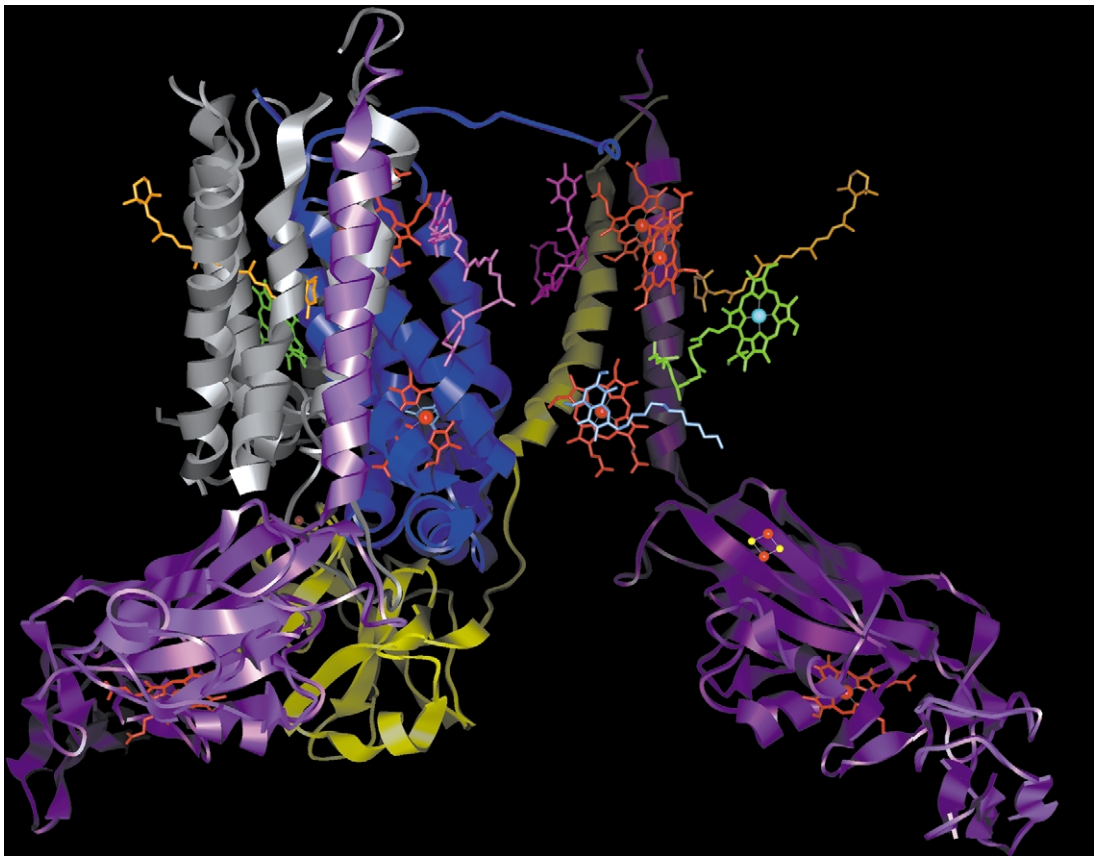

Figure 4. The structure of cytochrome b_6f from *Mastigocladus laminosus* derived from X-ray diffraction at 3.0 Å resolution, as determined by Kurisu *et al.* [2]. The view is parallel to the membrane plane, and 'up' is the stromal side (as in the schematic diagrams in Figure 2). The dimer is shown with some polypeptides of the right-hand monomer (Rieske Fe–S protein; subunits D, G, L, M, N) omitted to expose the cofactors (prosthetic groups). The dimer encloses a central, inner cavity in which the redox reactions of the quinone species all take place. Polypeptides: purple, cytochrome *f* (PetA); blue, cytochrome b_6 (PetB); yellow, Rieske Fe–S protein (Pet C); grey, subunit IV (PetD) and four smaller subunits (see later). Cofactors: red; haems with Fe atoms as spheres. The haems in the centre of the figure are b_L (lower) and b_H (upper; lying the left of the orange carotenoid). The haem just above b_H is haem c_i. To the left of these two haems is plastoquinone, depicted in magenta, occupying the Q_i site. The haem towards the bottom right of the figure is that of cytochrome *f*. Halfway between the cytochrome *f* haem and the b_L haem is the Fe_2S_2 cluster of the Rieske protein. The molecule in front of, and slightly obscuring haem b_L, is tridecylstigmatellin (depicted in cornflower blue), a quinone analogue that occupies the Q_o site. The location of Q_o permits it to transfer electrons both to b_L and to Fe_2S_2, (Figure 2). Chlorophyll *a* is depicted in green with the Mg atom depicted as a cyan sphere. β-carotene is represented in orange (to the right of b_H and above and behind the chlorophyll a). The assignment of small protein subunits as given by Kurisu *et al.* differs from that of Stroebel *et al.* in their paper [1] and in their coordinate file 1q90.pdb (Figure 3). In this figure, the small subunits (PetD, PetG, Pet L, PetM, PetN) are shown in grey, and are visible only in the left-hand monomer. It is likely that these detailed differences between the two structures (Figure 3) arise from differences in crystallographic interpretation rather than from true structural differences between *Chlamydomonas* cytochrome b_6f and *Mastigocladus* cytochrome b_6f. The graphic shown here was produced using UCSF Chimera [50], http://www.cgl.ucsf.edu/chimera, from the published file of atomic coordinates 1um3.pdb.

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_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 $Q\cdot^-$ 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_6f complexes, unlike bc_1 complexes, contain a chlorophyll molecule [26,27]. The structures give precise locations for the chlorophyll and for a familiar carotenoid in unusual surroundings, β-carotene [28]. As yet, there is no clear idea of the functions of these two pigments, although David Stroebel *et al.* [1] favour a role in regulation. The tip of the phytyl tail of chlorophyll protrudes into the Q_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_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_6f 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_6f complexes [30] that is absent from mitochondrial bc_1. This electron density lies near the Q_i site and close to the conventional, high-potential *b*-haem. Both Stroebel *et al.* and Kurisu *et al.* agree that this haem c_i ('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_i, through a thioether linkage to cysteine 35 of the cytochrome b_6 apoprotein, is also seen in *firmicutes* [33,34] – Gram-positive bacteria such as the non-photosynthetic *Bacillus subtilis* and the photosynthetic heliobacteria. The photosynthetic heliobacteria group contains only one kind of photosystem, resembling photosystem I. Thus, cytochrome b_6f might have had a long evolutionary participation in the cyclic chain of photosystem I, eventually replacing the cytochrome bc_1 associated with photosystem II-type and purple bacterial reaction centres, and destined to function in mitochondrial, respiratory electron transport. The haem c_i binding site of b_6f is occupied by a ubiquinone molecule in mitochondrial bc_1 [1]. This location suggests another role for haem c_i: rapidly to supply one of the two electrons required for concerted reduction of Q to QH_2 at the Q_i site. The Q_i semiquinone intermediate must be just as dangerous as that at the Q_o site. If the *b*-haems at the two sites, Q_o and Q_i, carry out the same chemistry but in opposite directions, then haem c_i at the Qi site might be the functional equivalent of the Rieske FeS centre.

Box 1. Guide to translation

David Stroebel *et al.* [1] report the structure of *Chlamydomonas* cytochrome b_6f (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 'Q_o' for 'outer' (lumen side) and 'Q_i' for 'inner' (stromal, cytoplasmic side); the cytochrome b_6 haems are designated b_L (for low potential) and b_H (for high potential). The novel haem is given the name 'haem c_i' (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 cyanobacterial cytoplasm (equivalent to the chloroplast stroma; 'inside') is the '*n*' (for 'negative')-phase; the lumen ('outside') is the '*p*' (for 'positive')-phase. Thus, the Q_o site is synonymous with the Q_p site and the nearby b_L haem is 'heme b_p'. Everything else follows: the Q_i site is synonymous with the Q_n site, and the nearby b_H is 'heme b_n'. In other literature, Q_o becomes 'Q_O' and stands for 'quinol oxidase', and Q_i becomes 'Q_R' 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_i' 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 'Fe_2S_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_6f 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 [36]. 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_6f 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 cytbc_1 complexes, cytochrome b_6f [1,2] confirms the true homology of protonmotive, 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_i) 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_6f probe occupancy of the Q_o site, and transmit that information across the membrane by some means, as Stroebel *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_L and to the iron–sulfur centre of the Rieske protein illustrated in Figure 2b.

Acknowledgements

I thank Jean-Luc Popot for discussions; Daniel Picot for correspondence on the structures and for the file 1q90.pdb in advance of public release; Derek S. Bendall, Antony R. Crofts, Fevzi Daldal and Sabeeha Merchant for suggestions and comments on the manuscript; and Antony R. Crofts, P. Leslie Dutton and Wolfgang Junge for manuscripts in press. Antony R. Crofts kindly provided his original Q_o animation, which is adapted, only slightly, for supplementary information; see http://www.life.uiuc.edu/crofts/bc-complex_site/ Supported by the Swedish Research Council (VR).

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