In photosynthetic organisms, photons are captured by light-harvesting antenna complexes, and energy is transferred to reaction centers where photochemical reactions take place. We describe here the isolation and characterization of a fully functional megacomplex composed of a phycobilisome antenna complex and photosystems I and II from the cyanobacterium Synechocystis PCC 6803. A combination of in vivo protein cross-linking, mass spectrometry, and time-resolved spectroscopy indicates that the megacomplex is organized to facilitate energy transfer but not intercomplex electron transfer, which requires diffusible intermediates and the cytochrome b$_6$f complex. The organization provides a basis for understanding how phycobilisomes transfer excitation energy to reaction centers and how the energy balance of two photosystems is achieved, allowing the organism to adapt to varying ecophysiological conditions.

Phycobilisomes Supply Excitation to Both Photosystems in a Megacomplex in Cyanobacteria

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In cyanobacteria and red algae, phycobilisomes (PBSs) (1–3) absorb light and transfer its energy to chlorophylls in photosystem II (PSII) and photosystem I (PSI), where charge separation occurs. This process of light capture by the PBS greatly expands the natural solar spectrum energy use under varying and sometimes extreme light conditions (4). Although spatial orientations of the chromophores in the PBS and chlorophylls in the reaction centers (RCs) dictate an efficient energy transfer, the exact PBS-RCs interactions are as yet unclear.

To address how the three protein complexes structurally interact, we examined chemically cross-linked PBS, PSII, and PSI by using liquid chromatography and tandem mass spectrometry (LC-MS/MS) (5–8) and analyzed the data by using two different searching methods (9, 10). Application of membrane-permeable, chemical cross-linkers to the living cells essentially captures the weak interactions between these components (5). This is made possible by the introduction of a polyhistidine tag on the C terminus of PSII subunit O (PsbO), in which, without cross-linking reactions, only PSII complexes are isolated (Fig. 1 and fig. S2).

Several observations are consistent with the formation of a larger, multicomponent complex. Key components from both PSII and PSI are present as putative protein-protein interactions (fig. S4, A and B); oxygen evolution (PSII) and oxygen consumption (PSI) activities were observed (table S2); not only PBS but PSI and PSII components (table S3) are also present, as demonstrated by LC-MS/MS; additionally, multiple cross-linking occurs between PBS-PSII and PBS-PSI (see below).

PSII isolation using affinity chromatography is routine and substantially reduces PSI contamination (fig. S5, A and B) (5). The blue-green band collected from the preparation (fig. S3A) shows characteristic fluorescence emission peaks from PBS, PSI (691 nm), and PSII (720 nm) (fig. S5). Taken together, these observations indicate that we have isolated a protein complex that contains PBS, PSII, and PSI. Considering the cumulative mass of PBS, PSII, and PSI is in the range of several megadaltons, we named this complex the PBS-PSII-PSI megacomplex (MCL).

LC-MS/MS identified all the major components from PBS, PSII, and PSI (table S3). Systematic analysis of the cross-linked MCL identified 26 protein interlinks (table S4). Notably, five interlinks were consistently found between the PSII components and ApcE (allophycocyanin E), a key component of the PBS (Fig. 2A and table S4F). [The PSII and PSI peptide sequence numbering used in this study (Synechocystis 6803) has its basis in the ARC and UB0 crystal structures, respectively (11, 12).] In PSII, Lys227 is in the loop D of PsbB (227K:PsbB) and is cross-linked to 87K:ApcE (Fig. 2A and figs. S5, S6, and S7) (5). Apce is a multidomain protein responsible for the assembly of the PBS core (13). The N-terminal portion of Apce (phycobilinprotein, or PB domain) shares high similarity to Apa (fig. S8) (4). The PB domain, however, is interrupted by a dispensable PB-loop insertion (13, 15). We also found that 23K:PsbD (or D2) is linked to 1354K:PsbD; additionally, multiple cross-linking occurs between PBS-PSII and PBS-PSI (see below).

We identified cross-links between 11K:PsaA (Psa for PSI and Psb for PSII) and 18K:Apce and between 48K:ApcD and 19K:Psad (Fig. 2E, table S4), in line with the previous observation that energy absorbed by the PBS is delivered to PSI as well as to PSII (17, 18). Our results locate Apce on the edge area of PSII through a domain formed by PsaA and PsaD (fig. 2C). Additionally, LC-MS/MS analysis showed cross-links between 17K:ApcB and 45K:Psad and between 13K:ApcB and 58K:Psad (Fig. 2, D and E; fig. S7; and table S4). These data support a docking model in which 17K:ApcB (β) is from one monomer (ApcBβ), and 58K:ApcB (β) is from another (ApcBα), instead of from one β subunit (fig. S10). These chemical cross-linking data in combination with results from protein modeling (fig. S10B) support a side-on orientation of the PBS core to PSI through a cove formed by PsaD and PsaA (fig. 2D). Our structural model predicts a close distance of about 22 Å from PBC (phycocyanobilin) to the cytoplasmic layer of chlorophylls in the PsaA (fig. S11). Although early studies demonstrated the involvement of Apce in energy transfer from PBS to PSI (19, 20), the route by which
Fig. 1. Schematic outline of the experimental workflow established for the genetic modification, isolation, and preliminary characterization of the MCL. (A) Genetic modification of PsbO protein at C terminus [PsbOH strain (5)]. (B) Luminal side of the PSII monomer, indicating the solvent-accessible PsbO C terminus with His6 tag (purple) introduction. PsbO is colored yellow; PsbU, orange; PsbV, light blue; and loop E of CP43, lime. (C) In vivo model of PBS and photosystems. NTA, nitrilotriacetic acid. (D) BN-PAGE (blue native polyacrylamide gel electrophoresis) analysis of isolated PSII (PsbOH), dimer (D), and monomer (M). (E) Ultracentrifugation isolation of MCL after affinity chromatography. (F and G) LC-MS/MS and TRF spectroscopy of the MCL.

Fig. 2. Identification of interprotein cross-links between PBS and two photosystems. (A) ApcE-PSII cross-links. The N-terminal domain of ApcE is the only phycobilin-attached region in linker proteins (Cys190). Five cross-links were found between ApcE and PSII. PsbB, sky blue; PsbC, wheat; PsbD, light gray; PsbI, orange. All lysines (227K:PsbB, 457K:PsbC, 35K:PsbI, and 23K:PsbD) are represented as spheres. (B) Docking model of ApcD to PSI trimmer (cytoplasmic view) based on the identified cross-links presented in (C). PsaA, marine; PsaB, wheat; PsaC, lime; PsaD, light pink; PsaE, yellow; PsaF, gray; PsaL, cyan; ApcD, green; ApcA, light blue; ApcB, teal. (D and E) Close-up views of the model shown in (C) and PCB (red sticks). Cross-links were found between 49K:ApcD (orange) and 11K:PsaA, 17K:ApcB and 30K:PsaA, 39K:ApcB and 49K:PsaD, and 49K:ApcD and 28K:PsaD. Lysine residues from PSI, ApcD, and ApcB are presented as red, orange, and chocolate spheres, respectively.
accompanied with fits. IRF, instrument response function. (D) Rise and decay of fluorescence at 720 nm (PSI) recorded for the MCL and the isolated PSI. (E) The MCL model of the PBS-PSII-PSI association, showing that PSII is fully covered by close association with the PBS core, whereas PSI is associated with ApcD through a side-on orientation.

the energy migrates from PBS to PSI has been elusive (21). Docking ApcD onto the core formed by PsaA and PsaD causes no apparent steric clashes on ferredoxin (fig. S12) (12).

We then applied time-resolved fluorescence (TRF) spectroscopy at 77 K to study energy transfer between structurally coupled components of the MCL (Fig. 3). Two-dimensional TRF profiles and representative TRF spectra taken at various delay times after excitation at 550 nm (Fig. 3, A and B) show three distinct emission bands with maxima of 645, 685, and 720 nm associated with fluorescence from phycocyanin (PC), PSI, and PSI, respectively, suggesting that energy transfer from PBS to both photosystems takes place. TRF of the MCL does not show a signature of APC680 (terminal energy emitter) fluorescence as in isolated PBS (fig. S13). This may not be surprising if the MCL is functionally intact. The absence of PBS fluorescence around this region and the almost instantaneous rise of the PSI chlorophyll a (Chl a) fluorescence (Fig. 3C) demonstrate efficient energy transfer from PBS to PSI, consistent with the PBS core sitting directly on top of the PSII dimer (Fig. 2A).

The rise of fluorescence associated with the PSI in MCL is not instantaneous (Fig. 3, C and D) but rather delayed compared with the others (figs. S14 to S20), suggesting that PBS-PSII and PBS-PSI energy transfer rates are different. Emission at ~720 nm originates from excitation traps—clusters of Chl a pigments with the strongest excitonic interactions located in the PsaA and PsaB PSI subunits (22). These traps effectively serve as excitation donors to the P700 dimer at physiological temperatures, demonstrating multidecay character at 77 K (23, 24) (figs. S18 and S19). If energy transfer from the PBS to PSI is slow, the rise of fluorescence at 720 nm should be delayed in the MCL with respect to the isolated PSI, as is demonstrated by the data in Fig. 3D. The delayed rise of the PSI fluorescence may be associated with decreased PBS-to-PSI excitation energy transfer owing either to side-on orientation of PBS and PSI or to a chemical cross-linking modification effect. We do not exclude the possibility of energy spillover from PSII (25). (Fig. 3E). However, undetectable long-lived PSI fluorescence may indicate that the delayed rise of PSI fluorescence is associated with slow excitation-energy transfer between the PBS and PSI, as indicated by the structural interactions between PsaA and ApcD (terminal energy emitter) (Fig. 2C).

Previous studies have suggested that physical interactions between the PBS core and PSII and I are essential for efficient excitation energy migration from low-energy allophycocyanin (APC) in the PBS core to Chl a in RCs (26, 27). However, the enigmatic “supercomplex” comprising PBS-RCs has never been consistently detected and isolated (21), owing to weak and easily disrupted interactions between these complexes. Our study demonstrates that if cells are gently cross-linked in vivo, the weak interactions can be captured and identified. The isolated complex contains PSI but not the cytochrome b6f complex, which connects PSI and PSII by mobile-electron carriers. Thus, the MCL is best considered as an energy-transfer complex that directs excitations to one or the other photosystem (Fig. 3E) and not as a complex that includes the complete electron-transport chain.

The isolation of the MCL allows us to witness the merger of three intensely studied photosynthetic events of light harvesting and two light-driven photochemistry reactions in one module. There are still many unresolved issues, including the molecular mechanism that governs the assembly of the MCL and the means by which the excitation energy is delivered to the two photosystems by the PBS. For instance, the orange carotenoid protein is directly involved in the fluorescence quenching of PBS and possibly in the regulation of energy transfer between PBS and the photosystems (28).

References and Notes
5. Materials and methods are available as supplementary materials on Science Online.
Long-Distance Integration of Nuclear ERK Signaling Triggered by Activation of a Few Dendritic Spines

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The late phase of long-term potentiation (LTP) at glutamatergic synapses, which is thought to underlie long-lasting memory, requires gene transcription in the nucleus. However, the mechanism by which signaling initiated at synapses is transmitted to the nucleus to induce transcription has remained elusive. Here, we found that induction of LTP in only three to seven dendritic spines in rat CA1 pyramidal neurons was sufficient to activate extracellular signal–regulated kinase (ERK) in the nucleus and regulate downstream transcription factors. Signaling from individual spines was integrated over a wide range of time (>30 minutes) and space (>80 micrometers). Spatially dispersed inputs over multiple branches activated nuclear ERK much more efficiently than clustered inputs over one branch. Thus, biochemical signals from individual dendritic spines exert profound effects on nuclear signaling.

Activity-dependent gene transcription is essential for the maintenance of long-term potentiation (LTP) and memory consolidation (1, 2). Induction of LTP in single dendritic spines activates signaling that can either be restricted to the stimulated spine or spread into the parent dendrite over 5 to 10 μm (3–5). However, it is not known whether signaling initiated at single dendritic spines can be transmitted into the nucleus to regulate gene transcription. Extracellular signal–regulated kinase (ERK) is important, both for signaling within the stimulated spine and adjacent dendrites (3, 6, 7) and for activating transcription factors in the nucleus during LTP (2, 8–11). Thus, ERK signaling may play an important role in relaying signals from the stimulated spines to the nucleus.

To monitor the activity of ERK in the nucleus, we ballistically transfected cultured organotypic hippocampal slices of rats with nuclear-targeted ERK activity reporter (EKARnuc) (12) and imaged CA1 pyramidal neurons with two-photon fluorescence lifetime imaging microscopy (2pFLIM). The expression of EKARnuc was highly localized to the nucleus (12). Using the weak ERK expression in the cytosol, we employed fluorescence intensity measurements to monitor structural plasticity of dendritic spines on secondary and tertiary apical dendritic branches (Fig. 1).

When a single spine was stimulated with a low-frequency train (1 Hz, 60 s) (Fig. 1A) of two-photon glutamate uncaging pulses in the absence of Mg2+, the spine volume increased rapidly by 275 ± 18% in 1 to 2 min (transient phase), which decayed to a sustained level 61 ± 4% larger than the original volume that lasted more than 1 hour (sustained phase) (Fig. 1, C and E), as expected (3–5, 13). The volume increase was similar to that induced in neurons expressing monomeric enhanced green fluorescence protein (mEGFP) (Fig. 1E). This structural LTP (sLTP) of spines is known to be associated with electrophysiological LTP (3–5, 13). Repeating this protocol in different spines one by one (at ~60-s intervals) (Fig. 1A), we induced sLTP sequentially in seven spines on three to five different dendritic branches (Fig. 1, B and C). After the seven-spine stimulation, we observed a slow and sustained elevation of ERK activity in the nucleus, as indicated by a gradual (over ~30 min) shortening of the fluorescence lifetime of EKARnuc (~0.02 ns) that was maintained for at least the following 40 min (Fig. 1, D and F). Pharmacological inhibition of ERK with ERK inhibitor FR180204 (50 μM) completely prevented the fluorescence lifetime decrease respectively (Fig. 1, F and G). Inhibition of the classical upstream molecules Ras and mitogen-activated protein kinase kinase (MEK) (14) with dominant-negative Ras (dnRas) expression and MEK inhibitor U0126 prevented the fluorescence lifetime decrease, respectively (Fig. 1J and fig. S5). Thus, the change in fluorescence lifetime of EKARnuc acted as a reliable reporter of ERK activation in the nucleus.

We further confirmed that our seven-spine stimulation protocol activates nuclear ERK by two methods independent of 2pFLIM imaging of EKARnuc. First, we performed immunostaining of phosphorylated ERK in CA1 neurons expressing mEGFP. Consistent with the EKARnuc results, the level of phosphorylated ERK in the nucleus was persistently elevated after seven-spine stimulation (15) (Fig. 1H and fig. S1). Second, we performed live imaging of mEGFP-tagged ERK2 in organotypic slices. Under basal conditions, mEGFP-ERK2 was localized predominantly to the cytoplasm but slowly translocated into the nucleus after seven-spine stimulation (Fig. 1I and fig. S2) (8, 15). Thus, nuclear ERK is activated by sequential activation of a few spines.

Next, we determined the source of intracellular Ca2+ elevation that leads to nuclear ERK activation. Uncaging-induced Ca2+ elevation was largely restricted to the stimulated spines, and spreading along the dendrite was limited to 2 to 3 μm (figs. S3 and S4) (15, 16). This local Ca2+ elevation was dependent mainly on N-methyl-D-aspartate-type glutamate receptors (NMDARs); there was negligible contribution from voltage-sensitive Ca2+ channels (VSCCs), metabotropic glutamate receptor (mGluR)–mediated internal Ca2+ release, or Ca2+-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptors (sGluCs). Inhibition of NMDARs with 2-amino-5-phosphonopentanoic acid (APV) (50 μM) completely prevented nuclear ERK activation (Fig. 1J and fig. S5A), as well as sLTP (Fig. 1, K and L) (13). In contrast, blockade of VSCCs with CdCl2 (200 μM) did not prevent nuclear ERK activation.

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