Received: 19 December 2020 | Accepted: 12 March 2021

DOI: 10.1111/ppl.13404

SPECIAL ISSUE ARTICLE

Physiologia Plantarum

Regulatory electron transport pathways of photosynthesis in cyanobacteria and microalgae: Recent advances and biotechnological prospects

Lauri Nikkanen | Daniel Solymosi | Martina Jokel | Yagut Allahverdiyeva

Molecular Plant Biology, Department of Life Technologies, University of Turku, Turku, Finland

Correspondence
Yagut Allahverdiyeva, Molecular Plant Biology, Department of Life Technologies, University of Turku, FI-20014 Turku, Finland.
Email: allahve@utu.fi

Funding information
Biotieteiden ja Ympäristön Tutkimuksen Toimikunta, Grant/Award Number: 315119; H2020 Future and Emerging Technologies, Grant/Award Number: 899576; NordForsk, Grant/Award Number: 82845

Edited by: C. Funk

Abstract

Cyanobacteria and microalgae perform oxygenic photosynthesis where light energy is harnessed to split water into oxygen and protons. This process releases electrons that are used by the photosynthetic electron transport chain to form reducing equivalents that provide energy for the cell metabolism. Constant changes in environmental conditions, such as light availability, temperature, and access to nutrients, create the need to balance the photochemical reactions and the metabolic demands of the cell. Thus, cyanobacteria and microalgae evolved several auxiliary electron transport (AET) pathways to disperse the potentially harmful over-supply of absorbed energy. AET pathways are comprised of electron sinks, e.g. flavodiiron proteins (FDPs) or other terminal oxidases, and pathways that recycle electrons around photosystem I, like NADPH-dehydrogenase-like complexes (NDH) or the ferredoxin-plastoquinone reductase (FQR). Under controlled conditions the need for these AET pathways is decreased and AET can even be energetically wasteful. Therefore, redirecting photosynthetic reducing equivalents to biotechnologically useful reactions, catalyzed by i.e. innate hydrogenases or heterologous enzymes, offers novel possibilities to apply photosynthesis research.

1 | INTRODUCTION

Cyanobacteria are a group of oxygenic photosynthetic Gram-negative bacteria that evolved at least 2.4 billion years ago. Importantly, they were the progenitors of chloroplasts, the endosymbiotic photosynthetic organelles in plants and algae, to which modern cyanobacteria still bear significant functional and structural similarities. In oxygenic photosynthesis, photons of light, harvested by protein complexes called phycobilisomes (PBS), excite P700 and P680 chlorophyll pigments at the reaction centers of photosystem (PS) I and II, respectively, embedded in the thylakoid membrane. This allows extraction of electrons from water, releasing oxygen as a byproduct. Electrons are conveyed via PSII, cytochrome (Cyt) b_6f, plastocyanin (PC)/Cyt c_6, and PSI to ferredoxin (Fd), which functions as a distribution hub for photosynthetic electrons. The photosynthetic electron transfer chain (PETC) also generates a difference in electrochemical potential over the thylakoid membrane, the proton motive force (*pmf*), which drives the production of ATP from ADP and P_i by the ATP synthase (Mullineaux, 2014).

Depending on environmental conditions and the metabolic state of the cell, electrons are conveyed from Fd via Fd-NAD(P)H-oxidoreductase (FNR), converting NADP+ to the electron carrier NADPH which is mainly used for CO_2 fixation in the Calvin–Benson–Bassham (CBB) cycle. However, if the sink capacity of the CBB cycle is saturated, the PETC may become excessively reduced, resulting in generation of reactive oxygen species (ROS). In natural

environments of cyanobacteria and algae, this may occur during fluctuations in light intensity or nutrient availability. Over-reduction and massive production of ROS can severely damage the photosynthetic machinery, which has necessitated the evolution of photoprotective mechanisms and auxiliary electron transport (AET) pathways (Figure 1). The photosystems are vulnerable to excessive reduction, and while PSII has a rapid repair cycle, recovery of PSI from damage is slow (Komenda et al., 2012; Sonoike, 2011). Photosynthetic organisms therefore invest heavily in protection of PSI by multiple mechanisms that aim at maintaining PSI and its P700 reaction center chlorophyll pair in an oxidized state.

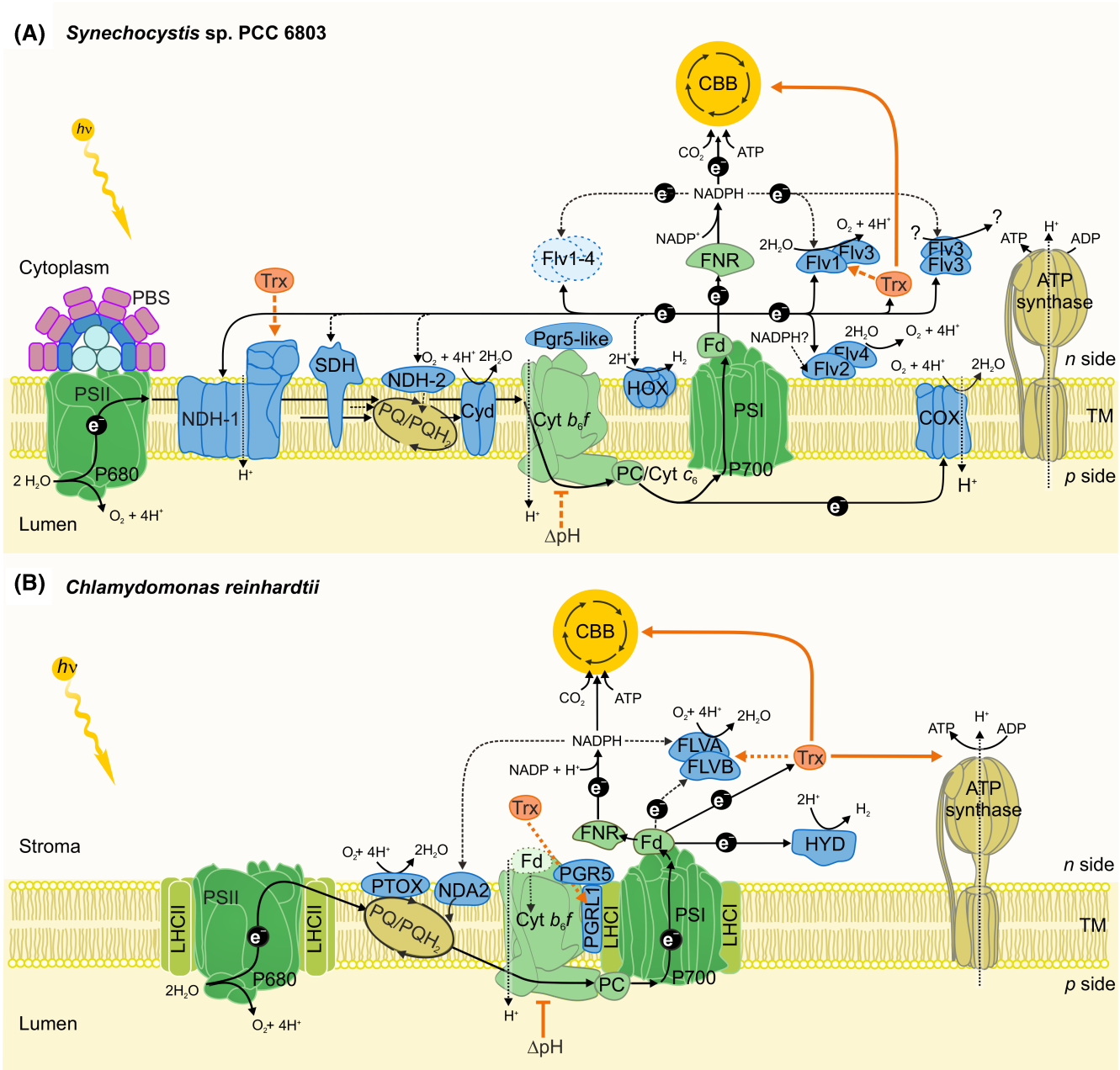

FIGURE 1 Schematics of photosynthetic light reactions and auxiliary electron transport pathways. The electron transport pathways in the thylakoids of cyanobacteria (A) and microalgae (B) branches into auxiliary electron transport routes, to distribute excess electrons when the sink capacity of the CBB cycle is insufficient. The major electron distribution hub is Fd, and the electron transport route toward the CBB-cycle converges there with auxiliary pathways driven by FDPs, RTOs, hydrogenases and in cyanobacteria, NDH-1. Electron fluxes and CO_2 fixation are adjusted to the redox state of the Fd-pool via Trx, which conveys redox signals to enzymes of the CBB-cycle, ATP synthase, and possibly, FDPs, PGRL1, and in cyanobacteria, NDH-1. In turn, the activity of these enzymes contributes to the pH gradient across the thylakoid membrane, a key regulatory component of photosynthesis, creating a dynamic system fine-tuned by feedback regulation. Dashed lines indicate putative electron transfer and dotted line marks proton transfer. HOX, Ni-Fe hydrogenase; HYD, Fe–Fe hydrogenase; LHCI and II, light harvesting complex; NDA2, NADH:Ubiquinone reductase; SDH, succinate dehydrogenase

The basic composition of PETC has remained largely unchanged over the course of evolution, with the exception of the substituting light harvesting complex proteins in place of PBS as the outer antennae of the photosystems in algae and plants. A major difference between prokaryotic cyanobacteria and eukaryotic algae and plants however, is that spatial separation of photosynthetic and respiratory bioenergetic pathways in chloroplasts and mitochondria has evolved in eukaryotes, while in cyanobacteria all bioenergetic and metabolic pathways exist in the same compartment (Figure 1). This has created distinct needs for coordination of the pathways by a variety of regulatory mechanisms. In this review, we summarize the recent advances in understanding these mechanisms in the unicellular cyanobacterial model species *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) and in the unicellular green alga *Chlamydomonas reinhardtii*.

We focus in particular on recent advances in understanding the mechanisms, physiological functions, and regulation of the Mehler-like reaction, catalyzed by flavodiiron proteins (FDPs), and the bioenergetic pathways mediated by NADH-dehydrogenase-like (NDH) complexes or the putative Fd-PQ-reductase (FQR)-mediated pathway. Moreover, we discuss the significance of the various terminal oxidases in thylakoid membranes, as well as processes contributing to the generation and regulation of the *pmf*. The photoprotective and AET pathways allow photosynthetic cells to survive in natural habitats where environmental conditions fluctuate. We also review recent advances in biotechnological modification of these mechanisms aimed at directing photosynthetic electrons into desired processes producing solar driven biochemicals.

2 | FLAVODIIRON PROTEINS

In oxygenic photosynthetic organisms apart from red or brown algae and angiosperms, a crucial mechanism to alleviate excessive reduction of the electron transfer chain involves FDPs (Alboresi et al., 2019; Allahverdiyeva et al., 2015). FDPs function as efficient release valves for excessive electrons in the PETC, reducing O_2 to H_2O—without the concomitant production of ROS—known as the Mehler-like reaction (Allahverdiyeva et al., 2013; Helman et al., 2003).

Cyanobacteria possess 2–6 isoforms of FDPs (Flv1-4, Flv1B,3B), Flv2 and Flv4 being unique to β cyanobacteria—such as *Synechocystis*—while Flv1B and Flv3B are found only in heterocysts of filamentous species (Allahverdiyeva et al., 2015). Based on the observation that Flv1 or Flv3 is unable to catalyze the Mehler-like reaction without the other in vivo, and the accumulation of these proteins are co-dependent at least to some extent (Allahverdiyeva et al., 2013; Mustila et al., 2016) a heterodimer is likely the active form of Flv1 and Flv3 in the cells (Allahverdiyeva et al., 2013; Helman et al., 2003). However, as Flv3 is substantially more abundant than Flv1 in *Synechocystis* (Allahverdiyeva et al., 2013), alternative oligomerization is likely to occur. Indeed, Flv3 homodimers were detected in *Synechocystis*, but the existence of Flv3 homotetramers or Flv1/3 heterotetramers cannot be excluded either. Flv3 homooligomers, being unable to reduce O_2 to H_2O in vivo, serve an as of yet unknown physiological function (Mustila et al., 2016). Moreover, the presence of Flv2/4 heterodimer in *Synechocystis* and Flv2 homodimer in the corresponding Δ*flv4* mutant was reported (P. Zhang et al., 2012).

Flv1/3 provides a strong transient response upon rapid changes in light conditions, whereas Flv2/4 facilitates weaker steady-state photoreduction of O_2. Curiously, Flv1/3 and Flv2/4 appear to rely on the other to some extent, as deleting either Flv1/3 or Flv2/4 decreases the O_2 photoreduction capacity of the cells. Therefore, coordinated inter-regulation likely exists between Flv1/3 and Flv2/4, possibly involving alternative heteromeric arrangements (Santana-Sanchez et al., 2019).

It has been established that Flv1/3 heterodimers operate on the acceptor side of PSI (Allahverdiyeva et al., 2013; Helman et al., 2003). In contrast, it was initially proposed Flv2/4 hetero-oligomers may extract electrons from PSII or the PQ pool, alleviating excitation pressure on PSII in order to avoid production of ROS (P. Zhang et al., 2012). However, while Flv2/4 hetero-oligomers contribute to the Mehler-like reaction under steady state and low carbon conditions, light-induced O_2 reduction is inhibited when electron transfer at Cyt b_6f is blocked by DBMIB in the mutant deficient in the cytochrome bd quinol oxidase (Cyd) (Santana-Sanchez et al., 2019). This indicates that the source of electrons for Flv2/4 induced Mehler-like reaction is not at PSII or at the plastoquinone pool, but rather downstream of the Cyt b_6f complex.

The green alga *C. reinhardtii* possesses two FDPs (FLVA and FLVB) which possibly function as heterodimers and share higher homology with Flv1 and Flv3 than Flv2 or Flv4 (Allahverdiyeva et al., 2015). FLVA and FLVB catalyze O_2 photoreduction during dark to light transitions (Chaux et al., 2017). The estimated maximal rate of O_2 uptake (V_{max}), in the presence of a plastid terminal oxidase (PTOX) inhibitor, was attributed to FDPs and was reported as 45 μmol O_2 mg Chl^{-1} h^{-1} (Saroussi et al., 2019). This is in the same range reported for FDPs in *Synechocystis* (Santana-Sanchez et al., 2019), although direct comparison is difficult due to different experimental conditions. Under fluctuating light intensities from low to high light *C. reinhardtii* FDPs represent the major photoprotective mechanism enabling cell growth by acting as fast and strong electron sinks downstream of PSI (Jokel et al., 2018). It is important to note that these studies were performed with *flvB* knockout mutants in which the accumulation of FLVA is also severely impaired (Chaux et al., 2017; Jokel et al., 2018), thus distinguishing between the function of homodimers or heterodimers is impossible. Interestingly, in addition to photoreduction of O_2, recent data show the involvement of FDPs in light-induced reduction of NO to N_2O, indicating a wider contribution of FDPs to the metabolism in *C. reinhardtii* (Burlacot et al., 2020).

3 | FLAVODIIRON PROTEINS ELECTRON DONOR(S)

The precise identities of the electron donors of FDPs have long remained uncertain. Class C FDPs contain a C-terminal NAD(P)H:flavin oxidoreductase-like domain and indeed, in vitro studies indicated

NADH and NADPH-dependent O_2 reduction activity of *Synechocystis* Flv1 and Flv3 (Brown et al., 2019; Vicente et al., 2002) as well as Flv4 (Shimakawa et al., 2015), suggesting NAD(P)H as the electron donor to FDPs. It is important to note however, that these enzymatic assays were carried out with homo-oligomers of FDPs, which do not catalyze photoprotective reduction of O_2 in vivo like the hetero-oligomeric forms (Mustila et al., 2016; Santana-Sanchez et al., 2019). Moreover, both Vicente et al. (2002) and Shimakawa et al. (2015) reported low reaction rates and low or no affinity of FDPs to NADPH as compared with NADH, albeit higher rates and relative NADPH affinities for Flv1 and Flv3 were measured by Brown et al. (2019). Thus, it remains questionable whether NADPH is an electron donor to FDPs in vivo. Furthermore, no in vitro enzymatic assays testing FDP activity with alternative electron donors, such as Fd or FNR, have been published.

Synechocystis Flv3 (Cassier-Chauvat & Chauvat, 2014) and its *C. reinhardtii* orthologue FLVB (Peden et al., 2013) have been shown to interact with Fd in two-hybrid tests, and *Synechocystis* Flv1 and Flv3 in a Fd-chromatographic assay (Hanke et al., 2011). Recently, it was also shown by near-infrared absorbance and fluorescence spectroscopy that absence of Flv1/3 results in impaired oxidation of the Fd pool in dark-adapted *Synechocystis* cells upon illumination, while NADPH redox kinetics are unaltered (Nikkanen et al., 2020; Sétif et al., 2020). These findings strongly support the hypothesis that Fd, instead of NADPH, is the primary electron donor to Flv1/3 hetero-oligomers in vivo, although possible involvement of F_AF_B iron–sulfur clusters of PSI cannot be excluded (Sétif et al., 2020). An intriguing possible explanation for the discrepancy between in vitro and in vivo results would be that the homo-oligomeric and hetero-oligomeric forms of FDPs would have distinct electron donors, allowing control between their distinct physiological functions based on the redox states of the cytosolic electron carriers. Identity of the electron donor to Flv2/4 hetero-oligomers also remains unresolved.

There are nine Fd isoforms in *Synechocystis* (Fed1–9) (Cassier-Chauvat & Chauvat, 2014). Little is known about the specific functions of or the potential redundancies between individual Fd isoforms, but Fed1 is likely the main isoform involved in photosynthetic electron transfer. Moreover, Fed2–9 are expressed at comparatively low levels, and even though Fed9 interacted with Flv3 in a two-hybrid test (Cassier-Chauvat & Chauvat, 2014), Fed2–9 are unlikely to be able to provide the reducing capacity to drive the large electron sink activity of the Mehler-like reaction. This leaves Fed1 as the most likely candidate to constitute the main electron donor to FDP hetero-oligomers.

4 | REGULATION OF FLAVODIIRON PROTEIN ACTIVITY

In *Synechocystis*, Flv1/3-mediated O_2 photoreduction is rapidly activated during the first seconds of illumination or upon sudden increases in light intensity, but diminished about 30 s thereafter (Nikkanen et al., 2020; Santana-Sanchez et al., 2019). In contrast, Flv2/4-dependent O_2 photoreduction is activated more slowly but persists at steady state at least for 5–10 min during illumination (Santana-Sanchez et al., 2019). Therefore, it is highly likely that the activities of FDP hetero-oligomers are subject to tight regulation, likely to avoid competition for reducing power with an activated CBB cycle.

Based on structural in silico modeling, it has been suggested that FLVA and FLVB hetero-oligomers of *Physcomitrella patens* could switch between an "open" active or a "closed" inactive conformation in response to a regulatory signal for example via redox regulation (Alboresi et al., 2019). Accordingly, *Synechocystis*, *Anabaena* sp. PCC7120, and *C. reinhardtii* FDPs all contain conserved cysteine residues (Alboresi et al., 2019; Jokel et al., 2018) that could potentially be targets to regulatory thiol-disulfide exchange, mediated by the thioredoxin (Trx) system. Interestingly, light-dependent redox modulation was observed in Cys33 and Cys226 of *Synechocystis* Flv1 and Cys207 of Flv3 in a redox proteomic assay, but no such changes were detected in Flv2 or Flv4 (Guo et al., 2014). The physiological significance of these redox exchanges is still being investigated, but coupling FDP activity to cellular thiol redox state could provide a rapid and reversible mechanism to coordinate the activity of the Mehler-like reaction according to environmental stimuli and the physiological state of the carbon metabolism, most importantly the CBB cycle.

While Flv2 and Flv4 contain no light-dependently redox-modulated cysteine residues, the activity of Flv2/4 hetero-oligomers may be controlled via *pmf* and Mg^{2+}-dependent association with the thylakoid membrane (P. Zhang et al., 2012), which would regulate the access of the hetero-oligomers to their photosynthetically produced reductant, be it Fd, NADPH, or FNR. Interestingly, regulation via the magnitude of the *pmf* could enable control of Flv2/4 via the activity of Flv1/3, given that during the first seconds of sudden light increases, up to 75% of *pmf* generation is dependent on Flv1/3 (Nikkanen et al., 2020). Moreover, the expression of Flv2 and Flv4 is strongly inhibited by elevated C_i concentration as well as alkaline pH. Interestingly, in high C_i where Flv2 and Flv4 are absent, the presence of Flv1/3 is sufficient to catalyze strong steady-state O_2 photoreduction (Santana-Sanchez et al., 2019).

Alternatively, FDP activity could be reversibly controlled by phosphorylation and de-phosphorylation, as a phosphoproteomic analysis of *Synechocystis* cells revealed phosphorylated serine residues in Flv3 and Flv4 (Angeleri et al., 2016).

5 | PHOTOPROTECTIVE RESPIRATORY TERMINAL OXIDASES IN CYANOBACTERIAL AND ALGAL THYLAKOIDS

Cyanobacterial photosynthetic thylakoid membranes harbor respiratory terminal oxidases (RTOs): Cyd and aa_3-type Cyt *c* oxidases (Cox), both reducing O_2 to H_2O. RTO-mediated dark respiratory O_2 uptake continues during illumination in *Synechocystis* (Santana-Sanchez et al., 2019), thus contributing to the oxidation of PETC. Cox and Cyd compensate to some extent for the loss of the other (Ermakova et al., 2016; Howitt & Vermaas, 1998; Pils & Schmetterer, 2001). Besides, Cyd contributes to ΔpH formation by reducing O_2 to water,

which alkalizes the cytosol. Although Cox primarily functions in the dark, it assists in the regulation of electron flow to PSI by transferring electrons from PC/Cyt c_6 to O_2 in light (Ermakova et al., 2016; Schmetterer, 2016). Cox is indispensable under low light (Kufryk & Vermaas, 2006) and contributes to photosynthetic control by likely pumping protons at a $4H^+/2e^-$ ratio. Mutant strains deficient in both Cox and Cyd cannot survive in a square-wave diurnal light regime (sharp alteration of 12 h high light and 12 h dark periods), but interestingly, are able to grow under sinusoidal diurnal regime (gradual changes in light intensity), under shorter square-wave light regime (5 min dark/5 min high light), or under constant illumination (Ermakova et al., 2016; Lea-Smith et al., 2013). The contribution of RTOs seems to depend on the length of the dark and length and intensity of light periods, and the amount of photodamage occurring during the light period. Importantly, light can induce O_2 uptake by RTOs when the linear electron transport is inhibited at Cyt b_6f with DBMIB (Berry et al., 2002) or at PSI by cultivating the Flv1/3-deficient mutant under mild fluctuating light (Allahverdiyeva et al., 2013; Ermakova et al., 2016). This activity however cannot rescue the fatal loss of Flv1/3 under strong fluctuating light (Allahverdiyeva et al., 2013) due to low responsiveness and sink capacity of RTOs (Ermakova et al., 2016). While RTOs only have a minor effect on the redox poise of the PETC in steady-state light conditions (Helman et al., 2005), they appear to tune the redox poise of cyanobacterial thylakoids when illumination ceases. Dark respiratory rates in *Synechocystis* are reportedly higher after illumination than before (Nikkanen et al., 2020; Santana-Sanchez et al., 2019). It is possible that relaxing photosynthetic control and/or increased concentration of O_2 stimulates RTOs.

Besides RTOs, PTOX can be found in some cyanobacteria (Schmetterer, 2016) and green algae. In chloroplasts of *C. reinhardtii*, auxiliary electron pathways branch off the PQ-pool via PTOX1 and PTOX2 to O_2. PTOX2 was shown to be the major plastidial terminal oxidase in *C. reinhardtii* preventing over-reduction of the PQ-pool in light, thus operating as a photoprotective electron sink (Houille-Vernes et al., 2011). Indeed, loss of PTOX2 in *C. reinhardtii* leads to excess excitation pressure and decreased growth rate under intermittent light periods but not under constant illumination (Nawrocki, Buchert, et al., 2019b).

6 | NADH DEHYDROGENASE-LIKE COMPLEXES

In cyanobacteria, the NADH dehydrogenase-like complex 1 (NDH-1) is a highly versatile multisubunit bioenergetic machinery. By incorporating specific subunits, NDH-1 functions in the main pathway of cyclic electron transport (CET) around PSI in cyanobacteria, respiratory electron transport, as well as the carbon-concentrating mechanism (CCM). Several cryo-EM structures of cyanobacterial NDH-1 complexes have been recently reported, providing detailed information on their composition (Laughlin et al., 2019, 2020; Schüller et al., 2019, 2020). The core complex consists of membrane-embedded subunits and the peripheral arm on the cytosolic side of the thylakoid membrane. Addition of D1 and F1, or D2 and F1 subunits to the core creates the NDH-1_1 and NDH-1_2 isoforms, respectively, which both catalyze cyclic as well as respiratory electron transport. Whether NDH-1_1 and NDH-1_2 have distinct physiological roles remains to be investigated. Low concentration of C_i triggers the expression of the D3/F3/CupA/CupS operon and the formation of the NDH-1_3 isoform, which functions in high-affinity conversion of CO_2 to HCO_3^- in the CCM. In contrast, the D4/F4/CupB operon is expressed constitutively, and incorporation of its protein products into the NDH-1 core creates the NDH-1_4 isoform that catalyzes CO_2 uptake but with low affinity (Peltier et al., 2016).

Functional and structural evidence has indicated that Fd (instead of NADPH) is the direct electron donor to photosynthetic NDH-1 complexes and binds to an oxidation site in the cytosolic peripheral arm of the complex likely formed by the O, I, H, V, and S subunits (Laughlin et al., 2020; Schüller et al., 2019; C. Zhang et al., 2020). A shared electron donor between FDPs and NDH-1 may, therefore, enable coordination of the two electron transfer pathways. Accordingly, we reported recently on partial redundancy between Flv1/3 and NDH-$1_{1/2}$ in protecting PSI by maintaining efficient oxidation of PSI in high light and air-level CO_2 concentration (Nikkanen et al., 2020). Similar functional redundancy was observed between FDPs and NDH-1 as well as PGRL1/PGR5 in the moss *P. patens* (Storti, Puggioni, et al., 2020a; Storti, Segalla, et al., 2020b). In *Synechocystis*, absence of both Flv1/3 and NDH-$1_{1/2}$ was even lethal when cells were moved from conditions of high CO_2 concentration and low light to air-level CO_2 concentration and high light, as in addition to exacerbated impairment of PSI oxidation, these mutants were unable to induce accumulation of proteins related to the CCM (Nikkanen et al., 2020). Interestingly, however, simultaneous loss of Flv1/3 and NDH-$1_{3\text{-}4}$, the forms associated with CCM, was not lethal in similar changes in growth conditions, suggesting that coordination of functions occurs specifically between Flv1/3 and NDH-1_{1-2}.

There is clear evidence that NDH-1 contributes to oxidation of PSI in both cyanobacteria and plants (Nikkanen et al., 2018, 2020; Shimakawa & Miyake, 2018; Storti, Puggioni, et al., 2020a). It is not immediately obvious why however, since cyclic and respiratory electron transport catalyzed by NDH-1 complexes merely shunts electrons from the acceptor side of PSI back to the donor side, and thus does not in itself increase oxidation of the PETC. Several mechanisms have however been proposed. Firstly, NDH-1 translocates protons to the thylakoid lumen with a $2H^+/e^-$ stoichiometry and thus generates ΔpH (Miller et al., 2021; Schüller et al., 2019). As acidification of the lumen limits the oxidation of PQH_2 in the Q-cycle in what is referred to as photosynthetic control (Malone et al., 2021), could the proton pumping activity of NDH-1 enhance PSI oxidation by limiting electron transfer from Cyt b_6f, thus trapping electrons in the PQ-pool? In vivo data suggest however that a loss of NDH-1 proton translocation activity is compensated by enhanced activity of FDPs, or down-regulation of the conductivity of the ATP synthase (Nikkanen et al., 2020). ATP deficiency could then limit the activity of the CBB cycle and result in impaired consumption of NADPH and oxidation of PSI.

NADPH consumption was not impaired, however, in *Synechocystis* cells lacking both Flv1/3 heterooligomer and NDH-1_{1-2} (Δ*flv3d1d2*), suggesting that an impairment of CBB cycle activity due to ATP deficiency is not the factor responsible for the diminished ability of these mutants to keep PSI oxidized. In contrast, in cells lacking only NDH_{1-2} (Δ*d1d2*), PSI oxidation and *pmf* generation are not impaired due to enhanced activity of FDPs, but activation of carbon fixation and NADPH consumption are delayed (Nikkanen et al., 2020). Hyperactive FDPs in Δ*d1d2* cells, while maintaining effective oxidation of PSI, may be out-competing the Trx system, whose reduction is required for activation of the CBB cycle (Tamoi et al., 2005), for electrons. When the competitive electron sink of Flv1/3 is removed in Δ*flv3 d1d2* cells, the CBB cycle is activated normally but PSI oxidation is severely impaired, resulting in photodamage. These studies with *Synechocystis* knockout strains aptly demonstrate how intricate coordination of the auxiliary electron transfer pathways is required to maintain cellular redox balance and integrity of the photosynthetic machinery in changing environmental conditions.

Coupling the activity of NDH-1 to that of thylakoid RTOs, would also enhance oxidation of PSI, but it is unlikely to be a major factor due to the low electron transfer capacity of RTOs (Ermakova et al., 2016; Helman et al., 2005). Lastly, the CCM-associated NDH-1_3 conformation utilizes reduced Fd to convert CO_2 to HCO_3^- (Schüller et al., 2020), and should therefore also enhance oxidation of PSI when its expression is induced in low CO_2 conditions.

NDH-1 is also present in chloroplasts of most seed plants, but interestingly, it has been lost in unicellular green algae (Peltier et al., 2016). Instead, NDH-2 (called NDA2 in *C. reinhardtii*), a single-subunit flavoenzyme, reduces PQ and mediates CET without pumping additional protons (Jans et al., 2008). Therefore, NDA2-mediated CET affects the NADPH/ATP ratio but is less effective in generating *pmf* than the cyanobacterial NDH-1 complex. The non-photochemical PQ-reduction via NDA2 is the point of entry for electrons that derive from glycolysis and other breakdown processes to PETC, and can subsequently feed alternative electron sinks such as hydrogenases (Mignolet et al., 2012; Milrad et al., 2021) or PTOX (Saroussi et al., 2016).

7 | REGULATION OF NDH-1 ACTIVITY

Recent structural cryo-EM reports suggested that the NdhV subunit, which is especially important in high irradiance, enhances Fd binding at the tip of the cytosolic arm of the complex, functioning as a positive regulator of cyclic and respiratory electron transfer mediated by NDH-1_{1-4}. Recruitment of the NdhV subunit is reversible, and is likely controlled by the NdhS subunit. Unlike previously suggested, NdhS is thus likely not directly involved in Fd binding but does nonetheless have an essential structural function in enhancing the affinity of the complex to Fd In contrast, presence of the NdhO subunit, which is also involved in forming the Fd binding site, was reported to have a negative effect on NDH-1 activity (Laughlin et al., 2020; C. Zhang et al., 2020).

This poses the question of how the reversible recruitment of NdhV by NdhS is controlled in order to adjust NDH-1 activity. Redox regulation of the chloroplast NDH-1 in plants by the Trx systems has been proposed, and the most likely putative target proteins of redox regulation identified by co-immunoprecipitation and bimolecular fluorescence complementation tests with Trx included several NDH-1 subunits around the Fd binding site, including NdhS, NdhH, and NdhO (Courteille et al., 2013; Nikkanen et al., 2018). *Synechocystis* NDH-1 subunits also contain several light-dependently redox modulated cysteine residues, which could potentially act as Trx targets. NdhK, NdhI, and NdhJ subunits all contained cysteines whose reduction was induced by light (Guo et al., 2014). Of these, NdhI is likely directly interacting with Fd, while NdhK and NdhJ are involved in forming the PQ binding site (Pan et al., 2020; Zhang et al., 2020). These findings make the hypothesis of redox regulation of NDH-1 activity via control of Fd binding affinity attractive, but obviously further studies are required to test the hypothesis and to elucidate how such regulation would be coordinated in vivo.

8 | FERREDOXIN–PLASTOQUINONE REDUCTASE PATHWAY

In plants and algae another CET pathway around PSI was suggested to involve proton gradient regulation 5 (PGR5) and PGR5-like photosynthetic phenotype 1 (PGRL1) (DalCorso et al., 2008). PGR5 is predicted to be a small extrinsic thylakoid protein while PGRL1 is integral to the thylakoid membrane. Involvement of PGRL1 and PGR5 in CET was proposed based on an in vitro assay, where the PGRL1-PGR5 complex catalyzed the reduction of PQ by Fd, thus suggesting that PGRL1 and PGR5 form the elusive Fd-plastoquinone reductase, FQR (Hertle et al., 2013). However, a direct participation of PGR5 or PGRL1 in FQR electron transfer in vivo could not be shown and different hypotheses of their function are still discussed.

In *C. reinhardtii*, several physiological and interaction studies have revived the old hypothesis that the FQR-pathway might be involved in a direct PQ reduction by the Cyt b_6f. The finding that the maximal rate and duration of CET is independent of PGRL1 suggests that PGRL1 is not directly involved in FQR electron transfer (Nawrocki, Bailleul, et al., 2019a). Thus, PGR5 and PGRL1 possibly do not directly mediate CET but rather regulate it under physiological conditions. Another piece of the puzzle was revealed with the finding that the loss of PGR5 and/or PGRL1 affects the association of FNR to the thylakoid membrane, and thus its proximity to PSI and/or Cyt b_6f (Mosebach et al., 2017). This would generate a switch between linear and cyclic mode. Additionally, under anoxic conditions PGR5 is functionally involved in a modified Q-cycle which is Fd-assisted and operates less efficiently in the *pgr5* mutant (Buchert et al., 2020). A modified Q-cycle acting as the FQR-pathway with PGR5 and PGRL1 regulating its activity by FNR recruitment is an attractive solution to the question of the molecular identity of the FQR-pathway. However, this theory needs more independent verification, especially in flowering plants and other photosynthetic organisms like cyanobacteria.

The presence and nature of the FQR-pathway in cyanobacteria is still poorly characterized, but possible involvement of a Pgr5 homolog in CET has been suggested, although its physiological role would be minor (Yeremenko et al., 2005) with NDH-1 constituting the main CET pathway in cyanobacteria (Miller et al., 2021). Recently, the *Synechocystis* Sll1217 protein with low sequence similarity to PGRL1 was proposed to be a functional analog of PGRL1 (Dann & Leister, 2019). The Sll1217 protein interacts in vitro with Pgr5 and its knockout mutant shows impaired P700 oxidation levels, which the authors suggested to be due to decreased CET. Although the physiological relevance of these findings is uncertain, they open up considerations about the evolution of the PGR5 and PGRL1 function in photosynthetic organisms.

9 | REGULATION OF THE PROTON MOTIVE FORCE

The proton motive force (*pmf*) consists of the difference in H^+ concentration over the thylakoid membrane (ΔpH) as well as an electric field ($\Delta\Psi$) generated by positive charges of protons, and influx of positively charged ions and efflux of negatively charged ions from the thylakoid lumen. The *pmf* is released mainly through the ATP synthase, which provides the rotational force for production of ATP. In cyanobacteria the ΔpH component is generated by (a) water oxidation, (b) the Q-cycle at Cyt b_6f, (c) H^+ pumping by the NDH-1 complex and by Cox. Moreover, oxidation of PQH_2 by Cyd or PTOX as well as consumption of H^+ in the cytosol by various processes such as the Mehler-like reaction mediated by FDPs and respiratory O_2 consumption by RTOs contribute to ΔpH formation. In plants and algae, the additional proton translocation through the FQR-CET around PSI also contributes to ΔpH formation (Shikanai & Yamamoto, 2017). The ΔpH component also has a crucial regulatory role in inducing photosynthetic control, a mechanism that inhibits excessive electron transfer at the Cyt b_6f complex in order to protect PSI. The physiological significance of photosynthetic control has been mostly discussed in plants and algae, but the mechanism most likely functions also in cyanobacteria, as evidenced by increased re-reduction rate of Cyt *f* in light due to addition of *pmf* uncouplers (Checchetto et al., 2012). It is likely, however, that high PSI/PSII ratio and the presence of FDPs makes photosynthetic control less essential for photoprotection of PSI in cyanobacteria than in angiosperms. In plants and algae ΔpH also induces non-photochemical quenching (NPQ), another photoprotective mechanism dissipating excessive excitation energy at PSII antennae as heat (Niyogi & Truong, 2013). In cyanobacteria NPQ induction does not depend on acidification of the lumen, but is induced by the orange carotenoid protein activated by strong light (Muzzopappa & Kirilovsky, 2020). Nonetheless, adjusting the magnitude of the *pmf* according to environmental conditions by controlling the influx and efflux of protons across the thylakoid membrane is of utmost importance for all photosynthetic organisms.

Flv1/3 is essential for effective generation of *pmf* at dark/light transitions. In *Synechocystis* at the onset of light up to 75% of thylakoid proton flux was attributable to the presence of Flv3 (Nikkanen et al., 2020), and similar albeit somewhat lower contributions to *pmf* have been reported for FLVA/B in *C. reinhardtii* (Chaux et al., 2017) and *P. patens* (Gerotto et al., 2016), and for Flv1 in the liverwort *Marchantia polymorpha* (Shimakawa et al., 2017). In part the FDP contribution to *pmf* is due to cytosolic consumption of H^+ in O_2 photoreduction, but perhaps even more importantly, FDPs enhance *pmf* generation by enabling a higher rate of linear electron transfer. Accordingly, absence of Flv1/3 or FLVA/B results in lowered gross O_2 production at the onset of light in *Synechocystis* (Santana-Sanchez et al., 2019) and *C. reinhardtii* (Chaux et al., 2017), respectively. Thus, less ΔpH is generated via water oxidation and the Q-cycle. After the first seconds of illumination, the lack of ΔpH generation in Flv3-deficient *Synechocystis* cells is compensated by downregulation of ATP synthase conductivity, resulting in WT level of *pmf*, but the thylakoid proton flux remains diminished for at least 30 seconds (Nikkanen et al., 2020). This was observed at dark-to-light transitions, corroborating the transient activity of Flv1/3 in O_2 photoreduction upon sudden change in illumination (Santana-Sanchez et al., 2019). Since dark to light transitions are rare in natural environments, contribution of FDPs could rather be significant when irradiation fluctuates and the generated *pmf* might contribute to photosynthetic control. However, FDP-driven electron transfer as a means of *pmf* generation is inefficient as it wastefully consumes reducing power unlike CET.

As the transfer of $2e^-$ from Fd to PQ by NDH-1 complexes is coupled to pumping of $3H^+$ (NDH-1_{3-4}; Schüller et al., 2020) or $4H^+$ (NDH-1_{1-2}; Schüller et al., 2019) into the lumen, as well as translocation of additional $4H^+$ in the Q cycle, NDH-1 also has a role in controlling the magnitude of *pmf*, and as a consequence, ATP synthesis. However, as high ΔpH inhibits the Fd-PQ reductase activity of NDH-1, its contribution to *pmf* has been suggested to be most significant under low irradiance (Shikanai & Yamamoto, 2017). It was recently shown that, in *Synechocystis*, NDH-1_{1-2} complexes could maintain up to 40% of lumenal acidification rate at dark/light transitions when electron transfer from PSII was inhibited, while NDH-1_{3-4} only contributed about 5% (Miller et al., 2021). This constitutes the first demonstration of NDH-1-mediated proton pumping in living cyanobacterial cells, and while these results were obtained in the presence of extensive inhibitor cocktails, which makes it difficult to assess their significance in more natural physiological conditions, they are in line with a recent report attributing a maximum of 35% of total electron transport at PSI to CET in living *Synechocystis* cells (Theune et al., 2021). Interestingly, in the M55 mutant (lacking all NDH-1 complexes) *pmf* generation was completely abolished at dark/light transitions (Miller et al., 2021). This suggests that the FQR-dependent CET pathway does not contribute to *pmf* formation at dark/light transitions in *Synechocystis*.

In order to adjust *pmf* during illumination and to prevent ATP hydrolysis in the dark, the *pmf* release rate must be controlled by regulating the activity of the ATP synthase. In plants and algae, including *C. reinhardtii*, reduction of a disulfide bond in the γ subunit of the chloroplast ATP synthase by Trx activates the ATP synthase at the onset of light. This mechanism is, however, absent in cyanobacteria

(Hisabori et al., 2013). Instead, reverse-activity of the ATP synthase and ATP hydrolysis in the dark are inhibited by the ϵ subunit (Imashimizu et al., 2011). The mechanisms of regulation of the conductivity of the ATP synthase and thylakoid ion channels during changes in light conditions in cyanobacteria and algae remain largely uncharacterized and further studies are required to elucidate them.

10 | IMPROVING BIOPRODUCTION BY ENGINEERING AUXILIARY ELECTRON TRANSPORT PATHWAYS

Photosynthetic microbes are promising chassis for biotechnological applications, due to their ability to convert light energy into chemical potential (Figure 2). However, economic feasibility of the system requires significant improvement. The theoretical photon-to-product conversion efficiency of photosynthesis is about 10–13%, whereas in reality efficiency barely exceeds 1–3% (Melis, 2009). Thus, identification and elimination of metabolic limiting factors and photosynthetic "waste points" can increase photosynthetic efficiency toward sustainable bioproduction. Even though light is the ultimate substrate for photosynthesis, any absorbed light exceeding the capacity of downstream metabolism, e.g. the CBB-cycle, has to be dissipated in order to avoid photodamage. Moreover, imbalance between photosynthetic light reactions and downstream processes triggers feedback regulations/inhibition to decrease photosynthetic efficiency. During the course of evolution photosynthetic organisms developed different photoprotective and alternative electron transport pathways in order to avoid photodamage of the photosynthetic apparatus. Under controlled conditions, these pathways can also be considered as "waste" of photosynthetic electrons or reducing power. Therefore, both elimination of "waste" points and/or introduction of strong heterologous sinks (to avoid feedback inhibition) are among main strategies for improvement of photosynthesis, optimization of reductant cofactor supply for desired biosynthetic reactions, and consequently, for increasing the efficiency of cyanobacterial and microalgal bioproduction platforms.

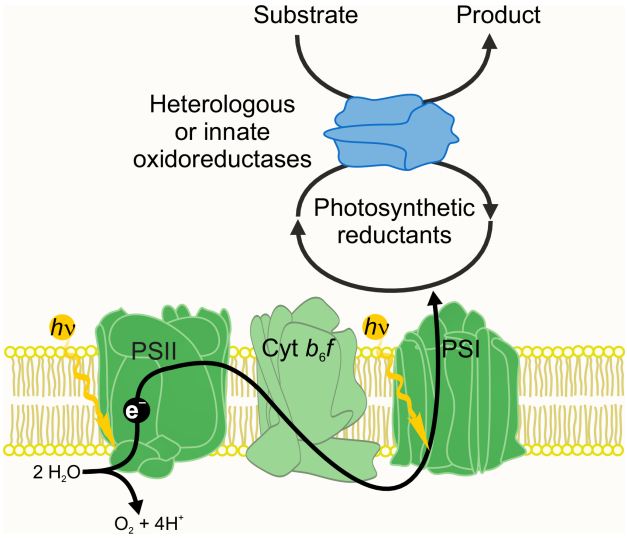

FIGURE 2 Harnessing photosynthetic reductants for production of targeted chemicals. Photosynthetic light reactions produce a large amount of reducing equivalents such as reduced Fd and NADPH. Photosynthetic microbes with engineered photosynthesis (eliminated competing routes) channel a majority of electrons to desired chemicals which are excreted from the cells. In this scenario the engineered photosynthetic microbes act as light-driven biocatalysts with a constantly recycled pool of reducing cofactors

11 | BIOPRODUCTION VIA ENZYMES DIRECTLY COUPLED TO PHOTOSYNTHETIC ELECTRON TRANSFER CHAIN AND VIA ELIMINATING COMPETING PATHWAYS

C. reinhardtii is capable of catalyzing molecular hydrogen (H_2) photoproduction by innate [Fe–Fe]-hydrogenase enzymes, which utilizes photosynthetically reduced Fd. The major obstacles of efficient H_2 photoproduction are the O_2 sensitivity of hydrogenases and strong competition with different pathways e.g. those toward CO_2 fixation, or FDPs. To overcome the competition between hydrogen production and downstream processes, hydrogenases can be fused to PSI, allowing photosynthetic electrons to be directly captured at the acceptor side of PSI (Appel et al., 2020; Kanygin et al., 2020). Alternatively, a "pulse illumination protocol" was developed recently, in which microalgae are exposed to a train of strong yet short light pulses superimposed on dark (or low light) background, channeling photosynthetic electrons originated from PSII water splitting to the hydrogenase instead of CO_2 fixation (Kosourov et al., 2018, 2020). Elimination of FDPs further increased H_2 production under "pulsing" light regime and constant illumination, possibly by providing more photosynthetic electrons for the hydrogenase (Chaux et al., 2017; Jokel et al., 2019). However, it is uncertain how significant the O_2 photoreduction activity of FDPs is under microoxic conditions. Since FDPs in *C. reinhardtii* contribute to photoreduction of NO to N_2O (Burlacot et al., 2020), the loss of FLVB could affect NO homeostasis and/or signaling mediated by reactive nitrogen species (Jokel et al., 2019). Moreover, delayed ΔpH generation in cells lacking FLVB (Chaux et al., 2017) could also contribute to the elevated H_2 production during pulse illumination. Indeed, proton uncouplers or the loss of PGRL1 reportedly increase H_2 photoproduction in *C. reinhardtii*. Delay in ΔpH compromises photosynthetic control and thus, enables electrons to be transferred toward the hydrogenase (Tolleter et al., 2011). Loss of FDPs or FQR function appears to have diverse effects on the metabolism that influences H_2 photoproduction. *C. reinhardtii* cells lacking FLVB, e.g. demonstrated higher respiratory activity compared to WT (Jokel et al., 2019), likely by providing a more optimal intracellular milieu for the [Fe–Fe]-hydrogenases that are reportedly O_2 sensitive. The *pgr5* and *pgrl1* mutants showed an increase in respiratory processes as well, together with enhanced PSII stability, resulting in higher H_2 photoproduction compared with WT (Steinbeck et al., 2015).

Light-driven whole-cell biotransformations, where heterologous oxidoreductases, imine reductases, mono-oxygenases, or ene-reductases are directly coupled to photosynthetic light reactions allow the sustainable utilization of photosynthetic reductants (NADPH or reduced Fd). Heterologous enzymes represent an additional intracellular electron sink, therefore an optimal balance between photosynthetic electron supply and consumption by the desired redox reactions is necessary for increased photosynthetic yield, optimal cell fitness and thus, prolonged production. Accordingly, a heterologously expressed Fd-dependent monooxygenase, Cyt P450, increased the photosynthetic capacity and ATP/NADPH ratio in *Synechococcus* sp. 7002 (Berepiki et al., 2018).

Elimination of photosynthetic competing pathways could positively impact productivity of the heterologous enzymes by providing an increased amount of photosynthetic reducing power (Figure 2). Indeed, elimination of Flv1/3 in *Synechocystis* increased the product formation rate and specific activity of the heterologous YqjM (Old Yellow Enzyme homolog) that catalyzes NAD(P)H-dependent ene reduction (Assil Companioni et al., 2020). Considering that NADPH is a limiting factor for the YqjM reactions, it appears convincing that the loss of Flv1 or Flv3 provided higher NADPH availability for the ene reductase. However, the specific product formation in the ΔFlv1 and ΔFlv3 mutant was already higher in the dark, when compared to the WT (Assil Companioni et al., 2020), suggesting that the loss of Flv1 or Flv3 provides additional advantages, likely by triggering rearrangements in the central carbon metabolic pathways. Similarly, deleting NdhD2, the subunit of NDH-1_1 involved in CET, enhanced the conversion of ethoxyresorufin to resorufin by Cyt P450 in *Synechococcus* sp. PCC 7002 (Berepiki et al., 2018).

Cyanobacteria and microalgae are able to discharge excessive photosynthetic reductants to the environment that could be potentially harnessed for conversion of light energy into renewable electricity in biophotovoltaic devices. Application of *Synechocystis* cells deficient in FDPs and RTOs (ΔFlv2/ΔFlv3/ΔRTOs) increased the power density fivefold (Saar et al., 2018), thus expanding utilization of photosynthetic microbes in emerging technologies.

12 | PHOTOPRODUCTION OF COMPOUNDS DERIVED FROM THE CENTRAL CARBON METABOLIC PATHWAYS

Photosynthetic production of carbon-derived chemicals by cyanobacteria and microalgae is a promising alternative for traditional petroleum-based synthesis of commodity and high value chemicals. In cyanobacteria, fixed CO_2 is allocated to biosynthetic pathways downstream the CBB-cycle, where innate or heterologous enzymes catalyze the production of target chemicals. Since photosynthesis provides the required reducing power and energy, engineering the photosynthetic electron transfer could potentially optimize cofactor supply for the desired reactions thus increasing productivity of the host cells.

Recently, heterologous D-lactate dehydrogenase was introduced to *Synechococcus* sp. PCC 7002 to produce D-lactate by utilizing the photosynthetically fixed carbon. Redirection of photosynthetic electrons was attempted toward D-lactate biosynthesis by deleting Flv1, Flv3, and a Pgr5 homolog. Loss of Flv1 and/or Flv3 increased D-lactate titers under constant illumination, mild temperature and atm. CO_2. However, ΔFlv1 and ΔFlv3 strains but not the WT underwent chlorosis during the production (Selão et al., 2020). Importantly, deletion of Pgr5 did not induce chlorosis and led to the highest D-lactate titer observed in the study. However, elevating either CO_2 level and/or temperature abolished the production advantage of each photosynthetic mutant over the D-lactate dehydrogenase-expressing reference strain (Selão et al., 2020).

An innate osmoprotectant, sucrose, was utilized as a marker product in salt-stressed *Synechocystis* overexpressing sucrose permease to evaluate whether more photosynthetic electrons could be channeled towards carbon-derived product in Flv3 deletion mutant of *Synechocystis* (Thiel et al., 2019). Indeed, sucrose titer and productivity under constant low or mild illumination and high carbon (1% CO_2) conditions was higher in the ΔFlv3 mutant compared to the reference strain. However, when higher light intensities were applied to increase the photosynthetic electron flux, the advantage of ΔFlv3 disappeared. The carbon flux under high light shifted toward polyhydroxybutyrate (PHB) instead of sucrose biosynthesis, and the loss of Flv3 exacerbated that shift. In silico model of the metabolism suggests that producing PHB is energetically more favorable than sucrose, thus carbon reallocation likely occurred as a compensatory mechanism (Thiel et al., 2019). Moreover, the alteration of the ATP/NADPH ratio can strongly affect carbon flux engineering in Synechocystis (Yao et al., 2020).

In conclusion, redirecting photosynthetic electrons toward carbon-derived products can be realized only when metabolic and photosynthetic engineering are combined and coupled with modeling and high-throughput omics studies on molecular mechanisms. Therefore, channeling photosynthetic electrons is more efficient towards enzymes associated directly with photosynthetic light reactions rather than the central carbon metabolic pathways.

13 | INTRODUCING FDPS TO CROPS

FDPs were lost in angiosperms during the course of evolution, presumably due to more sophisticated and energetically less costly regulatory mechanisms of photosynthesis. Introducing FDPs to chloroplasts is a tempting possibility to equip angiosperms with an additional photoprotective mechanism for improved robustness and ideally, higher crop yields.

FLVA/B of *P. patens* was expressed in chloroplasts of PGR5-deficient *A. thaliana*. In steady-state photosynthesis, FLVA/B operated in the *pgr5* background and transferred 25% of water-derived electrons to O_2, restoring the compromised *pmf* generation and rescuing the inability of *pgr5* to keep PSI oxidized. Curiously, FDPs remained inactive in the WT background, suggesting reciprocity

between PGR5 and FDPs. Nonetheless, exposure to fluctuating light triggered FDPs in the WT background and PSI was protected from overreduction that otherwise occurs at sudden increases in light intensity (Yamamoto et al., 2016). Heterologous FDPs thus can improve photoprotection in angiosperms and protect the photosynthetic machinery from excitation pressure. Hence, FLVA/B of *P. patens* was introduced to *Oryza sativa*, rice, a species of high economic importance. To test whether reciprocity between heterologous FDPs and PGR5 is a common feature in angiosperms, FLVA/B was expressed in chloroplasts of rice deficient of PGR5 and/or NDH assembly protein CRR6. Similar to what was observed in *A. thaliana*, FLVA/B restored *pmf* and rescued the impaired PSI oxidation in *pgr5* or *pgr5/crr6* rice, while in the WT background a positive effect was only demonstrated under intermittent periods of strong light (Wada et al., 2018). Nevertheless, FDPs wastefully dissipate absorbed energy via the protective Mehler-like reaction. Whether heterologously expressed FDPs could provide enhanced robustness for crops without trade-off in biomass yield remains to be elucidated.

ACKNOWLEDGMENTS

This work was supported by the NordForsk Nordic Center of Excellence "NordAqua" (no. 82845 to Y.A.), the Academy of Finland (project no. 315119 to Y.A), the Kone Foundation (no. 201710311 to M.J.), and EU FET Open (grant agreement no. 899576 to Y.A.).

AUTHOR CONTRIBUTIONS

Lauri Nikkanen, Daniel Solymosi, Martina Jokel, Yagut Allahverdiyeva: Wrote the article.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Lauri Nikkanen https://orcid.org/0000-0002-7192-9322
Daniel Solymosi https://orcid.org/0000-0003-3655-3654
Martina Jokel https://orcid.org/0000-0001-9195-7577
Yagut Allahverdiyeva https://orcid.org/0000-0002-9262-1757

REFERENCES

Alboresi, A., Storti, M., Cendron, L. & Morosinotto, T. (2019) Role and regulation of class-C flavodiiron proteins in photosynthetic organisms. *The Biochemical Journal*, 476, 2487–2498.

Allahverdiyeva, Y., Isojärvi, J., Zhang, P. & Aro, E. (2015) Cyanobacterial oxygenic photosynthesis is protected by Flavodiiron proteins. *Life (Basel, Switzerland)*, 5, 716–743.

Allahverdiyeva, Y., Mustila, H., Ermakova, M., Bersanini, L., Richaud, P., Ajlani, G. et al. (2013) Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 4111–4116.

Angeleri, M., Muth-Pawlak, D., Aro, E. & Battchikova, N. (2016) Study of O-phosphorylation sites in proteins involved in photosynthesis-related processes in *Synechocystis* sp. strain PCC 6803: application of the SRM approach. *Journal of Proteome Research*, 15, 4638–4652.

Appel, J., Hueren, V., Boehm, M. & Gutekunst, K. (2020) Cyanobacterial in vivo solar hydrogen production using a photosystem I–hydrogenase (PsaD-HoxYH) fusion complex. *Nature Energy*, 5, 458–467.

Assil Companioni, L., Büchsenschütz, H.C., Solymosi, D., Dyczmons, N.G., Bauer, K.K.F., Wallner, S. et al. (2020) Engineering of NADPH supply boosts photosynthesis-driven biotransformations. *ACS Catalysis*, 10, 11864–11877.

Berepiki, A., Gittins, J.R., Moore, C.M. & Bibby, T.S. (2018) Rational engineering of photosynthetic electron flux enhances light-powered cytochrome P450 activity. *Synthetic Biology*, 3, ysy009.

Berry, S., Schneider, D., Vermaas, W.F. & Rögner, M. (2002) Electron transport routes in whole cells of *Synechocystis* sp. strain PCC 6803: the role of the cytochrome bd-type oxidase. *Biochemistry*, 41, 3422–3429.

Brown, K.A., Guo, Z., Tokmina-Lukaszewska, M., Scott, L.W., Lubner, C.E., Smolinski, S. et al. (2019) The oxygen reduction reaction catalyzed by *Synechocystis* sp. PCC 6803 flavodiiron proteins. *Sustainable Energy & Fuels*, 3, 3191–3200.

Buchert, F., Mosebach, L., Gäbelein, P. & Hippler, M. (2020) PGR5 is required for efficient Q cycle in the cytochrome b6f complex during cyclic electron flow. *The Biochemical Journal*, 477, 1631–1650.

Burlacot, A., Richaud, P., Gosset, A., Li-Beisson, Y. & Peltier, G. (2020) Algal photosynthesis converts nitric oxide into nitrous oxide. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 2704–2709.

Cassier-Chauvat, C. & Chauvat, F. (2014) Function and regulation of Ferredoxins in the Cyanobacterium, Synechocystis PCC6803: recent advances. *Life (Basel, Switzerland)*, 4, 666–680.

Chaux, F., Burlacot, A., Mekhalfi, M., Auroy, P., Blangy, S., Richaud, P. et al. (2017) Flavodiiron proteins promote fast and transient O-2 photoreduction in Chlamydomonas. *Plant Physiology*, 174, 1825–1836.

Checchetto, V., Segalla, A., Allorent, G., La Rocca, N., Leanza, L., Giacometti, G.M. et al. (2012) Thylakoid potassium channel is required for efficient photosynthesis in cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 11043–11048.

Courteille, A., Vesa, S., Sanz-Barrio, R., Cazale, A., Becuwe-Linka, N., Farran, I. et al. (2013) Thioredoxin m4 controls photosynthetic alternative electron pathways in Arabidopsis. *Plant Physiology*, 161, 508–520.

DalCorso, G., Pesaresi, P., Masiero, S., Aseeva, E., Schuenemann, D., Finazzi, G. et al. (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. *Cell*, 132, 273–285.

Dann, M. & Leister, D. (2019) Evidence that cyanobacterial Sll1217 functions analogously to PGRL1 in enhancing PGR5-dependent cyclic electron flow. *Nature Communications*, 10, 5299.

Ermakova, M., Huokko, T., Richaud, P., Bersanini, L., Howe, C.J., Lea-Smith, D.J. et al. (2016) Distinguishing the roles of thylakoid respiratory terminal oxidases in the Cyanobacterium Synechocystis sp PCC 6803. *Plant Physiology*, 171, 1307–1319.

Gerotto, C., Alboresi, A., Meneghesso, A., Jokel, M., Suorsa, M., Aro, E. et al. (2016) Flavodiiron proteins act as safety valve for electrons in *Physcomitrella patens*. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 12322–12327.

Guo, J., Nguyen, A.Y., Dai, Z., Su, D., Gaffrey, M.J., Moore, R.J. et al. (2014) Proteome-wide light/dark modulation of thiol oxidation in cyanobacteria revealed by quantitative site-specific redox proteomics. *Molecular & Cellular Proteomics*, 13, 3270–3285.

Hanke, G.T., Satomi, Y., Shinmura, K., Takao, T. & Hase, T. (2011) A screen for potential ferredoxin electron transfer partners uncovers new, redox dependent interactions. *Biochimica et Biophysica Acta—Proteins and Proteomics*, 1814, 366–374.

Helman, Y., Barkan, E., Eisenstadt, D., Luz, B. & Kaplan, A. (2005) Fractionation of the three stable oxygen isotopes by oxygen-producing and oxygen-consuming reactions in photosynthetic organisms. *Plant Physiology*, 138, 2292–2298.

Helman, Y., Tchernov, D., Reinhold, L., Shibata, M., Ogawa, T., Schwarz, R. et al. (2003) Genes encoding a-type flavoproteins are essential for photoreduction of O-2 in cyanobacteria. *Current Biology*, 13, 230–235.

Hertle, A.P., Blunder, T., Wunder, T., Pesaresi, P., Pribil, M., Armbruster, U. et al. (2013) PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. *Molecular Cell*, 49, 511–523.

Hisabori, T., Sunamura, E., Kim, Y. & Konno, H. (2013) The chloroplast ATP synthase features the characteristic redox regulation machinery. *Antioxidants & Redox Signaling*, 19, 1846–1854.

Houille-Vernes, L., Rappaport, F., Wollman, F., Alric, J. & Johnson, X. (2011) Plastid terminal oxidase 2 (PTOX2) is the major oxidase involved in chlororespiration in Chlamydomonas. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 20820–20825.

Howitt, C.A. & Vermaas, F.J. (1998) Quinol and cytochrome oxidases in the Cyanobacterium Synechocystis sp. PCC 6803. *Biochemistry*, 37, 17944–17951.

Imashimizu, M., Bernat, G., Sunamura, E., Broekmans, M., Konno, H., Isato, K. et al. (2011) Regulation of F0F1-ATPase from Synechocystis sp PCC 6803 by gamma and epsilon subunits is significant for light/dark adaptation. *The Journal of Biological Chemistry*, 286, 26595–26602.

Jans, F., Mignolet, E., Houyoux, P., Cardol, P., Ghysels, B., Cuiné, S. et al. (2008) A type II NAD(P)H dehydrogenase mediates light-independent plastoquinone reduction in the chloroplast of Chlamydomonas. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 20546–20551.

Jokel, M., Johnson, X., Peltier, G., Aro, E. & Allahverdiyeva, Y. (2018) Hunting the main player enabling *Chlamydomonas reinhardtii* growth under fluctuating light. *The Plant Journal*, 94, 822–835.

Jokel, M., Nagy, V., Toth, S.Z., Kosourov, S. & Allahverdiyeva, Y. (2019) Elimination of the flavodiiron electron sink facilitates long-term-H-2 photoproduction in green algae. *Biotechnology for Biofuels*, 12, 280.

Kanygin, A., Milrad, Y., Thummala, C., Reifschneider, K., Baker, P., Marco, P. et al. (2020) Rewiring photosynthesis: a photosystem I-hydrogenase chimera that makes H_2 in vivo. *Energy & Environmental Science*, 13, 2903–2914.

Komenda, J., Sobotka, R. & Nixon, P.J. (2012) Assembling and maintaining the photosystem II complex in chloroplasts and cyanobacteria. *Current Opinion in Plant Biology*, 15, 245–251.

Kosourov, S., Nagy, V., Shevela, D., Jokel, M., Messinger, J. & Allahverdiyeva, Y. (2020) Water oxidation by photosystem II is the primary source of electrons for sustained H(2) photoproduction in nutrient-replete green algae. *Proceedings of the National Academy of Sciences of the United States of America*, 117, 29629–29636.

Kosourov, S., Jokel, M., Aro, E. & Allahverdiyeva, Y. (2018) A new approach for sustained and efficient H-2 photoproduction by *Chlamydomonas reinhardtii*. *Energy & Environmental Science*, 11, 1431–1436.

Kufryk, G.I. & Vermaas, W.F. (2006) Sll1717 affects the redox state of the plastoquinone pool by modulating quinol oxidase activity in thylakoids. *Journal of Bacteriology*, 188, 1286–1294.

Laughlin, T.G., Bayne, A.N., Trempe, J.F., Savage, D.F. & Davies, K.M. (2019) Structure of the complex I-like molecule NDH of oxygenic photosynthesis. *Nature*, 566, 411–414.

Laughlin, T.G., Savage, D.F. & Davies, K.M. (2020) Recent advances on the structure and function of NDH-1: the complex I of oxygenic photosynthesis. *Biochimica et Biophysica—Bioenergetics*, 1861, 148254.

Lea-Smith, D.J., Ross, N., Zori, M., Bendall, D.S., Dennis, J.S., Scott, S.A. et al. (2013) Thylakoid terminal oxidases are essential for the Cyanobacterium Synechocystis sp. PCC 6803 to survive rapidly changing light intensities. *Plant Physiology*, 162, 484–495.

Malone, L.A., Proctor, M.S., Hitchcock, A., Hunter, C.N. & Johnson, M.P. (2021) Cytochrome b6f: orchestrator of photosynthetic electron transfer. *Biochimica et Biophysica Acta—Bioenergetics*, 1862, 148380.

Melis, A. (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. *Plant Science*, 177, 272–280.

Mignolet, E., Lecler, R., Ghysels, B., Remacle, C. & Franck, F. (2012) Function of the chloroplastic NAD(P)H dehydrogenase Nda2 for H2 photoproduction in Sulphur-deprived *Chlamydomonas reinhardtii*. *Journal of Biotechnology*, 162, 81–88.

Miller, N.T., Vaughn, M.D. & Burnap, R.L. (2021) Electron flow through NDH-1 complexes is the major driver of cyclic electron flow-dependent proton pumping in cyanobacteria. *Biochimica et Biophysica Acta—Bioenergetics*, 1862, 148354.

Milrad, Y., Schweitzer, S., Feldman, Y. & Yacoby, I. (2021) Bi-directional electron transfer between H_2 and NADPH mitigates light fluctuation responses in green algae. *Plant Physiology*. https://doi.org/10.1093/plphys/kiab051.

Mosebach, L., Heilmann, C., Mutoh, R., Gäbelein, P., Steinbeck, J., Happe, T. et al. (2017) Association of Ferredoxin:NADP(+) oxidoreductase with the photosynthetic apparatus modulates electron transfer in *Chlamydomonas reinhardtii*. *Photosynthesis Research*, 134, 291–306.

Mullineaux, C.W. (2014) Co-existence of photosynthetic and respiratory activities in cyanobacterial thylakoid membranes. *Biochimica et Biophysica Acta*, 1837, 503–511.

Mustila, H., Paananen, P., Battchikova, N., Santana-Sanchez, A., Muth-Pawlak, D., Hagemann, M. et al. (2016) The flavodiiron protein Flv3 functions as a homo-oligomer during stress acclimation and is distinct from the Flv1/Flv3 hetero-oligomer specific to the O-2 photoreduction pathway. *Plant & Cell Physiology*, 57, 1468–1483.

Muzzopappa, F. & Kirilovsky, D. (2020) Changing color for Photoprotection: the Orange carotenoid protein. *Trends in Plant Science*, 25, 92–104.

Nawrocki, W.J., Bailleul, B., Cardol, P., Rappaport, F., Wollman, F. & Joliot, P. (2019a) Maximal cyclic electron flow rate is independent of PGRL1 in Chlamydomonas. *Biochimica et Biophysica Acta—Bioenergetics*, 1860, 425–432.

Nawrocki, W.J., Buchert, F., Joliot, P., Rappaport, F., Bailleul, B. & Wollman, F. (2019b) Chlororespiration controls growth under intermittent light. *Plant Physiology*, 179, 630–639.

Nikkanen, L., Santana Sánchez, A., Ermakova, M., Rögner, M., Cournac, L. & Allahverdiyeva, Y. (2020) Functional redundancy between flavodiiron proteins and NDH-1 in Synechocystis sp. PCC 6803. *The Plant Journal*, 103, 1460–1476.

Nikkanen, L., Toivola, J., Trotta, A., Guinea Diaz, M., Tikkanen, M., Aro, E. et al. (2018) Regulation of cyclic electron flow by chloroplast NADPH-dependent thioredoxin system. *Plant Direct*, 2, 1–24.

Niyogi, K.K. & Truong, T.B. (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Current Opinion in Plant Biology*, 16, 307–314.

Pan, X., Cao, D., Xie, F., Xu, F., Su, X., Mi, H. et al. (2020) Structural basis for electron transport mechanism of complex I-like photosynthetic NAD(P)H dehydrogenase. *Nature Communications*, 11, 610.

Peden, E.A., Boehm, M., Mulder, D.W., Davis, R., Old, W.M., King, P.W. et al. (2013) Identification of global ferredoxin interaction networks in *Chlamydomonas reinhardtii*. *The Journal of Biological Chemistry*, 288, 35192–35209.

Peltier, G., Aro, E. & Shikanai, T. (2016) NDH-1 and NDH-2 plastoquinone reductases in oxygenic photosynthesis. *Annual Review of Plant Biology*, 67, 55–80.

Pils, D. & Schmetterer, G. (2001) Characterization of three bioenergetically active respiratory terminal oxidases in the cyanobacterium Synechocystis sp. strain PCC 6803. *FEMS Microbiology Letters*, 203, 217–222.

Saar, K.L., Bombelli, P., Lea-Smith, D., Call, T., Aro, E., Müller, T. et al. (2018) Enhancing power density of biophotovoltaics by decoupling storage and power delivery. *Nature Energy*, 3, 75–81.

Santana-Sanchez, A., Solymosi, D., Mustila, H., Bersanini, L., Aro, E. & Allahverdiyeva, Y. (2019) Flavodiiron proteins 1-to-4 function in versatile combinations in O-2 photoreduction in cyanobacteria. *eLife*, 8, e45766.

Saroussi, S., Wittkopp, T.M. & Grossman, A.R. (2016) The type II NADPH dehydrogenase facilitates cyclic electron flow, energy-dependent quenching, and chlororespiratory metabolism during acclimation of *Chlamydomonas reinhardtii* to nitrogen deprivation. *Plant Physiology*, 170, 1975–1988.

Saroussi, S., Karns, D.A.J., Thomas, D.C., Bloszies, C., Fiehn, O., Posewitz, M.C. et al. (2019) Alternative outlets for sustaining photosynthetic electron transport during dark-to-light transitions. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 11518–11527.

Schmetterer, G. (2016) The respiratory terminal oxidases (RTOs) of cyanobacteria. In: Cramer, W. & Kallas, T. (Eds.) *Cytochrome complexes: evolution, structures, energy transduction, and signaling. Advances in Photosynthesis and Respiration (Including Bioenergy and Related Processes)*. Dordrecht: Springer.

Schüller, J.M., Birrell, J.A., Tanaka, H., Konuma, T., Wulfhorst, H., Cox, N. et al. (2019) Structural adaptations of photosynthetic complex I enable ferredoxin-dependent electron transfer. *Science*, 363, 257–260.

Schüller, J.M., Saura, P., Thiemann, J., Schüller, S.K., Gamiz-Hernandez, A. P., Kurisu, G. et al. (2020) Redox-coupled proton pumping drives carbon concentration in the photosynthetic complex I. *Nature Communications*, 11, 494.

Selão, T.T., Jebarani, J., Ismail, N.A., Norling, B. & Nixon, P.J. (2020) Enhanced production of D-lactate in cyanobacteria by re-routing photosynthetic cyclic and pseudo-cyclic electron flow. *Frontiers in Plant Science*, 10, 1700.

Sétif, P., Shimakawa, G., Krieger-Liszkay, A. & Miyake, C. (2020) Identification of the electron donor to flavodiiron proteins in Synechocystis sp. PCC 6803 by in vivo spectroscopy. *Biochimica et Biophysica Acta—Bioenergetics*, 1861, 148256.

Shikanai, T. & Yamamoto, H. (2017) Contribution of cyclic and pseudo-cyclic electron transport to the formation of proton motive force in chloroplasts. *Molecular Plant*, 10, 20–29.

Shimakawa, G., Ishizaki, K., Tsukamoto, S., Tanaka, M., Sejima, T. & Miyake, C. (2017) The liverwort, marchantia, drives alternative electron flow using a flavodiiron protein to protect PSI. *Plant Physiology*, 173, 1636–1647.

Shimakawa, G. & Miyake, C. (2018) Changing frequency of fluctuating light reveals the molecular mechanism for P700 oxidation in plant leaves. *Plant Direct*, 2, e00073.

Shimakawa, G., Shaku, K., Nishi, A., Hayashi, R., Yamamoto, H., Sakamoto, K. et al. (2015) FLAVODIIRON2 and FLAVODIIRON4 proteins mediate an oxygen-dependent alternative electron flow in Synechocystis sp PCC 6803 under CO2-limited conditions. *Plant Physiology*, 167, 472–U732.

Sonoike, K. (2011) Photoinhibition of photosystem I. *Physiologia Plantarum*, 142, 56–64.

Steinbeck, J., Nikolova, D., Weingarten, R., Johnson, X., Richaud, P., Peltier, G. et al. (2015) Deletion of proton gradient regulation 5 (PGR5) and PGR5-like 1 (PGRL1) proteins promote sustainable light-driven hydrogen production in *Chlamydomonas reinhardtii* due to increased PSII activity under sulfur deprivation. *Frontiers in Plant Science*, 6, 892.

Storti, M., Puggioni, M.P., Segalla, A., Morosinotto, T. & Alboresi, A. (2020a) The chloroplast NADH dehydrogenase-like complex influences the photosynthetic activity of the moss *Physcomitrella patens*. *Journal of Experimental Botany*, 71, 5538–5548.

Storti, M., Segalla, A., Mellon, M., Alboresi, A. & Morosinotto, T. (2020b) Regulation of electron transport is essential for photosystem I stability and plant growth. *The New Phytologist*, 228, 1316–1326.

Tamoi, M., Miyazaki, T., Fukamizo, T. & Shigeoka, S. (2005) The Calvin cycle in cyanobacteria is regulated by CP12 via the NAD(H)/NADP (H) ratio under light/dark conditions. *Plant Journal*, 42, 504–513.

Theune, M.L., Hildebrandt, S., Steffen-Heins, A., Bilger, W., Gutekunst, K. & Appel, J. (2021) In-vivo quantification of electron flow through photosystem I—cyclic electron transport makes up about 35% in a cyanobacterium. *Biochimica et Biophysica Acta—Bioenergetics*, 1862, 148353.

Thiel, K., Patrikainen, P., Nagy, C., Fitzpatrick, D., Pope, N., Aro, E.M. et al. (2019) Redirecting photosynthetic electron flux in the cyanobacterium Synechocystis sp. PCC 6803 by the deletion of flavodiiron protein Flv3. *Microbial Cell Factories*, 18, 189–192.

Tolleter, D., Ghysels, B., Alric, J., Petroutsos, D., Tolstygina, I., & Krawietz, D.. (2011) Control of hydrogen photoproduction by the proton gradient generated by cyclic electron flow in chlamydomonas reinhardtii. *The Plant Cell*, 23(7), 2619–2630. https://doi.org/10.1105/tpc.111.086876.

Vicente, J.B., Gomes, C.M., Wasserfallen, A. & Teixeira, M. (2002) Module fusion in an A-type flavoprotein from the cyanobacterium Synechocystis condenses a multiple-component pathway in a single polypeptide chain. *Biochemical and Biophysical Research Communications*, 294, 82–87.

Wada, S., Yamamoto, H., Suzuki, Y., Yamori, W., Shikanai, T. & Makino, A. (2018) Flavodiiron protein substitutes for cyclic electron flow without competing CO_2 assimilation in rice. *Plant Physiology*, 176, 1509–1518.

Yamamoto, H., Takahashi, S., Badger, M.R. & Shikanai, T. (2016) Artificial remodelling of alternative electron flow by flavodiiron proteins in Arabidopsis. *Nature Plants*, 22, 16012.

Yao, L., Shabestary, K., Björk, S.M., Asplund-Samuelsson, J., Jönsson, H.N., Jahn, M. et al. (2020) Pooled CRISPRi screening of the cyanobacterium Synechocystis sp PCC 6803 for enhanced industrial phenotypes. *Nature Communications*, 11, 1666.

Yeremenko, N., Jeanjean, R., Prommeenate, P., Krasikov, V., Nixon, P.J., Vermaas, W.F.J. et al. (2005) Open reading frame ssr2016 is required for antimycin A-sensitive photosystem I-driven cyclic electron flow in the Cyanobacterium Synechocystis sp. PCC 6803. *Plant & Cell Physiology*, 46, 1433–1436.

Zhang, C., Shuai, J., Ran, Z., Zhao, J., Wu, Z., Liao, R. et al. (2020) Structural insights into NDH-1 mediated cyclic electron transfer. *Nature Communications*, 11, 888.

Zhang, P., Eisenhut, M., Brandt, A., Carmel, D., Silen, H.M., Vass, I. et al. (2012) Operon flv4-flv2 provides Cyanobacterial photosystem II with flexibility of electron transfer. *Plant Cell*, 24, 1952–1971.

How to cite this article: Nikkanen L, Solymosi D, Jokel M, Allahverdiyeva Y. Regulatory electron transport pathways of photosynthesis in cyanobacteria and microalgae: Recent advances and biotechnological prospects. *Physiologia Plantarum*. 2021;1–12. https://doi.org/10.1111/ppl.13404