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Cytochrome *bc* complexes: a common core of structure and function surrounded by diversity in the outlying provinces

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The atomic-level picture of transmembrane protein complexes in the photosynthetic membrane has now been completed by the recent publication of crystal structures of cytochrome b_6f and photosystem II. The two structures of cytochrome b_6f, together with previously reported structures of the cytochrome bc_1 respiratory complex, provide a basis for understanding the central electron and proton transfer events of photosynthesis and respiration. The protein structures and charge transfer events within the core of the complexes are highly similar, but the complexes differ in subunit and chromophore composition in proportion to the distance from the central redox site within the membrane near the electropositive side.

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Abbreviations

ISP	iron-sulfur protein
n	electronegative
NQNO	*n*-nonyl-quinoline-N-oxide
p	electropositive

Introduction

The transduction of light or oxidative energy to chemical energy is essential to all living systems. Whether for photosynthesis or respiration, all biological energy transduction is accomplished by charge separation across an insulating membrane, which results in a transmembrane electrochemical potential gradient. Cytochrome b_6f, the central transmembrane protein complex of oxygenic photosynthesis, and cytochrome bc_1, its respiratory analog, carry out similar core electron and proton transfer steps (Figure 1).

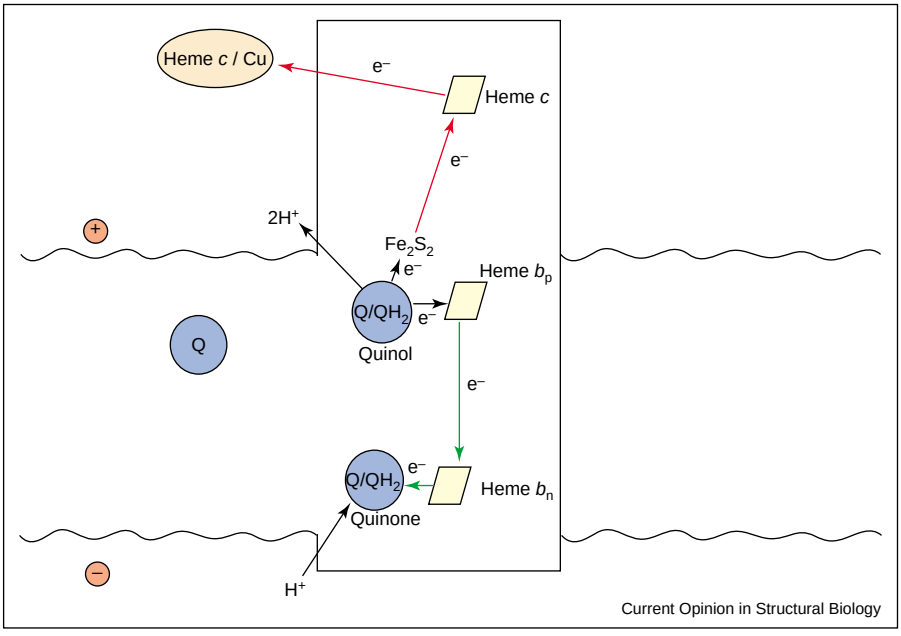
The subunit and chromophore compositions of the cytochrome b_6f and bc_1 complexes are similar in their essential features (Table 1). In each turnover of the complex, a reduced, lipid-soluble quinol carrier transfers one electron to the Fe_2S_2 center of a Rieske iron-sulfur protein (ISP) subunit, one electron to a 'heme b_p' in a cytochrome b_6 (or *b*) subunit and two protons to the aqueous phase on the electropositive (*p*) side of the membrane. In the high (redox) potential branch of the bifurcated electron transfer pathway, the ISP reduces a membrane-bound *c*-type cytochrome subunit at the membrane interface, which in turn reduces a soluble heme or copper protein carrier. In the low-potential electron transfer pathway, which traverses the complex, heme b_p reduces 'heme b_n', which in turn reduces a quinone carrier (Figure 1). Quinone reduction is accompanied by proton uptake from the aqueous phase on the electronegative (*n*) side of the membrane. This internal electron transfer cycle between *b* hemes and associated quinone reduction, known as the 'Q cycle', is one of the most intriguing aspects of the cytochrome b_6f and bc_1 complexes [1,2]. The Q-cycle mechanism can explain the stoichiometry of two protons transferred to the *p*-side aqueous phase for each electron transferred to the *p*-side protein carrier, which results in the higher efficiency of electrochemical energy transduction. Mechanistic details of *p*- and *n*-side charge transfer remain controversial.

Three-dimensional structures of cytochrome bc_1 have been available for some time [3–7]. The first crystal structures of the cytochrome b_6f complex were reported recently [8••,9••]. This review summarizes the new structural data on cytochrome b_6f, with an emphasis on comparisons to the cytochrome bc_1 complex. A large body of biochemical and sequence data points to substantial similarities in the structure and function of the b_6f and bc_1 complexes. Homology of the core subunits is clear from sequence. In the absence of structures of both complexes, cytochromes b_6f and bc_1 were expected to have identical mechanisms. With knowledge of the structures, it is clear that significant differences between the b_6f and bc_1 complexes overlay a common structure/function foundation.

Subunit composition

The b_6f and bc_1 complexes have a common core architecture (Table 1, Figure 2a). Both complexes are dimers of multisubunit monomer units. A central cavity is formed in the transmembrane region at the dimer interface. The three large (≥18 kDa) electron transfer subunits of the b_6f and bc_1 complexes have analogous functions and, except

Figure 1



Schematic of electron and proton transfer in a monomer unit of cytochrome *bc* complex. Q/QH_2 are the oxidized/reduced forms of the quinol — plastoquinol in cytochrome b_6f and ubiquinol in cytochrome bc_1. The soluble protein carrier is plastocyanin or cytochrome c_6 for the b_6f complex, and cytochrome *c* for the bc_1 complex. The wavy lines represent the boundaries of the membrane bilayer. Red arrows indicate the high-potential electron transfer pathway and green arrows the low-potential pathway.

for cytochromes *f* and c_1, are also homologous (Table 1). Thus, a common ancestral complex that functioned in energy transduction is assumed. However, there are also major differences in subunit content. Cytochrome bc_1 has several extrinsic subunits that do not exist in cytochrome b_6f, including core 1, core 2, subunit 9 and subunit 6 on the *n*-side of the complex, and subunit 8 on the *p*-side. Core 1 and core 2 comprise a mitochondrial processing peptidase [10], and subunit 9 is the processed signal peptide of the ISP [11]. The small transmembrane subunits of cytochrome b_6f are unrelated to those of cytochrome bc_1 and occupy different sites at the periphery of the complex, producing strikingly different profiles of the transmembrane regions (Figure 2b).

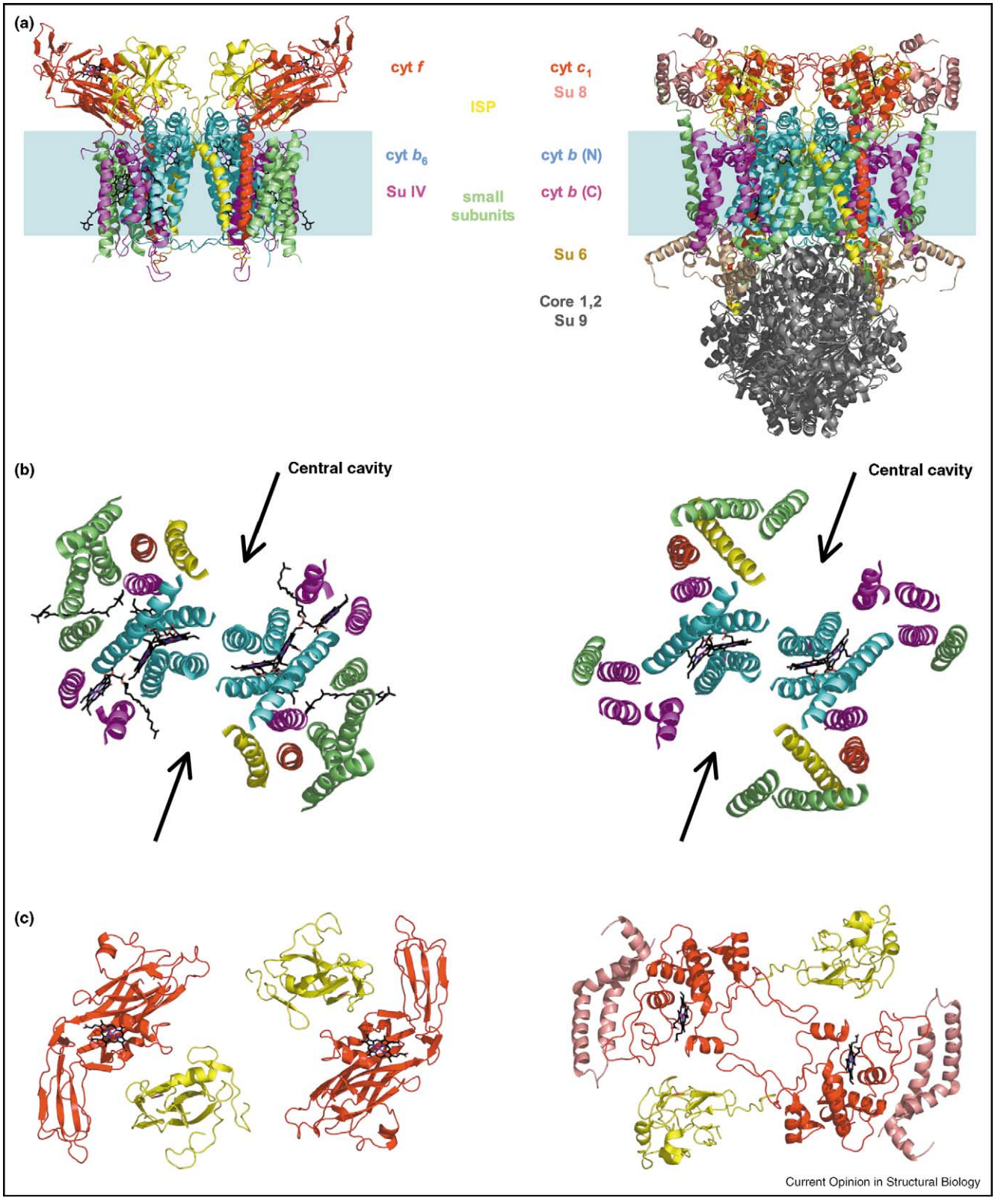
Table 1

Subunit composition and properties of cytochromes b_6f and bc_1.

Subunit	Cytochrome b_6f	Cytochrome bc_1	Relationship
c-type cytochrome	Cytochrome *f*: Heme *c* One TM helix Extrinsic domain	Cytochrome c_1: Heme *c* One TM helix Extrinsic domain	Analogous function, not homologous
b-type cytochrome	Cytochrome b_6: Two heme *b*, one heme *x* Four TM helices	Cytochrome *b*, N terminus: Two heme *b* Four TM helices	Homologous
Other	Subunit IV: Chlorophyll *a* Three TM helices	Cytochrome *b*, C terminus: No chromophores Four TM helices	Homologous
Rieske protein	ISP: Fe_2S_2 One TM helix Extrinsic domain	ISP: Fe_2S_2 One TM helix Extrinsic domain	Homologous
Small subunits	PetG, PetL, PetM, PetN: One TM helix apiece	Subunits 7, 10, 11: One TM helix apiece	Unrelated
Other large subunits	None	Core 1, core 2, subunits 6, 8, 9	
Crystal structures	Cyanobacterium, green alga	Bovine, chicken, yeast	

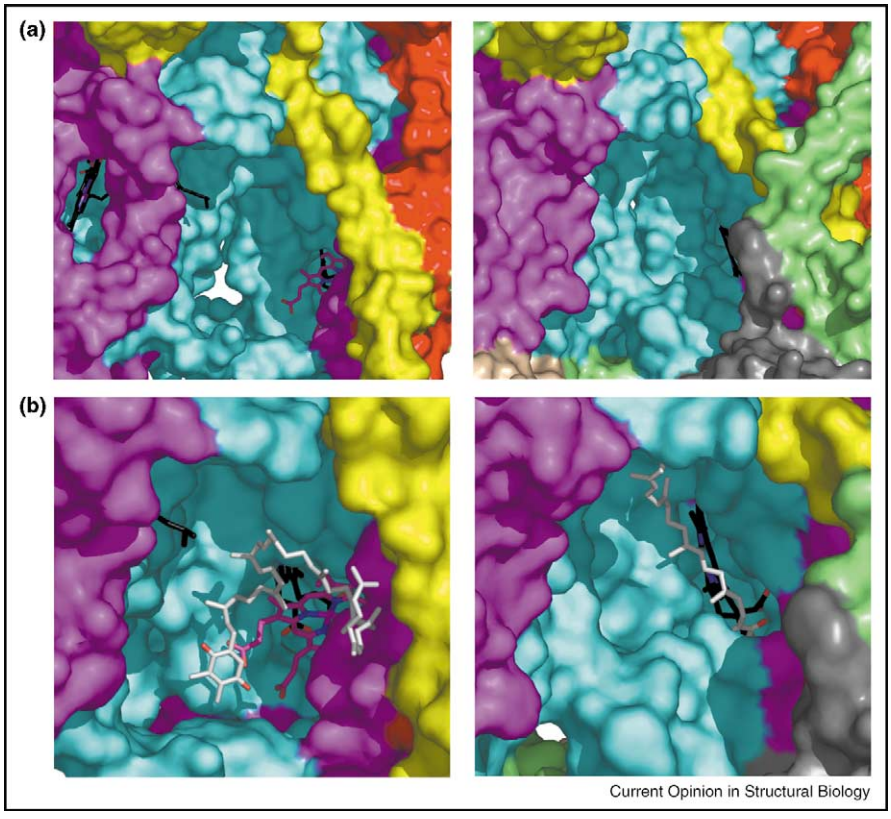
TM, transmembrane.

Figure 2



Subunit composition and placement. In each panel, cyanobacterial cytochrome b_6f [8••] (PDB code 1VF5) is shown on the left and a bovine cytochrome bc_1 complex [7] (PDB code 1LOL) is shown on the right. **(a)** Ribbon diagram of the overall structure of the b_6f and bc_1 complexes. Subunits are colored separately, with analogous/homologous subunits in the same color, as labeled. All atoms of the natural chromophores are shown,

Figure 3



Central cavity of cytochrome *bc* complexes. In each panel, cyanobacterial cytochrome b_6f [8••] (PDB code 1VF5) is shown on the left and the yeast bc_1 complex [6] (PDB code 1KB9) is shown on the right. **(a)** Overall view of the central cavity. The molecular surface of the protein is colored according to subunit, as in Figure 1. Heme *x* (magenta) is prominent in the b_6f complex and blocks access to heme b_n (black), which is further inside the cavity wall. In the yeast complex, the edge of heme b_n is accessible from the central cavity. At the top left, the phytyl tail of chlorophyll *a* is visible in cytochrome b_6f. **(b)** Q_n (site of quinone reduction) and quinone ligand. In cytochrome bc_1, ubiquinone (white carbon atoms) binds with its head in a niche in the cavity wall. In cytochrome b_6f, this volume is occupied by heme *x* and plastoquinone binds adjacent to heme *x* in the central cavity.

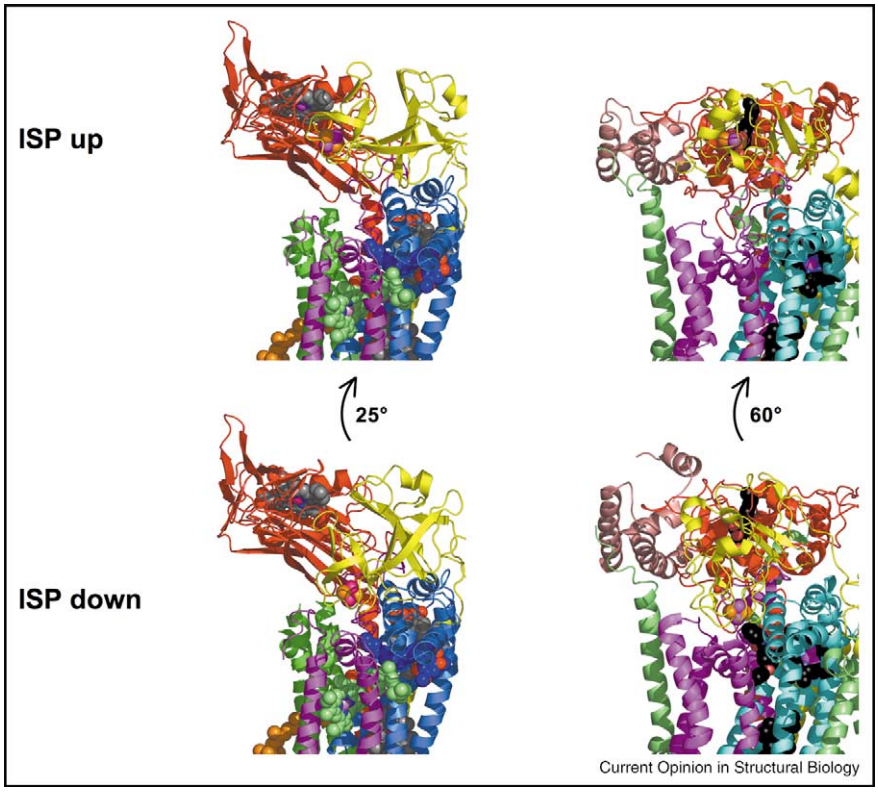
Cytochromes *f* and c_1, with *c*-type covalently bound hemes, have identical functions in the two complexes, but are unrelated proteins with radically different folds in their heme-binding extrinsic domains [12]. By contrast, their C-terminal transmembrane helices occupy identical positions in the b_6f and bc_1 complexes. Their *c* hemes are covalently bound by a sequence motif characteristic of *c*-type cytochromes (Cys-Xaa-Xaa-Cys-His) and occupy positions on the *p*-side of the complex in which the Fe locations differ by 12 Å with respect to the transmembrane core of cytochrome b_6 and subunit IV. The *p*-side aqueous phase profiles of the b_6f and bc_1 complexes are substantially different due to the structural differences between cytochromes *f* and c_1 and to the presence of subunit 8 in the bc_1 complex (Figure 2c). The cytochrome c_1 monomers contact one another in the dimeric bc_1 complex, but no such contacts exist between cytochrome *f* monomers in the b_6f complex. Thus, the structural details of the high-potential electron transfer chain are different in the b_6f and bc_1 complexes.

Chromophores

Of the seven natural prosthetic groups in the b_6f complex (four hemes, one Fe_2S_2 cluster, one chlorophyll *a* and one β-carotene), three (one heme, chlorophyll *a* and β-carotene) do not exist in the bc_1 complex. The heme *b*, heme *c* and Fe_2S_2 groups involved in electron transfer are similarly positioned in the b_6f and bc_1 complexes. A major finding from the structures of cytochrome b_6f is a new heme adjacent to heme b_n. This heme has been called

(Figure 2 Legend continued) with carbon atoms in black. Cyt, cytochrome; Su, subunit. **(b)** Transmembrane helices and chromophores. The different placement of the small transmembrane subunits (green) imparts different shapes to the b_6f and bc_1 complexes, as seen in this cross-section through the membrane bilayer. The central cavities of each complex are indicated by arrows. Transmembrane helices and chromophores within the bilayer are colored as in (a). **(c)** Extrinsic domains on the *p*-side of the membrane [coloring as in (a)].

Figure 4



ISP positions. The bottom panels show the ISP in a position near the Q_p site in the cyanobacterial b_6f complex (left) and the bovine bc_1 complex (right). The top panels show the ISP in positions adjacent to the *c* hemes of cytochrome *f*, as modeled for the b_6f complex [8••] and as observed for bovine cytochrome bc_1 [5] (PDB code 1BE3). Chromophores are shown as spheres, with atom colors as in Figure 2.

heme *x* [8••] (or heme c_i [9••]). Based on sequence conservation around its binding site, heme *x* is inferred to be present in all b_6f complexes. It is covalently linked to the protein by a single thioether bond to a cysteine sidechain, but has no Fe ligands from the protein. Because heme *x* contacts heme b_n, they are inferred to function as a single electron transfer unit. This unanticipated finding is the major biochemical difference between the b_6f and bc_1 complexes. Heme *x* and heme b_n fill a pocket in the wall of the central cavity, such that heme b_n is behind heme *x* and is inaccessible to molecules within the cavity (Figure 3a) [6,13]. The heme *x* binding pocket is analogous to the *n*-side site for quinone (Q_n site) in cytochrome bc_1 (Figure 3b, right). The Q_n site for plastoquinone in cytochrome b_6f is not a pocket, but rather the face of heme *x* exposed to the cavity (Figure 3b, left). Thus, some *n*-side biochemical events of the Q cycle must differ in the two complexes.

Long-standing biochemical observations highlighting differences in *n*-side electron transfer and the Q cycle now take on greater meaning. For example, the lack of a binding niche explains why cytochrome b_6f is not inhibited by antimycin A, a specific quinone analog inhibitor of *n*-side electron transfer in cytochrome bc_1 [14]. Antimycin binding to the Q_n site inhibits oxidation of heme b_n and also blocks all electron transfer through the high-potential chain to the soluble protein carrier, cytochrome *c*. This result is among the strongest evidence in support of a Q cycle in cytochrome bc_1. Similar evidence of a Q cycle in cytochrome b_6f does not exist. By contrast, NQNO (*n*-nonyl-quinoline-N-oxide), a weaker inhibitor of *b*-heme oxidation after reduction induced by a light flash, does not inhibit electron transfer through the high-potential chain of cytochrome b_6f [15]. The existence of a Q cycle in cytochrome b_6f remains controversial, but knowledge of heme *x* is critical to interpreting experiments that probe Q-cycle function.

New experiments and reinterpretation of existing data are underway to characterize the properties of heme *x* and to define its function ([16]; H Zhang, MK Bowman, DM Kramer, WA Cramer, unpublished). At present, the prime candidate for its function is as part of an elusive 'cyclic electron transfer pathway', whereby some of the electrons transferred through cytochrome b_6f and, subsequently, to the reducing *n*-side of photosystem I are returned to cytochrome b_6f. Heme *x* is close enough to the *n*-side surface of the b_6f complex to be reduced by *n*-side protein

carriers such as ferredoxin or $NADP^+$:ferredoxin reductase [17]. Cyclic electron transfer dependent on photosystem I is well documented in photosynthetic membranes, where it is thought to balance cellular needs for reducing equivalents and ATP, but of course it does not exist in respiratory membranes. Nor does it exist in the photosynthetic membranes of bacteria that have a bc_1 complex in lieu of a b_6f complex. In eukaryotic cytochrome bc_1, the large core 1 and core 2 subunits, which have a specific mitochondrial processing activity, block the *n*-side surface of the complex.

In addition to heme *x*, cytochrome b_6f has two chromophores of unknown function that are not present in cytochrome bc_1 (Figure 2a,b). A chlorophyll *a* molecule is bound between transmembrane helices of subunit IV and a β-carotene molecule is bound between two of the small transmembrane subunits. Differences in protein structure are associated with both of these chromophores. The chlorophyll is accommodated by a larger gap between two helices of subunit IV (b_6f) than between the homologous helices of cytochrome *b* (bc_1). The β-carotene is bound by small subunits of cytochrome b_6f that have no analog in cytochrome bc_1. It is of interest that small transmembrane subunits of the photosystem II reaction center also bind β-carotene [18•].

The b_6f and bc_1 complexes both possess prominent central cavities in the transmembrane region at the dimer interface (Figures 2b and 3). The cavity walls are composed of transmembrane helices from the core subunits of both monomer units, so that the cavity is created by dimer formation. The cavity connects a site for quinol oxidation (Q_p) on the *p*-side of one monomer unit with a site for quinone reduction (Q_n) on the *n*-side of the other monomer unit. Thus, if the Q cycle functions by exchange of quinol species between Q_p and Q_n sites, limited diffusion through the cavity necessarily involves the Q_p site of one monomer and the Q_n site of the other monomer. The cavity interiors of the b_6f and bc_1 complexes are similar in volume and hydrophobicity. However, the cavity entrance from the lipid phase is narrowed in the bc_1 complex by small transmembrane subunits adjacent to the ISP transmembrane helix (Figures 2b and 3). Relative to cytochrome bc_1, the wider cavity entrance in cytochrome b_6f may increase the frequency of quinone diffusion out of the complex. Thus, diffusion between the Q_p and Q_n sites may be a less frequent event in cytochrome b_6f than in cytochrome bc_1.

High-potential electron transfer chain

The cytochrome b_6f and bc_1 complexes share the problem of a long distance (25–30 Å) between the sites for reduction and oxidation of the ISP Fe_2S_2 cluster. Fe_2S_2 reduction by quinol takes place at the Q_p site within the *b* cytochrome, and oxidation by the membrane-bound *c* cytochrome takes place in the *p*-side aqueous phase (Figure 1).

In cytochrome bc_1, the problem of long-distance electron transfer is solved by shuttling of the ISP Fe_2S_2 cluster and ISP extrinsic domain between sites for reduction and oxidation (Figure 4). The positions of the Q_p site and *c* heme are invariant within the bc_1 complex in all reported crystal structures. Shuttle motion entails a 60° rotation of the ISP extrinsic domain and a 16 Å translation of the Fe_2S_2 cluster, achieved by a large conformational change in the hinge connecting the ISP extrinsic and transmembrane domains [5]. The function of the ten-residue hinge is sensitive to deletion, substitution and even insertion [19–21]. Residues in an adjacent conserved loop of cytochrome *b* (the 'ef' loop) also influence the operation of the hinge [22].

Cytochrome b_6f also seems to apply the principle of fixed Q_p and *c*-heme sites, and a moving Fe_2S_2 cluster and ISP domain, based on the limited structural data presently available. The two cytochrome b_6f crystal structures include three independent views of the monomer unit, with the ISP extrinsic domain in three positions differing by ~4 Å, and the Q_p and *c*-heme sites in identical positions within the b_6f complex. These positions most closely resemble the 'ISP down' position in cytochrome bc_1 structures. Although the ISP positions do not span the gap between the Q_p site and the cytochrome *f* heme, their variability demonstrates the existence of a functioning ISP hinge in cytochrome b_6f. In contrast to cytochrome bc_1, the ISP hinge of the b_6f complex is more tolerant of change, leading to the prediction that the amplitude of ISP motion is smaller in the b_6f complex than in cytochrome bc_1 [23]. A structural explanation for these observations is that the gap between Q_p and the *c* heme can be spanned by a far smaller hinge motion than that observed in cytochrome bc_1. A rotation of only 25° of the extrinsic domain could move the Fe_2S_2 cluster to within 14 Å of the cytochrome *f* heme and its His26 ligand (Figure 4). This distance could be bridged by intraprotein electron transfer [24,25]. At the membrane surface facing the ISP and cytochrome *f*, a conserved loop of subunit IV (the 'ef' loop) may affect the operation of the ISP hinge.

Cytochrome *f* presents to the ISP a relatively hydrophobic surface, including the conserved sidechains of Ala23, Leu27, Ala28 and Ile160. Just below this surface of cytochrome *f* lies the heme and a highly conserved, buried five-water chain [26]. The first water is hydrogen bonded to the backbone of Ala28 and the second to the heme Fe ligand His26. The function of the water chain is unknown, but sidechains that are hydrogen bonded to the waters are as conserved as those that interact with the *f* heme [27,28]. The hydrophobic surface and water chain appear to be positioned to interact directly with the ISP as it moves into position to reduce cytochrome *f*. Elsewhere in the extrinsic domain of cytochrome *f*, several conserved residues cluster around the membrane anchor. Many of these (Glu35, Gln38, Leu41, Glu246, Val248, Gln250,

Arg254) contact residues in or near the conserved ef loop of subunit IV.

The ISP occupies a cleft between the large and small domains of cytochrome *f*. The hydrophobic surface of cytochrome *f* and the underlying heme, His26 and water chain face the ISP (Figure 2c). The position of cytochrome *f* may be optimal for oxidation by the soluble protein carrier (plastocyanin or cytochrome c_6). The surface of the b_6f complex furthest from the membrane includes an edge of the heme and the conserved sidechain of Tyr1, the N-terminal heme ligand (Figure 2c). This surface of the complex also includes a positively charged region that is conserved in cytochrome *f* from higher plants and was predicted to be important for the electrostatic steering of plastocyanin towards cytochrome *f* [29], although such steering does not appear to occur *in vivo* [30].

Conclusions

The cytochrome b_6f and bc_1 complexes represent a hodgepodge of conservation, divergence and convergence. The complexes carry out analogous steps of electron and proton transfer in photosynthesis and respiration. The *b* cytochromes in the two complexes are homologous, but cytochrome b_6 binds a heme pair (heme b_n and heme *x*) on the *n*-side of the b_6f complex, whereas cytochrome *b* binds heme b_n in the analogous site in the bc_1 complex. The heme b_n–heme *x* pair probably functions in a photosynthetic cyclic electron pathway, which does not exist in respiratory membranes, and also mandates somewhat different electrochemical/biochemical events in any photosynthetic Q cycle. On the aqueous *p*-side of the membrane, the extrinsic domains of transmembrane subunits cytochrome *f* (b_6f) and cytochrome c_1 (bc_1) are an excellent example of evolutionary convergence; they bind identical *c* hemes, but have unrelated protein structures. The constellation of non-core subunits and chromophores differs markedly in the two complexes. In addition to heme *x*, cytochrome b_6f has chlorophyll and carotenoid chromophores, which are not present in the bc_1 complex and have no known function. Cytochrome bc_1 has extrinsic subunits that process mitochondrial target sequences. The b_6f and bc_1 complexes both have small subunits comprising single transmembrane helices without distinct extrinsic domains. However, these subunits differ in number and position in the two complexes. Long ago, Nature assembled an electron and proton transfer complex, and then used this core as a scaffold on which to build auxiliary functions and the structures to support them.

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