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Organization of transmembrane helices in photosystem II: comparison of plants and cyanobacteria

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Electron microscopy and X-ray crystallography are revealing the structure of photosystem II. Electron crystallography has yielded a 3D structure at sufficient resolution to identify subunit positioning and transmembrane organization of the reaction-centre core complex of spinach. Single-particle analyses are providing 3D structures of photosystem II–light-harvesting complex II supercomplexes that can be used to incorporate high-resolution structural data emerging from electron and X-ray crystallography. The positions of the chlorins and metal centres within photosystem II are now available. It can be concluded that photosystem II is a dimeric complex with the transmembrane helices of CP47/D2 proteins related to those of the CP43/D1 proteins by a twofold axis within each monomer. Further, both electron microscopy and X-ray analyses show that P_{680} is not a 'special pair' and that cytochrome *b*559 is located on the D2 side of the reaction centres some distance from P_{680}. However, although comparison of the electron microscopy and X-ray models for spinach and *Synechococcus elongatus* show considerable similarities, there seem to be differences in the number and positioning of some small subunits.

Keywords: photosystem II; structure; electron microscopy; transmembrane helices

1. INTRODUCTION

Essentially, the energy source for all living organisms on our planet is sunlight, which is absorbed and converted into chemical energy by the process of photosynthesis. At the heart of this energy-converting system is the water-splitting reaction. The breaking of the O–H bonds of water is thermodynamically very demanding and yields molecular oxygen and reducing equivalents, where the latter are used for the fixation of carbon dioxide to organic matter. The oxygen is released into the atmosphere where it is required to replace the oxygen consumed by combustion and respiration. Moreover, the continuous evolution of oxygen by photosynthetic organisms is needed to maintain the ozone layer and as such protect terrestrial life from damage by ultraviolet radiation. Approximately 300 000 different species of plants, together with the very large number of algal and cyanobacterial species, yield *ca.* 10^{14} kg of oxygen annually. Given that air contains 21% oxygen, amounting to a total mass of 10^{18} kg, it can be calculated that the mean residence time for a molecule of oxygen in the atmosphere is many thousands of years.

The choice of solar radiation as a power source, and water as the fundamental provider of reducing equivalents, clearly underpins the success of biology to prosper on an enormous scale since both are available in practically unlimited amounts. To be able to artificially mimic the photosynthetic process would similarly provide humanity with a supply of energy that would be continuous and non-polluting. It is therefore evident that one of the great challenges of present day science is to understand fully the reaction of the photosynthetic water-splitting process at a molecular level. The water-splitting reaction takes place in a multi-subunit protein complex, known as PSII, which is embedded in the thylakoid membrane of oxygenic photosynthetic organisms. A considerable knowledge base has been established over time for the biochemical and biophysical properties of PSII (Diner & Babcock 1996), but further progress requires an understanding of the structure of this multi-subunit protein complex. The reactions of PSII are initiated by the absorption of a photon of light by one of the many pigment molecules that make up its light-harvesting system. In higher plants, green algae and green oxyphotobacteria (prochlorophytes) these pigments are chlorophyll *a*, chlorophyll *b* and carotenoids, while phycobilins, such as phycoerythrobilin, phycocyanobilin and allophycocyanobilin replace chlorophyll *b* in red algae and cyanobacteria. The excitation is rapidly transferred from the light-harvesting pigments to the reaction centre where charge separation occurs. In PSII the primary electron donor is P_{680}, which, after being excited, passes an electron to an acceptor Pheo in a few picoseconds to form the radical pair state $P^{+}_{680}\cdot$ $Pheo\cdot^{-}$. An electron transfer follows this initial energy storage step from $Pheo\cdot^{-}$ to a plastoquinone molecule Q_A and then to another plastoquinone molecule Q_B. A second photochemical turnover yields a double-reduced Q_B, which is protonated and leaves the reaction centre, to be replaced by a fully oxidized plastoquinone

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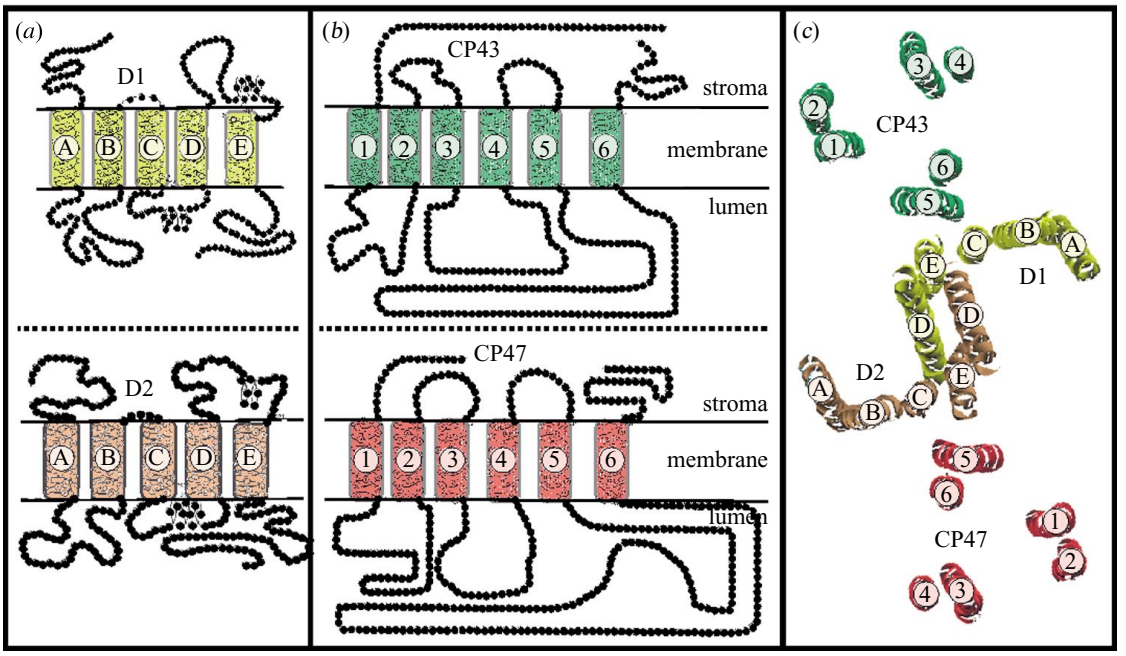

Figure 1. Transmembrane helical organization of the major subunits of PSII. (*a*) Predicted folding of the D1 and D2 proteins. (*b*) Predicted folding of CP43 and CP47. (*c*) Helix organization of the major subunits derived from electron microscopy (Hankamer *et al.* 2001*b*) and X-ray (Zouni *et al.* 2001) analyses showing that the 11 transmembrane helices of CP43/D1 proteins are related to the 11 transmembrane helices of CP47/D2 proteins by a twofold axis. The helix ordering is based on extrapolation from the high-resolution X-ray structures of the purple bacterial reaction centre (Deisenhofer *et al.* 1985) and PSI (Jordan *et al.* 2001).

molecule. Thus, PSII provides the reducing equivalents that are used, ultimately, to fix carbon dioxide. On the oxidizing side of the reaction centre, $P^{+}_{680}\cdot$ is reduced by a Mn atom, located in a cluster of four $(Mn)_4$, via a redox-active tyrosine, Y_Z. Four photochemical turnovers are needed to obtain the four oxidizing equivalents necessary to generate a dioxygen from two molecules of water. The electron transfer processes occur over a range of milliseconds to nanoseconds, depending on the oxidation state of the $(Mn)_4$ cluster. Our understanding of the chemistry of the $(Mn)_4$ cluster and the water oxidation reactions is advancing rapidly with the latest information and theories being presented in these proceedings.

All the redox-active centres of PSII are located in proteins. P_{680}, Pheo, Y_Z, Q_A and Q_B are bound within a heterodimer composed of the D1 (the product of *psbA* gene) and D2 (product of *psbD* gene) proteins. Moreover, it seems that the majority of the ligands for the $(Mn)_4$ cluster are also provided by the D1 protein (Nixon & Diner 1992). Thus, the D1 and D2 subunits are often referred to as the PSII reaction-centre proteins and, indeed, they are analogous in several ways to the L and M subunits of the reaction centre of anoxygenic purple photosynthetic bacteria (Barber 1987; Michel & Deisenhofer 1988). Closely associated with the D1 and D2 reaction-centre proteins are two similar chlorophyll *a* binding proteins, CP43 (product of the *psbC* gene) and CP47 (product of the *psbB* gene), which serve as an inner light-harvesting system (Barber *et al.* 2000). As shown in figure 1, the D1 and D2 proteins, which in the case of spinach have molecular masses of 38 kDa and 39 kDa, respectively, are predicted to have five transmembrane helices based on hydropathy analyses. Similarly, CP43 and CP47 are predicted to have six transmembrane helices each and are characterized by having very large loops joining the lumenal ends of helices 5 and 6. For the spinach proteins, their molecular masses are 50 kDa and 56 kDa, respectively. Genome and amino acid analyses have indicated the presence of a number of other low molecular weight subunits, PsbE, PsbF, PsbH, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbN, $PsbT_c$, PsbX, PsbY and PsbZ within the PSII reaction-centre core (Barber *et al.* 1997; Hankamer *et al.* 2001*a*; Swiatek *et al.* 2001). Each of these proteins is predicted to have a single transmembrane helix. In addition, PSII contains extrinsic proteins bound to the lumenal surface, which together with the intrinsic proteins make up the OEC. At least three OEC extrinsic proteins are present: the 33 kDa PsbO, 23 kDa PsbP and 17 kDa PsbQ in higher plants and green algae, while in cyanobacteria and red algae, PsbP and PsbQ are replaced by the 15 kDa PsbV and 11 kDa PsbU, respectively. In higher plants and green algae, there may also be two further extrinsic proteins associated with the OEC, 10 kDa $PsbT_n$ and 5 kDa PsbR (Barber *et al.* 1997).

2. STRUCTURE OF PSII

Low-resolution structures of PSII have been available for some time based on EM (Hankamer *et al.* 1997). By 1995, compelling evidence had accumulated which indicated that the PSII reaction-centre core was normally dimeric, having dimensions in negative stain of *ca.* 206 Å × 131 Å (Boekema *et al.* 1995). Until that time the concept of PSII being dimeric was well established for cyanobacteria (Rögner *et al.* 1996) but was a matter of debate for higher-plant PSII (Holzenberg *et al.* 1993; Boekema *et al.* 1995; Hankamer *et al.* 1997). Subsequently, significant advances have been made in determining the

structure of PSII using high-resolution electron cryomicroscopy and X-ray crystallography. The first details of the organization of the transmembrane helices came from the analyses of 2D crystals of a subcomplex of PSII isolated from spinach (Rhee *et al.* 1997, 1998). This subcomplex contained the D1, D2 and CP47 proteins and a number of low molecular weight proteins, including PsbE and PsbF, which are the α- and β-subunits of cytochrome *b*559. This electron crystallographic study confirmed that the 10 transmembrane helices of the D1/D2 heterodimer were arranged in a manner similar to those of the L and M subunit of the reaction centre of purple bacteria. The work also identified, for the first time, to our knowledge, that the six predicted transmembrane helices of CP47 were arranged in three pairs around a pseudo threefold axis. Additional densities in the 3D map were attributed to seven other transmembrane helices. At an 8 Å resolution, the map was sufficiently accurate to assign densities to the chlorin head groups of chlorophylls. Within the D1/D2 heterodimer six such densities were identified arranged around the same twofold axis which related the helices of the D1 and D2 proteins. By analogy with the bacterial reaction centre, two were suggested to be Pheo and the other four were assumed to be chlorophylls, with one or more being P_{680}. Although the arrangement was analogous to the bacterial system, the distance between the two chlorophylls, equivalent to the 'special pair' in the purple bacteria, was greater. Within the six helical bundle of CP47, 14 densities were assigned to chlorophyll, which tended to be layered towards the stromal and lumenal surfaces. Two of the other transmembrane helices were tentatively assigned to the PsbE and PsbF subunits of cytochrome *b*559 (Rhee 1998).

The 3D structure of the PSII subcomplex also indicated that the six transmembrane helices of CP43 would be positioned on the opposite side of the D1/D2 heterodimer to that of CP47. This was confirmed when 2D crystals of the complete spinach PSII reaction-centre core complex were grown and analysed, firstly to give a projection map (Hankamer *et al.* 1999) and later a 3D model (Hankamer *et al.* 2001*b*). At a resolution of *ca.* 10 Å, the 3D model of the PSII core complex was sufficient to detect the 22 transmembrane helices of the D1, D2, CP47 and CP43 proteins. In addition, 12 other transmembrane helices were identified as corresponding to the low molecular weight subunits present, including the two tentatively assigned to cytochrome *b*559 (see figure 3).

Complementing the above EM work have been the X-ray analyses of 3D crystals of the PSII reaction-centre core isolated from *Synechococcus elongatus* (Zouni *et al.* 2001), a thermophilic cyanobacterium. This work confirmed the arrangement of the transmembrane helices of the D1, D2, CP47 and CP43 proteins derived from EM analyses (Hankamer *et al.* 2001*b*). Moreover, the two studies concurred on the positioning of 11 of the small subunits. Because the X-ray map had a resolution of 3.8 Å, it confirmed unambiguously the tentative assignment of cytochrome *b*559, and also revealed the positioning of chlorophyll molecules both within the D1/D2 heterodimer and in CP43 and CP47 subunits. This method confirmed the absence of a special pair for P_{680}, and the general arrangement of chlorophylls within CP47 derived from electron crystallography (Rhee *et al.* 1998). Corresponding side views of the higher plant and cyanobacterial models are compared in figure 2. In the case of *S. elongatus*, the density for the extrinsic PsbV (cytochrome *c*550) was located, as was a portion of the PsbO protein based on the prediction that this protein has a high content of β-sheet (Bricker & Frankel 1998; De Las Rivas & Heredia 1999). The PSII complex isolated from spinach and used to grow 2D crystals was devoid of the PsbP and PsbQ OEC proteins and probably did not have a full occupancy of the PsbO. Therefore, the extrinsic density shown in figure 2*a* that protrudes from the lumenal surface is probably mainly due to the large extrinsic loops of CP43 and CP47 (see figure 2 legend for details).

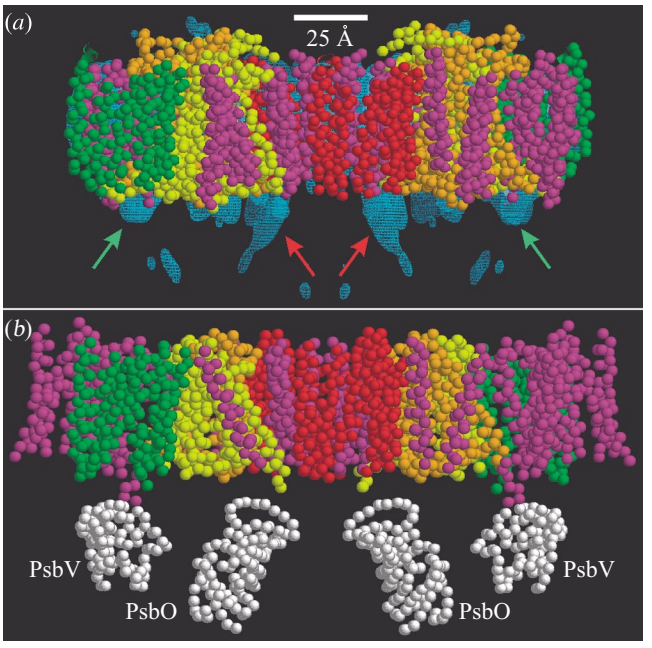

Figure 2. Side views of the 3D structure of the core dimer complex of PSII for (*a*) spinach derived from electron crystallography (Hankamer *et al.* 2001*b*) and (*b*) *Synechococcus elongatus* derived from X-ray crystallography (Zouni *et al.* 2001). In the spinach structure, the lumenal extensions (blue) are believed to be part of the large loops joining helices 5 and 6 of CP47 (red arrows) and CP43 (green arrows) (see figure 1*b*). In the case of *S. elongatus*, extrinsic densities have been assigned to the PsbV (cyt *c*550) and to a portion of the PsbO. Key: D1, green/yellow; D2, orange; CP43, green; CP47, red; small subunits, magenta.

Figure 3 compares the helix organization for structural models derived for spinach PSII by electron crystallography (Hankamer *et al.* 2001*a*,*b*) and *S. elongatus* PSII by X-ray crystallography (Zouni *et al.* 2001). Overall, they are remarkably similar with only a few differences. One difference is the apparent existence of an extra transmembrane helix on the periphery of the spinach complex (ringed in figure 3*a*) compared with the cyanobacterial system. However, four transmembrane helices have been identified adjacent to CP43 in the PSII of *S. elongatus*, whereas only one helix has been assigned in this same region in the case of spinach. Assignment of the five yellow transmembrane helices to the D1 protein of spinach PSII was made initially because there was evidence that this reaction-centre protein is adjacent to CP43 (Moskalenko *et al.* 1992). This assignment was confirmed by the 3.8 Å X-ray structure of *S. elongatus* PSII based on the assump-

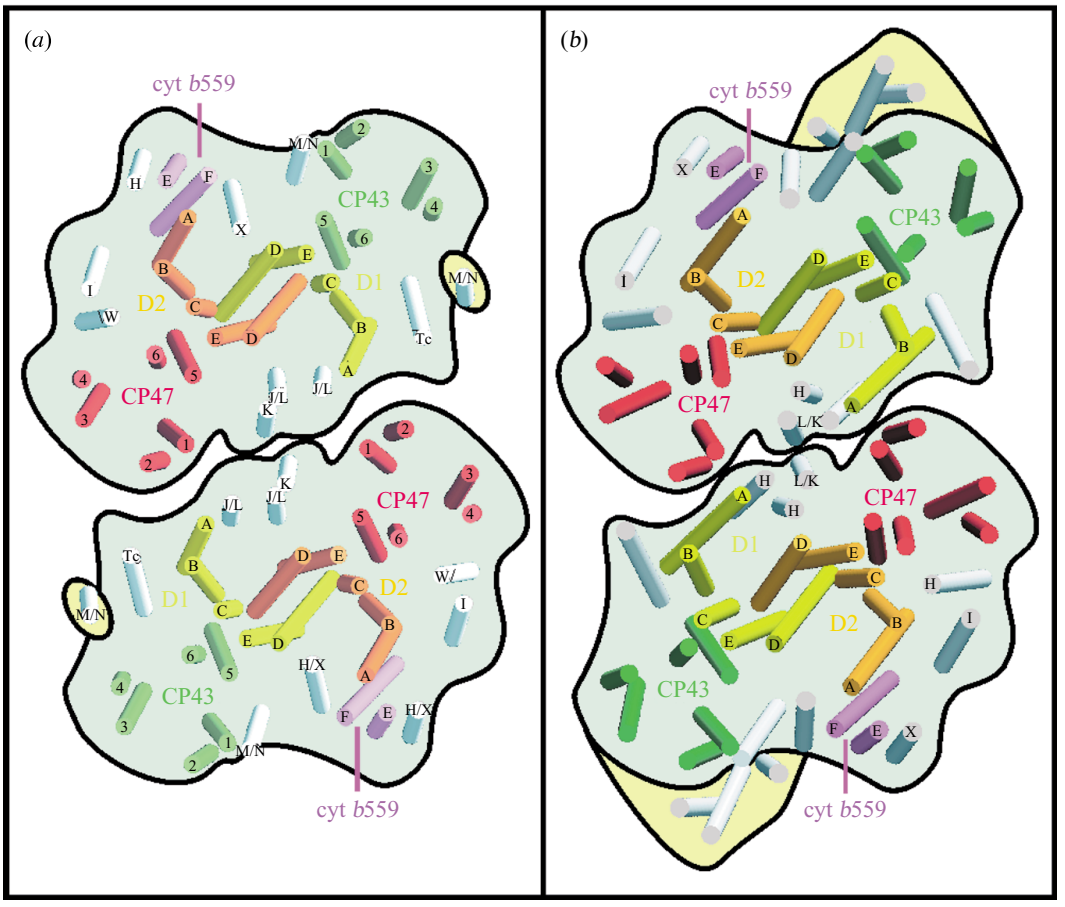

Figure 3. A comparison of the transmembrane helices in the core dimer of (*a*) spinach (Hankamer *et al.* 2001*a*) and (*b*) *Synechococcus elongatus* (Zouni *et al.* 2001). The helix organization of the D1 (green/yellow), D2 (orange), CP43 (green), CP47 (red), cyt *b*559 (purple) and nine further helices of the small subunits seem to be identical in the two models. The differences are highlighted by a yellow background fill, showing an additional helix in spinach (see (*a*)), while the *S. elongatus* structure has three additional helices adjacent to CP43 (*b*). The labelling of small subunits has been made, tentatively, for spinach (Hankamer *et al.* 2001*a*) and for *S. elongatus* (Zouni *et al.* 2001).

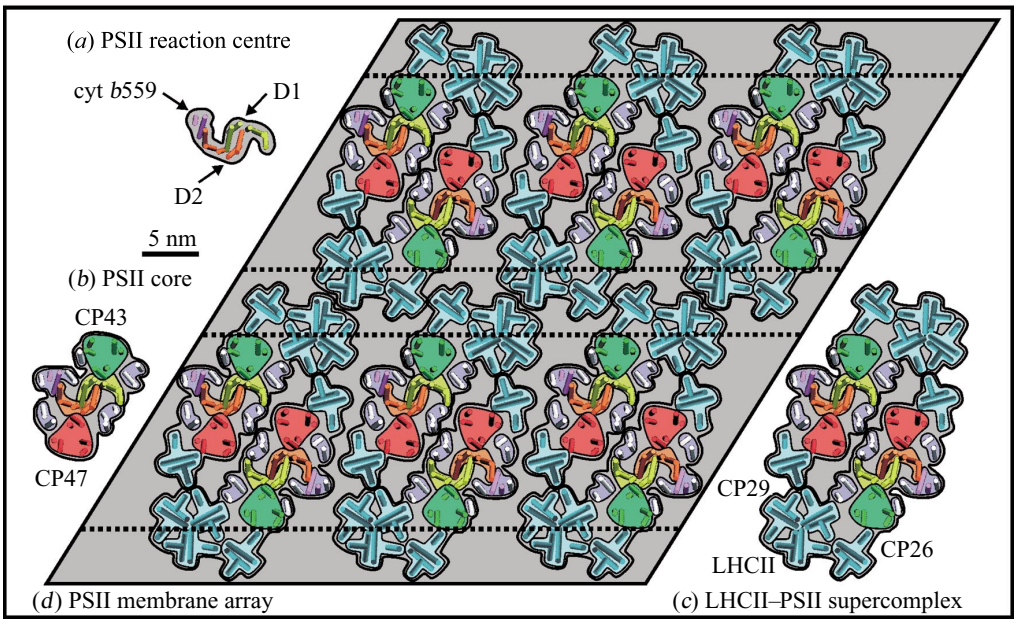

Figure 4. Diagrammatic representation of top views (lumenal side) of the LHCII–PSII supercomplex (*c*), its constituent subcomplexes (D1/D2/cyt *b*559 reaction-centre complex (*a*), PSII core monomer (*b*)) and its possible arrangement in an ordered 2D array (*d*) which may form under some conditions in the thylakoid membrane of higher plant chloroplasts. Colour coding of the core subunits is as given in figure 3. Helices of LHCII, CP29 and CP26 are shown in cyan.

tion that the $(Mn)_4$ cluster is ligated to the loop joining the lumenal ends of the C and D helices of this protein (Nixon & Diner 1992). By contrast, the assignment of the position of CP43 and CP47 comes from electron crystallography and difference mapping (Hankamer *et al.* 1999). The ordering of helices shown in figure 3*a* is derived from analogous structures; the L and M subunits for D1 and D2 proteins (Deisenhofer *et al.* 1985) and the N-terminal domains of the PSI PsaA/PsaB reaction-centre proteins (Jordan *et al.* 2001). In both cases, the ordering of the helices is unambiguous because the X-ray structures were, unlike that for PSII, at sufficient resolution to trace side chains.

The inability to trace side chains in the 3.8 Å map of PSII means that it is not yet possible to assign the small subunits to the remaining transmembrane helices, except for PsbE and PsbF that are characterized by the presence of a haem group. However, tentative assignments have been made (Hankamer *et al.* 2001*a*; Zouni *et al.* 2001) and are shown in figure 3. Perhaps of particular note is the work of Büchel *et al.* (2001) who showed that by using a Ni^{2+}–NTA–Au probe and PSII isolated from *Chlamydomonas reinhardtii*, that PsbH is located in a region close to cytochrome *b*559 and not at the interface between the two monomers, as suggested for the X-ray model (compare figure 3*a* and 3*b*).

3. WHAT HAVE WE LEARNT ABOUT THE STRUCTURE OF PSII TO DATE?

The high-resolution structure of PSII will ultimately be revealed by X-ray crystallography, and the 3.8 Å model published recently (Zouni *et al.* 2001) is the first step in that direction. Nevertheless, electron cryomicroscopy is a complementary technique suitable for obtaining intermediate resolution structures when ordered 3D crystals are not available. Indeed, this technique revealed significant amounts of structural information about PSII before the X-ray work and will continue to do so into the future. Together, the two techniques have so far revealed the following.

(i) The five transmembrane helices of the D1 and D2 proteins are arranged in a manner similar to those of the L and M subunits of purple photosynthetic bacterial reaction centres, as predicted from sequence homology studies (Barber 1987; Michel & Deisenhofer 1988).

(ii) The CP43 and CP47 proteins are each composed of six transmembrane helices as predicted (see Bricker 1990; Barber *et al.* 2000). These are arranged in a circular fashion as three pairs around a pseudo-threefold axis (see figure 1*c*).

(iii) The 11 transmembrane helices of CP47 and D2 are related to the corresponding 11 transmembrane helices of CP43 and D1 by a pseudo-twofold axis.

(iv) The present EM derived map indicates that the spinach PSII core complex contains a further 12 transmembrane helices while the X-ray data identified 14 transmembrane helices in the PSII core complex of *S. elongatus*. In both cases, two transmembrane helices are identified as the PsbE and PsbF subunits of cytochrome *b*559. The remaining helices are assigned to the other small subunits and are located in the same positions in both systems, with some exceptions.

(v) The four chlorophyll, two Pheo and two plastoquinone (Q_A and Q_B) molecules, which constitute the redox centre of PSII are arranged around the same twofold axis that relates the D1 and D2 proteins. The arrangement of these cofactors is similar to that of their counterparts in the purple bacterial reaction centre except there is no 'special pair'. The absence of a 'special pair' is probably due, in part, to the requirement of P_{680} to generate a high redox potential with implications for the mechanism of primary charge separation and vulnerability to photochemical damage (Barber & Archer 2001).

(vi) The X-ray structure also confirmed the existence of two further chlorophyll molecules located close to helix B of the D1 and D2 proteins, predicted to be ligated to the His118 of both subunits. The EM analyses also identified density in the same location that was initially assigned to transmembrane helices (Rhee *et al.* 1998) but subsequently modified (Hankamer *et al.* 2001*a*,*b*).

(vii) In addition, the EM analysis assigned density to 14 chlorophyll molecules bound to CP47 which was later confirmed and extended by the X-ray study. The X-ray study also identified at least 12 chlorophyll molecules bound to CP43. In both cases, the pigment molecules were located towards the stromal and lumenal surfaces.

(viii) X-ray diffraction analyses have unambiguously identified the positioning of the metal centres in the PSII complex of *S. elongates*, thus locating the position of the haems of cytochrome *b*559 and cytochrome *c*550 (PsbV) as well as the non-haem iron located midway between Q_A and Q_B. Importantly, the X-ray work has revealed the position of the $(Mn)_4$ cluster close to the CD lumenal helix of the D1 protein.

4. WHY IS PSII DIMERIC?

Although the highest resolution structure of PSII available to date lacks side chain information, the distances between the main redox-active centres are now known except for the location of the photooxidizable β-carotene (Telfer *et al.* 1993; Hanley *et al.* 1999). Also, yet to be revealed is the exact structure of the $(Mn)_4$ cluster and how this catalytic centre accommodates the Ca and Cl ions required for the water oxidation process. This detail will not emerge from EM but will require the production of highly ordered 3D crystals allowing X-ray diffraction analyses to 2.5 Å or better. However, high-resolution EM will play an important role in providing information about macromolecular organization. It has already produced a 3D structure of a supercomplex of PSII from spinach and from the green alga *C. reinhardtii*, consisting of the core dimer and peripheral domains containing Cab proteins CP29, CP26 and LHCII (Nield *et al.* 2000*a*,*b*). This LHCII–PSII supercomplex binds *ca*. 75 chlorophyll *a* and 25 chlorophyll *b* molecules per reaction centre. It seems to be a basic structure onto which additional Cab proteins

can bind, the extent of which depends on growth conditions e.g. low or high growth light. Under some conditions, the LHCII–PSII can form 2D crystals or arrays in the thylakoid membrane and again these have been studied by EM techniques (Boekema *et al.* 2000). The challenge is to improve the resolution of the 17 Å 3D structure of spinach LHCII–PSII in order to reveal, unambiguously, the positions of the Cab subunits and their transmembrane helices. Another challenge is to understand how the organization of the LHCII–PSII supercomplexes and the interconnecting LHCII complexes within the thylakoid membrane vary under different functional and environmental conditions. Using such information, the higher-resolution structural data obtained for LHCII (Kühlbrandt *et al.* 1994) and for the PSII core dimer (Hankamer *et al.* 2001*a*,*b*) can be built into the framework provided by the EM studies. Figure 4 is a diagrammatic representation of the LHCII–PSII supercomplex and its constituent parts. Under some conditions, the supercomplex can form native 2D arrays in the thylakoid membrane (Yakushevska *et al.* 2001) and figure 4 illustrates one such possible array.

In the case of cyanobacteria and red algae, the PSII supercomplex has the allophycocyanin core attached to its stromal surface. No such supercomplex has yet been isolated and studied in detail. This is an important challenge given that the highest resolution structure of PSII is being revealed by X-ray crystallography of a cyanobacterial complex. Not only do we need to understand how energy transfer is coupled between the chromophores within the allophycocyanin core and PSII, but also to establish the structural and functional implications of having a large protein mass attached to the stromal surface of PSII in the vicinity of the Q_A and Q_B binding pockets. Another challenge is to gain an insight into the coupling of the outer antenna systems of green oxyphotobacteria (prochlorophytes) where a CP43-like protein known as Pcb subunits, serves as the main light-harvesting system. Indeed, the recent discovery of a 18 Pcb subunit ring around the trimeric PSI reaction centre core in this class of organism (Bibby *et al.* 2001) has facilitated the investigation of the existence of a similar supercomplex of PSII.

5. WHAT ARE THE CHALLENGES FOR THE FUTURE?

Despite previous claims that the PSII complex within the thylakoid membrane was monomeric, it is now clear from structural studies on all types of oxygenic photosynthetic organisms that PSII is dimeric in its normal functional state. The reason for this is unclear. There is no evidence for electron transfer between the two monomers within the dimer. There is, however, a sharing of the light-harvesting antenna both at the level of the internal systems, composed of CP43 and CP47 and the outer, composed of Cab, Pcb or phycobilin proteins. This may facilitate better use of incident light under conditions of moderate intensity when one reaction centre within the dimer is closed or damaged. Another suggestion has been made which relates to a unique property of PSII, namely the turnover of the D1 protein. This turnover is linked to unavoidable damage of this protein due to the toxic nature of PSII photochemistry (Barber & Andersson 1992). However, *in vivo*, the degradation of the damaged D1 and its replacement by newly synthesized protein is synchronized (Komenda & Barber 1995). There is evidence that the exchange of the damaged D1 protein involves monomerization of the dimer followed by the detachment of the CP43 subunit (Barbato *et al.* 1992). It is possible therefore that the dimer is required for regulating the replacement process (Kruse *et al.* 1997). However, oligomerization of membrane protein complexes seems to be a common feature and there may be other reasons why PSII normally exists as a dimer *in vivo*.

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Discussion

C. Tommos (*Department of Biochemistry and Biophysics, Arrhenius Laboratories of Natural Science, Stockholm University, Stockholm, Sweden*). What is the distance between the Mn_4 cluster and the bulk solvent, i.e. how deeply buried is the metal site?

J. Barber. According to the single particle analysis that Jon Nield has been conducting with the LHCII–PSII supercomplex of spinach, we would estimate the distance to be in the range of 10–15 Å and, furthermore, according to our analysis (Nield *et al.* 2002), the large mass due to the OEC proteins are located to one side of the cluster and extend by about 50 Å into the lumen.

Additional reference

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GLOSSARY

Cab: chlorophyll *a*/*b* binding
CP: chlorophyll protein
EM: electron microscopy
LHCII: light-harvesting complex II
OEC: oxygen-evolving complex
Pheo: pheophytin
PSII: photosystem II