BBA - Bioenergetics 1860 (2019) 121–128

Contents lists available at ScienceDirect

BBA - Bioenergetics

journal homepage: www.elsevier.com/locate/bbabio

Radioprotective role of cyanobacterial phycobilisomes

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ARTICLE INFO

Keywords:
Space
Cosmic rays
Ionizing radiation
Cyanobacteria
Photosynthesis
Phycobilisomes
Phycobiliproteins
Orange carotenoid protein
Carotenoids
Reactive oxygen species

ABSTRACT

Cyanobacteria are thought to be responsible for pioneering dioxygen production and the so-called "Great Oxygenation Event" that determined the formation of the ozone layer and the ionosphere restricting ionizing radiation levels reaching our planet, which increased biological diversity but also abolished the necessity of radioprotection. We speculated that ancient protection mechanisms could still be present in cyanobacteria and studied the effect of ionizing radiation and space flight during the Foton-M4 mission on *Synechocystis* sp. PCC6803. Spectral and functional characteristics of photosynthetic membranes revealed numerous similarities of the effects of α-particles and space flight, which both interrupted excitation energy transfer from phycobilisomes to the photosystems and significantly reduced the concentration of phycobiliproteins. Although photosynthetic activity was severely suppressed, the effect was reversible, and the cells could rapidly recover from the stress. We suggest that the actual existence and the uncoupling of phycobilisomes may play a specific role not only in photo-, but also in radioprotection, which could be crucial for the early evolution of Life on Earth.

1. Introduction

It is well known that solar and cosmic radiation are lethal outside the ionosphere of the Earth, which is composed of ionized nitric oxide and dioxygen. Below the ionosphere, another thin atmospheric stratum, the ozone layer, blocks a significant amount of UV-C and UV–B light. Thus, di- and trioxygen essentially separate the surface of our planet from Space, but it has not always been like that. During the first two billion years of Earth's existence, its atmosphere contained no oxygen. The radiation dosage arriving on the Earth's surface exceeded today's level by approximately 15 times for UV and about 50 times for ionizing radiation (IoR) [1]. The situation changed after the appearance of photosynthetic organisms, which were able to use water as a source of electrons and evolved dioxygen as a side product. One must admit that this strategy was very successful since the amount of oxygen produced by microorganisms was sufficient to oxidize every mineral on the Earth's surface and to create the atmosphere [2–4]. Although the Great Oxygenation Event (GOE) took more than 900 million years to complete, it was a dramatic and game-changing incident, which affected biological diversity on our planet.

All modern geological and biological evidence indicates that communities of cyanobacteria are responsible for the GOE [5], and it is

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https://doi.org/10.1016/j.bbabio.2018.11.018
Received 8 August 2018; Received in revised form 7 November 2018; Accepted 18 November 2018
Available online 20 November 2018
0005-2728/

often assumed that modern and ancient cyanobacterial species are quite similar [6]. However, it is hard to believe that under different selection factors, namely, in the presence and in the absence of UV-C, UV-B and IoR, these primitive organisms remained unaltered over billions of years of evolution since then. Here, we would like to focus on the selection factors, which were crucial for cyanobacterial development before the GOE.

First of all, oxygenic photosynthesis requires effective bioenergetic conversion of light into the energy stored in separated charges and chemically stable equivalents. The first step of this complex process implies absorption of a photon by antenna pigments and transfer of the excitation energy to the reaction centers of the photosystem [7,8]. Thus, antennas which transfer excitation energy to the photosystems significantly increase the effective absorption cross-section of the reaction center. In cyanobacteria, light-harvesting antennas consist of large water-soluble pigment-protein complexes, phycobilisomes (PBs) [9–15], the absorption of which in the visible spectral region is accomplished by linear tetrapyrroles, which are covalently bound to specific cysteines of phycobiliprotein subunits of PBs. Different species of cyanobacteria utilize spectrally different phycobiliprotein types [16–18], with an overall quite similar structure, building up a megadalton complex harboring thousands of chromophores [19]. PBs are so large that they can be easily visualized in an electron microscope, and in some cyanobacteria, reach up to 60% of total cellular protein and up to 20% of dry cell mass [20]. Considering the efficiency of cyanobacterial photosynthetic apparatus and the robustness of their survival strategy, which was developed prior to the GOE, it appears surprising that most algae and all higher plants completely lack PBs, although it is generally accepted that their chloroplasts had evolved from cyanobacteria [21] after endosymbiosis. This suggests that utilization of this giant for light-harvesting only was not energetically favorable for algae and plants, since evolution in eukaryotic photosynthesis developed membrane-embedded light-harvesting complexes instead [22]. This immediately raises the question what else were PBs so necessary for, on top of light-harvesting in cyanobacteria?

Prior to the GOE, all living organisms were under pressure of other selection factors, and, in particular, protection of the integrity of DNA was vital. Several strategies were developed, including replication, storage of multiple copies of the same plasmid, chromosome, and genes [23], but from these, prevention of DNA damage was probably the most efficient. A general way to prevent excitation/ionization of DNA is to hide it behind some shield, which will be able to dissipate the energy into heat, to scavenge radicals, or to sacrifice itself in any other way for the greater good. This strategy probably became even more relevant due to the appearance of oxygenic photosynthesis, which significantly increases the probability of Reactive Oxygen Species (ROS) formation. Defense from UV and ROS includes several lines – from secondary metabolites like mycosporines and mycosporine-like amino acids [24], and phlorotannins [25] to complex enzymatic systems like superoxide dismutase, catalase, and glutathione peroxidase. Several examples of protective proteins containing hundreds of aromatic amino acids are known among different microorganisms. A particular (*e.g.*, enzymatic) role of such proteins is unknown, however, UV-protection and/or involvement in the storage of certain amino acids is possible. One such example is the S-layer carotenoid-binding protein of *Deinococcus radiodurans* (gene DR_2577), an organism that is known for its extreme ability to resist up to 200 times higher doses of IoR compared to *Escherichia coli* [26]. Thus, one can assume that the development of large protein complexes like PBs was beneficial for both, radiation protection and light-harvesting, however, the last one probably was not mandatory. This hypothesis is supported by the fact that several *Synechocystis* mutants completely lacking PBs [27] are able to grow autotrophically, although being much more susceptible to environmental stress. Very recently, a cyanobacteria living about 600 m under the surface of Earth in total darkness was described [28], which shows that adaptation mechanisms of these microorganisms know virtually no limits [29].

Importantly, utilization of PBs would also require the development of specific mechanisms regulating their energetic coupling with the photosystems. One such mechanism, called “state transitions”, involves redox-dependent redistribution of PBs between the two known types of photosystems [30–33]. Which molecular cascade triggers this reaction and how the mobility of PBs is regulated is poorly understood. Another mechanism requires carotenoid-binding proteins which can induce quenching of the excessive PBs photoexcitation, thus preventing excitation energy transfer (EET) to reach the photosynthetic reaction centers [34–40]. In some species like *Synechocystis*, PBs quenching is triggered by photoactivation of the Orange Carotenoid Protein (OCP) [41,42]; however, some species have multiple copies of full-length OCP-like genes and its fragments (Helical Carotenoid Proteins – HCPs [43,44] and C-terminal Domain Homologs – CTDHs [45]) which can bind carotenoids, but not all of them are able to induce PBs quenching. Recent studies demonstrated that carotenoid molecules, which are known for their excellent antioxidant properties, can be transferred from membranes into water-soluble HCPs *via* CTDH, with the state of the latter being regulated by redox conditions [45–47]. The presence of multiple completely different water-soluble carotenoid carriers, which are not involved in direct PBs quenching, raises the question about their present and past functional roles and their involvement into the photo- and other protection mechanisms. We note that phycobiliproteins also have antioxidant activity, and, as reported in some pilot studies, can even stimulate radioprotection in lymphocytes from nuclear power plant workers [48].

In this work, we present a study about one of the few biological species which were able to survive during Foton-M4 satellite mission [49], the cells of *Synechocystis* sp. PCC6803. In order to understand the observed effects of the space flight, we conducted a series of *in vivo* and *in vitro* model experiments using cyclotron-accelerated α-particles as a source of IoR, which allowed us to propose the radioprotective role of PBs.

2. Materials and methods

2.1. Cultivation of Synechocystis sp. PCC 6803 and the Foton-M4 satellite mission

To generate the ΔApcE mutant of *Synechocystis* sp. PCC 6803, the *apcE* gene was amplified together with 400 bp upstream and downstream DNA sequences and cloned into the pBluescriptKS/SK(+) plasmid vector. Then, the *Hind*III DNA fragment containing *apcE* gene and 5′-untranslated sequence was deleted and replaced with the gentamycin-resistance cassette. The CK mutant cells were transformed with a recombinant plasmid carrying the ΔApcE deletion to yield Km^R/Gm^R clones. The Km^R/Cm^R transformants were subcultured in the presence of increasing concentrations of gentamycin to segregate the mutant genome copies. Segregation was confirmed by PCR.

The cyanobacterial strains *Synechocystis* sp. PCC 6803 wild-type (WT) and the CK/ΔApcE mutant thereof were grown photoautotrophically for 4–5 days at 30 °C with a constant shaking in standard liquid BG-11 medium with the doubled concentration of sodium nitrate under continuous Phillips cool-white fluorescent light of 40 μM of photons $m^{-2} s^{-1}$. The CK/ΔApcE mutant was maintained in the presence of 10 μg/ml of gentamycin and 80 μg/ml of kanamycin.

The experiment “BIORADIATION-F” was conducted within the framework of a collaboration of Lomonosov Moscow State University and the Institute of Biological and Medical Problems of the Russian Academy of Sciences. The task of this experiment was to study the biologically significant characteristics of cosmic IoR and its impact on biological objects in open space and satellite environments.

Prior to sending to Baikonur Cosmodrome (Kazakhstan), liquid cell culture of *Synechocystis* sp. PCC 6803 was inoculated into 5-ml sterile tubes poured with agar (0.6%) BG-11 medium containing glucose and thoroughly mixed. Control samples were stored in the laboratory at

21 °C in the dark for the entire time of the flight of the satellite mission. At the Cosmodrome, samples were placed inside the satellite, into the thermostatted chambers maintaining temperature at 21 °C. The Foton-M4 satellite was sent into space on July 19, 2014, and spent 45 days on the orbit at the average height of 575 km (http://biosputnik.imbp.ru/eng/Foton4.html). Temperature inside the landing module was in the range from 14 to 22 °C [50]. At the beginning of the Foton-M4 mission, there was a communication failure, which was restored after eight days. This incident resulted in a reduction of orbital mission duration. After landing of the satellite, samples were delivered to Moscow and were studied by various optical methods. Part of the culture which returned from the space mission was placed into a liquid medium (BG-11) and cultivated under normal conditions. A similar procedure was carried out with the control samples kept in the laboratory.

2.2. Samples for in vitro experiments

PBs and phycocyanin (PC) were isolated from the wild-type *Synechocystis* sp. PCC 6803 according to Ref. [51]. OCP with the N-terminal His-tag was expressed in a carotenoid-producing *E. coli* strain and purified as described in Refs. [51, 52].

2.3. Exposure to ionizing radiation

In order to model the effect of IoR by high (relativistic) energy elements (HZE) of cosmic rays, we used a 120-cm cyclotron developed at the Lomonosov MSU Skobeltsyn Institute of Nuclear Physics, which allows obtaining accelerated helium nuclei (α-particles) with energies up to 30.4 MeV. A beam of α-particles from the cyclotron was extracted from the ion guide through 50-μm thick aluminum window into the air and directed through a replaceable diaphragm onto a sample in a 1.0 mm cell covered with 20 μm thick mylar film windows. The beam was monitored by the magnitude of the charge on the diaphragm and the cuvette with an absolute accuracy of absorbed dose determination estimated as 30%, relative accuracy - not worse than 10%. The energy loss of α-particles at the “window” of the ion guide, air layer, and mylar film was 4.2 MeV so that the α-particles energy on the inner surface of the film was about 26.2 MeV. The linear energy transfer (LET) of α-particles with such energy is about 25 keV/μm in water, and grows as the particles in suspension slow down by approximately an order of magnitude. The LET value of the particles at the entrance to the sample chamber is close to the value of LET of the relativistic neon-magnesium nuclei of galactic cosmic rays and then increases in the range, reaching 230 keV/μm corresponding to heavier nuclei (Si), which allows simulating the effect of heavy nuclei Solar Cosmic Rays and Galactic Cosmic Rays [53,54]. Since the mean free path of an α-particle with an energy of 26.2 MeV in water is equal to 0.45 mm, irradiation of the cuvette was carried out from both sides of the cell. The value of the absorbed dose was averaged over the entire volume of the cuvette and was equal to 30, 60, or 120 kGy.

2.4. Optical methods for the assessment of spectral and functional characteristics of photosynthetic complexes in vivo and in vitro

Fluorescence measurements were performed by time- and wavelength-correlated single photon counting system SPC-150EM with hybrid detector HMP-100-40 (Becker&Hickl, Berlin, Germany) as described in [55]. Excitation was performed with a pulsed 405 nm laser diode (InTop, St. Petersburg, Russia) and BDL-473-SMC (Becker&Hickl, Berlin, Germany) laser delivering picosecond excitation pulses, driven at repetition rates up to 50 MHz. Temperature of the sample was stabilized by a Peltier-controlled cuvette holder Qpod 2e (Quantum Northwest, Liberty Lake, WA). Fluorescence decay kinetics were approximated by a sum of exponential functions. Calculations were performed using SPCImage (Becker&Hickl, Germany) software packages.

Measurements of steady-state fluorescence spectra and fluorescence excitation spectra were carried out on a modified Fluorolog 3 (Horiba Jobin Yvon) spectrophotometer.

Absorption spectra were measured using a Lambda-25 spectrometer (Perkin Elmer).

Fluorescence induction curves were recorded using a PSI-3500Fl fluorimeter (PSI). Variable fluorescence (Fv/Fm) was calculated according to procedures described in [56–58].

All calculations were performed using Origin 9.1 (OriginLab Corporation, USA). Each experiment except Foton-M4 satellite mission was conducted at least 3 times.

2.5. ROS-induced bleaching of pigments in vitro

The photosensitizing activity of polycationic aluminum phthalocyanines (AlPCs) was used to study the effect of ROS on PBs and OCP. In order to induce ROS production, a mixture of AlPCs was illuminated by a 200 mW red LED with the emission maximum at 635 nm (Thorlabs, USA), which overlaps with the main absorption band of AlPCs. In order to prevent photoactivation of OCP by the red LED, the sample was illuminated through a 600 nm longpass filter (Thorlabs, USA). Upon illumination of the sample, absorption in the visible range was recorded using a MayaPro spectrometer (Ocean Optics, USA).

3. Results

3.1. Absorption measurements reveal that PBs are sensitive to ionizing radiation

The main absorption bands of *Synechocystis* cells in the visible/near-infrared (Vis-NIR) region of the spectrum are attributed to pigments of the photosynthetic apparatus, namely chlorophyll *a* (Chl, main peaks at 440 and 680 nm) of the photosystems 1 and 2, carotenoids (shoulder at ~500 nm) and phycobiliproteins of PBs, which are dominated by phycocyanin (PC, 623 nm). Fig. 1A shows characteristic changes in absorption spectra of *Synechocystis* sp. PCC6803 cells after exposure to IoR and space flight. Upon an increase of the absorbed dose, we observed a decrease of optical density in the regions of absorption of both Chl and PBs. This effect was characterized by saturation for the Chl component and was almost linear for the PBs component of the spectrum (*Fig. 1B*). *In vitro*, comparable concentrations of isolated PBs were 50% bleached already at 60 kGy, which may indicate that *in vivo* PBs are more stable, or that the effect of ionizing radiation is divided between different cell compartments. This was even more likely because of the layered structure of the thylakoids, where each layer shields a layer underneath. Such protection density might not be reached *in vitro*. In all experiments with cells, we observed a decrease of the PBs-to-Chl absorbance ratio (*Fig. 1C*), which indicates that PBs are more sensitive to IoR stress than Chl containing compartments. We note that storage of cells in the dark, even in the presence of glucose, resulted in a reduction of PBs concentration, which is typical of different types of starvation [59,60]. Reduction of PBs absorption was even more pronounced in the cells which traveled into space *via* the Foton-M4 satellite, in total resulting in a decrease of the PBs/Chl ratio comparable to the sum of the effects of starvation and exposure to IoR. We consider it to be a lucky coincidence that the duration of the space mission was shortened by two weeks. Otherwise, the longer starvation period could have eliminated the differences between the sample and the reference stored in the laboratory.

3.2. PBs and Chl fluorescence reveal that ionizing radiation affects energetic coupling of antenna and PS2

As we observed significant changes in concentrations of pigments in *Synechocystis* cells exposed to different stress factors (*Fig. 1*), we expected differences in fluorescence intensity of the samples, which (in an ideal case) would be proportional to the concentration and fluorescence

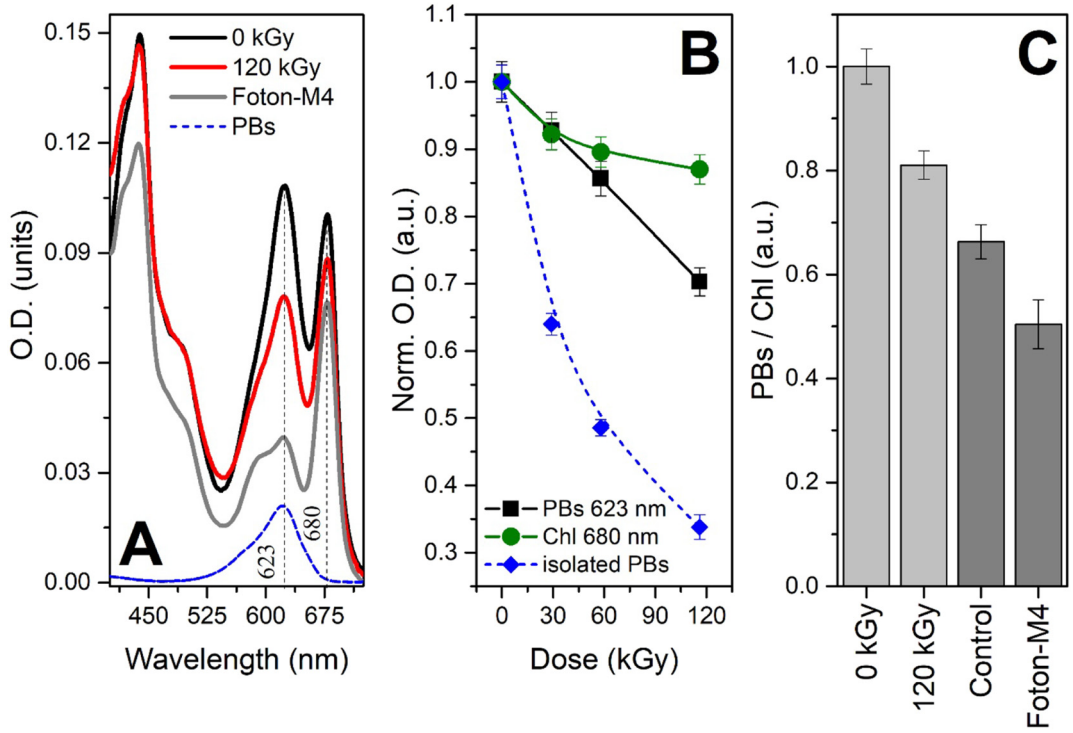

Fig. 1. (**A**) - Absorption spectra of *Synechocystis* sp. PCC6803 cells before and after exposure to 120 kGy of α-particles (black and red lines, respectively) or the Foton-M4 space mission (gray). The blue dashed line shows the absorption of isolated PBs. (**B**) – Dose dependency of the optical density of *Synechocystis* sp. PCC6803 cells (PBs absorption, black; and Chl absorption, green) and isolated PBs (blue) from irradiation with α-particles. Absorption was normalized to O.D. values at 0 kGy. (**C**) - Ratios of the optical density of *Synechocystis* sp. PCC6803 cells at 620 nm (PBs) and 680 nm (Chl) after different treatments. Control samples were stored in the laboratory at 21 °C in the dark for the entire time of flight of the satellite mission.

quantum yield of the pigments [61]. In order to separate the effects of IoR from concentration and the functional state of PBs, we used time-resolved fluorescence spectroscopy with picosecond time resolution (*Fig. 2AB*). Surprisingly, the reduction of PBs absorption was accompanied by an increase of the PBs fluorescence intensity, which is due to the increase of PBs fluorescence lifetime and, hence, quantum yield (*Fig. 2C*). Since under normal conditions PBs fluorescence is quenched due to highly efficient excitation energy transfer (EET) to the chlorophylls of the photosystems [9,20], we assume that the observed increase in PBs fluorescence lifetime is due to uncoupling of PBs from the photosystems. The decrease of the EET efficiency explains the characteristic changes in steady-state fluorescence spectra, in which the intensity of the PBs emission band (660 nm) significantly increases, while Chl emission at 685 nm decreases (*Fig. 2D*) after exposure to IoR or during the space flight. The decrease of Chl fluorescence intensity is probably caused by a combination of several factors (i) decrease of the EET efficiency form PBs, (ii) nonphotochemical quenching of Chl *a* excited states and (iii) decrease of Chl concentration in photosystems. Uncoupling of PBs from photosystems also explains the increase of the initial level of fluorescence intensity (F_O) observed in the fluorescence induction curves of the sample exposed to IoR (*Fig. 2E*). We found that the so-called photosynthetic efficiency of PS2 (*Fv/Fm*) significantly decreases with increasing radiation dose (*Fig. 2F*), which could be partially explained by an increase of F_O; however, the activity of PS2 is definitely affected, since no J-P phase could be observed at 120 kGy. Thus, under IoR, PS2 gets into the Q_B non-reducing state. Since the value of *Fv/Fm* of cells which returned from space was close to zero, the space flight also inactivated PS2. In contrast, the activity of PS2 of the control sample, which stayed in the dark during the time of the space flight, remained relatively high (*Fv/Fm* = 0.46), although the concentration of PBs antennas was significantly reduced (see *Fig. 1C*).

The most surprising effect was observed in experiments with PBs isolated from *Synechocystis* cells. Since EET in PBs proceeds from blue (PC, emitting at 647 nm) to red pigments (allophycocyanines (APC) and terminal emitters, at ~ 660 and 685 nm, respectively), we expected that α-particles IoR may cause decoupling of the PBs rods (mainly phycocyanines) and cores (mainly allophycocyanines), which would result in an increase of the PC fluorescence quantum yield. Such PBs decomposition would explain the observed increase of PBs lifetimes *in vivo*, and also this mechanism was tempting, since it was proposed previously to explain effects of γ-rays on *Synechocystis* cells [62], although these authors were relying mainly on the results obtained from steady-state spectroscopy. In contrast, we observed a decrease in PC and APC lifetimes in isolated PBs under IoR (*Fig. 2F*). This fact, together with the absence of a (significant) blue shift of PBs fluorescence emission *in vivo* (*Fig. 2D*) indicates that, under IoR, PBs uncouple from the photosystems; however, PBs do not dissociate, since EET from PC to APC remains effective. This shows that IoR-induced uncoupling of PBs occurs in a controlled (and probably reversible) manner, similar to state transitions. Since it is highly unlikely for cyanobacterial cells to have a specific receptor for α-particles, we assume that PBs uncoupling from photosystems could be a general protection mechanism which could be triggered by multiple stress factors, and most probably ROS.

3.3. Ionizing radiation and ROS can induce photoprotective mechanisms in vitro

It is known that the shape of the absorption spectrum, fluorescence lifetime and quantum yield of phycobilins is determined by interactions with the protein matrix, and denaturation of the protein causes significant reduction of the fluorescence lifetime [63,64]. If this happens when the phycobiliprotein is part of the PBs antenna, such a chromophore becomes a trap for excitation energy, which will be dissipated as heat. Since thermal dissipation of excitation energy is a major photoprotection strategy for all cyanobacteria, which are endowed with an OCP-dependent quenching mechanism of PBs fluorescence [41,65–67],

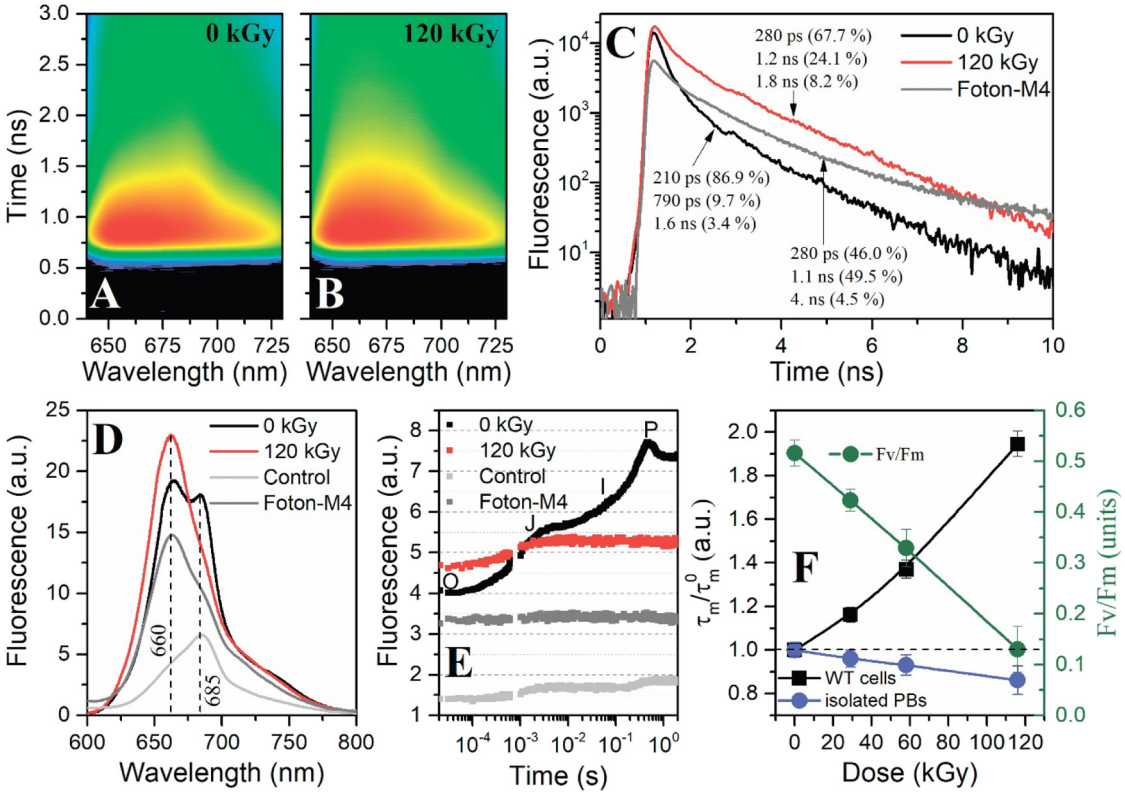

Fig. 2. (**A**) and (**B**) – Time-resolved emission spectra of *Synechocystis* sp. PCC6803 samples before and after exposure to 120 kGy of α-particles, respectively. The color represents the logarithm of the photon number in each time/spectral channel. Experiments were conducted at 25 °C. (**C**) – Fluorescence decay kinetics of *Synechocystis* sp. PCC6803 samples before (black) and after exposure to 120 kGy of α-particles (red), and after Foton-M4 space mission (gray, scaled) at 660 nm emission. (**D**) – Steady-state fluorescence spectra of *Synechocystis* sp. PCC6803 samples before and after exposure to 120 kGy of α-particles (black and red, respectively), Foton-M4 space mission (gray, scaled) and the control sample incubated in the dark for the duration of the space flight (light gray, scaled). Control samples were stored in the laboratory at 21 °C in the dark for the entire time of flight of the satellite mission. (**E**) – Fluorescence induction curves of *Synechocystis* sp. PCC6803 cells, color codes as in the previous panel. (**F**) – Dose dependency of fluorescence lifetimes of *Synechocystis* sp. PCC6803 cells and isolated PBs normalized to the corresponding values at 0 kGy. The right Y-scale shows the dose dependency of the Fv/Fm ratio calculated from fluorescence induction curves (see panel E).

we decided to test how IoR affects the properties of phycobiliproteins and OCP individually.

The absorption characteristic for PC gradually decreases under exposure to IoR (*Fig. S1A*). At 120 kGy, less than 5% of the initial absorption of PC remains in the region from 500 to 700 nm. Surprisingly, we found almost no change in fluorescence lifetime (data not shown), although fluorescence intensity decreased tenfold, which indicates that it was the concentration of chromophores that decreased. This is in line with the observed gradual decrease of the intact protein bands at 17 and 22 kDa [15] resolved by SDS-PAGE (*Fig. S1B*). We expected new bands on the gel, representing shorter fragments of peptide chains, or a gradual decrease of the molecular weight of PC polypeptides, but this was not the case. Most probably, the exposure stochastically disintegrates polypeptides, creating multiple protein forms which together represent a continuum ensemble non-detectable by Coomassie staining, instead of a set of discrete species like when a proteolytic degradation takes place. Thus, IoR reduces the concentration of PC in solution, but there was no evidence for accumulation of phycobilins released into solution or of denatured protein with phycobilins still bound and solvent-exposed. The bleaching of PC pigments upon exposure to IoR could be explained by two major reasons: (i) direct impact of α-particles and (ii) generation of ROS in traces of α-particles. Indeed, we observed similar gradual bleaching of PC in experiments in which ROS was generated by a photosensitizer (data not shown). However, even more interesting effect was found in experiments with OCP.

The OCP chromophore is also bleached upon generation of ROS *in vitro*, but during this process, the protein converts into a state with the red-shifted absorption spectrum (Fig. 3), typically accompanying its photoactivation process [42]. Upon illumination of OCP by the same red-light source in the absence of AlPCs as a photosensitizer, we observed no changes in the absorption spectrum (data not shown). However, in the presence of AlPCs, which are characterized by a high-yield ROS production [68], all absorption bands of OCP in the visible region of the spectrum gradually disappear (Fig. 3A). The changes in O.D. below 500 nm (*Fig. 2C*) could well be approximated by a single exponential decay component. At the red flange of OCP absorption, the time course is more complex and even exhibits a rising part at the beginning of the experiment. An O.D. increase in 500–600 nm spectral range is characteristic of the accumulation of the red active signaling state of OCP [55,66]. According to our results, a ROS-induced increase of O.D in 570 nm region is equivalent to 20% of changes which occur upon OCP photoactivation (Fig. 3B). Thus, ROS stress induces the transition of OCP from its stable orange state into the red form which by analogy with the well-studied red photoactivated OCP [52,69], is most likely capable of PBs binding and fluorescence quenching. Indeed, it is known that OCP could be activated "chemically" by high concentrations of chaotropes, for example, 1.5 M NaSCN [52,69], thus destabilization of the native OCP structure is expected to trigger conversion into the red state, equivalent to photoactivation. Along these lines, the nonspecific action of ROS can also destabilize and thereby activate OCP. This observation is important for the understanding of photoprotection in cyanobacteria since it shows that protective mechanisms could be activated not only by strong light but a variety of stresses in general, including ROS and cosmic radiation.

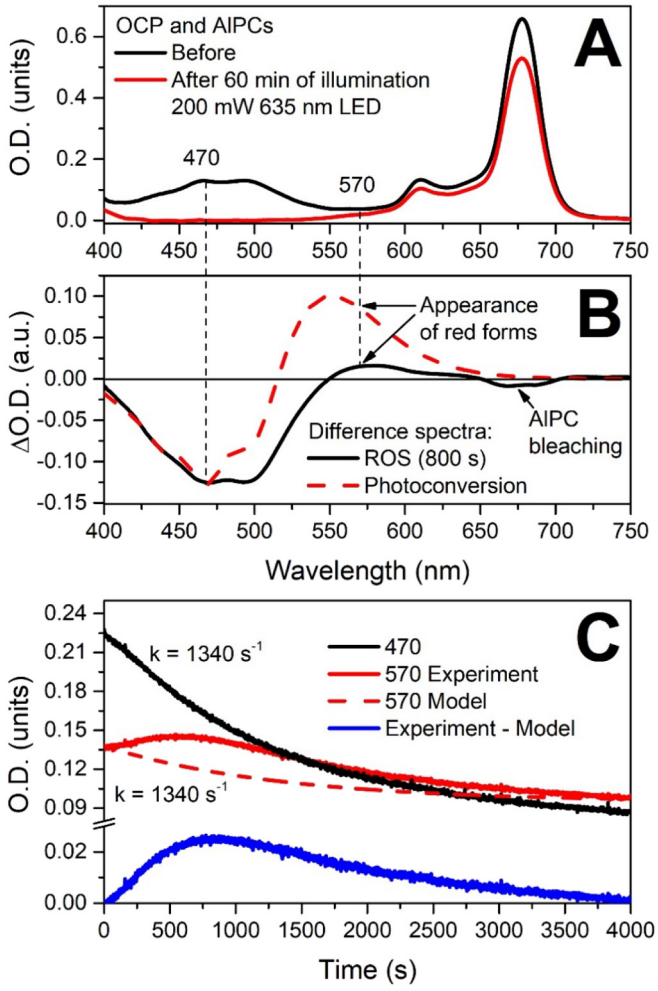

Fig. 3. Bleaching of the carotenoid chromophore of OCP under photoinduced ROS production by aluminum phthalocyanine (AlPC). (**A**) – Absorption spectrum of an OCP/AlPC mixture before and after illumination of the sample by red light triggering ROS production by AlPC. (**B**) – Difference spectrum indicating accumulation of red forms of OCP after 800 s of ROS production (black line) in comparison to photoactivation of OCP by actinic blue light (red dashed line). (**C**) – Kinetics of OCP bleaching, measured as changes of O.D. at 470 and 570 nm. Red dashed line shows how absorption of OCP would change at 570 nm if it was determined by bleaching with the same rate constant as at 470 nm. The blue curve represents kinetics of the red OCP accumulation (see text for details). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. PBs presence affects the viability of cyanobacteria but not the growth rate

In order to test our hypothesis about the radioprotective role of PBs, we exposed cells of WT *Synechocystis* and its mutants lacking PBs (CK and CK/ΔApcE) to 30 kGy of α-particles and further analyzed viability of the cultures by SYTOX Green *via* fluorescent microscopy and growing on solid media. Here we must note that cell death (lysis) immediately caused by absorption of α-particles and that followed by changes of viability are of completely different nature. The first is due to the damaging effects of ROS, while the second is a result of the DNA damage affecting the reproduction cycle. The difference in cell density on solid media is a combination of both effects and depends on the total number of viable cells, while fluorescent microscopy allows estimation of the fraction of the "damaged" culture which will be eliminated after several division cycles (*Fig. S3B*).

On solid BG-11 media, exposed cells of the mutants covered approximately the same space as a corresponding control sample, diluted 10 times (*Fig. S2A, Fig. S3A*), while the WT density was reduced only by half. This shows that mutants devoid of PBs are roughly 5 times more sensitive to ionizing radiation compared to WT *Synechocystis*. In the liquid medium, control samples and cells which survived after exposure to 30 kGy of α-particles had similar growth rates, although the photoautotrophic growth of CK/ΔApcE mutant was slightly slower compared to WT cells (*Fig. S2B*). Using SYTOX Green fluorescent dye and fluorescent microscopy, we found that the exposure to 30 kGy of α-particles causes a decrease in remaining cell viability (97 ± 3% in all control samples) to 76 ± 2% in WT, 46 ± 7% in the CK mutant and 76 ± 5% in CK/ΔApcE (*Fig. S3C*). As the main difference between the organization of photosynthetic membranes of the CK and CK/ΔApcE mutants is the connectivity of PBs with photosystem(s), which is lost in CK/ΔApcE, we can assume that the genetically induced detachment of PBs helped the CK/ΔApcE mutant to reduce negative effects of ionizing radiation on the cell cycle.

These observations support our suggestion that the presence and energetical coupling of large antennas with photosynthetic reaction centers could be crucial under stress conditions since PBs can be damaged and easily degraded and replaced (like S-layer proteins of *Deinococcus radiodurans*) preventing damage of DNA and other fragile cellular components, whereas under normal conditions, the main, but not obligatory role of PBs is light-harvesting.

4. Discussion

Both, the examination of the panspermia hypothesis and the exploration of possibilities for mankind to leave Earth and to send seeds of Life to other planets requires estimation of the influence of space flight conditions on major physiological functions and the viability of different species in general. Since relatively primitive life forms were able to successfully survive in harsh conditions during the early stages of Earth's history (and even today, see *e.g. Deinococcus radiodurans* [26]) and are considered as possible pioneers in colonization of other planets, their safe transportation is a challenge for future space missions. Among the stress factors, cosmic rays represent a major concern due to their ionizing ability. Thus, the evolution of the species preceding the formation of protective shields of the ozone layer and ionosphere obviously required the development of radioprotective mechanisms.

In this work, we analyzed the effects of space flight during the Foton-M4 mission and of direct irradiation by α-particles with energies of about 30 MeV equivalent to cosmic rays on the functional organization of the photosynthetic apparatus and primary stages of photosynthesis of the cyanobacterial strain *Synechocystis* sp. PCC6803. In order to study the action mechanism of irradiation with α-particles, we also performed experiments on the components of the photosynthetic apparatus at different levels of organization from whole cells over supercomplexes of light-harvesting proteins down to individual protein components. We found that the light-harvesting antennas - phycobilisomes - are extremely sensitive to IoR *in vitro*. The differences in effects observed *in vivo* and *in vitro* indicate that cyanobacteria (still) have general protection mechanisms and durability, which probably allowed them to survive and evolve during early stages of the Earth's history, long before the GOE. We found that *Synechocystis* sp. PCC6803 can uncouple PBs from photosynthetic membranes (*Fig. 2*), which could be the first step to prevent radiodamage and overexcitation of the photosystems. This event requires mobility of the PBs antennas, which is also observed during state transitions [66]; however, in this case, mobilization is rather triggered by ROS stress than by the cellular redox state. We also found signatures of OCP activation by ROS which may trigger OCP-dependent photoprotection under stress conditions. Such a combination of protective mechanisms allows to prevent damage to the photosystems and to keep PBs quenched while being detached, in order to prevent additional ROS production. Probably, water-soluble carotenoid-containing proteins from the OCP superfamily could be

involved in such regulatory processes. Some of the recently discovered types of helical carotenoid proteins (HCPs), which are homologs of the OCP N-terminal domain, do not interact with PBs and have no function assigned yet [43]. Also, at the moment it is completely unclear if HCP-induced PBs fluorescence quenching could be reversible and which system regulates this process. It is conceivable that HCPs could be remnants of ancient protective mechanisms, or that the site of their interaction with PBs is not available in the native state of the PBs. These hypotheses require more experimental scrutiny, since at the moment, even for the most comprehensively studied OCP from *Synechocystis*, the exact site of interaction with PBs remains unknown [70,71].

The production of huge amounts of proteins to sacrifice them in order to salvage DNA from damage is thought to be a general strategy for microorganisms to survive at high levels of IoR. Species like *Deinococcus radiodurans* are living proofs that this simple strategy works perfectly [72]. We can assume that extant cyanobacteria like *Synechocystis* inherited relics of this protective mechanism even in the absence of excessive IoR stress due to the involvement of PBs in photosynthesis, which gave them numerous other competitive advantages. It is not clear whether PBs had both radio- and, more general, ROS-protective as well as light-harvesting functions from the beginning since we know little about the evolution of PBs. However, we can postulate that in the absence of IoR as a selection factor both of these roles became advantageous, since both, optimization of light-harvesting and protection against ROS stress, are persistent issues for successful propagation of a species. Perhaps some of these questions could be answered by considering the reasons for the loss of PBs in most algae and all higher plants from the perspective of functional diversity of these mega-complexes.

After the space mission and exposure to high doses of IoR, *Synechocystis* sp. PCC6803 cells were able to grow autotrophically again and produced cultures with spectral and functional characteristics indistinguishable from the control samples. As approved space veterans, they are now introduced into the collection of microorganisms of Biological Faculty of Lomonosov Moscow State University.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

This work was supported by the Russian Foundation for Basic Research (projects 16-34-00394, 18-04-00554). This work was supported by Russian Ministry of Science (MK-951.2018.4).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbabio.2018.11.018.

References

[1] D. Morozova, R. Moeller, P. Rettberg, D. Wagner, Enhanced radiation resistance of *Methanosarcina soligelidi* SMA-21, a new methanogenic archaeon isolated from a siberian permafrost-affected soil in direct comparison to *Methanosarcina barkeri*, Astrobiology 15 (2015) 951–960.
[2] V. Beaumont, F. Robert, Nitrogen isotope ratios of kerogens in Precambrian cherts: a record of the evolution of atmosphere chemistry? Precambrian Res. 96 (1999) 63–82.
[3] A. Bekker, H.D. Holland, Oxygen overshoot and recovery during the early Paleoproterozoic, Earth Planet. Sci. Lett. 317-318 (2012) 295–304.
[4] A.L. Zerkle, S.W. Poulton, R.J. Newton, C. Mettam, M.W. Claire, A. Bekker, C.K. Junium, Onset of the aerobic nitrogen cycle during the Great Oxidation Event, Nature 542 (2017) 465.
[5] B.E. Schirrmeister, M. Gugger, P.C.J. Donoghue, Cyanobacteria and the Great Oxidation Event: evidence from genes and fossils, Palaeontology 58 (2015) 769–785.
[6] A.P. Nutman, V.C. Bennett, C.R.L. Friend, M.J. Van Kranendonk, A.R. Chivas, Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures, Nature 537 (2016) 535.
[7] N.Y. Kiang, A. Segura, G. Tinetti, R.E. Govindjee, M. Blankenship, J. Cohen, D. Siefert, V.S. Meadows Crisp, Spectral signatures of photosynthesis. II. Coevolution with other stars and the atmosphere on extrasolar worlds, Astrobiology 7 (2007) 252–274.
[8] M. Mamedov, Govindjee, A. Semenov, V. Nadtochenko, Primary electron transfer processes in photosynthetic reaction centers from oxygenic organisms, Photosynth. Res. 125 (2015) 51–63.
[9] H. Liu, H. Zhang, D.M. Niedzwiedzki, M. Prado, G. He, M.L. Gross, R.E. Blankenship, Phycobilisomes supply excitations to both photosystems in a megacomplex in cyanobacteria, Science 342 (2013) 1104–1107.
[10] M. Watanabe, M. Ikeuchi, Phycobilisome: architecture of a light-harvesting supercomplex, Photosynth. Res. 116 (2013) 265–276.
[11] D. Miao, W.L. Ding, B.Q. Zhao, L. Lu, Q.Z. Xu, H. Scheer, K.H. Zhao, Adapting photosynthesis to the near-infrared: non-covalent binding of phycocyanobilin provides an extreme spectral red-shift to phycobilisome core-membrane linker from Synechococcus sp. PCC7335, Biochim. Biophys. Acta 1857 (2016) 688–694.
[12] N. Adir, Elucidation of the molecular structures of components of the phycobilisome: reconstructing a giant, Photosynth. Res. 85 (2005) 15–32.
[13] E. Gantt, Phycobilisomes, Annu. Rev. Plant Physiol. 32 (1981) 327–347.
[14] A.N. Glazer, Light harvesting by phycobilisomes, Annu. Rev. Biophys. Biophys. Chem. 14 (1985) 47–77.
[15] R. MacColl, Cyanobacterial phycobilisomes, J. Struct. Biol. 124 (1998) 311–334.
[16] G. Bernat, U. Schreiber, E. Sendtko, I.N. Stadnichuk, S. Rexroth, M. Rogner, F. Koenig, Unique properties vs. common themes: the atypical cyanobacterium Gloeobacter violaceus PCC 7421 is capable of state transitions and blue-light-induced fluorescence quenching, Plant Cell Physiol. 53 (2012) 528–542.
[17] J. Mares, P. Hrouzek, R. Kana, S. Ventura, O. Strunecky, J. Komarek, The primitive thylakoid-less cyanobacterium gloeobacter is a common rock-dwelling organism, PLoS One 8 (2013) e66323.
[18] K. Tang, W.-L. Ding, A. Höppner, C. Zhao, L. Zhang, Y. Hontani, J.T.M. Kennis, W. Gärtner, H. Scheer, M. Zhou, K.-H. Zhao, The terminal phycobilisome emitter, LCM: a light-harvesting pigment with a phytochrome chromophore, Proc. Natl. Acad. Sci. 112 (2015) 15880–15885.
[19] J. Zhang, J. Ma, D. Liu, S. Qin, S. Sun, J. Zhao, S.-F. Sui, Structure of phycobilisome from the red alga Griffithsia pacifica, Nature 551 (2017) 57.
[20] N.K. Singh, R.R. Sonani, R.P. Rastogi, D. Madamwar, The phycobilisomes: an early requisite for efficient photosynthesis in cyanobacteria, EXCLI J. 14 (2015) 268–289.
[21] J.A. Raven, J.F. Allen, Genomics and chloroplast evolution: what did cyanobacteria do for plants? Genome Biol. 4 (2003) 209.
[22] D. Shevela Govindjee, Adventures with cyanobacteria: a personal perspective, Front. Plant Sci. 2 (2011) 28.
[23] R.P. Rastogi, A. Richa, M.B. Kumar, R.P. Sinha Tyagi, Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair, J. Nucleic Acids 2010 (2010).
[24] A. Oren, N. Gunde-Cimerman, Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol. Lett. 269 (2007) 1–10.
[25] I. Gomez, P. Huovinen, Induction of phlorotannins during UV exposure mitigates inhibition of photosynthesis and DNA damage in the kelp Lessonia nigrescens, Photochem. Photobiol. 86 (2010) 1056–1063.
[26] D. Farci, C. Slavov, E. Tramontano, D. Piano, The S-layer protein DR_2577 binds deinoxanthin and under desiccation conditions protects against UV-radiation in *Deinococcus radiodurans*, Front. Microbiol. 7 (2016).
[27] G. Ajlani, C. Vernotte, Construction and characterization of a phycobiliprotein-less mutant of *Synechocystis* sp. PCC 6803, Plant Mol. Biol. 37 (1998) 577–580.
[28] F. Puente-Sánchez, A. Arce-Rodríguez, M. Oggerin, M. García-Villadangos, M. Moreno-Paz, Y. Blanco, N. Rodríguez, L. Bird, S.A. Lincoln, F. Tornos, O. Prieto-Ballesteros, K.H. Freeman, D.H. Pieper, K.N. Timmis, R. Amils, V. Parro, Viable cyanobacteria in the deep continental subsurface, Proc. Natl. Acad. Sci. 115 (2018) 10702–10707.
[29] G.C. Papageorgiou, R. Govindjee, M. Govindjee, K. Mimuro, A. Stamatakis, N. Murata Alygizaki-Zorba, Light-induced and osmotically-induced changes in chlorophyll a fluorescence in two *Synechocystis* sp. PCC 6803 strains that differ in membrane lipid unsaturation, Photosynth. Res. 59 (1999) 125–136.
[30] J.F.A. Conrad, W. Mullineaux, Fluorescence induction transients indicate dissociation of Photosystem II from the phycobilisome during the State-2 transition in the cyanobacterium Synechococcus 6301, Biochim. Biophys. Acta 934 (1988) 96–107.
[31] H.-B. Mao, G.-F. Li, X. Ruan, Q.-Y. Wu, Y.-D. Gong, X.-F. Zhang, N.-M. Zhao, The redox state of plastoquinone pool regulates state transitions via cytochrome b6f complex in *Synechocystis* sp. PCC 6803, FEBS Lett. 519 (2002) 82–86.
[32] C.W. Mullineaux, D. Emlyn-Jones, State transitions: an example of acclimation to low-light stress, J. Exp. Bot. 56 (2005) 389–393.
[33] D. Kirilovsky, Modulating energy arriving at photochemical reaction centers: orange carotenoid protein-related photoprotection and state transitions, Photosynth. Res. 126 (2015) 3–17.
[34] M.G. Rakhimberdieva, I.N. Stadnichuk, I.V. Elanskaya, N.V. Karapetyan, Carotenoid-induced quenching of the phycobilisome fluorescence in photosystem II-deficient mutant of *Synechocystis* sp, FEBS Lett. 574 (2004) 85–88.
[35] A. Wilson, G. Ajlani, J.-M. Verbavatz, I. Vass, C.A. Kerfeld, D. Kirilovsky, A. Soluble Carotenoid, Protein involved in phycobilisome-related energy dissipation in cyanobacteria, Plant Cell 18 (2006) 992–1007.
[36] D. Kirilovsky, Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism, Photosynth. Res. 93

(2007) 7–16.
[37] N.V. Karapetyan, Protective dissipation of excess absorbed energy by photosynthetic apparatus of cyanobacteria: role of antenna terminal emitters, Photosynth. Res. 97 (2008) 195–204.
[38] E.G. Maksimov, F.J. Schmitt, E.A. Shirshin, M.D. Svirin, I.V. Elanskaya, T. Friedrich, V.V. Fadeev, V.Z. Paschenko, A.B. Rubin, The time course of non-photochemical quenching in phycobilisomes of *Synechocystis* sp. PCC6803 as revealed by picosecond time-resolved fluorimetry, Biochim. Biophys. Acta 1837 (2014) 1540–1547.
[39] E.E. Ostroumov, Y.R. Khan, G.D. Scholes, Govindjee, B. Demmig-Adams, G. Garab, W. Adams Iii, Photophysics of photosynthetic pigment-protein complexes, in: Govindjee (Ed.), Non-photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria, Springer Netherlands, Place Published, 2014, pp. 97–128.
[40] G.C. Papageorgiou, Govindjee, The non-photochemical quenching of the electronically excited state of chlorophyll a in plants: definitions, timelines, viewpoints, open questions, in: B. Demmig-Adams, G. Garab, W. Adams, IiiGovindjee (Eds.), Non-photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria, Springer Netherlands, Place Published, 2014, pp. 1–44.
[41] M. Gwizdala, A. Wilson, D. Kirilovsky, In vitro reconstitution of the cyanobacterial photoprotective mechanism mediated by the Orange Carotenoid Protein in *Synechocystis* PCC 6803, Plant Cell 23 (2011) 2631–2643.
[42] E.G. Maksimov, E.A. Shirshin, N.N. Sluchanko, D.V. Zlenko, E.Y. Parshina, G.V. Tsoraev, K.E. Klementiev, G.S. Budylin, F.J. Schmitt, T. Friedrich, V.V. Fadeev, V.Z. Paschenko, A.B. Rubin, The signaling state of orange carotenoid protein, Biophys. J. 109 (2015) 595–607.
[43] H. Bao, M.R. Melnicki, E.G. Pawlowski, M. Sutter, M. Agostoni, S. Lechno-Yossef, F. Cai, B.L. Montgomery, C.A. Kerfeld, Additional families of orange carotenoid proteins in the photoprotective system of cyanobacteria, Nat Plants 3 (2017) 17089.
[44] H. Bao, M.R. Melnicki, C.A. Kerfeld, Structure and functions of Orange Carotenoid Protein homologs in cyanobacteria, Curr. Opin. Plant Biol. 37 (2017) 1–9.
[45] F. Muzzopappa, A. Wilson, V. Yogarajah, S. Cot, F. Perreau, C. Montigny, C. Bourcier De Carbon, D. Kirilovsky, Paralogs of the C-terminal domain of the cyanobacterial orange carotenoid protein are carotenoid donors to helical carotenoid proteins, Plant Physiol. 175 (2017) 1283–1303.
[46] M. Moldenhauer, N.N. Sluchanko, D. Buhrke, D.V. Zlenko, N.N. Tavraz, F.-J. Schmitt, P. Hildebrandt, E.G. Maksimov, T. Friedrich, Assembly of photoactive orange carotenoid protein from its domains unravels a carotenoid shuttle mechanism, Photosynth. Res. (2017) 1–15.
[47] E.G. Maksimov, N.N. Sluchanko, Y.B. Slonimskiy, K.S. Mironov, K.E. Klementiev, M. Moldenhauer, T. Friedrich, D.A. Los, V.Z. Paschenko, A.B. Rubin, The unique protein-to-protein carotenoid transfer mechanism, Biophys. J. (2017), https://doi.org/10.1016/j.bpj.2017.1006.1002.
[48] K.G. Ivanova, K.G. Stankova, V.N. Nikolov, R.T. Georgieva, K.M. Minkova, L.G. Gigova, I.T. Rupova, R.N. Boteva, The biliprotein C-phycocyanin modulates the early radiation response: a pilot study, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 695 (2010) 40–45.
[49] A. Slobodkin, S. Gavrilov, V. Ionov, V. Iliyin, Spore-forming thermophilic bacterium within artificial meteorite survives entry into the earth's atmosphere on FOTON-M4 satellite landing module, PLoS One 10 (2015) e0132611.
[50] V.B. Ponizovskaya, M.Y. Dyakov, A.B. Antropova, E.N. Bilanenko, V.L. Mokeeva, V.K. Ilyin, The survival of micromycetes exposed to space conditions, Mosc. Univ. Biol. Sci. Bull. 72 (2017) 6–12.
[51] N.N. Sluchanko, K.E. Klementiev, E.A. Shirshin, G.V. Tsoraev, T. Friedrich, E.G. Maksimov, The purple Trp288Ala mutant of *Synechocystis* OCP persistently quenches phycobilisome fluorescence and tightly interacts with FRP, Biochim. Biophys. Acta 1858 (2017) 1–11.
[52] E.G. Maksimov, M. Moldenhauer, E.A. Shirshin, E.A. Parshina, N.N. Sluchanko, K.E. Klementiev, G.V. Tsoraev, N.N. Tavraz, M. Willoweit, F.-J. Schmitt, J. Breitenbach, G. Sandmann, V.Z. Paschenko, T. Friedrich, A.B. Rubin, A comparative study of three signaling forms of the orange carotenoid protein, Photosynth. Res. 130 (2016) 389–401.
[53] E. Fermi, On the origin of the cosmic radiation, Phys. Rev. 75 (1949) 1169–1174.
[54] M. Ralf, R. Marina, L. Stefan, B. Thomas, H.C. Elisabeth, F. Akira, O. Ryuichi, H. Gerda, a.t.S.R. Group, STARLIFE—an international campaign to study the role of galactic cosmic radiation in astrobiological model systems, Astrobiology 17 (2017) 101–109.
[55] E.G. Maksimov, N.N. Sluchanko, Y.B. Slonimskiy, E.A. Slutskaya, A.V. Stepanov, A.M. Argentova-Stevens, E.A. Shirshin, G.V. Tsoraev, K.E. Klementiev, O.V. Slatinskaya, E.P. Lukashev, T. Friedrich, V.Z. Paschenko, A.B. Rubin, The photocycle of orange carotenoid protein conceals distinct intermediates and asynchronous changes in the carotenoid and protein components, Sci. Rep. 7 (2017) 15548.
[56] E.G. Maksimov, K.S. Mironov, M.S. Trofimova, N.L. Nechaeva, D.A. Todorenko, K.E. Klementiev, G.V. Tsoraev, E.V. Tyutyaev, A.A. Zorina, P.V. Feduraev, S.I. Allakhverdiev, V.Z. Paschenko, D.A. Los, Membrane fluidity controls redox-regulated cold stress responses in cyanobacteria, Photosynth. Res. 133 (2017) 215–223.
[57] A. Stirbet, Govindjee, Chlorophyll a fluorescence induction: a personal perspective of the thermal phase, the J-I-P rise, Photosynth. Res. 113 (2012) 15–61.
[58] R. Kana, O. Prasil, O. Komarek, G.C. Papageorgiou, Govindjee, Spectral characteristic of fluorescence induction in a model cyanobacterium, Synechococcus sp. (PCC 7942), Biochim. Biophys. Acta 1787 (2009) 1170–1178.
[59] C. Richaud, G. Zabulon, A. Joder, J.-C. Thomas, Nitrogen or sulfur starvation differentially affects phycobilisome degradation and expression of the nblA Gene in *Synechocystis* strain PCC 6803, J. Bacteriol. 183 (2001) 2989–2994.
[60] A. Baier, W. Winkler, T. Korte, W. Lockau, A. Karradt, Degradation of phycobilisomes in *Synechocystis* sp. PCC6803: evidence for essential formation of an NblA1/NblA2 heterodimer and its codegradation by a Clp protease complex, The J. Biol. Chem., 289 (2014) 11755–11766.
[61] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Springer Science & Business Media, Place Published, 2013.
[62] L.R. Dartnell, M.C. Storrie-Lombardi, C.W. Mullineaux, A.V. Ruban, G. Wright, A.D. Griffiths, J.P. Muller, J.M. Ward, Degradation of cyanobacterial biosignatures by ionizing radiation, Astrobiology 11 (2011) 997–1016.
[63] R.H. Goldsmith, W.E. Moerner, Watching conformational- and photo-dynamics of single fluorescent proteins in solution, Nat. Chem. 2 (2010) 179–186.
[64] E.G. Maksimov, F.J. Schmitt, P. Hätti, K.E. Klementiev, V.Z. Paschenko, G. Renger, A.B. Rubin, Anomalous temperature dependence of the fluorescence lifetime of phycobiliproteins, Laser Phys. Lett. 10 (2013) 055602.
[65] E.G. Maksimov, K.E. Klementiev, E.A. Shirshin, G.V. Tsoraev, I.V. Elanskaya, V.Z. Paschenko, Features of temporal behavior of fluorescence recovery in *Synechocystis* sp. PCC6803, Photosynth. Res. 125 (2015) 167–178.
[66] D. Kirilovsky, C.A. Kerfeld, Cyanobacterial photoprotection by the orange carotenoid protein, Nature Plants 2 (2016) 16180.
[67] E.A. Shirshin, E.E. Nikonova, F.I. Kuzminov, N.N. Sluchanko, I.V. Elanskaya, M.Y. Gorbunov, V.V. Fadeev, T. Friedrich, E.G. Maksimov, Biophysical modeling of in vitro and in vivo processes underlying regulated photoprotective mechanism in cyanobacteria, Photosynth. Res. 133 (1–3) (2017) 261–271.
[68] E.G. Maksimov, D.A. Gvozdev, M.G. Strakhovskaya, V.Z. Paschenko, Hybrid structures of polycationic aluminum phthalocyanines and quantum dots, Biochem. Mosc. 80 (2015) 323–331.
[69] J.D. King, H. Liu, G. He, G.S. Orf, R.E. Blankenship, Chemical activation of the cyanobacterial orange carotenoid protein, FEBS Lett. 588 (2014) 4561–4565.
[70] L. Tian, I.H. van Stokkum, R.B. Koehorst, A. Jongerius, D. Kirilovsky, H. van Amerongen, Site, rate, and mechanism of photoprotective quenching in cyanobacteria, J. Am. Chem. Soc. 133 (2011) 18304–18311.
[71] I.N. Stadnichuk, M.F. Yanyushin, E.G. Maksimov, E.P. Lukashev, S.K. Zharmukhamedov, I.V. Elanskaya, V.Z. Paschenko, Site of non-photochemical quenching of the phycobilisome by orange carotenoid protein in the cyanobacterium *Synechocystis* sp. PCC 6803, Biochim. Biophys. Acta 1817 (2012) 1436–1445.
[72] M.J. Daly, *Deinococcus* prospects, Nat. Rev. Microbiol. 7 (2009) 476.