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Domain organization of photosystem II in membranes of the cyanobacterium *Synechocystis* PCC6803 investigated by electron microscopy

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Abstract The supramolecular organization of photosystem II (PSII) complexes in the photosynthetic membrane of the cyanobacterium Synechocystis 6803 was studied by electron microscopy. After mild detergent solubilization, crystalline PSII arrays were extracted in which dimeric PSII particles associate in multiple rows. Image processing of the arrays shows that the PSII dimers are tightly packed at distances of 12.2 and 16.7 nm. The domains are considered to be an important type of association for preventing either spill-over energy from PSII towards photosystem I (PSI) or direct energy flow from phycobilisomes to PSI, because the latter can only be at periphery of the arrays.
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Keywords: Electron microscopy; Photosystem II; Single particle analysis; Membrane organization; *Synechocystis* 6803

1. Introduction

On a global scale the cyanobacteria and green plants are the most important groups of oxygenic photosynthetic organisms. Oxygenic photosynthesis is mediated by two large membrane-embedded photosystems, named photosystem I (PSI) and photosystem II (PSII). The green plant and cyanobacterial photosystems have several core subunits which are rather homologous, but the peripheral light-harvesting antenna proteins which are associated to PSI and PSII are very different in sequence and organization. Green plants have membrane-bound antenna proteins, belonging to the LHCII family. These proteins are lacking in most cyanobacteria. Instead, water-soluble phycobiliproteins are organized into giant (3–7 MDa) phycobilisome complexes, which are primarily associated to PSII, but also can serve excitation energy to PSI [1].

The organization of the photosynthetic thylakoid membrane of green plant chloroplasts is very special, because it consists of two main domains: the grana, which are stacks of thylakoids, and the stroma lamellae, which are unstacked thylakoids and connect the grana stacks. PSII and LHCII reside mainly in the grana membranes, while PSI and ATP synthase reside predominantly in the stroma (see [2] for a review). One of the main consequences of stacking is the physical separation of PSI and PSII. It has been argued [3] that the separation of the antenna systems of PSI and PSII is essential for efficient photosynthesis, because the kinetics of the trapping of excitation energy is much faster in PSI than in PSII, and a location of both antenna systems at short distances would lead to an uncontrolled flow of energy from PSII to PSI. The stacking not only prevents this spill-over of excitation energy, but it also provides the chloroplast the means to fine-regulate the light need for photosynthesis [4]. The stacking also provides PSII a very large functional antenna, in which excitation energy can flow within a thylakoid membrane and between two stacked membranes. In addition, it provides an easy means to adapt to low-light conditions, in which both the amount of LHCII and the extent of stacking have been shown to increase [5]. Within the membrane PSII is sometimes forming highly ordered semi-crystalline domains [2]. The cyanobacteria do not show a tight stacking of membranes, because of the presence of the phycobilisomes. There is no evidence for an extensive domain organization in cyanobacteria, but many electron microscopy studies have reported the presence of row-like associations of PSII dimers [6–9]. In one case small semi-crystalline PSII arrays were reported [10].

State transitions form a physiological adaptation mechanism that changes the interaction of the peripheral antenna proteins with the PSI and PSII core complexes. In cells adapted to state 1, phycobilisomes transfer energy primarily to PS II. In cells adapted to state 2 a significant proportion of phycobilisomes transfer energy to PS I instead. The main trigger for state transitions is reduction or oxidation of plastoquinone or a closely associated electron carrier. However, the signal transduction pathway that links a change in plastoquinone redox state to a change in the binding properties of phycobilisomes remains unknown [11]. On the larger scale level of the membrane the process is also not fully understood. It was found that in the cyanobacterial membrane large scale organization changes occur under state transition. It was found in *Synechocystis* 6803 and 6714 that under state 1 light the PSII complexes are arranged in row-like superstructures, whereas under state 2 light the orientation of PSII complexes was more random [9,12]. Based on this a ''spill-over model'' was proposed. It predicts that excess energy absorbed by pigments associated with PSII (either in the phycobilisome or the Chl *a* core antenna) spills over to PSI in state 2 (see [13] for an extensive discussion). The spill-over model considers that a change in the membrane organization of PSI and PSII, as observed by electron

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microscopy (EM), is important. More recently, however, it was considered that the phycobilisomes play a specific role to (re)-direct part of the excitation energy either towards PSI or PSII. It was found with FRAP measurements that the phycobilisomes diffuse quite rapidly, but that PSII is immobile on the timescale of the measurement, indicating that the linkage between phycobilisomes and the photosystems is unstable. Based on these data it was proposed that the lateral diffusion of phycobilisomes is involved in regulation of photosynthetic light-harvesting (states 1–2 transitions). Perhaps the best evidence for a connection between phycobilisome diffusion and state transitions comes from experiments with changing the osmotic strength. As the osmotic strength of the medium increases the phycobilisomes slow down and then they stop. Over the same range of osmotic strengths state transitions slow down and then they stop as well [14]. Other research claimed that light-induced state transition depends completely on a movement of phycobilisomes toward PSI or PSII [15].

The above discussed higher-order membrane organization of PSII and the cyanobacterial membrane morphology was mostly found by electron microscopy performed on freeze-fracture replicas. Such specimens have, however, a low intrinsic resolution. To understand processes like state transition in more detail, there is a strong need for higher resolution data. Single particle averaging based on negatively stained specimens can provide much higher resolution data, in the range of 2 nm or better, because this averaging technique efficiently retrieves the signal from noisy EM projections. In a previous EM study of purified PSII small numbers of double dimers were found as a contaminant of the standard single dimers [16] and provided a first 2D map. One way to improve this map could be to purify double dimers for EM analysis. The purification approach is sometimes frustrating, because many of the higher order associations of photosynthetic membrane complexes are fragile and are difficult to purify, if possible at all. As an alternative way, a state transition complex between green plant PSI and LHCII was characterized without purification [17]. The assignment was possible because single particle analysis can classify heterogeneous data sets of protein complexes. Recently this procedure was used to assign the position of the loosely bound CupA protein on the cyanobacterial membrane complex NDH-1 [18]. In this study we followed the same strategy. Thylakoid membranes from the cyanobacterium *Synechocystis* 6803 were partially solubilized with the mild detergent digitonin and EM specimens were prepared directly after solubilization.

2. Materials and methods

2.1. Cultivation of cyanobacteria and preparation of Thylakoid membranes

Synechocystis sp. PCC 6803 WT was grown in BG-11 medium under continuous light of 50 μmol photons m^{-2} s^{-1} at air level of CO_2. The thylakoid membranes of *Synechocystis* were isolated from 100 ml cell culture according to [19].

2.2. Solubilization of membranes

For preparing EM specimens, membranes from *Synechocystis* were solubilized with 2% digitonin at a 0.5 mg/ml final chlorophyll concentration. The suspension was stirred for 30 min using a small magnet stirrer, at 4 °C and followed by 15 min centrifugation at 15,000 g. The unsolubilized material was discarded and the supernatant used for electron microscopy analysis.

2.3. Electron microscopy and single particle analysis

Samples were made from solubilized membranes by dilution in buffer with detergent and subsequent negative staining using 2% uranyl acetate on glow- discharged carbon-coated copper grids. Electron microscopy was performed on a Philips CM120 equipped with a LaB_6 tip operating at 120 kV. The "GRACE" system for semi-automated specimen selection and data acquisition [20] was used to record 2048 × 2048 pixel images at 66850× calibrated magnification (0.375 nm) with a Gatan 4000 SP 4 K slow-scan CCD camera. A total of 50 PSII crystals and 500 single particle projections of PSII double dimers were collected from about 4000 images. Single particle analysis [21–23] was performed using Groningen Image Processing ("GRIP") software packages on a PC cluster. Crystal fragments and single particles were repeatedly aligned and classified and finally the best 70% of the data set was taken for the final sums.

3. Results

Partial membrane solubilization and subsequent electron microscopy on negatively stained specimens revealed the projections of many single particles and small membrane fragments. Most of the membrane fragments consisted of rectangular-shaped crystalline arrays (Fig. 1). The most regular arrays were collected and submitted for image analysis. The arrays consist of several rows of dimeric PSII complexes, most of them comprised of two rows (left five examples, Fig. 1). The upper right frame displays an array that is part of a larger piece of membrane which is partly non-crystalline. In the lower right frame the crystal is built up of five rows of PSII dimers. The average 2D map, obtained by merging small crystal fragments from 50 crystals, shows that the arrays contain equidistant spaced PSII dimers. The spacing is 12.2 nm in the vertical direction (along the rows) and 16.7 nm between the rows, as indicated by the unit cell (blue box, Fig. 2A).

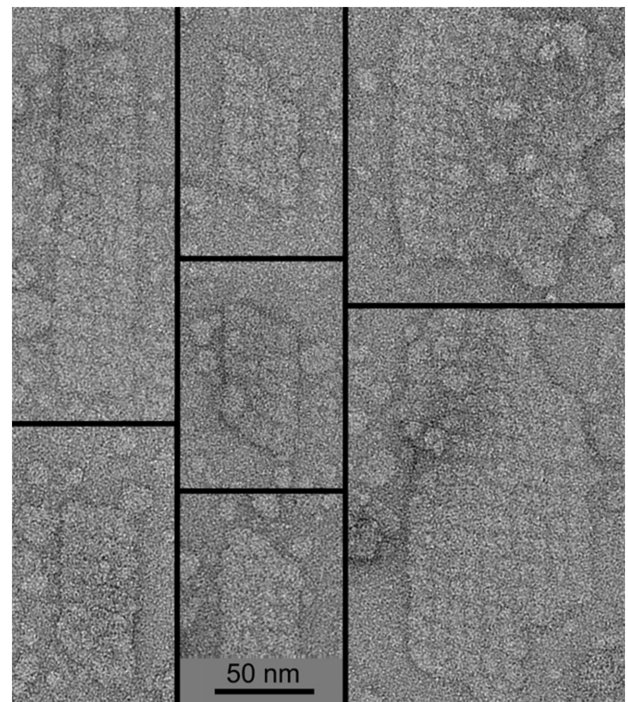

Fig. 1. A gallery of PSII crystals, negatively stained with 2% uranyl acetate.

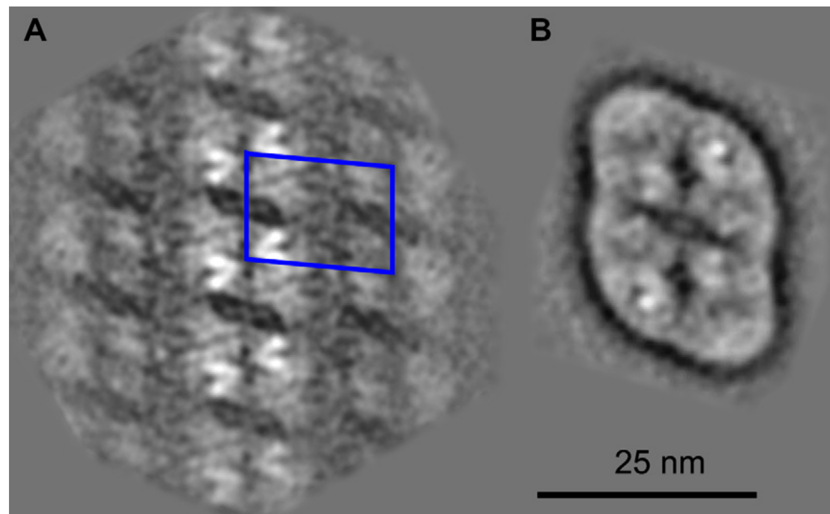

Fig. 2. Image analysis of PSII crystals and single particles. (A) 2D projection map of averaged crystalline membrane fragments (B) sum of 500 projections of double PSII dimers.

Electron microscopy of solubilized membranes shows a large spectrum of single particle complexes, some of them easily recognizable by their particular shape. Among them, the most abundant were trimeric PSI, the ATP synthase and, NDH-1 complexes [18] and dimeric PSII. Moreover, double PSII dimers were found and a final 2D map was obtained from a sum of 500 projections (Fig. 2B). The features of the double dimer map appear to match very well with those of the dimers within the 2D arrays and with single dimers, analyzed previously [16,23]. The center-to-center distance between dimers in the double dimers is the same as in the crystals. But at the periphery the dimers are larger than the dimers in the crystals. The difference is caused by the detergent shell. Integral membrane proteins are only soluble as protein-detergent micelles and the detergent shell is not penetrated by the negative stain and thus significantly contributes to the total surface in projection (see also [16] for details). In the rectangular arrays PSII dimers are tightly packed in the lipid membrane, without detergent between them.

In order to see if the double PSII dimer has the same overall features as the singular dimers, the high-resolution X-ray structure [24] was fitted in the EM 2D map of the double dimer and the crystal (Fig. 3). The match is reasonably good, if we take in account that the periphery has the detergent contribution. At the interface, however, the EM map is larger in surface, indicated by red asterices (Fig. 3). It is possible that these sites contain subunits which are lacking in the purified dimers from which the X-ray structure was solved.

4. Discussion

The process of photosynthesis cannot be understood without a detailed knowledge of the structure of its single components. Detailed knowledge about the subunits and pigments of cyanobacterial PSI and PSII has been derived from high-resolution structures obtained by X-ray diffraction studies [24,25]. For insight of the interactions of PSI and PSII detailed knowl-

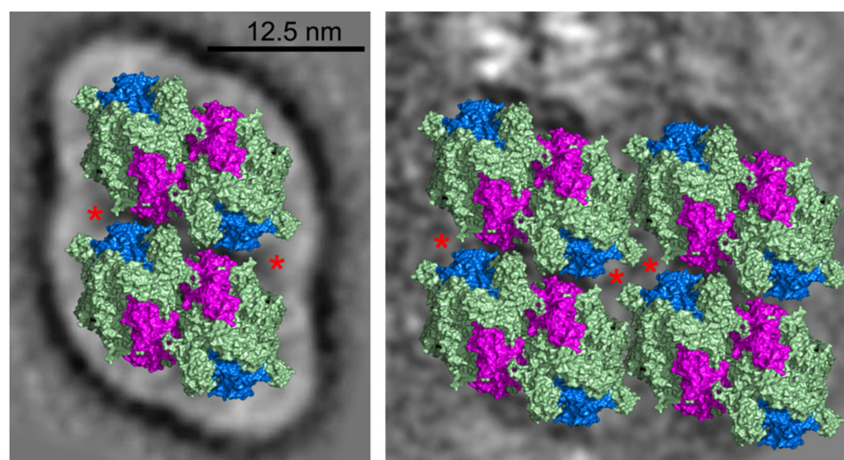

Fig. 3. Comparison of PSII double dimer map with the high-resolution X-ray structure. The high-resolution structure (PDB entry 2axt, [24]) was fitted in and is seen from the lumenal side of the membrane. Red asterices space for possible additional protein components not present in the X-ray structure.

edge of the supramolecular organization of these photosynthetic complexes within the cyanobacterial membrane is also crucial.

EM performed on partially purified *Synechocystis* 6803 membranes indicated that the only larger structures are PSII double dimers and the PSII crystalline arrays. A similar, but lower-resolution 2D map of double dimeric PSII particles was presented earlier for *Thermosynechococcus elongatus* [16], but the fact that the PSII is packed in well-ordered arrays is a novel finding. The double dimers are considered to be break-down products of rows of PSII dimers. We did not observe intact single rows, and just a few triple dimers were found. Apparently the rows break down more often in double dimers. The distance between the dimers was found to be 12.1 nm and the same spacing was found in freeze-fracture EM specimens before [7,8]. We measured a center-to-center distance between dimers in the double dimer of 12.8 nm. This distance is compatible but slightly smaller than the width of a phycobilisome, which is close to 15 nm for *Synechocystis* 6803 (A. A. Arteni, I. M. Folea, G. Ajlani, E.J. Boekema, unpublished results). This means that a 1:1 match of phycobilisomes on top of rows of PSII is possible as proposed before [7,13,26]. A good match is especially possible when the phycobilisomes make an angle with the PSII rows, as will be discussed below in our model.

The domains of multiple rows of PSII as observed in this study are unexpected, because most studies indicated only the presence of single rows of PSII, although small PSII arrays were found in the cyanobacterium *Synechocystis* BO8404 with a spacing of about 18 nm between the rows [10]. The distance between the rows is 16.7 nm whereas the length of a phycobilisome in this direction is about 6 nm. In a freeze-fracture EM study of *Synechocystis* 6803, the distance between the rows was 50 nm in the wild-type, 23 nm in a rod-less mutant and 16 nm in a PBS-less mutant [9]. The large size of the phycobilisome implies that in the PSII arrays only one third of the PSII dimer can be attached to a phycobilisome. It is not known, however, if the PSII were functionally attached to phycobilisomes because they are not present anymore. If fully present on PSII, the rows of phycobilisomes may keep the PSII rows at a distance, as in the study of [9]. It is not clear to what extend the PSII-phycobilisomes complexes will provide a sterical hindrance for PSI complexes. At their cytoplasmic sides the PsaC-E extrinsic subunits are protruding about 4–5 nm from the membrane surface [25].

The issue of the sterical hindrance is also raising the questions what the function of the PSII arrays might be and how the other membrane proteins are dispersed in the membrane. We present a model in Fig. 4 which shows the main membrane protein complexes on scale. In the model the ratio of PSI to PSII (compared as monomers) is 2:1; this is within the ratio of 3:0 found for *Synechocystis* 6803 grown under PSII light and 1.5:1 found for PSI light [27] and also close to a situation for other species such as *Synechococcus* sp. PCC 7002 cells, where a ratio of 1.86:1 was measured [28]. The arrangement of PSII in our model, where the arrays keep PSI at a distance, could reflect an arrangement close to State 1, in which it is supposed that direct energy transfer from phycobilisomes to PSI is impaired or lowered. In the arrays the PSII complexes are arranged in such a way that along the long side of the arrays the edge is formed by the small, non-pigment containing subunits such as PsbD, -F, -K and -Z. This will imply that

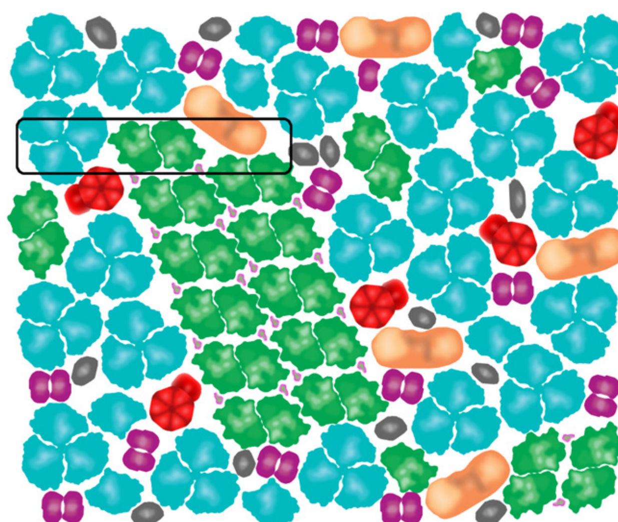

Fig. 4. A model for the organization of the photosynthetic membrane of the cyanobacterium *Synechocystis* PCC6803, densily packed with single and multi-subunit protein complexes [29]. PSII arrays consisting of several PSII rows (green) are surrounded by small numbers of randomly distributed PSII monomers and dimers (green), larger numbers of PSI complexes (trimers and some monomers; in blue), dimeric cytochrome b_6f complexes (purple), monomeric ATP synthase (red), NDH-1 complexes (ochre) and various other types of membrane proteins, such as cytochrome oxydase (grey). Small connecting masses (pink/green) between the PSII complexes are hypothesized to trigger and maintain dimer to dimer interactions. In the model the core PSI to PSII ratio is 2:1, which is within the ratio of 3:0 found for *Synechocystis* 6803 grown under PSII light and 1.5:1 found for PSII light [27] and the monomer ratio of cytochrome b_6f to PSII is 0.8:1, which is fairly constant under variable growth conditions [31]. The black rectangle, on top of a PSII dimer, indicates the size of a phycobilisome.

the possibility of excitation transfer via these sides towards a neighbouring PSI trimer or monomer will be very low or zero. This means that the PSII arrays could have the same function as in the green plants: to keep the excitation energy away from PSI. This is important because the kinetics of the trapping of excitation energy is much faster in PSI than in PSII, and a location of both antenna systems at short distances would lead to an uncontrolled flow of energy from PSII to PSI [3]. In this context it is also relevant to mention that in the experiments with *Synechocystis* 6803 on long term light adaptation [27], discussed above, it was found that PSI is the variable component in changes in the PSI to PSII stoichiometry. This could also implicate that the number and size of PSII arrays are rather fixed and that during long-term changes in the light regime only the randomly ordered PSI complexes change in numbers.

The more rapid changes of membrane reorganization under state transitions remain to be solved. Three overall structural events seem or may take place at the same moments: (1) a rearrangement of the PSII rows or arrays into a more random organization; (2) a rearrangement of the phycobilisome positions from PSII towards PSI or vise versa; and (3) smaller changes in the PSII and/or phycobilisome structure yet to be discovered. It is possible that a small membrane component, hypothesized in our model to connect the dimers in the rows and arrays, could play a role in array formation. Because of sterical hindrance of the PSI extrinsic subunits it is not likely that the phycobilisomes have unlimited freedom to diffuse on

the membrane surface, unless a kind of disconnection takes place. A refined spillover model of the cyanobacterial photosynthetic membrane was proposed [13]. It describes the redistribution of the Chl a excitation energy and the phycobilosme excitation energy. An important aspect of the model is the idea that the Chl *a* pools of CP43 and CP47 inside PSII are separated. Changes in the distribution of Chl *a*- absorbed light are accomplished by a spillover mechanism characterized by excitation energy transfer from only one component of PSII (CP47) to PSI. Energy reaching the PSII reaction center from the phycobilisomes either directly or via CP43 would have a relatively low probability of visiting CP47 pigments. This energy would thus not be lost via spillover to PSI. In other words, the refined spillover model also implies that it matters how the PSI and PSII complexes are associated.

In our model of Figs. 3 and 4 the positions of CP43 (blue) and CP47 (purple) in the PSII double dimer and arrays are highlighted. It shows that in the supramolecular structures they are in close proximity. On the other hand, CP43 is not exposed at the periphery and neighbouring PSI trimers can not become in close connection, which means that there is no possibility for energy transfer from CP43 to PSI. This means that there is a large difference in the possibility of energy transfer between single dimers and PSI the PSII arrays and PSI. This indicates that the arrays might be relevant for preventing the spillover process. There is also a second explanation for the array formation possible, which is a lowering of direct energy transfer from phycobilisomes to PSI. Phycobilisomes attached to PSII dimers will be close to several other complexes (black circumference, Fig. 4), but within an array they will have a lower change to be close to PSI complexes. This second possibility is realistic as well because there are some lines of evidence to suggest that spillover is not particularly prevalent in cyanobacteria specifically grown under physiological conditions [29].

In conclusion, we have shown in this paper that single particle averaging is able to detect the PSII superstructures without any purification step. Further work along these lines should focus on the cause of the array formation by membrane analysis of cyanobacteria lacking specific small PSII subunits. Second, it would be worth to see if the same arrays are present in other species, because cyanobacteria form a heterogeneous group of organisms with different types of overall membrane organisations [30]. Third, on the longer time scale it should be very relevant to directly establish the structure of the PSII-phycobilisome supercomplex.

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References

[1] Adir, N. (2005) Elucidation of the molecular structures of components of the phycobilisome: reconstructing a giant. Photosynth. Res. 85, 15–32.

[2] Dekker, J.P. and Boekema, E.J. (2005) Supermolecular organization of the thylakoid membrane proteins in green plants. Biochim. Biophys. Acta 1706, 12–39.

[3] Trissl, H.-W. and Wilhelm, C. (1993) Why do thylakoid membranes from higher plants form grana stacks? Trends Biochem. Sci. 18, 415–419.

[4] Horton, P. (1999) Are grana necessary for regulation of light harvesting? Aust. J. Plant Physiol. 26, 659–669.

[5] Anderson, J.M. (1986) Photoregulation of the composition, function and structure of thylakoid membranes. Ann. Rev. Plant Physiol. Plant Mol. Biol. 37, 93–136.

[6] Giddings, T.H., Wasman, C. and Staehelin, L.A. (1983) Structure of the thylakoids and envelope membranes of the cyanelles of Cyanophora paradoxa. Plant Physiol. 71, 409–419.

[7] Mörschel, E. and Schatz, G.H. (1987) Correlation of photosystem II complexes with exo-plasmatic freeze-fracture particles of thylakoids of the cyanobacterium *Synechococcus sp*. Planta 172, 145–154.

[8] Vernotte, C., Astier, C. and Olive, J. (1990) State-1-State-2 adaptation in the cyanobacteria *Synechocystis*-PCC-6714 wild-type and *Synechocystis*-PCC-6803 wild-type and phycocyanin-less mutant. Photosynth. Res. 26, 203–212.

[9] Olive, J., Ajlani, G., Astier, C., Recouvreur, M. and Vernotte, C. (1997) Ultrastructure and light adaptation of phycobilisome mutants of *Synechocystis* PCC 6803. Biochim. Biophys. Acta 1319, 275–282.

[10] Westermann, M., Ernst, A., Brass, S., Böger, P. and Wehrmeyer, W. (1994) Arch. Microbiol. 161, 222–232.

[11] Mullineaux, C.W. (2008) Phycobilisome-reaction centre interaction in cyanobacteria. Photosynth. Res. 95, 175–182.

[12] Olive, J., M'Bina, J., Vernotte, C., Astier, C. and Wollman, F.A. (1986) Randomization of the EF particles in thylakoid membranes of *Synechocystis* 6714 upon transition state from state 1 to state 2. FEBS Lett. 208, 308–312.

[13] McConnell, M.D., Koop, R., Vasil'ev, S. and Bruce, D. (2002) Regulation of the distribution of chlorophyll and phycobilin-absorbed excitation energy in cyanobacteria. A structure-based model for the light state transition. Plant Physiol. 130, 1201–1212.

[14] Joshua, S. and Mullineaux, C.W. (2004) Phycobilisome diffusion is required for light-state transitions in cyanobacteria. Plant Physiol. 135, 2112–2119.

[15] Li, H., Li, D.H., Yang, S.Z., Xie, H. and Zhao, J.Q. (2006) The state transition mechanism – simply depending on light-on and -off in Spirulina platensis. Biochim. Biophys. Acta 1757, 1512–1519.

[16] Kuhl, H., Rögner, M., van Breemen, J.F.L. and Boekema, E.J. (1999) Localization of cyanobacterial PS II donor-side subunits by electron microscopy and the supramolecular organization of PS II in the thylakoid membrane. Eur. J. Biochem. 266, 453–460.

[17] Kouřil, R., Zygadlo, A., Arteni, A.A., de Wit, C.D., Dekker, J.P., Jensen, P.E., Boekema, E.J. and Scheller, H.V. (2005) Structural characterization of a complex of photosystem I and light-harvesting complex II of *Arabidopsis thaliana*. Biochemistry 44, 10935–10940.

[18] Folea, I.M., Zhang, P., Nowaczyk, M.M., Ogawa, T., Aro, E.M. and Boekema, E.J. (2008) Single particle analysis of thylakoid proteins from *Thermosynechococcus elongatus* and *Synechocystis* 6803: Localization of the CupA subunit of NDH-1. FEBS Lett. 582, 249–254.

[19] Zhang, P., Battchikova, N., Jansen, T., Appel, J., Ogawa, T. and Aro, E.-M. (2004) Expression and functional roles of the two distinct NDH-1 complexes and the carbon acquisition complex NdhD3/NdhF3/CupA/Sll1735 in *Synechocystis* sp. PCC 6803. Plant Cell 16, 3326–3340.

[20] Oostergetel, G.T., Keegstra, W. and Brisson, A. (1998) Automation of specimen selection and data acquisition for protein electron crystallography. Ultramicroscopy 74, 47–59.

[21] Frank, J. (2002) Single-particle imaging of macromolecules by cryo-electron microscopy. Annu. Rev. Biophys. Biomol. Struct. 31, 309–319.

[22] Ludtke, S.J., Chen, D.-H., Song, J.-L., Chuang, D.T. and Chiu, W. (2004) Seeing GroEL at 6 Å resolution by single particle electron cryomicroscopy. Structure 12, 1129–1136.

[23] Arteni, A.A., Nowaczyk, M., Lax, J., Kouřil, R., Rögner, M. and Boekema, E.J. (2005) Single particle electron microscopy in combination with mass spectrometry to investigate novel complexes of membrane proteins. J. Struct. Biol. 149, 325–331.

[24] Loll, B., Kern, J., Saenger, W., Zouni, A. and Biesiadka, J. (2005) Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. Nature 438, 1040–1044.
[25] Jordan, P., Fromme, P., Witt, H.T., Kuklas, O., Saenger, W. and Krauss, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 angstrom resolution. Nature 411, 909–917.
[26] Bald, D., Kruip, J., Barber, J. and Rögner, M. (1996) Supramolecular architecture of cyanobacterial thylakoid membranes: How is the phycobilisome connected with the photosystems? Photosynth. Res. 49, 103–118.
[27] Aizawa, K., Shimizu, T., Hiyama, T., Satoh, K., Nakamura, Y. and Fujita, Y. (1992) Changes in composition of membrane proteins accompanying the regulation of PSI/PSII stoichiometry observed with Synechocystis PCC 6803. Photosynth. Res. 32, 131–138.
[28] Zhao, J., Shen, G. and Bryant, D.A. (2001) Photosystem stoichiometry and state transitions in a mutant of the cyanobacterium *Synechococcus* sp. PCC 7002 lacking phycocyanin. Biochim. Biophys. Acta 1505, 248–257.
[29] Mullineaux, C.W. (1999) The thylakoid membranes of cyanobacteria: structure, dynamics and function. Aust. J. Plant Physiol. 26, 671–677.
[30] Liberton, M., Berg, R.H., Heuser, J., Roth, R. and Pakrasi, H.B. (2006) Ultrastructure of the membrane systems in the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. Protoplasma 227, 129–138.
[31] Fujita, Y. and Marakami, A. (1997) Regulation of electron transport composition in cyanobacterial photosynthetic system: stoichiometry among photosystem I and II complexes and their light-harvesting antenna and cytochrome b_6/f complex. Plant Cell Physiol. 28, 1547–1553.