Thylakoid membranes are typical and essential features of both chloroplasts and cyanobacteria. While they are crucial for phototrophic growth of cyanobacterial cells, biogenesis of thylakoid membranes is not well understood yet. Dark-grown Synechocystis sp. PCC 6803 cells contain only rudimentary thylakoid membranes but still a relatively high amount of phycobilisomes, inactive photosystem II and active photosystem I centers. After shifting dark-grown Synechocystis sp. PCC 6803 cells into the light, “greening” of Synechocystis sp. PCC 6803 cells, i.e. thylakoid membrane formation and recovery of photosynthetic electron transport reactions, was monitored. Complete restoration of a typical thylakoid membrane system was observed within 24 hours after an initial lag phase of 6 to 8 hours. Furthermore, activation of photosystem II complexes and restoration of a functional photosynthetic electron transport chain appears to be linked to the biogenesis of organized thylakoid membrane pairs.

Thylakoid membranes are typical and essential features of both chloroplasts and cyanobacteria. The intracellular thylakoid membranes of cyanobacteria harbor the protein complexes of the photosynthetic electron transport chain (Nowaczyk et al., 2010; Bernat and Rögner, 2011). The photosynthetic electron transport chain is composed of three large membrane protein complexes, i.e. PSII, the cytochrome $b_{6}f$ complex, and PSI. Excitation energy trapping by PSII results in water splitting at the PSII donor side within the thylakoid lumen and transport of electrons to the primary and secondary electron accepting quinone molecules $Q_{A}$ and $Q_{B}$ respectively. Following double reduction and protonation, $Q_{A}$ is released from PSII into the plastoquinone (PQ) pool and delivers electrons to the cytochrome $b_{6}f$ complex. The cytochrome $b_{6}f$ complex transfers the electrons to the soluble electron carrier plastocyanin or cytochrome $c_{6}$ which subsequently reduces PSI. For efficient light harvesting, cyanobacteria contain soluble light-harvesting antenna proteins, the phycobilisomes (PBSs), which transfer light energy to the photosynthetic reaction centers. In cyanobacteria, the PSI-to-PSII ratio is controlled by light and by the redox state of the PQ/PQH$_2$-pool (Fujita et al., 1987), and under high-light growth conditions, typically less PSI is present in cyanobacterial thylakoid membranes compared with low-light growth conditions (Fujita et al., 1994).

Thylakoid membranes and photosynthetic electron transport are essential for phototrophic growth of cyanobacterial cells. Despite their importance for survival of cyanobacteria, biogenesis of thylakoid membranes is yet not well understood. It still is an ongoing debate whether the internal membrane systems (cytoplasmic and thylakoid membranes) are connected in cyanobacteria or not, and thus whether thylakoids represent a completely separated membrane entity (Liberton et al., 2006; van de Meene et al., 2006, 2012; Schneider et al., 2007). Up to now, only few proteins have been described to be involved in thylakoid membrane biogenesis. Among them the Vipp1 protein (vesicle inducing protein in plastids 1) seems to play an important role in thylakoid membrane biogenesis, as in chloroplasts of Arabidopsis (Arabidopsis thaliana) and in the cyanobacterium Synechocystis sp. PCC 6803 (hereafter referred to as Synechocystis), depletion of Vipp1 results in a reduced thylakoid membrane system (Kroll et al., 2001; Westphal et al., 2001). While the exact physiological function of the protein is not yet known (Vothknecht et al., 2012), depletion of Vipp1 in Synechocystis not only results in reduced thylakoid membrane formation, but also affects the activity and structure of components of the photosynthetic electron transport chain (Fuhrmann et al., 2009; Gao and Xu, 2009).
As complexes of the respiratory electron transport chain are also localized in cyanobacterial thylakoids, the photosynthetic and respiratory electron transport pathways are highly interconnected and both contribute to formation of an electrochemical gradient across the thylakoid membrane and energy production. Due to this, *Synechocystis* is able to grow completely heterotrophically under light-activated photoheterotrophic growth (LAHG) conditions in the presence of high Glc concentrations (Anderson and McIntosh, 1991; Smart et al., 1991).

In this study, we have used dark-grown *Synechocystis* cells to investigate “greening” of *Synechocystis* cells, i.e. thylakoid membrane formation and recovery of photosynthetic electron transport reactions. Following transfer of *Synechocystis* cells into the light, complete restoration of a typical thylakoid membrane system was observed within 24 h. While dark-grown *Synechocystis* cells contained only rudimentary thylakoid membranes, they still contained a high concentration of PBSs, active PSI as well as inactive PSII complexes. Activation of PSII complexes appears to be linked to the biogenesis of organized thylakoid membrane pairs.

**RESULTS**

**Ultrastructure of Greening *Synechocystis* Cells**

After cultivation of the cyanobacterium in the dark for more than 2 weeks, the majority of internal thylakoid membranes was lost but reassembled again after the cells had grown in the light. To directly visualize remodeling of the thylakoid membrane system, we analyzed ultra-thin sections of dark-grown *Synechocystis* cells as well as of cells that had been shifted into the light for various time periods by electron microscopy (Fig. 1). When grown in the dark (*t = 0 h*), the cells showed merely rudimentary thylakoid membranes, which mostly appeared as not-well-organized, cloudy structures within individual *Synechocystis* cells. During the first hours after light exposure, the ultrastructure of the thylakoid membrane system did not change dramatically; whereas approximately 8 h after light exposure, an increasing number of organized internal thylakoid membrane pairs became visible, and further incubation led to increasing amounts of thylakoids. After approximately 24 h, the thylakoid membrane network had recovered completely. Note-worthy, while we observed dramatic changes in the internal thylakoid membrane content, changes in the cell size or structure were not observed.

**Cellular Content and Composition of Pigment-Containing Protein Complexes Involved in Photosynthesis**

As the thylakoid membranes harbor the chlorophyll-containing photosystems, we next determined the relative cellular chlorophyll autofluorescence in greening *Synechocystis* cells (Fig. 2A). Chlorophyll fluorescence emission, mainly originating from PSII, was analyzed at room temperature on a per cell basis using a fluorescence microscope, as described in “Materials and Methods.” The observed increase in the relative chlorophyll autofluorescence during greening is completely in line with the observations described above. The chlorophyll fluorescence started to increase approximately 6 to 8 h after shifting the dark-grown cells into the light and reached a plateau after approximately 20 h, indicating that biogenesis of (mainly) PSII has reached a steady state. As these observations indicate a direct correlation between the amount of internal thylakoid membranes and the chlorophyll content per cell, we additionally determined the cellular chlorophyll content in greening *Synechocystis* cells (Fig. 2B). After an initial lag phase, the cellular chlorophyll content increased considerably about 8 h after shifting dark-grown cells into the light and reached a plateau already after approximately 16 h, once again indicating that biogenesis

![Figure 1. Formation of thylakoid membranes as visualized by electron microscopy. Electron micrographs of *Synechocystis* cells are shown using dark-grown cells grown in the light for the indicated times.](https://www.plantphysiol.org)
of chlorophyll-containing complexes has reached a steady state by that time. Absorbance spectra of whole *Synechocystis* cells were measured at various time points after shifting the cells from dark into the light (Fig. 3A). Both typical chlorophyll absorbance maxima at around 440 and 680 nm are clearly visible in all cells, as well as the PBS absorbance maximum at 625 nm. While dark-grown cells also show a typical absorbance spectrum, the amplitude of the chlorophyll absorbance peaks, and thus the content of chlorophyll-containing protein complexes, was severely reduced per cell, in line with the data shown in Figure 2. Although the PBS content was also reduced in dark-grown cells, the PBS $A_{625}$ was significantly increased relative to the chlorophyll absorbance peaks in the dark. To analyze this relative increase more quantitatively, the phycocyanin/chlorophyll ratio was calculated from the absorbance spectra shown in Figure 3A. This ratio was high and just about constant during the first 8 to 12 h in light; however, it decreased dramatically thereafter. Thus, in dark-grown cells and during the initial light adaptation period, the PBS content per cell is relatively high compared with the chlorophyll-containing photosystems (Fig. 3B).

As the relative PBS content was significantly increased in dark-grown cells, we next analyzed PBS fluorescence emission, as well as energy transfer from PBSs to the respective photosystems by 77 K fluorescence spectroscopy, upon exciting PBS at 580 nm (Fig. 4A). In dark-grown *Synechocystis* cells, an atypical fluorescence emission spectrum, dominated by a strong fluorescence emission at 685 nm, was recorded upon PBS excitation, resembling spectra observed for *Synechocystis* cells containing uncoupled PBS antenna proteins (Rakhimberdieva et al., 2007; Tamary et al., 2012). As dark-grown *Synechocystis* cells have an excess of PBSs compared with chlorophyll-containing protein complexes (Figs. 2 and 3), the strong fluorescence emission at 685 nm most likely originates from the PBS terminal emitter, which cannot transfer the excitation energy to the photosynthetic reaction centers. After shifting the cells into the light, the fluorescence emission at 685 nm gradually decreased, most likely due to an increased energy transfer from PBSs to the photosystems.

To analyze the photosystems in greening *Synechocystis* cells in more detail, we recorded low-temperature (77 K) fluorescence emission spectra also after chlorophyll excitation (excitation wavelength $l_{ex}$ = 435 nm). Upon chlorophyll excitation (Fig. 4B), all samples showed an intense, typical PSI fluorescence emission peak at 725 nm. Furthermore, three minor fluorescence peaks were detected at around 685 nm, 720 nm, and 730 nm, which correspond to the fluorescence emission peaks of the PSI, PSII, and PSIII complexes, respectively. The relative fluorescence intensities of these peaks were calculated from the fluorescence spectra shown in Figure 4B. The results are shown in Figure 5C. As the relative PBS content was significantly increased in dark-grown cells, we next analyzed PBS fluorescence emission, as well as energy transfer from PBSs to the respective photosystems by 77 K fluorescence spectroscopy, upon exciting PBS at 580 nm (Fig. 4A). In dark-grown *Synechocystis* cells, an atypical fluorescence emission spectrum, dominated by a strong fluorescence emission at 685 nm, was recorded upon PBS excitation, resembling spectra observed for *Synechocystis* cells containing uncoupled PBS antenna proteins (Rakhimberdieva et al., 2007; Tamary et al., 2012). As dark-grown *Synechocystis* cells have an excess of PBSs compared with chlorophyll-containing protein complexes (Figs. 2 and 3), the strong fluorescence emission at 685 nm most likely originates from the PBS terminal emitter, which cannot transfer the excitation energy to the photosynthetic reaction centers. After shifting the cells into the light, the fluorescence emission at 685 nm gradually decreased, most likely due to an increased energy transfer from PBSs to the photosystems.

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emission bands were pronounced at 677, 685, and 695 nm, respectively. While the 695-nm emission solely originates from PSII (Boardman et al., 1966; Murata et al., 1966), the 685-nm emission is caused by two signals having different origins: from PSII and from the PBS terminal emitter (Rakhimberdieva et al., 2007). The 677-nm emission most likely originates from partially assembled PSII (Keren et al., 2005). As can be seen in Figure 4B, the individual amplitudes of these emission maxima strongly depend on the light-growth conditions. While the 685-nm fluorescence emission maximum is pronounced in dark- as well as light-grown cells, the typical PSII fluorescence emission maximum at 695 nm did not appear to be clearly defined in dark-grown cells and only appeared within time. By contrast, the fluorescence emission peak at 677 nm disappeared gradually after shifting the cells into the light. While both the increase in the 695-nm fluorescence emission and the decrease in the 677-nm fluorescence emission indicate formation of active PSII complexes, changes in the fluorescence emission at 685 nm might result from an increasing PSII together with decreasing PBS fluorescence yields. Together, the spectra indicate activation of partially assembled PSII centers or de novo synthesis of fully active PSII complexes.

The existence and the relative amount of PSII centers in dark-adapted Synechocystis cells was analyzed by determining the relative abundance of PSI and PSII core subunits in dark- versus light-grown cells by western-blot analyses. As can be seen in Figure 5, the relative amounts of the analyzed PSI (PsaA/PsaB) and PSII proteins (PsbA, PsbB, PsbC, and PsbO) were similar in cells grown in the light or dark, respectively. Thus, while the aforementioned results clearly demonstrate a general increase of chlorophyll-containing membrane protein complexes after shifting cells from light into the dark, the PSI-to-PSII ratio does not differ dramatically in dark- versus light-grown cells, and the changes observed before (Fig. 4B) most likely reflect maturation of preexisting (inactive) PSII centers, as further analyzed and discussed below.

Activity of Photosynthetic Complexes

As the previous analyses have indicated that (at least) PSII is altered in dark-grown Synechocystis cells, we next analyzed the photosynthetic activity of intact Synechocystis cells at various time points after shifting the cells from the dark into the light by measuring oxygen evolution rates. During the first 6 h, only oxygen consumption was observed in greening Synechocystis cells, and only 6 to 8 h after light exposure, increasing oxygen production was monitored (Fig. 6). Thus, after 6 to 8 h, thylakoid membranes are sufficiently organized and active PSII centers have sufficiently

![Figure 4. Seventy-seven degrees Kelvin fluorescence emission spectra of intact Synechocystis cells at various times after shifting the cells from dark into the light upon PBS excitation at 580 nm (A) and chlorophyll excitation at 425 nm (B). The spectra were normalized at 650 nm in A and at 725 nm in B. Synechocystis cells were cultivated in the light for given times, as indicated in Figure 3B. a.u., Arbitrary units.](image)

![Figure 5. Immunoblot analyses of selected PSI and PSII subunits from Synechocystis. Cells were grown in the dark (D) or 24 h in the light (L), respectively, before total cellular extracts were separated by SDS-PAGE (0.5 μg chlorophyll per lane), transferred to nitrocellulose membranes, and immunostained with antibodies recognizing the indicated subunits, which were all raised in rabbits.](image)
developed to allow a net oxygen production. Maximal oxygen evolution was reached 20 to 24 h after transferring the cells into the light. Noteworthy, the oxygen consumption rate remained about constant (approximately 0.16–0.20 μmol [h 10^5 cells]^{-1}) in the dark and in the light. A very similar pattern, as observed in the oxygen evolution measurements, was obtained by chlorophyll fluorescence measurements after determining the PSII-mediated linear electron transport rates (ETRs) by chlorophyll fluorescence measurements (for a review, see Schreiber, 2004; Fig. 7). These measurements also indicate a virtually zero photosynthetic activity during the first 12 h of light acclimation followed by a rapid increase. Noticeably, the time shift between the oxygen evolution rates and the ETR indicates that in an early stage of light-growth, oxidation of PQH_{2} by PSI is much more efficient than reduction of PQ by PSII, resulting in an apparently zero quantum yield of PSII (compare below). This assumption is supported by the low oxygen production (Fig. 6) of dark-grown cells.

Fluorescence induction curves are widely used to assess the photosynthetic activity of oxygenic photoautotrophs from cyanobacteria to higher plants. While it essentially probes the redox state of the primary electron acceptor (Q_{A}) of PSII, it also provides information on the status of the entire electron transport chain as well as on the antenna system. As we have observed that PSII centers are present even in dark-grown cells, we next analyzed the PSII activity in more detail. In spite of an apparent lack of organized thylakoid membranes in dark-grown Synechocystis cells, we observed PSII fluorescence rise after onset of actinic light (Fig. 8, 0 h). However, compared with light-grown cultures, fluorescence induction curves of dark-grown Synechocystis cells differed in many aspects (Fig. 8, 0 h versus 24 h). At first, the “minimal” fluorescence yield, F_{o}, was significantly higher compared with light-grown cells. As the applied measuring light (λ_{ex} = 620 nm) was absorbed not only by chlorophyll molecules but also by PBSs, whose fluorescence emission could also significantly contribute to F_{o}, the high F_{o} value was most likely caused by an elevated fluorescence emission of PBSs. An increased relative PBS content in dark-grown cells and elevated PBS fluorescence emission have been observed in the UV/visible absorbance and 77 K fluorescence spectra (Figs. 3 and 4).

Strikingly, in dark-grown cells, a saturating light pulse induced a negative spike (F_{m}). As this negative spike was also observed in the presence of the PSII inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethyleurea (DCMU; data not shown), transient oxidation of the PQ pool as the main cause for fluorescence quenching could be excluded. The observations rather indicate light-induced formation of a strong fluorescence quencher, most likely formation of the oxidized PSII reaction center pigment P_{680}^{+}, upon the saturation pulse (Shinkarev and Govindjee, 1993). Switching on the actinic light after 40 s induced a slow gradual increase (timescale of minutes) of both the steady-state fluorescence (F_{s}) and the F_{m}′ fluorescence yields, which still manifested as negative spikes. This indicates an increasing PBS-to-PSII excitation energy transfer.

After transferring dark-grown cells into the light, a gradual transition toward the spectroscopic characteristics of a “classical” induction curve was observed. Antiparallel to the increasing chlorophyll concentration (Fig. 2B), F_{s} fluorescence yields (as well as the F_{i} and F_{m} values) decreased considerably and dropped to approximately 65% after 24 h. In line with the results presented above, these changes occurred rapidly after 8 to 16 h of light acclimation. While the induction curves obtained with cells grown for only 4 h in the light were still similar to the curves obtained using dark-grown cells, the F_{m} values (negative spikes) became smaller (Fig. 8), indicating reduced fluorescence quenching by P_{680}^{+} and thus activation of PSII. The negative spikes disappeared after 8 to 12 h, where F_{m} and F_{o} and F_{m}′ and F_{o}′, respectively, were approximately identical. After 12 h, both a saturating pulse in the dark and the onset of the actinic light induced a transient PQ reduction. Twenty-four hours after shifting the Synechocystis cells from dark into the light, the fluorescence induction curves did not differ any more from the curves observed with light-grown cells.

**Figure 6.** Oxygen evolution of intact *Synechocystis* cells at various times after shifting the cells from dark into the light in presence of 10 mM HCO_{3}-. The presented rates are the average of at least three measurements, and the SD is given.

**Figure 7.** PSII-mediated ETR values of intact *Synechocystis* cells derived by using fluorescence parameters recorded during rapid light curves (see “Materials and Methods”). a.u., Arbitrary units.
A plot of \( \frac{F_v}{F_o} \) values versus time is shown in Figure 9. This curve shows a slow increase (lag phase) during the first 8 h of recovery, again, a fast (exponential) increase between 8 and 20 h and a subsequent saturation phase. Together, these data indicate efficient remodeling of PSII centers after light exposure.

In contrast to PSII, which has a low activity in the dark, the overall PSI activity is virtually identical in both dark- and light-grown cells. As shown in Figure 10, A and B (black lines), the oxidation reduction pattern of \( \frac{P_{700}^+}{P_{700}} \) is very similar in dark- and light-grown cells, with a rereduction half-time of about 7 ms (Fig. 10C). Thus, PSI-mediated electron flow appears to be independent of the growth light conditions. Contrary to the kinetics, the maximal \( P_{700} \) signal intensity (\( P_{700}^+ \)), which is proportional to the PSI content of the sample, changes remarkably during light adaptation (Fig. 10D). Similar to the chlorophyll autofluorescence and cellular chlorophyll content (Fig. 2), the amounts of PSI centers per cell started to increase only 6 to 8 h after shifting the dark-grown cells into light.

As the PSII activity is largely impaired in dark-grown cells, the observation of a normal PSI activity (Fig. 10, A and B) implies a high-yield respiratory electron flow. Addition of DCMU blocks PQ reduction by PSII, and thus PQ reduction from other sources, such as respiratory complexes or cyclic electron flow around PSI, can be monitored (Bernát et al., 2009). Addition of 10 \( \mu M \) DCMU did not result in any changes of the \( P_{700}^+ \) decay kinetics in dark-grown *Synechocystis* cells (Fig. 10A) but increased the halftime of the \( P_{700}^+ \) decay about 3.5-fold in light-grown cells (Fig. 10, B and C). The apparent lack of any DCMU effect on dark-grown cells further supports the assumption that the existing PSII centers are inactive in dark-grown cells. By contrast, in light-grown cells, approximately 70% of electrons hitting \( P_{700}^+ \) originate from PSII.

### DISCUSSION

#### The Dark-Grown *Synechocystis* Cell

Several cyanobacteria are able to grow chemoheterotrophically in the dark (Rippka, 1972), and about two decades ago, the ability of *Synechocystis* cells to grow under LAHG conditions was described for the first time (Anderson and McIntosh, 1991). Heterotrophic growth is known to cause structural and functional alterations in several cyanobacterial species. Dark-grown cells of the cyanobacterium *Synechocystis* sp. PCC 6714 still contained thylakoid membranes, which, however, were less abundant and less organized than in light-grown cells (Vernotte et al., 1992). Furthermore, the chlorophyll content per cell dropped to about 10% after growing the cells in the dark for several months. By contrast, growing the cyanobacterium *Anabaena variabilis* ATCC 29413 in the dark for 1 year did not affect thylakoids or the cellular pigment content dramatically (Mannan and Pakrasi, 1993). In dark-grown *Synechocystis* cells, organized thylakoid membrane structures were essentially not observed after 2 weeks of LAHG. The chlorophyll content per cell was about 4-fold decreased in dark-grown cells, in line with earlier observations (Smart et al., 1991). However, while thylakoids were severely affected and the chlorophyll content was significantly reduced in *Synechocystis*, the apparent chlorophyll autofluorescence...
per cell was reduced only about 2-fold in dark-grown cells, compared with light-grown cultures (Fig. 2A). Noteworthy, while chlorophyll fluorescence at room temperature mostly originates from PSII in light-grown cultures, uncoupled PBSs also contribute to the observed fluorescence emission, especially in dark-grown cells (Fig. 4A). Therefore, the alleged chlorophyll fluorescence emission of dark-grown cells might be overestimated. Changes in the fluorescence emission per cell during greening are influenced by both recoupling of PBSs to PSII and increasing PSII auto-fluorescence.

All cyanobacteria, which have been analyzed after grown in the dark thus far, appear to contain photosystems within their (rudimentary) thylakoid membranes. In dark-grown *Synechocystis* sp. PCC 6714 cells, oxygen evolution was about 80% reduced, resembling the situation in *Chlorogloea fritschii* (Evans and Carr, 1975; Vernotte et al., 1992). By contrast, in *A. variabilis*, a fully functional electron transport chain was preserved in the dark, and the PSI activity even appeared to be increased by about 20% (Mannan and Pakrasi, 1993). While the 77 K fluorescence emission spectra (Fig. 4) might indicate a reduced ratio of active PSII to active PSI in dark-grown *Synechocystis* cells, the western-blot analyses (Fig. 5) suggest that the ratio of PSI and PSII core subunits is not dramatically altered in the dark. However, as mentioned above, the fluorescence measurements have to be interpreted with great caution due to the increased fluorescence emission originating from uncoupled PBSs (see above). A generally reduced content of chlorophyll-binding protein complexes per cell has been observed in dark-grown *Synechocystis* cells (Figs. 2 and 3), and after transfer into the light, the amount of both photosystems steadily increased, accompanied by activation of pre-existing PSII centers. In addition, the absorbance spectra indicate that dark-grown *Synechocystis* cells contain relatively more PBSs than light-grown cells, as the PBS/chlorophyll ratio is increased by more than 20% in the dark compared with the light (Fig. 3B). Although the cellular content of PSI centers was decreased in dark-grown *Synechocystis* cells (Fig. 10D), the remaining PSI centers were photosynthetically fully active (Fig. 10, A and B). Thus, the electron transport activity of the PSI centers is not impaired in the dark, in contrast to PSII.

Oxygen evolution measurements have indicated inactive PSII complexes in dark-grown cells, and saturating pulses induce strong fluorescence quenching in dark-adapted cells. This indicates accumulation of P700**, which, in turn, implies at least an inactive PSII donor side. The observations of state 2-to-state 1 transition in dark-adapted *Synechocystis* cells (Fig. 8) and of fast P700 rereduction after saturating pulses (Fig. 10) clearly demonstrate that the PQ pool is functionally linked to PSI in the remaining thylakoid membrane structures. However, blockage of the PSII activity by addition of DCMU did not affect the PSI rereduction kinetics, which strongly indicates that the PQ pool is not reduced by PSII in dark-grown cells, most likely due to a nonfunctional PSII donor side. Notably, assembly of the catalytically active tetramanganese cluster at the PSII donor side, the so-called photo-activation process, is light dependent (Becker et al., 2011), and the observed PSII complexes might represent a manganese-depleted, inactive PSII precursor form (Nixon et al., 2010; Nowaczyk et al., 2010).

The observation that dark-grown cells contain active PSI and PBSs in relatively high abundance might include
Thylakoid Membrane Biogenesis Is Linked to PSII Activation in Greening Synechocystis Cells

After shifting dark-grown Synechocystis cells into the light, we observed remodeling of the thylakoid membrane as well as reappearance of the photosynthetic electron transport chain. While in dark-grown cells no structured internal thylakoid membrane pairs were visible, reassembly of a structured internal thylakoid membrane system was completed after approximately 24 h of light growth. Two to 8 h after the light shift, thylakoids were still visible mainly as a cloudy, unstructured membrane system appearing within individual cyanobacterial cells, and first structured thylakoid membrane pairs became visible within 8 to 12 h. In line with the observed development of internal thylakoid membranes (Fig. 1), the chlorophyll auto-fluorescence per cell increased gradually after light exposure. This indicates that thylakoid membrane formation is closely linked to biosynthesis of chlorophyll-containing photosystems, especially PSII. In line with this, the chlorophyll content per cyanobacterial cell increased about 4-fold after shifting the cells into the light. Similarly, in Synechocystis sp. PCC 6714, thylakoid membranes were also reduced after dark growth, and it took at least 24 h to rereach a chlorophyll concentration per cell equal to light-grown cells (Vernotte et al., 1992). However, in Synechocystis sp. PCC 6714, pigment biosynthesis appeared to set in immediately after light exposure, whereas in Synechocystis, we observed an initial lag phase of about 6 to 8 h prior to a steep increase of the chlorophyll content per cell. The parallel increase in chlorophyll concentration and development of thylakoids (Figs. 1–3) indicates that in Synechocystis, biogenesis of the photosystems is tightly coupled to thylakoid membrane biogenesis. Note-worthy, PSII-less Synechocystis cells have wild-type-like thylakoid structures (Nilsson et al., 1992), and thus presence of (active) PSII appears to not influence biogenesis or maintenance of Synechocystis thylakoid membranes. In dark-grown Synechocystis cells, a fluorescence emission, having a maximum at around 677 nm, was visible, which gradually disappeared after shifting cells into the light. The exact nature of this emission peak is currently unclear. However, a similar peak has been observed in preparations enriched in inactive, only partly assembled PSII (Keren et al., 2005), and thus the 677-nm fluorescence emission most likely originates from PSII assembly intermediates. Dark-grown cells had decreased chlorophyll and PBSs contents but an increased PBS/chlorophyll ratio, which decreased after shifting cells into the light. This decrease set in about 8 to 12 h after the light shift, parallel to the development of the thylakoid membrane system and PSII activation. Nevertheless, as mentioned above, the dark-grown cells still contained PSI and PSII, although PSII was inactive, most likely due to an inactive donor side. Oxygen evolution, fluorescence induction, and P700 measurements indicate that after an initial lag phase, PSII activity set in 8 to 12 h after shifting the cells into the light.

Taken together, biogenesis of internal thylakoid membranes and remodeling of an active photosynthetic electron transport chain, especially activation of PSII, appear to be tightly coupled in greening Synechocystis cells.

CONCLUSION

While Synechocystis cells strongly reduce their internal thylakoid membrane system in the dark, rudimentary thylakoids remain preserved and a complete loss and rebuilding of thylakoids has never been described for any cyanobacteria yet. This observation might indicate that thylakoid membranes are not built de novo, and biogenesis of thylakoids requires existing thylakoid structures, which are restructured in the light. Another interesting observation was that PSI and PSII complexes are still present in the rudimentary thylakoids. Retaining fully functional PSI and inactive PSII complexes, which can serve as a scaffold for rapid biogenesis of active PSII centers, might be advantageous in dark-grown Synechocystis cells, because a functional cyclic photosynthetic electron transport chain can be generated immediately, and a linear electron transfer chain rapidly, in the light. Such an assumption is further supported by the observation that PBSs are present in significant amounts in dark-grown cells, and these PBSs might immediately be involved in efficient light harvesting after shifting Synechocystis cells into the light. Thus, although biogenesis of active PSII appears to be linked to thylakoid membrane biogenesis, dark-grown Synechocystis cells appear to be prepared to immediately adapt to light-growth conditions.

MATERIALS AND METHODS

Growth Conditions

A Glc-tolerant Synechocystis sp. PCC 6803 wt strain was cultivated photomixotrophically in liquid BG11 media (Rippka et al., 1979) supplemented with 10 mM Glc in Erlenmeyer flasks on an orbital shaker at 150 rpm at 30°C under fluorescent white light at a light intensity of 30 μmol photons m−2 s−1. For LAHG cultures, light-grown cells were diluted to an optical density at 750 nm (OD750) of about 0.2 and grown in the dark in BG11 media supplemented with 60 mM Glc in a dark cabinet on orbital shakers on an orbital shaker at 120 rpm. Cultures were illuminated with white light (10 μmol photons m−2 s−1) for 15 min per day (Anderson and McIntosh, 1991). When LAHG cultures had reached an OD670 of 1 or above, cultures were diluted to OD670 of 0.2 in fresh medium. LAHG cultures were grown in the dark for at least 2 weeks, during which, cultures were diluted at least five times in fresh medium.
Chlorophyll Concentration Determination

Cell numbers were counted by a light microscope using a counting chamber (Thoma scale). Chlorophyll concentration was determined according to Porra et al. (1989) after extraction of whole cells or membrane fractions with 100% methanol.

SDS-PAGE and Immunoblot Analysis

S. elongatus cells grown under light or LAHG conditions were harvested in the exponential growth phase (OD₆₅₀ of 0.5–2.0). Cells were resuspended in buffer (50 mM HEPES, pH 7.0, 25 mM CaCl₂, 5 mM MgCl₂, 10% (v/v) glycerol, and proteinase inhibitor mix) and broken with glass beads (0.25–0.5 mm in diameter) in a Mini Beadbeater. Glass beads and unbroken cells were removed by centrifuging twice at 1,600g. After SDS-PAGE, proteins were transferred onto polyvinylidene difluoride membranes for subsequent western-blot analysis. Individual PSI and PSII subunits were detected with primary rabbit and goat-anti-rabbit secondary antibodies (Sigma), respectively.

PsbA, respectively. A goat anti-rabbit secondary antibody (Sigma) was used in a dilution of 1:10,000. Membranes were developed using the enhanced chemiluminescence kit from Pierce.

Electron and Fluorescence Microscopy

A S. elongatus cell pellet obtained from a 10-mL cell suspension was washed three times with buffer (50 mM K₂HPO₄/Na₂HPO₄, pH 7.0), and ultrastructural investigations were performed as described previously (Sven et al., 2008).

Fluorescence microscopy was performed using a Zeiss Axioskop 20 microscope (Carl Zeiss MicroImaging), using a CCD camera (F-View, Olympus). Cells were mounted on 1% (w/v) agarose pads containing growth medium. Chlorophyll autofluorescence was excited at 480 to 550 nm, and auto-fluorescence emission was detected using d570 and e590 filters (from Chroma Technology). After 500 ms of exposure time, the relative fluorescence intensity of approximately 100 cells was determined using the MetaVue software (BioVision Technologies) and averaged. When indicated, membranes were stained for 10 min with 200 nM Mitotracker green (Invitrogen; Schneider et al., 2007).

Absorbance Spectra

UV/Vis absorbance spectra of whole cells were recorded using a Perkin-Elmer Lambda 25 spectrophotometer equipped with an integrating sphere. Cell suspensions were adjusted to a constant value of 100,000 cells mL⁻¹.

Phycocyanin and chlorophyll contents were calculated from whole-cell absorbance spectra using the molar extinction coefficients determined by Bennett and Bogorad (1973).

Low-Temperature (77 K) Fluorescence Spectra

Low-temperature (77 K) fluorescence emission spectra were recorded using an Aminco Bowman Series 2 spectrophotometer. Both monochromators were set to a slit width of 4 mm. Cell suspensions were adjusted to an OD₆₅₀ of 1.0 in BG11 medium and frozen in liquid nitrogen. Chlorophylls and PBs were excited at 435 nm and 580 nm, respectively. Fluorescence emission was recorded from 630 to 760 nm.

Oxygen Evolution

Oxygen evolution rates of intact S. elongatus cells were measured in a 1-mL chamber using a fiber-optic oxygen meter (PreSens) at a light intensity of 600 µmol photons m⁻² s⁻¹. Cells were harvested and adjusted to OD₆₅₀ of 2.0 in BG11 medium prior to the measurement. Photosynthetic rates were determined in the presence of 10 mM NaHCO₃.

Chlorophyll Fluorescence Induction Transients

Fluorescence induction curves were recorded at room temperature with a Dual-PAM-100 measuring system equipped with Dual-E and DUAL-DR modules (Walz) using the instrumental default trigger file. Fluorescence was probed by weak measuring light (0.024 µmol photons m⁻² s⁻¹), and fluorescence induction was induced by switching on red actinic light with an intensity of 95 µmol photons m⁻² s⁻¹. Six hundred-millisecond saturating pulses (10,000 µmol photons m⁻² s⁻¹) were applied once during the initial dark phase (40 s) and then at 30-s intervals to probe the maximal fluorescence yields F₅₀ and F₅₀', respectively. Minimal (F₀₅₀) and variable (F₅₀') fluorescence values (van Kooten and Snel, 1990) were obtained during the first flash.

ETRs

ETRs were derived from the steady-state (F₅₀) and maximal (F₅₀') chlorophyll fluorescence yield parameters recorded with a Dual-PAM-100 measuring system at defined light intensities according to Genty et al. (1989). Steady-state fluorescence yields were determined by rapid light curves with 30-s adaptation periods at stepwise-increased (logarithmic increment) actinic light intensities from 0 to 850 µmol photons m⁻² s⁻¹. Maximal fluorescence yields were obtained by saturating light pulses at the end of each 30-s period.

Maximal P₇₀₀ Signal Intensity and P₇₀₀ Reduction Kinetics

Maximal P₇₀₀ signal intensities (F₅₀₉) and P₇₀₀ oxidation and reduction kinetics were recorded with a Dual-PAM-100 measuring system, and averages of 10 individual traces were taken. Complete P₇₀₀ oxidation was achieved by a 20-ms saturation pulse (I = 10,000 µmol photons m⁻² s⁻¹). Traces were recorded without any addition as well as in the presence of 10 µM DCMU. P₇₀₀ decay kinetics were fitted with single exponential functions to determine decay half-times (t₁/₂).

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LITERATURE CITED


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