Marine cyanobacteria tune energy transfer efficiency in their light-harvesting antennae by modifying pigment coupling

Yuval Kolodny, Hagit Zer, Mor Propper, Shira Yochelis, Yossi Paltiel, and Nir Keren

Keywords
energy transfer; photosynthesis; phycobilisome; quantum biology

Correspondence
Y. Paltiel, Applied Physics Department, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
Tel: +972 54 8820919
E-mail: nir.ke@mail.huji.ac.il
and
N. Keren, Department of Plant and Environmental Sciences, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
Tel: +972 50 7215184
E-mail: paltiel@mail.huji.ac.il

(Received 23 January 2020, revised 24 April 2020, accepted 15 May 2020)
doi:10.1111/febs.15371

Introduction

Photosynthetic light harvesting is the first step in harnessing sunlight toward biological productivity. To operate efficiently under a broad and dynamic range of environmental conditions, organisms must tune the harvesting process according to the available irradiance. The marine cyanobacteria *Synechococcus* WH8102 species is well-adapted to vertical mixing of the water column. By studying its responses to different light regimes, we identify a new photo-acclimation strategy. Under low light, the phycobilisome (PBS) is bigger, with extended rods, increasing the absorption cross-section. In contrast to what was reported in vascular plants and predicted by Forster resonance energy transfer (FRET) calculations, these longer rods transfer energy faster than in the phycobilisomes of cells acclimated to a higher light intensity. Comparison of cultures grown under different blue light intensities, using fluorescence lifetime and emission spectra dependence on temperature at the range of 4–200 K *in vivo*, indicates that the improved transfer arises from enhanced energetic coupling between the antenna rods’ pigments. We suggest two physical models according to which the enhanced coupling strength results either from additional coupled pathways formed by rearranging rod packing or from the coupling becoming non-classical. In both cases, the energy transfer would be more efficient than standard one-dimensional FRET process. These findings suggest that coupling control can be a major factor in photosynthetic antenna acclimation to different light conditions.

Abbreviations

APC, allophycocyanin; EET, exciton energy transfer; FRET, Forster resonance energy transfer; PBS, phycobilisome; PC, phycocyanin; PCB, phycocyanobilin; PE, phycoerythrin; PEB, phycoerythrobilin; PSI, photosystem I; PSII, photosystem II; PUB, phycourobilin; TCSPC, time-correlated single-photon counting.
[3,4] have raised much interest in the possible role delocalized exciton states may play in the excitation energy transfer (EET) of light-harvesting processes [5,6]. Most photosynthetic complexes exhibit strong inter-pigment coupling, where Redfield theory has been used with great success to explain EET. In the large and complex antennae systems of cyanobacteria and red algae, phycobilisomes (PBS), exciton-exciton coupling is thought to be weaker than exciton-phonon coupling. In this weak-coupling regime, semi-classical Forster theory is applicable and usually used to describe the energy transfer dynamics [6–8]. Nevertheless, it has been postulated that in PBS, small conformational changes may enhance the coupling strength and drive the system into a stronger coupling regime [9,10].

It is well established that the sizes of photosynthetic units can reach hundreds of pigments. Recent structural studies resolved an intact phycobilisome (PBS) antenna with thousands of pigments [11,12]. Elucidating energy transfer dynamics in such large-scale systems will benefit from studies of intact systems, within their biological context. However, the study of ultrafast processes by advanced spectroscopy is usually done on isolated pigment–protein complexes [3,13,14]. The benefits of working with small numbers of pigments are clear, but the biological relevance of such experiments is limited by their small scale. In vivo measurements present more complex scenarios that cannot be fully captured in simplified isolates. As phrased by Aristotle, ‘which have several parts and in which the totality is not, as it were, a mere heap, but the whole is something beside the parts’.1 An example for this approach is the study of desiccation-tolerant desert crust cyanobacteria [15]. That study demonstrated the link between small changes in PBS structure and large quenching effects in the PBS antenna directly, under a desiccation/rehydration scenario where water availability triggers quick energy transfer tuning. Since in desert environments light intensities are usually high, it is interesting also to examine organisms that are adapted to life where light is a limiting factor.

In this study, we looked for an organism which is adapted to an environment where (a) light can be limiting to the extreme, and (b) the intensity and quality of light varies, requiring acclimation of photosynthetic systems to a broad range of light regimes. Marine Synechococcus spp. are obvious candidates for such a study. The marine environment provides a matrix of light and environmental conditions that are even more complex than those of a terrestrial desert. In the marine environment, where as much as 50% of the world’s primary productivity takes place [16], light is the major factor limiting the abundance of photosynthetic organisms. Light intensity attenuates exponentially as it penetrates deeper into the water column and its spectrum is narrowed to blue (~490 nm) [16,17].

An important perturbation of the open oceans water column is vertical mixing, which greatly affects the dynamics of Synechococcus populations [18]. Mixing of a stratified water column, typically to a depth of a few hundred meters, homogeneously distributes the nutrients and the planktonic organisms along the water column. The only resource that cannot be ‘mixed’ is sunlight. As a result, an organism that lives near the surface with plenty of available light may rather abruptly find itself with very little light if it reaches the deeper layers [19]. The response to varying light conditions (photon flux density and spectral distribution) triggers a cascade of photo-acclimation responses affecting the form and function of light-harvesting systems [20–22].

Synechococcus are small unicellular coccolid cyanobacteria responsible for an estimated 25% of global primary productivity. They use phycobilisomes (PBSs) as their light-harvesting antenna. Among them, Synechococcus WH8102 represents an abundant clade that is well adapted to mixing regime and can be found throughout the water column [18]. Studies of marine Synechococcus, including WH8102, demonstrated their ability to acclimate to the wide range of light intensities experienced along the water column [23].

Synechococcus WH8102 PBS consists of phycobiliprotein (PBP) αβ dimers organized into trimer rings which, in turn, couple to create the hexamers which form the basic subunit of the core and stacked rods [24]. The core is made of allophycocyanin (APC) and the rods of phycocyanin (PC) and phycoerythrin (PE). These PBPs consist of an apo-protein and contain 1–3 open-chain tetrapyrrole bilin chromophores. APC and PC both bind phycocyanobilin (PCB). The different protein environments generate a bathochromic shift in APC absorption [25], 620 nm in PC and 650 nm in APC. The rod extremities are constituted by two structurally different forms of PE: PEI and PEII. PEI binds either only phycoerythrobilin (PEB, absorption peak 545 nm), or PEB and phycourorbilin (PUB, absorption peak 495 nm). PEII binds both PEB and PUB, but with fewer PEB than does PEI [23]. Synechococcus demonstrates chromatic adaptation, able to modify PEB into PUB and reversibly according to the available light spectrum [26–28].

---

1Aristotle, Metaphysics, book VIII part 6 (350 B.C.E).
Importantly, *Synechococcus* WH8102 has the highest reported content of phycourobilin, the pigment which best absorbs the blue light that penetrates ocean waters [29,30]. The fact that it possesses particularly large PBSs challenges energy transfer processes, since energy transfer occurs over longer distances. According to one-dimensional classical mechanics approach, energy transfer efficiency is expected to decrease with an increase in the number of pigments and rod length. The optimal length was calculated to be very short, on the order of a few trimers [31]. For longer rods, therefore one would expect stronger coupling that may result from different coupling mechanics.

Photo-physiological studies conducted on the response of marine *Synechococcus* species to light intensity gradients [23] demonstrated a large dynamic range of photosynthetic activity. In this study, we take advantage of these photo-acclimation processes, to ask how photosynthetic units tune their energy transfer rates. Using *Synechococcus* WH8102, we generated a testing ground for studying ultrafast energy transfer processes in large antenna structures with complex pigment compositions *in vivo*. Our analysis includes physiological, biochemical, and spectroscopic measurements of energy transfer dynamics, conducted while comparing acclimated states of this marine cyanobacterium to different light regimes.

**Results**

In order to compare the function of photosynthetic units within cells acclimated to light conditions representing shallower and deeper water column depths, we grew *Synechococcus* WH8102 cultures under blue LED light illumination, which resembles the light spectrum that penetrates the ocean (Fig. 1A). We compared cultures grown at different blue light intensities: 10 and 150 µmol photons m\(^{-2}\)s\(^{-1}\) denoted ‘low light’ and ‘medium light’, respectively. It is important to note that both intensities are low with respect to this species, to avoid photoinhibitory processes. Six *et al.* [23] determined maximal growth rate for this species at 207 µmol photons m\(^{-2}\)s\(^{-1}\) under continuous white light and determined it remained quite high up to 650 µmol photons m\(^{-2}\)s\(^{-1}\). Measures of the photosynthetic functionality under a range of actinic light intensities [light curves obtained by oxygen evolution and pulse amplitude modulation (PAM) fluorometry, see below] indicate that photoinhibition takes place in both cultures only at higher intensities than the growth conditions. Therefore, non-photochemical quenching is not expected under our experimental conditions [32].

**Growth and primary productivity parameters**

*Synechococcus* WH8102 underwent photo-acclimation when grown under the two different blue light intensities. They exhibited extensive morphological and physiological modifications, linked to changes in their photosynthetic units. Immediately noticeable to the naked eye is a change in the cultures’ color (Fig. 2A). The acclimation process is evident within 2 days, as seen by several physiological parameters (Figs 1B,C and 2B) and is completed in about 7–10 days. *Synechococcus* WH8102 was able to grow under both low and medium light intensities. Cell numbers increased at a slower rate under low light.

The photosynthetic traits of the cultures were evaluated by several methods. Oxygen evolution per cell is shown in Fig. 2D. The maximal rate of the low light-grown cells is reached at illumination of about 120 µmol photons m\(^{-2}\)s\(^{-1}\). For the medium light-grown cells, values are approaching saturation at approximately 400 µmol photons m\(^{-2}\)s\(^{-1}\), with a small and steady increase beyond that. For both, this maximum light intensities for photosynthesis are higher than the light intensities in which the cultures were grown, quantitatively justifying the ‘low light’ and ‘medium light’ notations, as both are lower than optimum and do not induce light stress. The maximal photosynthetic rate, P\(_{\text{max}}\), per cell, is three times higher in low light compared to medium light. When comparing on a chlorophyll basis (Fig. 2E), we observe that up to ~400 µmol photons m\(^{-2}\)s\(^{-1}\), where the low light-grown cells experience a reduction in photosynthetic activity (probably photoinhibition), the photosynthetic rate, per chlorophyll, is higher in low light-grown cells. Similar results are presented in Fig. 2C from light response curves obtained by PAM fluorometry, showing effective PSII quantum yield (Y\(_{\text{II}}\)) as a function of actinic light intensity. In addition, maximal apparent photosynthetic yield values, measured as F\(_{\text{v}}\)/F\(_{\text{m}}\) (Table 1), show that the low light culture exhibits significantly higher values (more than twice).

All of the measured photosynthetic parameters point to the same conclusion that the low light-acclimated photosynthetic apparatus is more efficient under low light intensities. In the following sections, we will try to elucidate what structural changes lead to the increased performance at low light intensities, and compare in more detail the EET in both systems.

**Cellular morphology**

The side scatter area parameter (SSC-A) of fluorescence-activated cell sorting (FACS) measurement
raised the possibility that cell sizes were larger when grown under lower light. This prompted us to examine cellular morphology more closely. Confocal fluorescence microscopy and transmission electron microscopy (TEM) confirmed that cells change their shape, size, and internal structure in response to the different blue light intensities. Under low light, the median cell size increased, and the size distribution was broader, as seen from the SSC-A statistics (Fig. 3A) and the quantification of confocal images (Fig. 3B). In addition, TEM revealed that the cells changed their structure from round coccids under medium light to elongated under low light (Fig. 3D). The elongated shape of the cells under low light, along with the larger average size, is consistent with their slower growth rate. Lastly, under medium light cells typically had one thylakoid membrane while under low light three to four membranes were observed, organized in uniformly distributed distances. Photosynthetic units are embedded in the thylakoid membranes, so an increase in the number of membranes indicates an increase in the number of photosynthetic complexes per cell. These results are similar to photo-acclimation changes observed in other marine cyanobacterial species [33].

The increased cellular thylakoid area has the advantage of a higher light absorption cross-section, which is beneficial when light is limiting, provided that the
energy transfer efficiency remains high. It is important to note that an increase in cell size has the disadvantage of a reduced surface area per volume ratio, which may limit the assimilation of nutrients [34]. The fact that nutrients are typically more abundant deeper within the ocean water column mitigates this risk. Under our experimental conditions, however, nutrients were abundant under both conditions.

**Photosynthetic complexes**

As implied by the higher number of thylakoid membranes, under low light the number of photosynthetic units is higher. This is supported by the fact that chlorophyll \( a \) absorption, a component of both photosystems, was increased 8-fold at low light as compared to medium light on a per-cell basis (Fig. 1D). Similarly, on a protein basis, chlorophyll content increased by 7.4-fold (Table 1). The ratio of PSI to PSII was slightly increased under low light, as demonstrated by protein immunoblotting (Fig. 1E).

A prominent change is evident in the composition of the photosynthetic antennae, the PBS. The differences, seen in Fig. 1D and summarized in Table 1, indicate that the PBS composition changes under different blue light intensities are similar to those reported by Six et al. [23,35] for the same species under different white light illumination intensities. In their detailed study, they found that the size of the PBS complexes increased at lower light, in consistence with the higher
intermembrane distance we observed in the TEM images (Fig. 3D). They reported on the following changes in pigment content which are also in agreement with our data: The PE content is higher under lower light; the PUB : PEB ratio increases at low light, leading to the conclusion that PEII : PEI ratio increases (since PEII has higher PUB content); they clearly showed that most of the variation arises from the increase in PEII at low light, while PEI and PC content do not change much. The absence of PC rod linker (LR) genes such as cpcC or cpcD in Synechococcus WH8102 implies that under all light conditions there is only one hexameric PC disk per PBS rod [35,36]. Taking all those finding into account, they conclude that PBS rods under lower light are longer, probably containing more PEII disks. They suggested a model of a PBS rod in which each rod is composed of six disks at low light, namely three PEII disks, two PEI disks, and one PC disk.

The increase in PBS size under low light could be explained by a few structural models. While the data presented here and in the work of Six et al. support longer rod structures, there is still an open question on the spatial organization of these rods into a PBS structure. In Fig. 4, we present two possible models, in one the increase in PBS size arises only from the extension of the rods, and in the other it also arises from a larger number of rods in each PBS. The functional implications of these structures will be discussed in the conclusions section.

**Table 1.** Differences in photosynthetic units’ composition and function. Phycobiliprotein ratios were extracted from absorption spectra. Maximal PSII quantum efficiency, \( F_{v}/F_{m} \), was measured in dark-adapted cultures using Imaging-PAM. Similar results were obtained using kinetic PAM-2500 and JTS-10. \( F_{m} \) values were recorded in the presence of the PSII electron transfer inhibitor DCMU. Protein concentrations determined using the Bradford assay. Next to each mean value appears SD, and the number of independent repetitions from which it was obtained.

|                      | Low light | Medium light | LL/ML ratio |
|----------------------|-----------|--------------|-------------|
| Chl/protein (\( \mu g \) chl/(mg protein))\(^{-1} \) | 274       | 37           | 7.4         |
| PUB/PC               | 11.7 ± 1.3 | 4.6 ± 1.3    | 2.6         |
|                      | (n = 4)   | (n = 4)      |             |
| PEB/PC               | 6.1 ± 0.9 | 2.8 ± 0.8    | 2.2         |
|                      | (n = 4)   | (n = 4)      |             |
| PUB/PEB              | 1.93 ± 0.09 | 1.66 ± 0.05 | 1.16        |
|                      | (n = 4)   | (n = 4)      |             |
| \( F_{v}/F_{m} \)   | 0.36 ± 0.1 | 0.17 ± 0.05  | 2.1         |
|                      | (n = 6)   | (n = 6)      |             |

**Fig. 3.** Cellular morphology. (A) Histogram of side scatter area (SSC-A) measured by FACS. Cultures grown under low light exhibited larger scattering values. Their mean size is about twice the size of cells grown under medium light and their scattering distribution is broader. These results indicate larger cell sizes. (B) Quantification of cell sizes from fluorescence confocal microscopy images. The box plot central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles. The whiskers extend to the most extreme data points not considered outliers, shown as red pluses. (C) Fluorescence confocal imaging of low light (L15) and medium light (L150) grown cells. (D) Transmission electron microscopy of acclimated cells. Typically, the low light-grown cells are elongated and contain about three thylakoid membranes, while the medium light-grown cells are round, with one thylakoid membrane (TM) circling the cell. The presented images are representative of over 200 cells recorded from each treatment.
These structures raise an interesting problem. While bigger rods containing additional PUB have an increased absorption cross-section, especially in the light region that best penetrates the deeper water, they generate a longer pathway for energy transfer to the reaction centers. Theoretical models using FRET demonstrated that an increase in rod length is expected to result in a lower energy transfer efficiency [31]. However, we found the functionality of each photosynthetic unit is improved under low light, as shown earlier in the primary productivity parameters section. To further probe the issue of energy transfer efficiency, we examined fluorescence spectra and lifetime characteristics.

**Energy transfer properties**

Steady-state fluorescence spectra of *Synechococcus* WH8102, at room temperature, allow us to compare the energy flow through the photosynthetic apparatus. Figure 5A shows the emission spectra of cultures treated with the PSII electron transfer inhibitor DCMU. It allows us to eliminate photochemistry and concentrate on exciton energy transfer to the reaction center. Excitation was set at 497 nm, the absorption peak of the PUB at the extremity of the rod. The spectra were normalized to the emission at 652 nm, the peak of the PC (since PC content per rod is constant, only one disk per rod). Interestingly, the emission in the spectral range of PC, APC, and PSII is identical for both cultures (650–700 nm). This implies that the cells have maintained energy transfer homeostasis by regulating energy flow, so that the same flux that passes through the PC disks reaches the reaction center under both low light and medium light.

The emission peak of the PE was shifted (565 nm at low light, 572 nm at medium light), consistent with the higher PUB : PEB ratio under low light (Fig. 1D and Table 1). Moreover, PE emission was more than twice stronger in low light. An increase is expected considering the higher PE content. The major increase may further imply that multiple PE units on the same rod cannot move energy simultaneously through the single PC disk.
To further investigate the rate of energy transfer following excitation of PUB, we performed measurements of the excited state’s lifetime. Time-correlated single-photon counting approach (TCSPC) was used to measure the decay lifetime of the different components of the photosynthetic unit. Excitation was set at 485 nm targeting the extremities of the PBS rod, and emission was collected in three spectral windows, covering the phycobilins (PBS window, 500–675 nm), the APC terminal emitter and photosystem chlorophylls (PS window, 675–750 nm), and the overall system’s lifetime (Wide window, 500–750 nm). The measured lifetime, \( \tau \), is the typical lifetime of the system’s excited state and is composed of different pathways which can be related to three major components: radiative \( (1/R) \), heat dissipation \( (1/k) \), and energy transfer \( (1/ET) \). Their combined effective value is formulated as a harmonic average:

\[
\frac{1}{\tau} = \frac{1}{\tau_R} + \frac{1}{\tau_k} + \frac{1}{\tau_{ET}}.
\]

The PBS average lifetime at room temperature was shorter for low light than for medium light-grown cells (102 ps compared to 150 ps; Fig. 5B). The fact that the lifetime is shortened under lower light is surprising. The opposite phenomenon was reported in plants, where acclimation to lower light triggered an increase in lifetime [37]. The shorter lifetime indicates that in each photosynthetic unit, the absorbed energy decays faster. This could either be due to higher dissipation of the energy, or be due to faster energy transfer rate. Non-photochemical quenching (NPQ) is not expected under our experimental conditions, but even if it existed it should have been stronger in medium light-grown cells, leading to shorter lifetime in this culture, while we see the opposite trend. The excitation

![Steady-state fluorescence spectra](image1)

![Fluorescence decay at different temperatures](image2)

![Fluorescence decay in PBS and Chlorophyll](image3)

![Fluorescence decay in isolated PBS](image4)

Fig. 5. Energy transfer in photosynthetic apparatus. (A) In vivo emission spectra (excitation 497 nm). Emission was normalized to the peak of phycocyanin (652 nm). Samples treated with 5 \( \mu \)M DCMU, to inhibit PSII photochemistry. PSI fluorescence is not observed at room temperature. (B) Time-resolved fluorescence spectroscopy in vivo (excitation at 485 nm, absorbed mainly by PE). Emission was collected in spectral windows of 500–675 nm (PBS window) or 675–750 nm (mainly chlorophyll, PS window). Insert shows the rise time of the chlorophyll peak. In both low and medium light, a 90 ps delay between the PS window and the PBS windows was observed. (C) Time-resolved fluorescence of isolated PBS fractions of PBS (in vitro). Emission integrated over both windows (500–750 nm). Insert shows the fluorescence spectra of the isolated PBS fractions when excited at 497 nm. The peaks are ascribed to (left to right) PE, PC and APC terminal emitter. The shorter low light lifetime was observed in three independent PBS isolation procedures. Panels A–D present representative curves of measurements repeated in four independent experiments with comparable results. Data analysis of the TCSPC curves appear in supporting information.
intensity is low in both cases; therefore, multi-exciton processes are not relevant. We hypothesized that higher energy transfer rates within the PBS (τEET) are responsible for the shorter lifetime.

The 90 ps shift between the peak of the PBS window trace and the photosystem (PS) window trace (Fig. 5B insert) gives a rough estimation of the transfer rate between the phycobilisome and the reaction center. The fact that this timing is similar for both samples although the number of pigments per system is different supports the hypothesis that PBS energy transfer is accelerated under low light.

The average lifetime in the PS window was 267 ps under medium light compared to 336 ps under low light. Examining the components of these decay curves indicates that the contribution of the fast decay component in the medium light is larger. The fast component (~ 150 ps) is probably dominated by the direct excitation of photosystem chlorophylls. While chlorophyll a absorption peak is around 430 nm, they do not absorb, with lower efficiency, also at 497 nm (Fig. 1D). Under medium light where chlorophyll absorption is not as masked by PBS absorption as in low light, the proportion of direct chlorophyll excitation will be higher.

A similar PC/PSII steady-state emission ratio is measured for both samples, as seen in Fig. 5A. However, a prominent difference is evident in the PE/PC ratio, the rod pigments. Likewise, the major difference in lifetime between the two samples is evident in the PBS fluorescence lifetime. These two findings support the hypothesis that the energy transfer step which is enhanced is in the PBS.

Figure 5C shows that at 5 K, where contributions of thermal vibrations are negligible, the lifetime at low light is still shorter (629 ps in low light compared to 927 ps in medium light). In fact, the difference in lifetimes increases with decreasing temperatures (Fig. 6A). Since radiation decay rates are intrinsic, it implies the energy transfer from the PBS rod extremity (the PUB) is indeed faster.

To examine whether this phenomenon originates from the PBS itself, or is rather related to the energy transfer rate between the PBS to the membrane-embedded reaction centers, we conducted TCSPC measurements on PBS fractions isolated from the acclimated cells. We selected the sucrose gradient fraction that exhibited the closest fluorescence spectra to the one recorded in vivo for analysis. Figure 5D shows that the isolated PBS fraction of the low light-grown cells exhibited a shorter lifetime compared to the PBS fraction of the medium light-grown cells (503 ps vs. 905 ps, respectively). The fact that we observe the same trend in vitro and in vivo supports the conclusion that, indeed, within the PBS of low light-grown cells energy migrates faster toward the terminal emitter.

This finding is surprising. As mentioned above, FRET calculations predict a decrease with increasing number of pigments [31]. Such a decrease was indeed demonstrated in plant systems, where longer lifetimes were reported in the large antenna of low light-acclimated samples [37].

Pigment coupling

Faster energy transfer over a larger pigment system will require stronger energetic coupling between these pigments. To explore this hypothesis and inspect the coupling strength between PBS pigments, we measured the temperature dependence of both steady-state and time-resolved fluorescence, from 300 K down to 4 K. Below the freezing point, photochemistry is blocked. However, the photosynthetic complexes remain intact, so their structure could still be studied. Thermal vibrations strongly affect the coupling between the chromophores in a system, causing thermal broadening and heat dissipation. Hence, the dependence of fluorescence lifetime and emission spectrum on temperature could be used to probe the inter-pigment coupling. Figure 6A shows the average fluorescence lifetime as a function of temperature. An interesting feature is the abrupt rise in lifetime at the temperature range of 250–265 K. This phenomenon was reported in vitro in isolated phycobiliproteins [38]. It was related to photoisomerization of the billions into a form more prone to quenching, due to interactions of the solvent with the chromophores that are influenced by temperature. Maksimov and coworkers reasoned that the interaction between monomeric subunits of phycobiliprotein trimers prevents photoisomerization of the chromophores. Once this interaction is weakened due to the decrease in temperature, photoisomerization takes place. In our case, the measurement was done in vivo, where linker proteins occupy the center of the trimer. We observe the same phenomenon at slightly lower temperatures, either due to the in vivo PBS water content and conformation being different from the isolated protein scenario, or due to an offset in the temperature measurement (see Materials and methods). Nevertheless, we were able to detect this anomaly in intact cell samples. The in vivo temperature anomaly had a different trend in medium and low light cells. While in medium light cells the rapid rise toward the 250–265 K peak began immediately upon cooling (highlighted in inset of Fig. 6A), low light-grown cells maintained a constant lifetime down
to lower temperatures (about 280 K), and their rise in lifetime was more gradual. According to the proposed explanation for the rise in lifetime, this difference indicates stronger interaction between the chromophores in the low light-grown PBS, supporting our hypothesis. Another noticeable difference between the medium and low light lifetime trend is a different jump size seen around 230 K. As we could not determine the origin of this jump, we speculate that it may be related to confined water phase transition, that are known to be present in the center of the phycobiliprotein hexamers [39].

Figure 6B,C shows the emission spectra at a few chosen temperatures along the gradient. In both medium and low light cells, emission intensity increases dramatically with the decrease in temperature, as expected when decreasing the heat dissipation component. The main difference was the change in the ratio between the PUB emission (560 nm) to PEB emission (574 nm). At room temperature, the emission of both pigments comprised one visible peak due to thermal broadening. At lower temperatures, the two spectral lines narrowed and the emissions of the two pigments were resolved. While under low light the PUB : PEB emission peak ratio decreases significantly with temperature (down to 0.45 at 5 K), under medium light the ratio is closer to 1 (reaching 0.8 at 5 K). Since the PUB and PEB content remained constant while cooling down, it implies that under low light a larger fraction of the energy absorbed by PUB was transferred to PEB and emitted from PEB, even at 5 K, proving stronger coupling between the PUB–PEB pigments. It is interesting to note that the PBS under low light has a higher PUB:PEB ratio, so even though the PUB content under low light was higher, we observe less PUB emission than in medium light. These results further support the hypothesis that in cells acclimated to low light, the coupling between the chromophores in the PBS is stronger. Enhanced coupling is the basis for the higher energy transfer rate.

Discussion
Taking advantage of the extensive photo-acclimation capacity of *Synechococcus* WH8102, we were able to study physical mechanisms governing the acclimation of the photosynthetic apparatus. Cells displayed extreme morphological flexibility, acclimating to the
irradiance regime within days. When experiencing low blue light, growth rates were slower, cells were bigger and contained more thylakoid membranes. The number of photosynthetic units increased, but what we find most fascinating is that each of these units was more efficient under a low light regime.

The PBS was bigger under low light, with longer rods containing additional PUB pigments which increase the absorption cross-section at 495 nm. Surprisingly, instead of decreasing the rate of energy transfer, as was reported in plant systems [37], the longer rods exhibit shorter lifetime, indicating faster energy transfer. In cyanobacterial photosynthetic apparatus, the slow energy transfer rate is from the PBS to the reaction centers [40–42]. However, under low light conditions the limitation is the photon flux density. Hence, efficiently transferring the absorbed excitation energy to the terminal emitter of the PBS becomes advantageous, even when the rate of transfer toward the reaction centers is unchanged.

Several lines of evidence support the hypothesis that improved energy transfer is achieved by stronger coupling between the pigments. The question that remains open at this point is what structural or conformational changes lead to this enhanced coupling. In our first suggested model, assuming constant number of rods in each PBS (illustrated in Fig. 4 as ‘low light 1’), the coupling strength is expected to increase between the chromophores within the same PE disk or between disks of the same rod. Coupling strength according to the semi-classical FRET theory is a function of dipole strength, distances, orientation, and energetic overlap between the pigments in the exciton transfer network. Conformational changes in the phycobilins themselves may result in an improvement to some extent. Such changes were recently shown in vitro in phycocyanin [43]. It is possible that phycoerythrin has a similar switching mechanism. An increased efficiency over a long pigment network may also suggest the involvement of coupling that is stronger than predicted by FRET calculations. Improving energy transfer within a rod, by either of these mechanisms, will require rearrangements of the pigment network. The possibility for such a rearrangement is limited by space, preventing a significant degree of hexamer condensation along the z-axis of a rod.

In the second model, the PBS is enlarged not only due to the extension of the rods but also due to higher density of rods on the core surface (illustrated in Fig. 4 as ‘low light 2’). This opens the possibility for achieving stronger coupling by launching new pathways for the excitation energy: between chromophores of adjacent rods. A theoretical model that can be applied to this situation was suggested by Mendoza-Arenas et al. [44]. They showed how energy transfer rates could be enhanced when transport is possible not only along one-dimensional quantum conductors but also by incoherent coupling between them, resembling the case of the PBS rods. The long inter-chromophore distances between the phycobilin pigments and the short distances between neighboring rods [10] make this model plausible. There is experimental evidence to support energy transfer perpendicular to the z-axis of rods. We have evidence for such transfer in arrays of pure phycocyanin rods in vitro. Absorbed energy was shown to transfer not only vertically down the rod but also between rods [45]. Cryo-electron tomography imaging of the three-dimensional architecture of PBSs in Synechocystis sp. PCC6803 [46] revealed close connections between adjacent phycobilisome units, in vitro. In essence, this hypothesis is an extension of the concept of excitation energy transfer between PSII units, introduced for the first time by Juliot and Joliot in 1964 [47]

The two mechanisms described, enhanced intra-rod-coupling and additional inter-rod-coupling, utilize incoherent delocalization that is stronger than simple FRET dipole–dipole interactions. Both mechanisms are not mutually exclusive, and both may contribute to the overall enhanced transfer rate. Further structural studies of the PBS are required to determine the extent to which these mechanisms contribute to the enhanced coupling strength we discovered in low light-acclimated PBS. Unveiling the mechanism by which cyanobacteria tune energy transfer will help better understand photo-acclimation, and may pave the way to bio-inspired nano-scale energy-transducing devices with a broad and controllable dynamic range.

Materials and methods

Growth conditions and cellular morphology measurements

*Synechococcus* WH8102 cultures were grown in ASW medium under continuous blue light (spectrum in Fig. 1A), in 10 and 150 µmol photons·m⁻²·s⁻¹, referred to as ‘low light’ and ‘medium light’, respectively. For FACS analysis, 1 mL samples were collected every second day, preserved in 0.2% glutaraldehyde grade II (Sigma-Aldrich, Saint Louis, MO, USA), frozen in liquid nitrogen, and stored at −80 °C. Cells were counted using FACS Aria III flow cytometer-sorter (BD Biosciences, San Jose, CA, USA) using 488 nm laser. The flow rate was 14 µL·min⁻¹, data were collected for 1 min. Confocal microscopy was performed using an
Olympus FV-1200 confocal microscope, Olympus Corporation, Tokyo, Japan.

**Transmission electron microscopy**

Acclimated cells were harvested by centrifugation at 8000 g for 5 min, washed twice in ASW, and chemically fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Sample preparation followed standard procedures. Sections were sequentially stained with uranyl acetate and lead citrate for 3 min each and viewed with Tecnai 12 TEM 100kV (Phillips, Eindhoven, the Netherlands) equipped with MegaView II CCD camera.

**PBS isolation**

Following 7 days in ML or LL, 1 L cells were harvested by centrifugation at 6300 g for 10 min. Cells were resuspended in 0.8 M phosphate buffer pH7, broken twice using French Press (Constant Systems Ltd, 20 000 Psi; Warwick, UK), and then centrifuged for 45 min at 18 514 g. The pellet was resuspended in 2% Triton X-100 (W/V) in 0.8 M phosphate buffer pH7. Following 1-h incubation in darkness, the resuspension was centrifuged for 2.5 h at 147 000 g. After resuspending the resulting pellet in 0.8 M phosphate buffer pH7, it was loaded on sucrose gradient (0.25–2 M sucrose – 40% in buffer A) and centrifuged 16 h at 221 630 g (SW41) in 4°C. The supernatant was collected to a new test tube and centrifuged for 1 h using 95 000 g in 4°C. The pellet containing thylakoids was resuspended in small volume of buffer A. Protein concentration was determined using Bradford reagents. Solubilization of membrane pigment–protein complexes was done using dodecyl-maltoside (DDM 1.5% per 1 mg protein). Thylakoids were incubated than for 30 min in ice. Following incubation, thylakoids were centrifuged for 10 min using 17 000 g in 4°C. The supernatant was loaded on sucrose gradient (0–40% in buffer A with the addition of 0.05% DDM). Gradients were centrifuged 16 h at 221 630 g (SW41) in 4°C.

**Physiological parameters, pigment, and protein composition**

Oxygen evolution was measured using Clark-type electrode (Hansatech Instruments Ltd, King’s Lynn, Norfolk, UK). Measurements were performed using the same blue LED illumination under which the sample grew. Light intensities were altered by changing the distance of the light source from the culture. NaHCO₃ was added to the samples to a final concentration of 5 mM as a terminal acceptor.

Chlorophyll fluorescence kinetic parameters were measured in three different methods. Maximal PSII quantum yield, Fv/Fm values, was measured using a PAM 2500 (Walz, Effeltrich, Germany), a Joliot-type spectrophotometer (JTS-10; Bio-Logic, Grenoble, France), and Imaging-PAM fluorometer (Walz imaging-PAM MAXI version). Maximal fluorescence, Fm, was measured following the addition of 5 µM DCMU. Light response curve was measured using the imaging-PAM.

Hydrophobic pigments were extracted in 100% methanol, and the pellet containing hydrophilic phycobilins was suspended in Tris-magnesium–chloride NaCl pH 7.5. Protein concentration determined using Bradford reagent according to Zor and Selinger [48]. Thylakoid preparation and photosystem complex separation were done following 7-day incubation under medium or low blue light. Cells were centrifuged (12 800 g for 7 min) and washed twice with buffer A (50 mM Tris/HCl pH8; 10 mM NaCl; 1 mM EDTA; 200 mM Sorbitol). Finally, cells were resuspended in 5 mL buffer A with the addition of anti-protease cocktail (Roche, Indianapolis, IN, USA). Cells were broken using French Press, centrifuge for 3 min using 1160 g in 4°C. The supernatant was collected to a new test tube and centrifuge for 1 h using 95 000 g in 4°C. The pellet containing thylakoids was resuspended in small volume of buffer A. Protein concentration was determined using Bradford reagents. Solubilization of membrane pigment–protein complexes was done using dodecyl-maltoside (DDM 1.5% per 1 mg protein). Thylakoids were incubated than for 30 min in ice. Following incubation, thylakoids were centrifuged for 10 min using 17 000 g in 4°C. The supernatant was loaded on sucrose gradient (0–40% in buffer A with the addition of 0.05% DDM). Gradients were centrifuged 16 h at 221 630 g (SW41) in 4°C.

**Spectroscopic measurements**

Steady-state absorption and fluorescence were performed within an integrated sphere to reduce scattering, using a Horiba PTI Quantamaster (Kyoto, Japan). Temperature-dependent time-resolved fluorescence measurements were carried out using a time-correlated single-photon counting (TCSPC) setup built on attoDRY800 cryo-optical table (atocube, Munich, Germany). Excitation was done with a Fianium WhiteLase SC-400 (NKT Photonics, Birkerød, Denmark) supercontinuum laser monochromatized at 485 nm at a repetition rate of 40 MHz, with a spot size of 1 mm² and intensity of 10 µW. Detected with MPD PD-100-CTE-FC photon counter and PicoHarp300. The measured instrument response function (IRF) appears in Fig. 5. All measurements were done in reflection mode, to minimize self-absorption effect. The samples were trapped between two sapphire windows with a Teflon O-ring in between, in their original medium with no additives. This sample holder was put inside a vacuum chamber, thermally coupled to a cold plate that is gradually cooled down to 4 K. Sample temperature was measured along the process with a Cernox DT-670-SD attached to the sapphire window. There may be an offset of up to a few degrees between the temperature recorded by the sensor and the actual temperature of the sample. TCSPC data were analyzed using decayFIT 1.4 software (Fluorescence Decay Analysis Software 1.4, FluorTools, www.fluortools.com) to deconvolute with the measured IRF, and fit to a three exponential decay models. Temperature-dependent emission spectra were recorded at the same cryogenic setup: excited
with a CPS450nm laser diode and emission detected with Flame spectrometer (Ocean Optics, FL, USA).

Acknowledgements

The TEM imaging was done at the imaging facility in the Institute for Life Sciences, HUJI, by Dr Yael Friedmann. The structural models in Fig. 4 were created by Dr Ido Eisenberg. Work was supported by ISF grant 1182/19 and ISF-NSFC grant 2466/18.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

YK lead the research, and performed experiments and data analysis. HZ performed experiments and data analysis. MP conducted experiments. SY performed data analysis. YP and NK supervised the study and analysis. YK lead the research, and performed experiments and analysis. YP and NK supervised the study and analysis. MP conducted experiments. SY performed data analysis. HZ performed experiments and data analysis. YK lead the research, and performed experiments and analysis.

References

1 Mullineaux CW (2014) Electron transport and light-harvesting switches in cyanobacteria. Front Plant Sci 5, 7.
2 Croce R & van Amerongen H (2014) Natural strategies for photosynthetic light harvesting. Nat Chem Biol 10, 492–501.
3 Engel GS, Calhoun TR, Read EL, Ahn TK, Mančal T, Cheng YC. Blankenship RE & Fleming GR (2007) Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. Nature 446, 782–786.
4 Collini E, Wong CY, Wilk KE, Curmi PMG, Brumer P & Scholes GD (2010) Coherently wired light-harvesting in photosynthetic marine algae at ambient temperature. Nature 463, 644–647.
5 Fassoli F, Dinshaw R, Arpin PC & Scholes GD (2014) Photosynthetic light harvesting: excitons and coherence. J R Soc Interface 11, 20130901.
6 Marais A, Adams B, Ringsmuth AK, Ferretti M, Gruber JM, Hendriks R, Schuld M, Smith SL, Sinayskiy I, Krüger TPJ et al. (2018) The future of quantum biology. J R Soc Interface 15, 20180640.
7 Suter GW & Holzwarth AR (1987) A kinetic model for the energy transfer in phycobilisomes. Biophys J 52, 673–683.
8 Chenu A & Scholes GD (2015) Coherence in energy transfer and photosynthesis. Annu Rev Phys Chem 66, 69–96.
9 Keren N & Paltiel Y (2018) Photosynthetic energy transfer at the quantum/classical border. Trends Plant Sci 23, 497–506.
10 Adir N, Bar-Zvi S & Harris D (2019) The amazing phycobilisome. Biochim Biophys Acta Bioenerg 1861, 148047.
11 Zhang J, Ma J, Liu D, Qin S, Sun S, Zhao J & Sui S-F (2017) Structure of phycobilisome from the red alga Griffithsia pacifica. Nature 551, 57–63.
12 Ma J, You X, Sun S, Wang X, Qin S & Sui S (2020) Structural basis of energy transfer in Porphyridium purpureum phycobilisome. Nature 579, 146–151.
13 Staleva H, Komenda J, Shukla MK, Slouf V, Kanà R, Polivka T & Sobotka R (2015) Mechanism of photoprotection in the cyanobacterial ancestor of plant antenna proteins. Nat Chem Biol 11, 287–291.
14 Valkunas L, Chmeliov J, Krüger TPJ, Illiaia C & van Grondelle R (2012) How photosynthetic proteins switch. J Phys Chem Lett 3, 2779–2784.
15 Bar Eyal L, Choubeh RR, Cohen E, Eisenberg I, Tamburu C, Dorogi M, Unnep R, Appavou MS, Nevo R, Raviv U et al. (2017) Changes in aggregation states of light-harvesting complexes as a mechanism for modulating energy transfer in desert crust cyanobacteria. Proc Natl Acad Sci USA 114, 9481–9486.
16 Falkowski PG & Raven JA (2013) Aquatic Photosynthesis. Princeton University Press, Princeton, NJ.
17 Eyal G, Eyal-Shaham L, Cohen I, Tamir R, Ben-Zvi O, Sinninger F & Loya Y (2016) Euphyllyia paradivisa, a successful mesophotic coral in the northern Gulf of Eilat/Aqaba, Red Sea. Coral Reefs 35, 91–102.
18 Post AF, Penno S, Zandbank K, Paytan A, Huse SM & Welch DM (2011) Long term seasonal dynamics of Synechococcus population structure in the Gulf of Aqaba, Northern Red Sea. Front Microbiol 2, 131.
19 Carlson DF, Fredj E & Gildor H (2014) The annual cycle of vertical mixing and restratification in the Northern Gulf of Eilat/Aqaba (Red Sea) based on high temporal and vertical resolution observations. Deep Sea Res Part I Oceanogr Res Pap 84, 1–17.
20 Falkowski PG & LaRoche J (1991) Acclimation to spectral irradiance in algae. J Phycol 27, 8–14.
21 Grébert T, Doré H, Partensky F, Farrar GK, Boss ES, Picheral M, Guidi L, Pesant S, Scanlan DJ, Wincker P et al. (2018) Light color acclimation is a key process in the global ocean distribution of Synechococcus cyanobacteria. Proc Natl Acad Sci USA 115, E2010–E2019.
22 Gutu A & Kehoe DM (2012) Emerging perspectives on the mechanisms, regulation, and distribution of light color acclimation in cyanobacteria. Mol Plant 5, 1–13.
23 Six C, Thomas JC, Brahamsha B, Lemoine Y & Partensky F (2004) Photophysiology of the marine cyanobacterium *Synechococcus* sp. WH8102, a new model organism. *Aquat Microb Ecol* **35**, 17–29.

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

25 Stirbet A, Lázár D & Papageorgiou GC (2019) Chlorophyll a fluorescence in cyanobacteria: relation to photosynthesis. In Cyanobacteria, pp. 79–130. Academic Press, Cambridge, MA.

26 Shukla A, Biswas A, Blot N, Partensky F, Karty JA, Hammad LA, Garzarek L, Gutu A, Schluchter WM & Kehoe DM (2012) Phycocerythrin-specific bilin lyase-isomerase controls blue-green chromatic acclimation in marine *Synechococcus*. *Proc Natl Acad Sci USA* **109**, 20136–20141.

27 Sanfilippo JE, Nguyen AA, Karty JA, Shukla A, Schluchter WM, Garzarek L, Partensky F & Kehoe DM (2016) Self-regulating genomic island encoding tandem regulators confers chromatic acclimation to marine *Synechococcus*. *Proc Natl Acad Sci USA* **113**, 6077–6082.

28 Mahmoud RM, Sanfilippo JE, Nguyen AA, Streit JA, Partensky F, Garzarek L, Abo El Kassem N, Kehoe DM & Schuchter WM (2017) Adaptation to blue light in marine *Synechococcus* requires MpeU, an enzyme with similarity to phycerythrobilin lyase isomerases. *Front Microbiol* **8**, 243.

29 Fuller NJ, Marie D, Partensky F, Vaulot D, Post AF & Scanlan DJ (2003) Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. *Appl Environ Microbiol* **69**, 2430–2443.

30 Toledo G, Palenik B & Brahamsha B (1999) Swimming marine *Synechococcus* strains with widely different photosynthetic pigment ratios form a monophyletic group. *Appl Environ Microbiol* **65**, 5247–5251.

31 Chenu A, Keren N, Paltiel Y, Nevo R, Reich Z & Cao J (2017) Light adaptation in phycobilisome antennas: influence on the rod length and structural arrangement. *J Phys Chem B* **121**, 9196–9202.

32 Soitamo A, Havurinne V & Tyystjärvi E (2017) Photoinhibition in marine picocyanobacteria. *Physiol Plant* **161**, 97–108.

33 Kana TM & Gilbert PM (1987) Effect of irradiances up to 2000 µE m⁻² s⁻¹ on marine *Synechococcus* WH7803-I. Growth, pigmentation, and cell composition. *Deep Sea Res Part A Oceanogr Res Pap* **34**, 479–495.

34 Lis H, Shaked Y, Kranzler C, Keren N & Morel FM (2015) Iron bioavailability to phytoplankton: an empirical approach. *ISME J* **9**, 1003–1013.

35 Six C, Thomas JC, Thion L, Lemoine Y, Zal F & Curie M (2005) Two novel phycocerythrin-associated linker proteins in the marine. *J Bacteriol* **187**, 1685–1694.

36 Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen EE, McCarren J et al. (2003) The genome of a motile marine *Synechococcus*. *Nature* **424**, 1037–1042.

37 Wientjes E, van Amerongen H & Croce R (2013) Quantum yield of charge separation in photosystem II: functional effect of changes in the antenna size upon light acclimation the migration of LHClII from PSII to PSI has. *J Phys Chem B* **117**, 11200–11208.

38 Maksimov EG, Schmitt F-J, Hätti P, Klementiev KE, Paschenko VZ, Renger G & Rubin AB (2013) Anomalous temperature dependence of the fluorescence lifetime of phycobiliproteins. *Lasor Phys Lett* **10**, 055602.

39 Kurzweil-Segev Y, Popov I, Eisenberg I, Yochelis S, Keren N, Paltiel Y & Feldman Y (2017) Confined water dynamics in a hydrated photosynthetic pigment-protein complex. *Phys Chem Chem Phys* **19**, 28063–28070.

40 Tian L, Gwizdala M, van Stokkum IHM, Koechhorst RBM, Kirilovsky D & van Amerongen H (2012) Picosecond kinetics of light harvesting and photoprotective quenching in wild-type and mutant phycobilisomes isolated from the cyanobacterium *Synechocystis* PCC 6803. *Biophys J* **102**, 1692–1700.

41 Akhtar P, Biswas A, Petrova N, Zakar T, Van Stokkum IJM & Lambrev PH (2020) Time – resolved fluorescence study of excitation energy transfer in the cyanobacterium *Anabaena* PCC 7120. *Photosynth Res* **144**, 247–259.

42 Mullineaux CW & Holzwarth AR (1991) Kinetics of excitation energy transfer in the cyanobacterial phycobilisome-photosystem II complex. *Biochim Biophys Acta Bioenerg* **1098**, 68–78.

43 Gwizdala M, Krüger TPJ, Wahadoszamen M, Gruber JM & Van Grondelle R (2018) Phycocyanin: one complex, two states, two functions. *J Phys Chem Lett* **9**, 1365–1371.

44 Mendoza-Arenas JJ, Mitchison MT, Clark SR, Prior J, Jaksch D & Plenio MB (2014) Transport enhancement from incoherent coupling between one-dimensional quantum conductors. *New J Phys* **16**, 053016.

45 Eisenberg I, Yochelis S, Ben-Harosh R, David L, Faust A, Even-Dar N, Taha H, Haegele NM, Adir N, Keren N et al. (2014) Room temperature biological quantum random walk in phycocyanin nanowires †. *Phys Chem Chem Phys* **16**, 11245.

46 Rust A, Schaffer M, Albert S, Wan W, Pfefier S, Beck F, Pitzko JM, Nickelsen J & Engel BD (2019) Biogenic regions of cyanobacterial thylakoids form contact sites with the plasma membrane. *Nat Plants* **5**, 436–446.
47 Joliot P & Joliot A (2003) Excitation transfer between photosynthetic units: the 1964 experiment. *Photosynth Res* **76**, 241–245.

48 Zor T & Selinger Z (1996) Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. *Anal Biochem* **236**, 302–308.

49 Arteni AA, Ajlani G & Boekema EJ (2009) Structural organisation of phycobilisomes from *Synechocystis* sp. strain PCC6803 and their interaction with the membrane. *Biochim Biophys Acta Bioenerg* **1787**, 272–279.

**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Data analysis of the TCSPC (time correlated single photon counting) curves which appear in Figure 5.