constitute at least one-third of the total population of GRBs. With the measurement of the distance to two XRFs, it has been shown (D. Lamb, Univ. Chicago) that these events follow the same relationship between isotropic energy and peak energy as do GRBs[12] — which strongly suggests that GRBs and XRFs share the same origin. One possibility is that an XRF is a GRB seen off-axis, rather than head-on. In the collapsar model, in addition to the collimated, high-energy jet that gives rise to the GRB, a considerable fraction of energy is ejected at wider angles and could produce a burst that peaks at X-ray wavelengths, rather than in γ-rays.

The evidence in favour of a connection between supernovae and GRBs — and probably also XRFs — seems overwhelming. But there are still some inconsistencies in the picture: a radio survey of a particular sample of supernovae (called type Ib and Ic) thought to be connected with GRBs shows that basically none of these objects has properties similar to those of GRB afterglows or of SN1998bw, the first supernova to be linked to a GRB (E. Berger, Caltech); this makes it difficult to understand how there can be simple underlying physics across the board for GRBs and supernovae. And despite the efforts made to assemble the observational evidence into a single model, not all the pieces of the puzzle fit. For instance, the observed evolution of most of the afterglows is awkward to explain in the collapsar model (R. Chevalier, Univ. Virginia), although not a problem for the supranova model (A. Konigl, Univ. Chicago).

GRBs are among the most distant sources that we can observe in the Universe, because they are so bright. For cosmology, this opens the exciting perspective of GRBs as probes of the unobserved history of the Universe, looking back to when the first population of stars and primordial galaxies formed (G. Djorgovsky, Caltech). Present estimates indicate that although a substantial fraction of GRBs actually lie in this region of the Universe (technically, beyond a 'redshift' of five; A. Loeb, Harvard Univ.), they are not visible at optical wavelengths because this radiation is absorbed by hydrogen in the intergalactic medium. Interestingly, several GRBs have no detected optical counterpart[13]. And because most distance (redshift) measurements are derived from optical data, analyses are at present biased against the most distant GRBs: X-ray and infrared measurements are needed as well.

The SWIFT observatory, launching in May 2004, will deliver hundreds of precise, fast localizations of GRBs at γ-ray wavelengths and track within minutes their X-ray and optical afterglows (N. Gehrels, NASA Goddard Space Flight Center), improving considerably on the pioneering method first used by the BeppoSAX mission (F. Frontera, Univ. Ferrara). In addition, the HETE-2 satellite, currently in orbit, should continue to provide complementary X-ray localizations of GRBs (G. Ricker, Massachusetts Inst. Technology). For the study of GRBs, the future is certainly bright. ■

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1. Costa, E. *et al. Nature* **387,** 783–785 (1997).
2. van Paradijs, J. *et al. Nature* **386,** 686–689 (1997).
3. Frail, D. A. *et al. Nature* 389, 261–263 (1997).
4. Metzger, M. R. *et al. Nature* 3**87,** 878–880 (1997).
5. Mészáros, P. *Annu. Rev. Astron. Astrophys.* **40,** 137–169 (2002).
6. Pian, E. *et al. Astrophys. J.* **536,** 778–787 (2000).
7. Galama, T. J. *et al. Nature* **395,** 670–672 (1998).
8. Bloom, J. S. *et al. Nature* **401,** 453–456 (1999).
9. Piro, L. *et al. Science* **290,** 955–958 (2000).
10. Vietri, M. & Stella, L. *Astrophys. J.* **507,** L45–L48 (1998).
11. Heise, J. *et al.* in *Gamma-Ray Bursts in the Afterglow Era* (eds Costa, E., Frontera, F. & Hjorth, J.) 16–21 (Springer, Berlin, 2001).
12. Amati, L. *et al. Astron. Astrophys.* **390,** 81–89 (2003).
13. de Pasquale, M. *et al. Astrophys. J.* **592,** 1018–1024 (2003).

Structural biology

Dual approach to a light problem

Werner Kühlbrandt

The structure of the last of the major pigment-containing protein complexes involved in photosynthesis is now revealed. The details complete our picture of electron shuttling in this vital process.

It is not uncommon for notable scientific progress to be made simultaneously by two independent teams of researchers. The latest example concerns the long-awaited structure of the cytochrome b_6f complex, described by Stroebel *et al.* on page 413 of this issue[1] and by Kurisu *et al.* in *Science*[2]. Cytochrome b_6f mediates the flow of electrons between photosystems II and I in the photosynthetic membranes of plants and cyanobacteria. It is the last of these large, pigment-containing protein complexes to yield to detailed crystallographic analysis, and the structures offer some surprising insights into how it works.

Stroebel and colleagues[1] examined the b_6f complex found in the chloroplasts — the photosynthetic organelles — of a unicellular alga[1], whereas Kurisu and co-workers[2] studied the same complex from a cyanobacterium. Both complexes have essentially the same structure. This in itself is astonishing, given that the two types of organisms are separated by an evolutionary distance of roughly 1,000 million years.

Plants and cyanobacteria have the unique

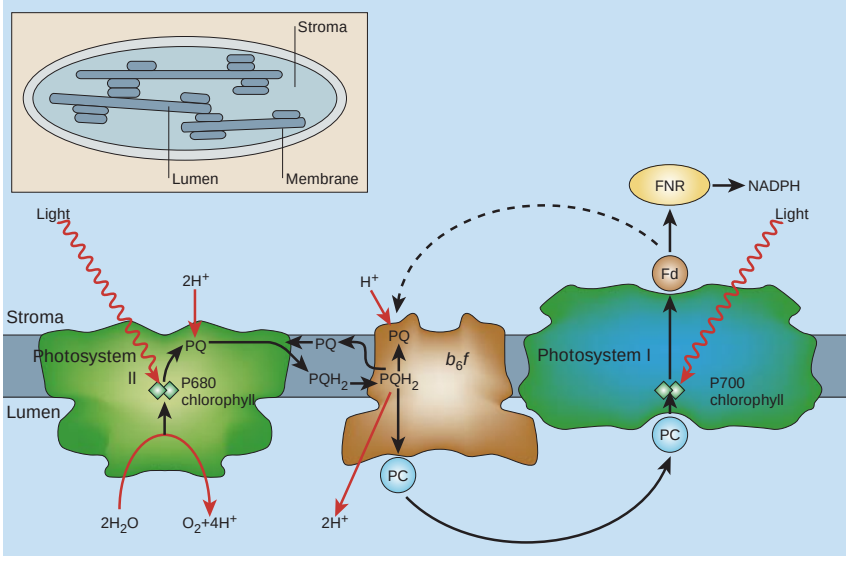

Figure 1 **Electron transport in oxygenic photosynthesis. Photosystem II uses solar energy to withdraw electrons from water, generating oxygen as a waste product. Two electrons are accepted by plastoquinone (PQ), which then binds two protons for electroneutrality. The reduced plastoquinone (PQH_2) diffuses in the membrane to the cytochrome b_6f complex. One electron is transferred to plastocyanin (PC); the other passes to another PQ molecule. The PC diffuses to photosystem I, which uses solar energy to propel the electron against a potential gradient across the membrane. The electron is accepted by ferredoxin (Fd) and transferred to an enzyme (ferredoxin:$NADP^+$ reductase, FNR) that converts NADP into NADPH, the primary product of photosynthesis. X-ray structures of the cytochrome b_6f complex[1,2] suggest that Fd can also deliver its electrons back to b_6f in a cyclic electron flow (dashed line). Black arrows, electron transfer; red arrows, proton transfer. The inset shows a chloroplast.**

ability to use solar energy to withdraw electrons from water, the most abundant substrate on Earth. Unlike the purple and green bacteria, which use more energy-rich substrates, plants and cyanobacteria need two big protein complexes — the photosystems — to bridge the large energy gap between water and the stable reducing agent NADPH. This agent enables the synthesis of organic substances such as sugars and starch.

Photosystem II is the complex that uses solar energy to withdraw electrons from water, generating oxygen as a waste product (Fig. 1). The immediately useful product of this photosystem is the unstable, reduced form of plastoquinone, a lipid-soluble molecule that has been endowed with two electrons by photosystem II. The cytochrome b_6f complex recycles this reduced plastoquinone, stripping it of its electrons and releasing two protons that contribute to the electrochemical gradient across the photosynthetic membrane. This gradient is ultimately used to make ATP, the main energy-storing molecule in living cells. The oxidized plastoquinone goes back to photosystem II to be reduced again. Meanwhile, the b_6f complex feeds one of the stripped electrons into photosystem I, which uses solar power to transfer it to the opposite membrane surface for NADPH synthesis. The other electron passes through the b_6f complex and reduces another plastoquinone molecule.

In addition to this linear mode of electron flow, the system can switch to a cyclic mode, in which photosystem I returns some of its electrons to the b_6f complex rather than feeding them into biosynthesis. This is necessary to balance the activities of the two photosystems, which depend on the variable amounts of solar energy absorbed by each. The cyclic mode of electron flow is poorly understood, but the X-ray structures of the b_6f complex[1,2] suggest a plausible mechanism.

Cytochrome b_6f comprises several membrane-spanning proteins, namely cytochrome b_6, cytochrome *f*, and a protein that harbours an iron–sulphur cluster. The complex owes its deep brown colour to pigment molecules attached to the proteins; these include four haems, a chlorophyll and a β-carotene. Stroebel *et al.* and Kurisu *et al.* now find that the membrane-embedded part of the b_6f complex has an extensive hydrophobic cavity, which is partly filled with membrane lipids, but allows access to the two plastoquinone-binding sites of the complex.

As expected from knowledge of a related structure, the mitochondrial cytochrome bc_1 complex[3–6], the two *b*-type haems are located next to the plastoquinone-binding sites (Fig. 2). Unexpectedly, however, one of these sites contains an extra haem group, not present in the bc_1 complex. This pigment (which Stroebel *et al.* call haem c_i and Kurisu *et al.* call haem *x*) is covalently attached to the cytochrome b_6 polypeptide — making it all the more astonishing that it has escaped discovery until now. Given its position, the most likely function of this extra haem is in cyclic electron flow (Fig. 1). The X-ray structures now make it possible to design experiments to study this elusive process in detail.

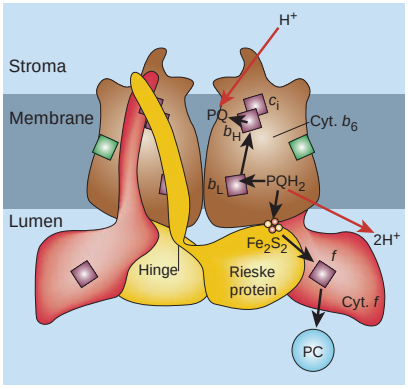

Figure 2 **Electron-transfer routes in the cytochrome b_6f complex, incorporating the new findings[1,2]. The complex is a dimer, with two identical sets of components. At the PQH_2-binding site, two protons are released on the lumenal side of the membrane. The iron–sulphur cluster (Fe_2S_2) takes one electron from PQH_2 and passes it to haem *f* in cytochrome *f*, where it is picked up by PC. The Fe_2S_2 cluster is attached to an iron–sulphur protein (Rieske protein; ISP in ref. 2), which moves on a hinge to bridge the gap between haem b_L and *f*. The second electron passes via haem b_L and b_H (b_p and b_n in ref. 2) to a PQ bound at a site near the stromal membrane surface. PQ binds a proton, adding to the pH gradient across the membrane. The newly discovered haem c_i (*x* in ref. 2) is well placed for the re-uptake of electrons in cyclic electron flow. Purple squares, haems; green squares, chlorophylls; black arrows, electron transfer; red arrows, proton transfer.**

Together, the haems mark the path that electrons take between the two plastoquinone-binding sites. The other path to the surface-exposed cytochrome *f* leads via an iron–sulphur cluster, which is found in a protein domain that moves on a hinge to deliver the electron to haem *f* (Fig. 2). This movement has been inferred from the different positions of the analogous iron–sulphur domains in bc_1 complexes[3–6]. Kurisu and colleagues' structure[2] provides evidence for the same mechanism in the b_6f complex.

The new haem is not the only surprising feature of the b_6f complex. Even though their function in the complex was not clear, it was widely expected that the chlorophyll and carotene would be in close contact, allowing the carotene to defuse the chlorophyll — excitation of an isolated chlorophyll molecule by light would produce oxygen radicals. But both structures show that the two pigments are too far apart to prevent this potentially lethal reaction. It was also hoped that the structure would explain why the chlorophyll is there, but its role remains a mystery. Kurisu *et al.*[2] suggest that it may simply be a space filler (although it would be a highly dangerous one!), whereas Stroebel *et al.*[1] propose that it acts as a sensor in the interaction with photosystem I — which might then provide the absent carotene. Such large functional assemblies, or 'supercomplexes', of cytochrome b_6f and the photosystems have been postulated[7,8], and might indeed exist in the crowded photosynthetic membrane, but they have not yet been seen.

Both teams[1,2] have been working towards a high-resolution structure of the b_6f complex for well over ten years. Why did it take so long? Crystallizing membrane proteins is nearly always problematic, because detergents are required, which often make it difficult to grow diffraction-quality crystals. To make matters worse, the b_6f complex is easily disrupted once it is removed from the photosynthetic membrane. Each team came up with a different ruse to get around this problem. Stroebel *et al.*[1] engineered a tag into the complex, using it to get hold of the complex and purify it quickly — so avoiding prolonged exposure to damaging detergents. Kurisu *et al.*[2] chose the inherently more stable complex from a thermophilic organism, but found that they needed to add back lipids removed during purification before they could obtain well-ordered crystals[9].

The structure of cytochrome b_6f completes our picture of the molecular events that produce the oxygen in the air we breathe. This is one of the first membrane processes that we can follow in its entirety. In the future, we can hope to understand many other, no less fundamental and fascinating membrane systems, including those that enable us to see, hear, taste and think. For all of these we will need information on the structure of membrane proteins at similarly high resolution. But this information cannot be obtained without similar high-risk, long-term efforts. ■

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1. Stroebel, D., Choquet, Y., Popot, J.-L. & Picot, D. *Nature* 426, 413–418 (2003).
2. Kurisu, G., Zhang, H., Smith, J. L. & Cramer, W. A. *Science* 302, 1009–1014 (2003).
3. Xia, D. *et al. Science* 277, 60–66 (1997).
4. Zhang, Z. *et al. Nature* 392, 677–684 (1998).
5. Iwata, S. *et al. Science* 281, 64–71 (1998).
6. Hunte, C. *et al. Structure Folding Design* 8, 669–684 (2000).
7. Zhang, H. *et al. J. Biol. Chem.* 276, 38159–38165 (2001).
8. Joliot, P. & Joliot, A. *Proc. Natl Acad. Sci. USA* 99, 10209–10214 (2002).
9. Zhang, H., Kurisu, G., Smith, J. L. & Cramer, W. A. *Proc. Natl Acad. Sci. USA* 100, 5160–5163 (2003).