The Bidirectional NiFe-hydrogenase in *Synechocystis* sp. PCC 6803 Is Reduced by Flavodoxin and Ferredoxin and Is Essential under Mixotrophic, Nitrate-limiting Conditions*

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**Background:** Cyanobacterial hydrogenases are claimed to produce hydrogen via NAD(P)H, which contradicts thermodynamic considerations; the physiological function of these hydrogenases is unresolved.

**Results:** Flavodoxin/ferredoxin reduce cyanobacterial hydrogenases, which are essential under mixotrophic, nitrate-limiting conditions.

**Conclusion:** Cyanobacterial bidirectional hydrogenases are electron sinks for reduced flavodoxin/ferredoxin.

**Significance:** This study provides a basis for a target-oriented enhancement of hydrogen production and explains the aquatic distribution of cyanobacterial hydrogenases.

Cyanobacteria are able to use solar energy for the production of hydrogen. It is generally accepted that cyanobacterial NiFe-hydrogenases are reduced by NAD(P)H. This is in conflict with thermodynamic considerations, as the midpoint potentials of NAD(P)H do not suffice to support the measured hydrogen production under physiological conditions. We show that flavodoxin and ferredoxin directly reduce the bidirectional NiFe-hydrogenase of *Synechocystis* sp. PCC 6803 in vitro. A merodiploid ferredoxin-NADP reductase mutant produced correspondingly more photohydrogen. We furthermore found that the hydrogenase receives its electrons via pyruvate:flavodoxin/ferredoxin oxidoreductase (PFOR)-flavodoxin/ferredoxin under fermentative conditions, enabling the cells to gain ATP. These results strongly support that the bidirectional NiFe-hydrogenases in cyanobacteria function as electron sinks for low potential electrons from photosystem I and as a redox balancing device under fermentative conditions. However, the selective advantage of this enzyme is not known. No strong phenotype of mutants lacking the hydrogenase has been found. Because bidirectional hydrogenases are widespread in aquatic nutrient-rich environments that are capable of triggering phytoplankton blooms, we mimicked those conditions by growing cells in the presence of increased amounts of dissolved organic carbon and dissolved organic nitrogen. Under these conditions the hydrogenase was found to be essential. As these conditions close the two most important sinks for reduced flavodoxin/ferredoxin (CO₂-fixation and nitrate reduction), this discovery further substantiates the connection between flavodoxin/ferredoxin and the NiFe-hydrogenase.

Hydrogenases are enzymes that catalyze the reduction of protons to hydrogen and the reaction vice versa. Cyanobacterial bidirectional NiFe-hydrogenases belong to a group termed bidirectional NAD(P)-linked hydrogenases (1). They are made up of the five subunits HoxEFUYH. Whereas *hoxY* and *hoxH* code for the small and the large subunit of the hydrogenase module holding the active NiFe site, HoxEFU form the module interacting with NAD(P)H and mediating the electron transfer to the hydrogenase part. The physiological role of the cyanobacterial bidirectional NiFe-hydrogenase is still under debate. It was shown that the enzyme functions as an electron sink, storing surplus electrons coming from PSI² in the form of hydrogen (2, 3). The electrons were considered to take the route via PSI-ferredoxin-FNR-NADPH to the hydrogenase. The hydrogenase additionally helps to balance the redox poise under fermentative conditions (4) as e.g. observed at night in microbial mats when cells consume photosynthates that were accumulated during the previous day (5, 6). The wide distribution of cyanobacterial bidirectional hydrogenases in natural aquatic environments (7) is, however, still not fully understood, and until now no strong phenotype of mutants lacking the enzyme has been discovered.

The direct redox partner of the bidirectional NiFe-hydrogenase in cyanobacteria has remained ambiguous for 30 years. Multiple attempts to identify the electron donor to the enzyme were undertaken either with the purified hydrogenase or in cell-free extracts.

Artificial electron donors such as reduced methylviologen (MV) were early shown capable of transferring electrons to the purified enzyme (8, 9). When the bidirectional hydrogenase of the cyanobacterium *Synechocystis* sp. PCC 6803 (thereafter *Synechocystis*) was sequenced in 1996, the homology of *hoxEFU* to the NADH binding module of NDH-I pointed to NAD(P)H as the direct redox partner (10, 11). The first evidence that

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NAD(P)H is able to induce hydrogen production came from measurements in cell-free extracts (11) which was confirmed in other cell-free extracts later on (3, 12, 13). Attempts to reduce the purified hydrogenase with NAD(P)H either failed (8, 9) or resulted in hydrogen production at low rates (14, 15) whereas addition of reduced MV induced hydrogen evolution at high rates.

It was observed that pentameric HoxEFUYH hydrogenases tend to dissociate into their NAD(P)H binding module (HoxEFU) and hydrogenase (HoxYH) parts upon purification (15–17). Remarkably, the hydrogenase part alone has H₂-evolving activity with reduced MV (15, 18). Analyses concerning the stoichiometry of the purified hydrogenase in our laboratory revealed only substoichiometric levels of HoxE (19). HoxE deletion mutants are devoid of hydrogen production in vivo, and the enzyme no longer evolves hydrogen upon addition of NAD(P)H in cell-free extracts (13). Addition of reduced MV to cell-free extracts of HoxE deletion mutants, however, still induces hydrogen production, and the catalytic activity of their hydrogenases as measured by H-D exchange is unaltered (13). This shows that the catalytic activity of a purified hydrogenase as measured by MV does not allow any conclusions about the integrity of the holoenzyme. Therefore, activity measurements with redox partners on the purified enzyme need to be interpreted cautiously concerning statements about electron transport processes in vivo as long as the integrity of the enzyme was not verified. As the bidirectional hydrogenase might be membrane-bound with HoxE anchoring the enzyme, it might even be impossible to isolate this enzyme in its native form (14, 20, 21). To the best of our knowledge, reduced ferredoxin was only tested once as electron donor in an essay with a purified hydrogenase, where it was shown to be ineffective to induce H₂ production (14). Even though all five subunits were present in this assay, conclusions concerning the integrity of the holoenzyme cannot be made due to the lack of a thorough analysis of its stoichiometry.

Reduced ferredoxin was not tested in any of the cell-free extracts (3, 11–13). One exception is an assay in which a hydrogen-dependent NAD⁺ reduction was measured that was found to be enhanced upon the addition of oxidized ferredoxin, indicating that the hydrogenase might be able to reduce ferredoxin directly (22).

It has been shown lately that NADPH is a poor electron donor to the enzyme compared with NADH concerning H₂ production (12, 13, 15). This is in conflict with the documented function of the hydrogenase as electron sink storing surplus electrons coming from PSI via ferredoxin, FNR, and NADPH (2, 3).

In addition and most important, the midpoint potentials of NAD(P)H/NAD(P)⁺ (−320 mV) and flavodoxin/ferredoxin (−440 mV) compared with the H₂/H⁺ couple (−414 mV) indicate that an electron transfer from NAD(P)H to the hydrogenase is energetically unfavorable. This uphill reaction would demand high NADH/NAD⁺ ratios in the cells. If Synechocystis is grown autotrophically it produces approximately 12 μM H₂ under fermentative conditions (23). This corresponds to a redox potential of −360 mV and a ratio of NADH/NAD⁺ of 26. Depending on the growth conditions, much higher H₂ concentrations can be produced. Hydrogen concentrations produced by the bidirectional hydrogenase can be as high as 450 μM as reported for the cyanobacterium Lyngbya (24). To support this measured H₂ production with NADH alone, NADH/NAD⁺ ratios of close to 1000 would be necessary. This is far above the measured NADH/NAD⁺ ratios of 0.03 under heterotrophic conditions in Escherichia coli (25, 26) and of 0.09 – 0.2 in Clostridium acetobutylicum (27) or of 0.5 – 0.7 under fermentative conditions in E. coli and Synechococcus (26, 28). The NAD⁺ pool in living cells is generally substantially (95%) oxidized (25). NADH/NAD⁺ ratios of 1000 have never been measured and are not physiological as they would slow down metabolic flow to rates ultimately causing cell death (25). It is therefore difficult to reconcile the measured hydrogen production in cyanobacteria with a sole electron donation by NADH.

However, flavodoxin and ferredoxin have a more negative midpoint potential than NADH. Therefore, ratios of reduced/oxidized flavodoxin and ferredoxin of 0.1 – 0.3 would suffice to induce hydrogen production as measured for wild type cells (23). These ratios are certainly reached in living cells. Reduced flavodoxin/ferredoxin could thus be able to promote hydrogen production as electron donor under physiological conditions.

This prompted us to reinvestigate the direct redox partner of the hydrogenase in an in vitro hydrogen production assay in Synechocystis and to test our observations in vivo. Our results guided us to discover conditions under which the cyanobacterial NiFe-hydrogenase is essential for survival, which not only supports our enzymatic results in vivo but also provides an explanation for the distribution of cyanobacterial NiFe-hydrogenases in its natural aquatic environment.

**EXPERIMENTAL PROCEDURES**

**Hydrogenase Purification**—Hydrogenase was purified as described (19). Briefly, a mutant containing an N-terminal Strep tag fused to HoxF was harvested. After breaking the cells by a French press, an ammonium sulfate precipitation was performed with the soluble fraction. After spinning down the precipitate, the supernatant was directly applied to a Strep-Tactin column (IBA, Göttingen, Germany). The fractions containing the highest activity were pooled and concentrated by centrifugal filter units (Amicon Ultra, Merck Millipore) with a cut-off of 10 kDa.

**Preparation of Cell-free Homogenate**—For preparation of the homogenate, cells were harvested and broken as described (29) except that the buffer contained 100 mM Tris, pH 8.0, and 150 mM NaCl. Cells were broken by vortexing with glass beads (diameter 0.17 – 0.18 mm) three times for 3 min in the cold with intermittent cooling on ice. After centrifuging at 800 × g for 1 min at 4 °C, the liquid phase was centrifuged at 1300 × g for 10 min at 4 °C. The last step was repeated if the homogenate was not cell-free.

**Hydrogen and Hydrogenase Measurements**—Hydrogen was measured at the hydrogen electrode as described (23) except that 10 mM glucose, 40 units/ml glucose oxidase, and 50 units/ml catalase were used to induce anaerobiosis. Ferredoxins and flavodoxin were reduced by adding 10 μl of 100 mM sodium dithionite in 100 mM Tris, pH 8.0, 150 mM NaCl, 10 mM...
Regarded on 10 mM glucose, 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, and 5 μE light.

**Growth Conditions**—For hydrogen measurements, fnr/Δfnr mutant and WT were grown on BG-11 medium with 10 mM glucose for comparison. Conditions for growth measurements were as described (23, 30). If BG-11 medium was used it was supplemented with 5 mM arginine and 10 mM glucose. The cultures were grown in a light-dark (12-h:12-h) cycle. They were bubbled with air in the light. At the onset of darkness cultures were sparged 15 min with N₂ gas to induce anaerobiosis without further bubbling during the rest of the dark phase.

**RESULTS**

**Determination of the Redox Partner of the Bidirectional NiFe-hydrogenase in Vitro**—The hydrogenase of Synechocystis was purified as already described (19) and subjected to different electron donors. Hydrogen was only produced upon addition of dithionite-reduced MV, whereas all natural electron carriers as NADH, NADPH, reduced flavodoxin, and reduced ferredoxin failed. As analysis of the purified hydrogenase in our laboratory revealed stoichiometric levels of HoxE (19) we concluded

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**Table 1**

| Primer name       | Sequence                                    | Name used in this study |
|-------------------|---------------------------------------------|-------------------------|
| ssl0020-N         | CATGTTGAGCCACCCGAGATTTGAGAAGAAGAGGCTGATGTTTACGTTAATG    | fdx1                    |
| ssl0120-C         | GAATCTCTTCTCTTCCTCTCCGATCATGTTTACGTTAATG    | fdx2                    |
| sll1382-N         | CATGTTGAGGCAACCCGAGATTTGAGAAGAAGAGGCTGATGTTTACGTTAATG    | fdx3                    |
| sll1382-C         | GAATCTCTTCTCTTCCTCTCCGATCATGTTTACGTTAATG    | fdx4                    |
| slr0150-N         | CATGTTGAGCAGCCCCGAGATTTGAGAAGAAGAGGCTGATGTTTACGTTAATG    | flv                     |
| slr1828-N         | CATGTTGAGCAGCCCCGAGATTTGAGAAGAAGAGGCTGATGTTTACGTTAATG    |                         |
| sll1382-C         | GAATCTCTTCTCTTCCTCTCCGATCATGTTTACGTTAATG    |                         |

**Table 2**

| Primer name       | Sequence                                    | Resistance cassette used | Name of deletion construct |
|-------------------|---------------------------------------------|--------------------------|---------------------------|
| petHin1           | AGCGAGCAGATATAGACGCGGCAGATGGGTTTACT         | Gm                       | pFNR                      |
| petHin2           | GCGCTATTACCTCCGTCCGTTTGGTTTACTGGTGTTAATGGTGTTAT    | Em                       | pPFOR                     |
| petHout1          | CGGCTATTACCTCCGTCCGTTTGGTTTACTGGTGTTAATGGTGTTAT    | Sp                       | pFDX1                     |
| petHout2          | CCGCTATTACCTCCGTCCGTTTGGTTTACTGGTGTTAATGGTGTTAT    | Km                       | pFDX2                     |
| fdx1in1           | ATGGGAAACATTGCCGTTAATGGGTTTACTGGTGTTAATGGTGTTAT    | Cm                       | pFDX3                     |
| fdx1in2           | ATGGGAAACATTGCCGTTAATGGGTTTACTGGTGTTAATGGTGTTAT    | Em                       | pFDX4                     |
| fdx2in1           | ATGGGAAACATTGCCGTTAATGGGTTTACTGGTGTTAATGGTGTTAT    | Gm                       | pFLV                      |

**Construction of Mutants**—Constructs for the deletion mutation of nifJ encoding the pyruvate:ferredoxin/ferredoxin oxidoreductase (PFOR) (ssl0741), petH encoding the FNR (slr1643), the different ferredoxins (ssl0020, sll1382, slr0150, and slr1828), and isisI encoding the flavodoxin (sll0248) were made by a PCR fusion method as described (30). The primers used are shown in Table 2. The respective constructs were either cloned into pCR1-TOPO (Invitrogen) or pGEM-T (Promega). Mutants were checked by PCR and Southern hybridization (30). The mutant of the ferredoxin:NADP reductase (fnr/Δfnr) was segregated on 10 mM glucose, 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, and 5 μE light.
that the holoenzyme is not intact. Therefore, we decided to use cell-free extracts to measure the enzyme as closely as possible to its native form.

*Synechocystis* holds four different plant-like ferredoxins (Fdx1–4) and a flavodoxin (Flv). Flavodoxin and a ferredoxin (Fdx1–4) and a flavodoxin (Flv). Flavodoxin and a ferredoxin (Fdx1–4) and a flavodoxin (Flv) were overexpressed in *E. coli* and affinity-purified. As we did not succeed to overexpress Fdx1 and Fdx3 in sufficient quantities, Fdx1 from spinach was used.

Concentrations of 150 μM NAD(P)H or lower failed to induce measurable H₂ production in the cell-free extract. 1.5 mM NADPH did still not induce H₂ production whereas the addition of 1.5 mM NADH led to the production of H₂ that became already saturated at low levels (0.25 μM) (Fig. 1), being well in line with earlier studies (3, 11–13). Addition of Flv, Fdx3, and Fdx1 at concentrations of 3 orders of magnitude lower than NADH (Flv, 1.8 μM; Fdx3, 1.5 μM; Fdx1, 1.3 μM), however, led to the production of similar or higher rates and high levels of hydrogen without saturation in the measured time frame. Because dithionite was used to reduce these electron shuttles the hydrogen production was first measured in the presence of dithionite alone. The *Kₘ* of Flv in the cell homogenate was determined to 5.3 μM ± 3, and the *Kₘ* of Fdx3 was determined to 15 μM ± 6. These values are close to those measured for the flavodoxin of *C. acetobutylicum* with its FeFe-hydrogenase of 8.8 μM (31) and of ferredoxin with the NiFe-hydrogenase of *Methanococcus barkeri* of 7.5 μM (32) or ferredoxin with the FeFe-hydrogenase of the green alga *Chlamydomonas reinhardtii* of 21 μM (33). The higher H₂ production rate with flavodoxin as electron carrier found in our experiments is probably attributable to a higher turnover number of the flavodoxin-hydrogenase complex compared with the ferredoxin-hydrogenase complex.

Some FeFe-hydrogenases from anaerobic heterotrophic bacteria have recently been shown to be electron-bifurcating enzymes that strictly require both NADH and reduced ferredoxin for hydrogen production (34). We did not see a strict requirement of both substrates for H₂ production, but since a cell homogenate was used in our assay we cannot rule out that NAD(P)H has a stimulating effect on the electron transfer via flavodoxin/ferredoxin to the hydrogenase.

**Evidence for Flavodoxin/Ferredoxin as Electron Donor to the Bidirectional NiFe-hydrogenase in Vivo**—To verify these results *in vivo*, ferredoxin (ssl0020, sll1382, slr0150, slr1828) and flavodoxin (ssl0248) deletion mutants were constructed. According to earlier works, it was not possible to delete Fdx1 (*ssl0020*) from the genome (35). Probably due to the compensatory effect of Fdx1 and the remaining ferredoxins in the single mutants, hydrogen production was not significantly affected in any of them (data not shown).

As photohydrogen was claimed to be produced via ferredoxin-FNR-NADPH hydrogenase, a FNR mutant was constructed to interrupt the electron transfer from flavodoxin/ferredoxin at PSI to NADPH (see Fig. 3B). The cells were grown on glucose obtaining merodiploid mutants (Fig. 2A). A reduced expression of FNR was verified by immunoblotting (Fig. 2B).

Photohydrogen production was not diminished in the fnr/Δfnr mutant as expected (Fig. 3A). The mutant even showed an enhanced and prolonged photohydrogen production and did not consume all of the hydrogen produced. Dark-adapted WT cells react with a short burst of hydrogen production when
brought to light. The hydrogen is consumed by the cells as soon as the Calvin-Benson cycle is activated and accepts electrons. The prolonged photohydrogen production of the fnr/Δfnr mutant is thus well in line with the partly interrupted electron transfer from flavodoxin/ferredoxin via FNR to NADP and the Calvin-Benson cycle and confirms ferredoxin as a direct electron donor in vivo. This conclusion is further supported by the inability of NADPH to induce significant H₂ production (Fig. 1).

Besides Photohydrogen Production, Synechocystis Produces Hydrogen Fermentatively under Anaerobic Conditions—As hydrogenase-free mutants of Synechocystis were not found to exhibit a strong phenotype it was repeatedly supposed that bidirectional hydrogenases are primarily relics of early history of Earth. The wide distribution of these enzymes in contemporary cyanobacteria (7) indicates, however, that it is advantageous to currently hold a bidirectional NiFe-hydrogenase.

The discovery that nifJ (sll0741) co-occurs with the genes of the bidirectional hydrogenase also in those genomes of cyanobacteria that are not able to fix nitrogen (7), prompted us to test whether this enzyme might be functionally connected to the hydrogenase. NifJ encodes the PFOR, which is known to reduce flavodoxin/ferredoxin to provide electrons for the fixation of nitrogen (36).

Deletion of pfor in Synechocystis resulted in a completely segregated mutant (Fig. 4) with a severely reduced fermentative hydrogen production (Fig. 5A). Similar results were obtained for the cyanobacterium Synechococcus sp. PCC 7002 (37). As the fnr/Δfnr mutant was not impaired in its fermentative hydrogen production (Fig. 3A) and as NADPH is an uncommon electron acceptor under fermentative conditions and also a poor electron donor to the hydrogenase (Fig. 1), we claim that hydrogen is not produced via PFOR-ferredoxin-FNR-NADPH under fermentative conditions but directly receives its electrons via PFOR from ferredoxin or flavodoxin (Fig. 5B). Taken together, our results suggest that ferredoxin/flavodoxin are the principal electron donors to the bidirectional NiFe-hydrogenase in cyanobacteria both in transition states from dark anaerobic conditions to photosynthesis in the light as well as under dark fermentative conditions.

**Physiological Significance of the Bidirectional NiFe-hydrogenase**—Bidirectional NiFe-hydrogenases are absent from the open ocean but are widespread in marine coastal regions and fresh-
Ferredoxin Reduces Cyanobacterial NiFe-hydrogenase

**DISCUSSION**

Our results show both *in vitro* and *in vivo* that photohydrogen production and fermentative hydrogen production in *Synechocystis* are directly coupled via ferredoxin/flavodoxin to PSI and PFOR. Reduced ferredoxin/flavodoxin were superior electron donors to the hydrogenase compared with NAD(P)H in an *in vitro* cell-free extract (Fig. 1). Furthermore a *fnr/fnr* mutant was not impaired in its fermentative hydrogen production and showed elevated levels of photohydrogen production (Fig. 3A) excluding NADPH as electron donor under these conditions. Due to the midpoint potentials of ferredoxin/flavodoxin (−440 mV) compared with the midpoint potentials of NAD(P)H (−320 mV) we claim that reduced ferredoxin/flavodoxin is the principal electron donor to the bidirectional NiFe-hydrogenase and that it is responsible for the bulk of hydrogen production both in dark-light transition states and under fermentative conditions. We cannot, however, rule out that NAD(P)H additionally transfers electrons to the enzyme as NAD(P)H alone, although at low rates, was able to induce hydrogen production in purified hydrogenases (14, 15) and in cell-free extracts (Fig. 1 and Refs. 3, 11–13). It was furthermore shown that fermentative hydrogen production in *Synechococcus* corresponds to rising NADH/NAD⁺ ratios (37). Beside its role as substrate, NAD(P)H was shown to activate the hydrogenase in H-D exchange measurements (3, 13, 15).

Remarkably, in all *in vitro* assays with either the purified hydrogenase (14, 15) or cell-free extracts (3, 11–13) that tested NAD(P)H as substrates, high NAD(P)H concentrations (0.8–5 mM) were used that are far above physiological concentrations. Assays, however, in which NAD(P)H was tested as an enzyme activator to the hydrogenase came along with catalytic concentrations (0.3 mM, 100 μM, and 12 μM) (3, 15). It is therefore plausible to assume that NAD(P)H functions to activate the hydrogenase under high intracellular NAD(P)H/NAD(P) ratios and that it may also account for a minor hydrogen production, but that the bulk of hydrogen production is due to an electron transfer via reduced flavodoxin/ferredoxin, which are able to promote the amount of hydrogen production measured in cyanobacterial cells under physiological conditions (23).

We found that the hydrogenase is essential for survival under mixotrophic, nitrate-limiting conditions (Fig. 6). It has been known for a while that cyanobacteria are able to take up amino acids (43, 44) and that several strains can grow on glucose (45), providing them with a mixotrophic lifestyle. Evidence accumulates that mixotrophy in photoautotrophic plankton was long underestimated in marine and freshwater environments, as it is widespread and provides the organisms with a significant selective advantage (46–49). The hydrogenase probably functions as an electron sink for reduced ferredoxin/flavodoxin under mixotrophic, nitrate-limiting conditions. It has been repeatedly observed that nitrate limitation elevates hydrogen production which was assumed to be due to a general competition between nitrate reduction and hydrogen production for electrons (23).
According to our results, nitrate reductase and hydrogenase directly compete for reduced flavodoxin/ferredoxin.

Senescent blooms are accompanied by anoxic conditions, elevated concentrations of organic carbon, and nitrate limitation (38). To hold a bidirectional NiFe-hydrogenase might thus be of selective advantage for cyanobacteria under these conditions. Consistent with this was the observation in enclosure experiments with diatom blooms, that Synechococcus abundance peaked shortly after nitrate depletion (50).

Eukaryotic cells possess, dependent upon their lifestyle, chloroplasts, mitochondria, or hydrogenosomes harboring the enzymatic equipment for photosynthesis, respiration, or the anaerobic breakdown of pyruvate via PFOR, ferredoxin, and H₂ production (51). All of these processes supply the cells with ATP. Our findings show that in addition to photosynthesis and respiration, cyanobacteria contain a third ATP-producing pathway found in hydrogenosomes, providing for a highly flexible energy metabolism. Synechocystis gains additional ATP by oxidizing pyruvate to acetate via PFOR (Fig. 5B).

Besides mixotrophic conditions, this might be important during the fermentation of photosynthetically produced carbohydrates during night. Accordingly, it was observed that H₂ evolution of microbial mat layers dominated by cyanobacteria is most pronounced under anoxic conditions at night and correlates strongly with sunlight exposure from the previous day (5). Metatranscriptomic analyses from microbial mats revealed that cyanobacterial PFOR and hydrogenase genes are highly expressed at night (52), supporting the finding of this study that PFOR and bidirectional NiFe-hydrogenases in cyanobacteria are functionally intertwined.

Our study corrects a long held misbelief about the direct electron donor to the bidirectional NiFe-hydrogenase in cyanobacteria that has lasted for approximately 30 years. The finding that flavodoxin/ferredoxin are the principal electron donors to the enzyme has far reaching implications for biotechnological approaches aiming to enhance sunlight-driven hydrogen production in cyanobacteria but also for the conception of these enzymes concerning metabolic and evolutionary aspects.

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