Heterocyst-specific flavodiiron protein Flv3B enables oxic diazotrophic growth of the filamentous cyanobacterium Anabaena sp. PCC 7120

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Flavodiiron proteins (FDPs, also called A-type flavoproteins) belong to a large family of proteins originally discovered and investigated in strict or facultative anaerobic bacteria, archaea, and some eukaryotic parasites (1). They have been proposed to help anaerobic species in coping with oxidative and/or nitrosative stress and to play an important role in maintaining the anaerobic metabolism. Homologs of genes encoding FDPs were later found in the genomes of oxygenic photosynthetic organisms: cyanobacteria, green algae, mosses, and lycophytes (2). Initial in vitro studies with recombinant Flv3 protein from Synechocystis provided evidence that it may function as an NAD(P)H oxidoreductase reducing O2 directly to water (4). Afterward, Helman et al. (7) demonstrated that the Δflv1 and Δflv3 mutants lack the light-induced O2 uptake and proposed that Flv1 and Flv3 reduce molecular O2 to water with NADPH produced on the acceptor side of PS I without formation of reactive oxygen species (ROS). Under certain conditions, up to 60% of electrons originating from water-splitting PS II could be forwarded to O2 via Flv1 and Flv3 proteins (8). The importance of Flv1 and Flv3 for the survival of cyanobacteria was unambiguously proven only recently by application of fluctuating light to mimic the constantly changing natural illumination conditions in aquatic environments (9).

We have demonstrated the existence of two “extra” genes that represent copies of flv1 and flv3 in Anabaena sp. strain PCC 7120 (hereafter Anabaena), a model filamentous N2-fixing, heterocyst-forming cyanobacterium. We have detected in the genome of Anabaena the following flv genes: flv1A (all3891), flv1B (all0177),

Significance

Cyanobacterial flavodiiron proteins (FDPs) comprise a protein family with unique modular structure and photoprotective functions in an oxygenic environment. It is conceivable that FDPs have made the development of oxygenic photosynthesis possible in cyanobacteria. Here, we report the ability of specific FDPs to reduce O2 directly to water in heterocyst-forming filamentous cyanobacteria, not only to support the photosynthetic machinery, but also to prevent oxidative damage of the N2-fixing enzyme nitrogenase. Whilst in the ancient environment, N2 fixation was secured from O2 inhibition, the later increase of atmospheric O2 may have initiated an important role for FDP-mediated protection of nitrogenase in maintaining the N2-fixing activity of cyanobacteria.


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flv2 (all4444), flv3A (all3895), flv3B (all0178), and flv4 (all4446) (10). *Anabaena* forms long filaments comprised only of vegetative cells when grown in medium containing nitrate or ammonium. In the absence of combined nitrogen, some vegetative cells differentiate into heterocysts, cells with specific morphology and metabolism providing the conditions for fixation of atmospheric N₂ (11). Oxygenic photosynthesis in *N₂*-fixing filaments is restricted to vegetative cells. Heterocysts, in contrast, bear an extra, O₂-impermeable envelope with glycolipid and polysaccharide layers outside of the outer membrane, so that diffusion of gases, including N₂, occurs mainly through terminal pores connecting heterocysts and vegetative cells (12). Vegetative cells also supply heterocysts with energy for N₂ fixation, mainly in the form of sucrose, whereas heterocysts, in turn, provide the whole filament with fixed nitrogen.

An obligatory condition for the activity of nitrogenase, the key enzyme in N₂ fixation, is a low partial pressure of O₂ inside the cells (13). O₂ in heterocysts is thought to be eliminated immediately by respiratory complexes (12). Two clusters encoding respiratory terminal oxidases, cox2 and cox3, were found to be expressed specifically in heterocysts and shown to be essential for diazotrophic growth in *Anabaena* (14, 15). Additionally, several other systems of protection against O₂ and ROS were recently found to be required to maintain the activity of nitrogenase or diazotrophic growth, emphasizing the ultimate importance of microoxic conditions for proper functioning of the *N₂*-fixing machinery (16–19).

Fluorescent protein tagging revealed that the duplicates of Flv1 and Flv3 are spatially segregated in the filaments of *Anabaena* (10). Flv1A and Flv3A were detected only in vegetative cells, whereas Flv1B and Flv3B were expressed only after combined nitrogen stepdown and were found exclusively in heterocysts. Flv1B and Flv3B could possibly form an operon, and the expression of Flv3B may be activated by NtcA, a global regulator of heterocyst differentiation (20, 21). Here, we investigate *Anabaena* strains lacking the heterocyst-specific flavodiiron proteins and demonstrate the existence of Flv-mediated light-induced O₂ uptake inside heterocysts, which is important for N₂ fixation and diazotrophic growth of this filamentous cyanobacterium.

**Results**

**Growth Phenotype of the Mutants Lacking Heterocyst-Specific FDPs.**

To clarify the roles of Flv1B and Flv3B in heterocysts, we constructed mutant strains Δflv1B and Δflv3B, as well as the double mutant Δflv1B/Δflv3B (Fig. S1 and Table S1). In the presence of combined nitrogen, wild-type (WT) and mutant strains grew similarly (Fig. 1A, +N). Next, N₂ prototrophy of the mutants was addressed by shifting the nitrate-grown filaments to nitrate-free medium. Whereas the Δflv1B mutant grew similarly to WT cells, the strains lacking Flv3B protein demonstrated approximately 50% reduced growth rate (0.40 ± 0.06, P < 0.001) compared with WT (0.79 ± 0.08) based on OD₅₇₀ measurements (Fig. 1A, -N). Additionally, Flv3B-depleted strains had approximately 50% lower chlorophyll (Chl) a and protein ratio compared with OD₅₇₀ after 4 d in N₂-fixing conditions (Fig. S2). To test the role of O₂ in slowing growth in the absence of Flv3B, the growth of the Δflv3B mutant was next examined in the absence of O₂, i.e., while flushing with a gas mixture of 99.96% N₂ and 0.04% CO₂. The O₂-depleted atmosphere rescued the growth of the Δflv3B mutant in N₂-fixing conditions similar to that of the WT (Fig. 1B). Furthermore, the protein amounts of the nitrogenase subunit NifH were similar in the mutant and the WT in microoxic conditions (Fig. 1B, Inset), which is in contrast to heavily reduced amount of NifH in the Δflv3B mutant underoxic conditions (Fig. 1A, Inset).

To test for possible modifications in the morphology of the mutant strains, the phenotype of the filaments under N₂-fixing conditions in oxic environment was investigated in more detail.

Bright field microscopy revealed that single and double mutant strains lacking Flv3B consisted of only short pale-green chains of vegetative cells, typically with one or two terminal heterocysts, whereas the Δflv1B strain had long filaments similar to the WT in color and heterocyst content (Fig. 1C and Table S2). Simultaneous monitoring of Chl a autofluorescence from WT and Δflv3B mutant filaments confirmed that vegetative cells of the mutant contained lower amounts and uneven distribution of Chl a (Fig. 1D).

**Gas Exchange in Filaments and Heterocysts of WT and the flv Mutants.**

We previously demonstrated that Flv1A and Flv3A proteins, located exclusively in vegetative cells, are essential for the growth of *Anabaena* under fluctuating light (9). Importantly, after nitrogen stepdown, under both the steady-state (Fig. 1A) and fluctuating light conditions (Fig. S3), the growth of Δflv1B did not differ from that of the WT. However, the Δflv3B mutant had approximately 50% lower growth rates under both light conditions compared with WT cells. This observation suggested a different role for the Flv1B and Flv3B proteins in heterocysts from that of the Flv1A and Flv3A in vegetative cells under fluctuating light conditions. The next question to address was whether Flv1B and Flv3B, like their homologs Flv1A and Flv3A, are involved in O₂ photoreduction. To this end, membrane inlet mass spectrometry (MIMS) was applied to monitor online gas exchange from the whole filaments incubated in N₂-fixing conditions. The method allows simultaneous measurement of the amount of naturally abundant ^16O₂ evolved by PS II and the heavy isotope ^18O₂ injected into the experimental medium before the analysis, thus making it possible to specifically distinguish the O₂ uptake.

As demonstrated by the MIMS gas exchange measurements (Fig. 2A and Table 1), illumination of the dark-adapted WT filaments with white light strongly stimulated the rate of O₂ uptake (69.5 μmol-[mg of Chl a]⁻¹·h⁻¹), hereafter rates are average; details in Table 1) compared with that in darkness (respiration, 20.3 μmol-[mg of Chl a]⁻¹·h⁻¹). The fraction of O₂ consumption during illumination, after subtraction of the dark O₂ uptake rate, is referred to as light-induced O₂ uptake (49.1 μmol-[mg of Chl a]⁻¹·h⁻¹). In contrast
to the WT, both Δflv3B and Δflv1B/3B mutants in darkness consumed more O₂ (32.4 and 30.4 μmol·[mg of Chl a]⁻¹·h⁻¹), but upon transfer to the light showed less light-induced O₂ uptake (21.8 and 25.4 μmol·[mg of Chl a]⁻¹·h⁻¹). The Δflv1B mutant showed dark respiration and light-induced O₂ uptake rates similar to those of WT.

To specifically address the O₂ exchange reactions in heterocysts, we isolated heterocysts from WT and mutants and checked their ability to take up O₂ by using a Clark-type O₂ electrode. WT heterocysts demonstrated a dark-respiration rate of 3.4 μmol·[mg of Chl a]⁻¹·h⁻¹ and also a substantial light-induced O₂ uptake (2.2 μmol·[mg of Chl a]⁻¹·h⁻¹; Fig. 2B). The origin of the light-induced O₂ uptake was tested by supplementation with well-known inhibitors of respiratory chain components. Incubation of WT heterocysts with potassium cyanide (KCN), salicylhydroxamic acid (SHAM), or antimycin A resulted in significant inhibition of dark O₂ uptake, whereas the light-induced O₂ uptake was stimulated by the presence of these inhibitors (Fig. 2B and Table 1). The Δflv1B heterocysts exhibited a 44% higher dark O₂ uptake rate than the WT, whereas the light-induced O₂ uptake rate was not significantly different from that in the WT. In contrast to Δflv1B, the heterocysts of the Δflv3B and Δflv1B/3B mutants showed notably higher rates (approximately twofold) of dark respiration than the WT, whereas light-induced O₂ uptake was completely absent (Fig. 2C and Table 1).

Light-saturated photosynthesis was measured by MIMS as O₂ evolution from whole filaments incubated in the absence of combined nitrogen. The WT and the Δflv1B mutant showed comparable rates of net O₂ evolution (approximately 118 μmol·[mg of Chl a]⁻¹·h⁻¹), whereas in the Δflv3B strain, the rate was reduced by 25% (Table 1). Simultaneous analysis of CO₂ uptake in the presence of 10 mM NaHCO₃ revealed significantly lower light-induced CO₂ uptake in mutants lacking Flv3B compared with the WT and Δflv1B (Table 1). As to the yields of PS I and PS II, the values in the Δflv3B mutant under normal growth light conditions were similar to those of the WT, but upon increasing light intensity, the yields of PS I and PS II dropped more rapidly in Δflv3B than in the WT (Fig. S4).

### Lack of Heterocyst-Specific Protein Flv3B Modulates the Transcriptome of Whole Filaments

To get insights into the role of the Flv1B and Flv3B proteins in cellular metabolism, we next compared the gene expression profiles of the WT and the Δflv1B, Δflv3B, and Δflv1B/3B mutant strains. To identify the genes with differential expression, we first isolated total RNA from whole filaments incubated under N₂-fixing conditions and subjected it to RNA sequencing. The genes with prominent differences in expression between the strains were then subjected to more precise transcript analysis by quantitative real-time PCR (RT-q-PCR). In Fig. 3, the RT-q-PCR results are presented as a ratio of the gene transcript abundance in each mutant over the transcript abundance in the WT.

The filaments of both mutants lacking Flv3B (the Δflv3B and Δflv1B/3B mutants) demonstrated similar changes in the transcript profile in comparison with the WT. Notably, we observed decreased amounts of nifH1 mRNA (0.54 ± 0.13, hereafter data indicated is only for the Δflv3B mutant), but elevated transcript abundance of nifH2 (2.09 ± 0.68). The latter gene represents a copy of nifH1 with nearly identical sequence but is not accompanied by copies of nifD and nifK genes. The physiological role of nifH2 is not clear; however, it might be involved in biosynthesis of the Fe-Mo cofactor of nitrogenase (22). The transcript abundance of heterocyst-specific ferredoxin, considered to be a donor of electrons for nitrogenase (23), was also up-regulated in Flv3B-deficient mutants (flxH, 1.73 ± 0.13). Importantly, genes encoding proteins involved in O₂ and H₂O₂ reduction were significantly up-regulated in the absence of Flv3B under N₂-fixing conditions (coxA3, 3.47 ± 1.58; rbcA, 1.98 ± 0.47; Table 1).
lox, 2.34 ± 0.28; katB, 3.88 ± 1.01). On the contrary, a specific group of photosynthesis-related genes was down-regulated (psbB1, 0.53 ± 0.01; psbA1, 0.67 ± 0.05; rbcL, 0.53 ± 0.02; fb3A, 0.51 ± 0.15), and the most prominent drop in transcript level was observed for genes encoding components of phycobilisomes (PBS): both the core (apcA, 0.53 ± 0.06) and the rods (pecA, 0.19 ± 0.01). However, the transcript amount of fh4, a homolog of the gene encoding the flavodiiron protein that is specifically connected to PS II and PBS in photoprotection of Synechocystis (6), was higher in the mutants lacking Flv3B than in WT (2.54 ± 0.49).

In the Δflv1B mutant, the transcript data obtained on the level of whole N₂-fixing filaments demonstrated no prominent differences from that of the WT with regard to light harvesting, photosynthesis, and N₂ fixation-related genes. However, nifHΔ mutant was down-regulated compared with the WT (0.66 ± 0.26), whereas expression of the nifHΔ gene was higher (2.15 ± 0.21).

**Protein Composition of Heterocyst-Specific flv Mutants.** To track changes on the protein level, we compared Δflv1B, Δflv3B, and WT, using both the whole filaments incubated in the absence of combined nitrogen and heterocyst-enriched cell fractions. For protein comparison, the differential gel electrophoresis (DIGE) approach was taken to identify differentially abundant proteins (Fig. S5). The difference in expression of proteins between Δflv3B and WT is hereafter shown as a ratio of the relative protein amount in the mutant compared with the WT; a full list of proteins and ratio values are presented in Table S3. Whole filaments of the Δflv3B mutant completely lacked the PecA and PecC proteins, which are the phycoerythrocyanin component of PBS rods and its linker protein, respectively. The down-regulated proteins included the large subunit of ribulose bisphosphate carboxylase (RbcL, 0.6 ± 0.1), fructose bisphosphate aldolase (Fda II, 0.7 ± 0.1), glyceraldehyde-3-phosphate dehydrogenase (Gap2, 0.6 ± 0.0), d-fructose-1,6-bisphosphatase class 2/sedoheptulose-1,7-bisphosphatase (GlpX, 0.7 ± 0.0), thioredoxin reductase (TrxB, 0.6 ± 0.1), and several proteins involved in various biosynthesis pathways: sulfolipid biosynthesis protein (Akr1744), ketal-acid reductoisomerase (Ivc), protocellulohyde oxidoreductase (Por), and glucose-1-phosphate adenylytransferase (GlcC).

In the heterocyst fraction of Δflv3B, the most prominent decrease was observed for subunits of nitrogenase (NifH, 0.5 ± 0.1; NifD, 0.5 ± 0.1; NifK, 0.6 ± 0.0) and uptake hydrogenase (HupL, 0.3 ± 0.0). Further, down-regulation was recorded for GlpX (0.7 ± 0.1), 6-phosphogluconate dehydrogenase (6-PGD, 0.5 ± 0.1), aconitate hydratase (AcmB), TrxB, enzymes of Chl biosynthesis geranylgeranyl hydrogenase (ChlP) and Por, and several other proteins. As expected, heterocysts lacking Flv3B protein, but Flv1B was detected in a higher amount than in WT (3.3 ± 0.5). Among other up-regulated proteins, we identified Fda I (1.9 ± 0.1), transaldolase (Tal, 1.5 ± 0.0), succinate-semialdehyde dehydrogenase (1.8 ± 0.3), and alamine dehydrogenase (Ald, 3.5 ± 0.4). Likewise, two porins, Akr0834 and All4499, possibly involved in cell wall remodeling of heterocysts (11), were found in higher amounts in the Δflv3B mutant.

In contrast to the Δflv3B mutant, the Δflv1B strain, which did not differ much in growth characteristics from the WT (Fig. L4), demonstrated only subtle changes in protein amounts in both heterocysts and whole N₂-fixing filaments (Fig. S5 and Table S4). However, several proteins up-regulated in whole filaments of the Δflv1B mutant were Fda I, Fda II, and Gap2. PecA and 6-PGD showed decreased amounts in Δflv1B filaments. In heterocysts of the Δflv1B mutant, Flv1B was absent, as expected, but the amount of Flv3B was similar to that in the WT. Decreased amounts of HupL, TrxB, and 6-PGD were also observed in Δflv1B heterocysts, but the mutation did not have an effect on protein amounts of the nitrogenase subunits.

**Nitrogenase Activity of the Mutant Strains.** In oxic acetylene reduction assays, the WT showed a nitrogenase activity rate of 19.6 ± 0.2 μmol [mg of Chl]⁻¹ h⁻¹. All mutants studied had a significantly lower nitrogenase activity compared with the WT: 10.3 ± 4.1, 11.1 ± 4.6, and 8.9 ± 1.5 μmol [mg of Chl]⁻¹ h⁻¹ by Δflv1B, Δflv3B, and the double mutant, respectively (mean ± SD, n = 3, *P < 0.05").

**Discussion**

**Flavodiiron Protein Flv3B Is Responsible for Light-Induced O₂ Uptake in Heterocysts.** N₂ fixation in heterocysts is an energetically expensive process that requires large amounts of reducing equivalents and ATP, which are ultimately provided by photosynthesis occurring in vegetative cells during illumination. It is conceivable that the efficient diffusion of PS II-generated O₂ into the heterocyst occurs mainly through terminal pores according to the concentration gradient (12).

Besides respiratory O₂ uptake pathways, this extra O₂ might be taken in charge by the PS I-driven light-induced O₂ system in heterocysts (13). Such light-induced O₂ uptake of unknown nature has been reported and discussed in respect to protection of the nitrogenase activity against oxidative damage (24, 25). Here, we demonstrate O₂ uptake by isolated heterocysts in darkness (Fig. 2B), i.e., respiratory activity that has been postulated as a means to decrease partial O₂ pressure in heterocysts (12). We also show that O₂ uptake increases 1.6-fold in WT heterocysts upon a dark-to-light transition. Whereas dark O₂ uptake is inhibited by respiratory electron transfer inhibitors, light-induced O₂ uptake remains unaffected (Fig. 2B). However, light-induced O₂ uptake is completely eliminated by disruption of the flv3B gene (Fig. 2C). This unambiguously indicates that the flavodiiron protein Flv3B is responsible for O₂ photoreduction in heterocysts.

Flv3B-dependent O₂ reduction in heterocysts appears to be crucial for growth and photosynthesis of *Anabaena* filaments upon removal of combined nitrogen (Fig. 1). Only the Δflv3B mutant, but not Δflv1B, has lost the capability for light-induced O₂ uptake in heterocysts. In line with this observation, the presence of the Flv1B protein in Δflv3B heterocysts did not rescue the severe Δflv3B phenotype. We suggested earlier that flavodiiron proteins in cyanobacteria function as heterodimers, but in the case of heterocyst-specific FDPs, Flv3B is clearly capable of functioning independently. Therefore, the function of Flv1B remains unclear.
Extra FDP-coding genes are present in genomes of all heterocyst-forming cyanobacteria sequenced to date (10). Thus, Flv3B seems to have a unique role in O$_2$ quenching in heterocysts. It is dispensable for N$_2$ fixation-dependent growth when _Anabaena_ is incubated under microoxic conditions (Fig. 1B). However, its absence leads to strong growth impairment in oxic conditions, even if dark O$_2$ uptake appears stimulated in mutants. Therefore, Flv3B function cannot be replaced by up-regulation of common respiratory pathways, which likewise consume O$_2$. Although terminal oxidases are concentrated in the membranes near to cell junctions (26), Flv3B protein is equally distributed across the heterocysts (10), likely following the localization of the nitrogenase enzyme. By eliminating molecular O$_2$, Flv3B may participate in the control of the redox status of the cytosol and provide appropriate conditions for the function of nitrogenase, and probably many other enzymes, under illumination.

Possible Donors of Electrons for Flv3B. Identification of Flv3B as a responsible protein for light-induced O$_2$ uptake in heterocysts raises a question about its electron donor. NADPH and NADH are both known to donate electrons to Flv3B in vitro (4). However, the electron donor might also be ferredoxin, because Fdx, one of ferredoxins of _Chlamydomonas reinhardtii_, was recently found to interact with Flv3, a homolog of cyanobacterial-like flavodiron proteins (27). Current knowledge of heterocyst-specific metabolism does not allow us to make a direct distinction between possible electron donors to Flv3B. Nevertheless, it is known that heterocysts harbor only the “short” form of ferredoxin:NADPH oxidoreductase (FNR), i.e., the form that lacks the PBS-binding domain and is active in oxidation of NADPH, making it unlikely that NADP$^+$ is reduced on the reducing side of PS I in heterocysts (28). Therefore, it is conceivable that both NADPH and NADH are produced by glycolysis and oxidative pentose phosphate pathway (OPPPP) equally in darkness and under the light. As to ferredoxin serving as an electron donor to Flv3B, two heterocyst-specific ferredoxins are known in _Anabaena_. The fdxN gene within the main nif gene cluster encodes a “bacterial-type” ferredoxin (29). Although its role remains unclear, the disruption of fdxN did not affect the diazotrophic growth of _Anabaena variabilis_ (30). Another ferredoxin, encoded by fdxH, has been shown to function as a physical donor of electrons to nitrogenase in _Nostoc_, _Scenedesmus_, and _Chlorella_ (28). Although its role remains unclear, the disruption of fdxN did not affect the diazotrophic growth of _Anabaena variabilis_ (30). Another ferredoxin, encoded by fdxH, has been shown to function as a physical donor of electrons to nitrogenase in _Nostoc_, _Scenedesmus_, and _Chlorella_ (28). However, in the case of nitrogenase subunits, the lower protein amount might simply result from the transcript scarcity. Control of gene expression in heterocysts under steady-state conditions has not yet been thoroughly investigated. However, it is conceivable that once nitrogenase is active, nitrogen deprivation is no more an ultimate driving factor for modulating nitrogenase gene expression, and other mechanisms might take over. Unlike the direct transcriptional regulation by O$_2$ of the nitrogenase genes in nonphotosynthetic diazotrophs (34), the expression of _nifHDK_ in _Anabaena variabilis_ has been considered to be mainly subject to developmental control (35). In line with this idea, the WT level of _nif_ gene expression was observed in developing heterocysts of a double _nifHDK_ mutant of _Anabaena variabilis_ (15) despite the apparent resistance to light-induced nitrogenase down-regulation. However, we cannot exclude that regulation of gene expression in heterocysts is carried out by O$_2$ indirectly, through subsequent O$_2$ interactions in a cell. Indeed, O$_2$-induced modification of cytosolic redox status or degradation products of the nitrogenase protein might trigger a signaling cascade resulting in strong transcriptional changes of genes encoding nitrogenase, O$_2$ scavenging enzymes, and other proteins. In accordance with this notion, up-regulation of _coxA3_, part of an operon encoding a heterocyst-specific terminal oxidase; _lax_, which encodes lactate oxidase that is thought to reduce O$_2$; and genes encoding ruberythrin and Mn catalase, both reducing H$_2$O$_2$, were found in the mutants lacking Flv3B (Fig. 3). Lactate oxidase and ruberythrin have been shown to be important for diazotrophic growth and nitrogenase activity, respectively (17, 18).

Our data also demonstrate that Flv3B is crucial for protein composition in _Anabaena_. Many proteins were present in different amounts in the _Anabaena_ Flv3B mutant compared with WT, especially in the heterocyst-enriched fraction. The profound adjustment of protein composition likely allows the mutant to partially overcome the severe effects of the mutation and maintain a level of nitrogenase activity. Our findings reiterate the importance of mechanisms for gene and protein regulation in mature heterocysts that are yet to be investigated.

Concluding Remarks. Understanding the ultimate importance of flavodiron proteins for the well-being of cyanobacteria has emerged...
only during the past few years. It is likely that evolution of FDPs made the appearance of oxygenic photosynthesis possible by providing protection at multiple sites: the Flv2/Flv4 heterodimer protects against photodamage of PS I. Here, we demonstrate that a heterocyst-specific flavodiiron protein Flv3B is crucial for elimination of O₂ in illuminated heterocysts, thereby contributing to solve the long-lasting problem of O₂ protection of nitrogenase in the light. The ability of FDPs to transfer light-driven electrons to chloroplasts of microalgae. Photosynth Res 106(1-2):19-31.


