Review

Regulation of electron transport in microalgae ☆

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ARTICLE INFO

ABSTRACT

Unicellular algae are characterized by an extreme flexibility with respect to their responses to environmental constraints. This flexibility probably explains why microalgae show a very high biomass yield, constitute one of the major contributors to primary productivity in the oceans and are considered a promising choice for biotechnological applications. Flexibility results from a combination of several factors including fast changes in the light-harvesting apparatus and a strong interaction between different metabolic processes (e.g. respiration and photosynthesis), which all take place within the same cell. Microalgae are also capable of modifying their photosynthetic electron flow capacity, by changing its maximum rate and/or by diverting photogenerated electrons towards different sinks depending on their growth status. In this review, we will focus on the occurrence and regulation of alternative electron flows in unicellular algae and compare data obtained in these systems with those available in vascular plants. This article is part of a Special Issue entitled: Regulation of Electron Transport in Chloroplasts.

1. Introduction

Eukaryotic photosynthesis is an extremely ancient process, the primary endosymbiotic event considered to be at the origin of all photosynthetic eukaryotes being traceable back at least 1.8 billion years [1]. Since this event, different groups of photosynthetic eukaryotes have evolved, characterized by distinct thylakoid membrane topologies and different light-harvesting accessory pigments. The basic mechanisms of oxygenic photosynthesis—light-harvesting by specialized pigments, electron transfer between photosystems II and I (PSII and PSI), synthesis of ATP and of reducing power (NADPH), carbon assimilation—are however basically conserved in these groups. Conversely, different strategies have evolved to adapt photosynthesis to biotic/abiotic constraints. They involve photoprotection mecha-

nisms [2], carbon assimilation [3], acclimation to change in nutrient availability [4] and electron diversion towards alternative sinks, which will be described in this review (see also reference [5]).

In oxygenic photosynthesis, carbon assimilation is mainly driven by linear electron flow (LEF), which requires the in series activity of the two photosystems. ATP and NADPH are produced in this process, although in a ratio probably not sufficient to support the formation of glyceraldehyde-3-phosphate, the export product of the Calvin Benson Bassham (CBB) cycle [6]. And, in no case, the ATP/NADPH ratio coupled with LEF can overcome the value of 1.5, i.e. the stoichiometry required for CO_2 fixation. This means that photosynthesis cannot fuel nitrogen metabolism, synthesis of lipids, amino acids, pigments, proteins and gene expression (which also require reducing equivalents and ATP in variable stoichiometries) at the same time as the CBB cycle (the 'energy balance' issue, [7,8]). Various abiotic and biotic stresses often modify the efficiency and/or the ratio of ATP and NADPH generation in the light, further exacerbating the difficulty of managing the cellular "energy stocks." Therefore, other mechanisms must operate in the light to provide 'extra' ATP for the carbon assimilatory metabolism (the 'ATP shortage' problem, [7]). In plants and algae, different alternative electron flow pathways exist, which can compete with linear electron flow for absorbed quanta. They include oxygen reduction by photosynthesis, through various processes namely photorespiration [9], the Mehler reaction [10], respiration (owing to activity of the malate–oxaloacetate and/or the malate–aspartate shuttles [11]) and PTOX not only in some alpine [12]

and halotolerant [13] plants but also in some marine organisms [14]. Some of these pathways can increase the ATP synthesis capacity. Another process able to produce ATP without net NADPH generation is cyclic electron flow (CEF) around PSI, the extent of which is extremely important in the green alga *Chlamydomonas reinhardtii*, under specific metabolic conditions [15]. Eventually, electrons can be directed towards other metabolic and/or regulatory pathways [16]. All these processes have been extensively studied in the past, leading to the conclusion that the efficiency of electron diversion away from carbon assimilation can be extremely high. Therefore, this capacity must be tightly regulated, in order to avoid an excessive decrease of the overall quantum yield of photosynthesis. Recent research has identified some key factors, which control the diversity of the electron transfer pathways, responding to changes in the environmental stimuli in terms of changing their concentrations and/or activity (reviewed by Eberhard et al. [17]).

Microalgae represent an ideal system to study the mechanisms and regulation of electron flow because they grow faster than plants; they can be isolated as axenic populations and easily manipulated. They have been instrumental in elucidating the mechanism of important processes in photosynthesis like water oxidation (*Chlorella*, [18]), state transitions (changes in the light absorption capacity of the two photosystems; *Chlorella pyrenoidosa*, [19]) and chlororespiration (the respiratory activity taking place in the chloroplast; *Chlorella sorokiniana* [20]). A comparative analysis of algal responses to changing environments has led to the establishment of the general rules governing acclimation to light and nutrient changes (changes in the antenna size versus changes in the number of reaction centers; *Dunaliella tertiolecta* and *Skeletonema costatum* [21]). More recently, studies with Chlamydomonas have revealed essential regulatory processes governing light acclimation (e.g. the role of LHCSR proteins in NPQ [22]) and cyclic electron flow around PSI [23]. As described below, unicellular algae also show an extreme flexibility in adapting their electron flow capacity to changes in their environment.

2. Regulation of photosynthetic electron flow at the PSI acceptor side

The acceptor side of PSI is the place where photosynthesis faces other metabolisms. There, electrons are not only used to feed CO_2 assimilation in the CBB cycle but also to aliment other metabolic processes including nitrate metabolism, biosynthesis of lipids, amino acids and pigments, etc. The PSI acceptor side is also the place where most abiotic and biotic stresses modify the capacity to generate ATP and NADPH, by redirecting electrons towards alternative electron sinks.

2.1. Photorespiration and the Mehler reaction

In C3 plants, oxygenation of ribulose-1,5-bisphosphate by RuBisCo is a major alternative sink for electrons (reviewed by Wingler et al. [24]) (Fig. 1). It employs the same electron flow machinery as CO_2 assimilation. This process is no longer considered just a wasteful phenomenon but rather a physiologically relevant process capable of reducing the redox pressure in the stroma—when CO_2 assimilation is low [9]. On the other hand, photorespiration is probably less important in algae than in plants. While early experiments have suggested that photorespiration could be active in Scenedesmus [25], more recent data have reinterpreted this conclusion, showing that oxygen consumption probably results from the reduction of molecular oxygen at the PSI acceptor side [26], i.e. from the occurrence of the Mehler reaction [27] (Fig. 2). This process results in the production of superoxide, which is rapidly converted into H_2O_2 by the activity of the superoxide dismutase (SOD) enzyme. The hydrogen peroxide produced by the SOD is converted back into O_2 and H_2O by a catalase in the peroxisome (when present) during photorespiration. In the case of the Mehler reaction, the H_2O_2 is efficiently scavenged by a chloroplast-associated ascorbate peroxidase (APX) before it diffuses

out of the chloroplast, leading to the production of a monodehydroascorbate radical (MDA), starting from ascorbate and H_2O_2 [28]. The MDA species is extremely reactive and can be reduced by photogenerated PSI electrons at a rate comparable with that of NADP reduction [29]. This pathway of electron transport, called the "Mehler–ascorbate reaction", shares the same electron transfer carriers as LEF until the acceptor side of PSI and is coupled to ATP synthesis with the same ATP/electrons ratio as NADP reduction [30]. Alternatively, MDA can be reduced by photosynthesis through the MDA reductase enzyme, which uses NADPH as a substrate or by another indirect pathway involving the glutathione/NADPH system (reviewed by Asada [10]).

The Mehler reaction could provide a major contribution to the "extra" ATP needed for CO_2 assimilation in plants, so accounting for the observation that O_2 uptake occurs simultaneous to O_2 evolution in steady state photosynthesis, at the rate of 15%–30% [31]. In Chlamydomonas, the existence of an ascorbate peroxidase activity similar to that of plants has been reported [32]. In this alga, where the ascorbate concentration is very low, the ascorbate peroxidase enzyme has an extremely high affinity for this metabolite [32]. Other enzymatic systems than APX could be involved in the Mehler reaction. In cyanobacteria, flavodiiron (Flv) proteins [33] provide a molecular frame for oxygen reduction. Because of the significant sequence conservation with their cyanobacterial counterparts, it has been recently proposed that Flv proteins of microalgae could catalyze the Mehler reaction in these organisms [5].

In principle, the ROS intermediates of the Mehler reaction are responsible for the observed photodamage induced by light exposure [34]. However, an additional role for these ROS has been recently proposed in Chlamydomonas. In this alga, expression of a nuclear-encoded reporter gene coupled to an H_2O_2 sensitive promoter was shown to respond not only to the levels of exogenously added H_2O_2 but also to the light. The higher induction of the reporter gene seen in light–H_2O_2 treated cells was correlated with a lower H_2O_2 scavenging activity [35]. Therefore, the authors concluded that the enhanced H_2O_2 concentration observed upon light exposure would represent a molecular switch to activate a specific ROS signaling pathway within the cell.

Another regulatory role for the Mehler reaction has been recently invoked in Chlamydomonas. This process enhances the reoxidation of the PQ pool during a dark to light transition [36]. By doing so, it could modulate the occurrence of state transitions (a process leading to changes in the relative absorption cross section of PSII and PSI in relationship to redox changes in the plastoquinone pool, reviewed by Rochaix [37]) and consequently the efficiency of cyclic electron flow (see section below).

2.2. Cyclic electron flow around PSI (CEF)

Electrons generated at the PSI reducing side can be re-injected into its donor side via cyclic electron flow around this complex (Fig. 3). Discovered in the 50s by Arnon, this process is now considered as a prominent phenomenon to counterbalance over-reduction of the PSI acceptor side in different photosynthetic organisms [38] and in particular in some unicellular algae [15]. Despite the large number of studies devoted to CEF, the actual pathway by which electrons are transferred back from the acceptor side of PSI to the electron transport chain is still largely unknown. At least two major routes for CEF have been proposed. The first one involves the activity of a chloroplast NAD(P)H dehydrogenase (Ndh) complex. In plants, this enzyme has similar characteristics as the mitochondrial complex I [39–41]. Although its real contribution to CEF is still under debate (see e.g. reference [38]), recent work suggests that the Ndh-mediated CEF can be important in Arabidopsis [42,43]. In most microalgae species, including green algae (with the exception of some *Prasinophytes*), red algae and diatoms, genes encoding this complex have disappeared
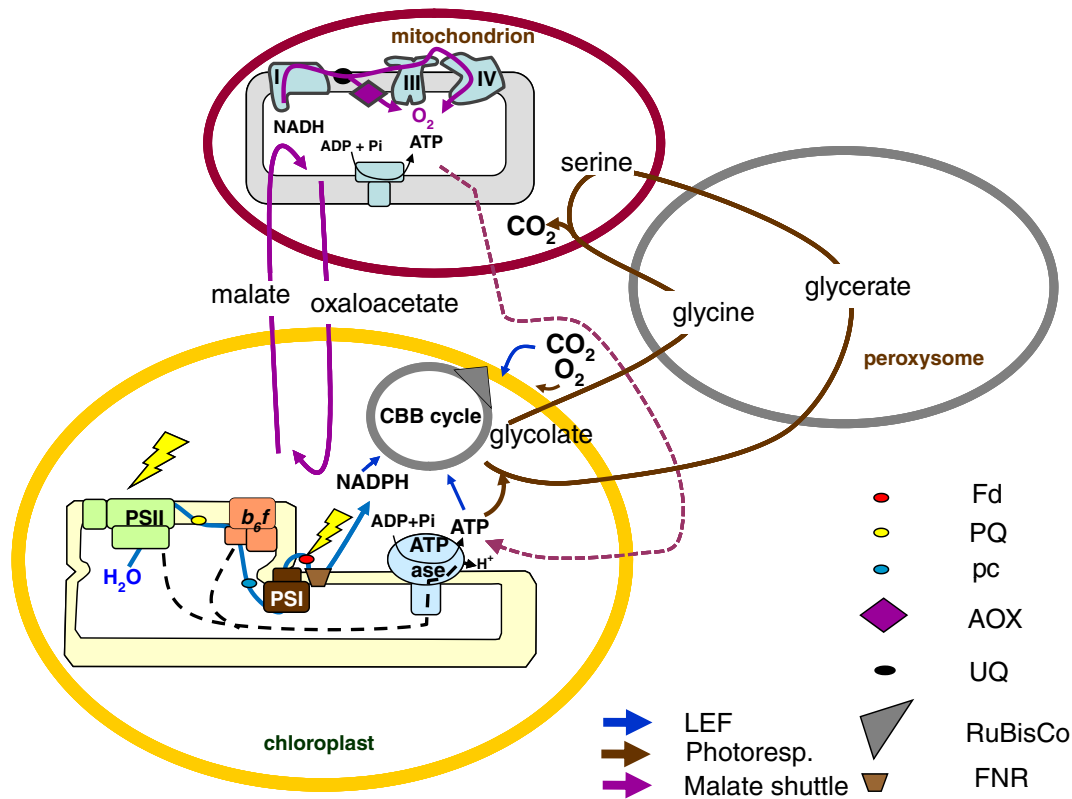
Fig. 1. The photorespiratory and the chloroplast–mitochondrion exchange pathways. The full photorespiratory carbon oxidation cycle of C3 plants involves three separate plant cell organelles. The initial production is phosphoglycolate by the RuBisCo-catalyzed oxygenation of ribulose bisphosphate. After dephosphorylation, glycolate leaves the chloroplast reaching the peroxisome (when present). There, a series of reactions produce glycine by, which is further converted into, serine in the mitochondrion. The final product (glycerate) is generated again in the peroxisome and enters chloroplast and subsequently the CBB cycle by the same transporter that exports glycolate. One ATP is consumed to convert glycerate into 3-phosphoglycerate (PGA), within the chloroplast, which is then free to re enter the CCB cycle [9]. Reducing power can be exported from the chloroplast across the envelope in illuminated leaves. The malate–oxaloacetate shuttle is considered to function as an efficient exporter of excess reductants from the chloroplast. Malate is exported and can be converted into oxaloacetate in the cytosol. In the mitochondrion, a malate–oxaloacetate shuttle is active at the level of the inner membrane. The malate–aspartate shuttle also functions as a reductant transporter. In Fe limited *Phaeodactylum* cells, part of the mitochondrial electron flow chain is shunted by an enhanced AOX (alternative oxidase) activity [61].

at least from the chloroplast genome [44]. Other types of enzymes exist, however, which ensure the same activity. For example, non photochemical reduction of the plastoquinone pool in Chlamydomonas is catalyzed by the Nda2 enzyme, which is localized in the thylakoid membranes [45].

Alternative to the Ndh pathway, the FQR (ferredoxin–quinone reductase) would catalyze the reduction of the plastoquinone pool using ferredoxin as a substrate (Fig. 3). The existence of FQR was deduced from in vitro experiments using antimycin A, a putative inhibitor of this enzyme [46]. However, the nature of this enzyme is still unknown. The FQR could correspond to the cytochrome b_6f complex itself, which could mediate electron flow from Fd to the PQ pool via the recently discovered c′ heme reviewed in reference [47]. Alternatively, the FQR would correspond to a new membrane complex, containing at least two gene products recently discovered in Arabidopsis: PGR5 [48] and PGRL1 [49]. The first one could participate to some extent to cyclic electron flow [40], while the second one would provide a membrane anchor for the complex [49]. PGR5 and PGRL1 gene copies have been identified in all of the genomes from photosynthetic eukaryotes sequenced so far [5,50], suggesting a conserved role for this complex. Expression of PGRL1 is highly increased in iron starved Chlamydomonas cells, where RNAi knockdown mutants have been instrumental to define a role of this protein in both CEF regulation and in iron sensing [51].

The LEF and CEF pathways share a large part of the electron carriers (from the plastoquinone pool to ferredoxin at least). Therefore, a number of models have been proposed in the past to explain how the two paths may operate at the same time without wasting absorbed

energy quanta (reviewed in Eberhard et al. [17]). The two most extreme views predict either a fully dynamic competition between the two pathways for reducing equivalents at the acceptor side of PSI or their complete thermodynamic and kinetic segregation within tight compartments generated by protein–protein interactions. According to the first model, which is mainly supported by studies in plants [43,52], competition would favor linear flow under steady state illumination conditions for instance because of its higher efficiency in Fd reduction. Conversely, CEF would prevail under particular conditions, where the redox state of the electron transfer chain is more reduced. The observed changes in the rates of CEF versus LEF in different experimental conditions would simply reflect a change in the amount of reducing equivalents stored in the PSI donors' pool, providing a flexible system to achieve efficient CO_2 assimilation. According to the second model, segregation of photosynthetic complexes in the grana and/or stroma lamellae and protein–protein interaction would generate diffusion barriers, capable of preventing any mixing between LEF and CEF and of maintaining redox homeostasis in the photosynthetic chain [53]. The extreme view of this model, in which cyclic flow occurs within tightly bound PSI–cyt b_6f supercomplexes, containing stoichiometric amounts of plastocyanin and Fd, has recently received a strong experimental support by data obtained in Chlamydomonas cells acclimated to state 2 [23]. This state corresponds to a condition where PSI absorption is largely increased (at least in this alga), owing to the recruitment by PSI of most of the PSII antennas, LHCIIs (reviewed in Wollman, [54]). The enhancement of CEF activity that accompanies transition to state 2 in Chlamydomonas (e.g. reference [55]) is paralleled by the appearance of a supercomplex
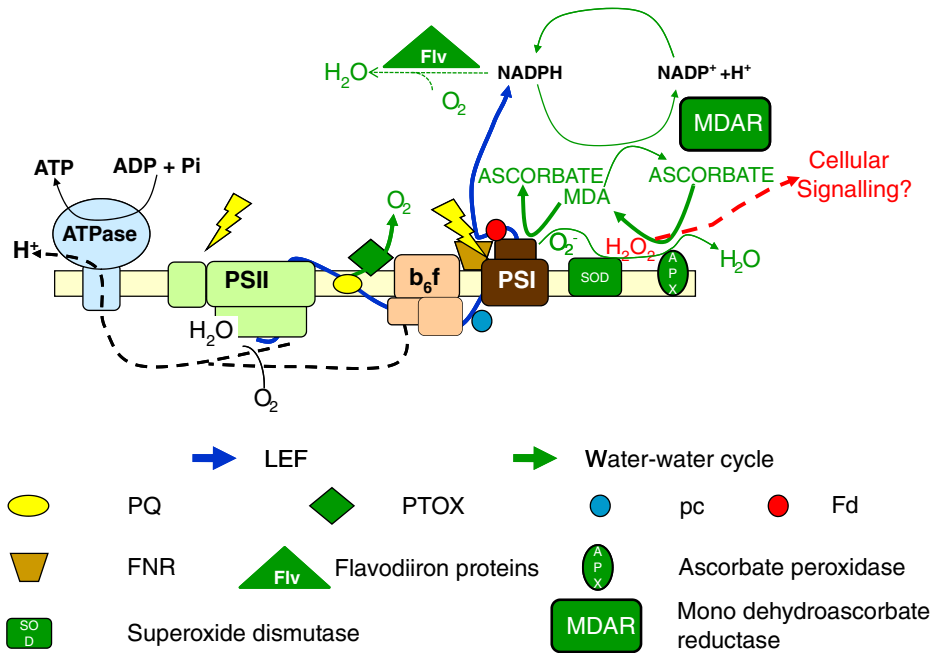
Fig. 2. The water–water cycle. During the Mehler reaction, a superoxide radical anion (O_2^-) is generated by reduction of molecular oxygen (O_2) at the PSI reducing side. A thylakoid-membrane-attached superoxide dismutase (SOD) catalyzes the disproportionation of this compound into hydrogen peroxide (H_2O_2) and O_2. The H_2O_2 is reduced by ascorbate to form water and monodehydroascorbate (MDA) in a reaction catalyzed by ascorbate peroxidase (APX). MDA is a very efficient electron sink, which can be reduced either by electrons form PSI or in a reaction mediated by monodehydroascorbate reductase (MDAR). Recently, the possible involvement of flavodiiron (Flv) protein in reduction of molecular oxygen using NADPH as an electron donor has also been proposed in microalgae. In some organisms, linear electron flow is diminished when electrons are prevented to reach PSI by the activity of a plastoquinone terminal oxidase PTOX. This enzyme catalyzes the reduction of oxygen back to water using electron from reduced plastoquinones. The PTOX-mediated water to water cycle reduces the quantum yield of NADPH production. However, the combined PTOX plus PSII activity results in the net transfer of protons from the stroma to the lumenal space. This leads to the generation of a ΔpH, capable of maintaining ATP synthesis.
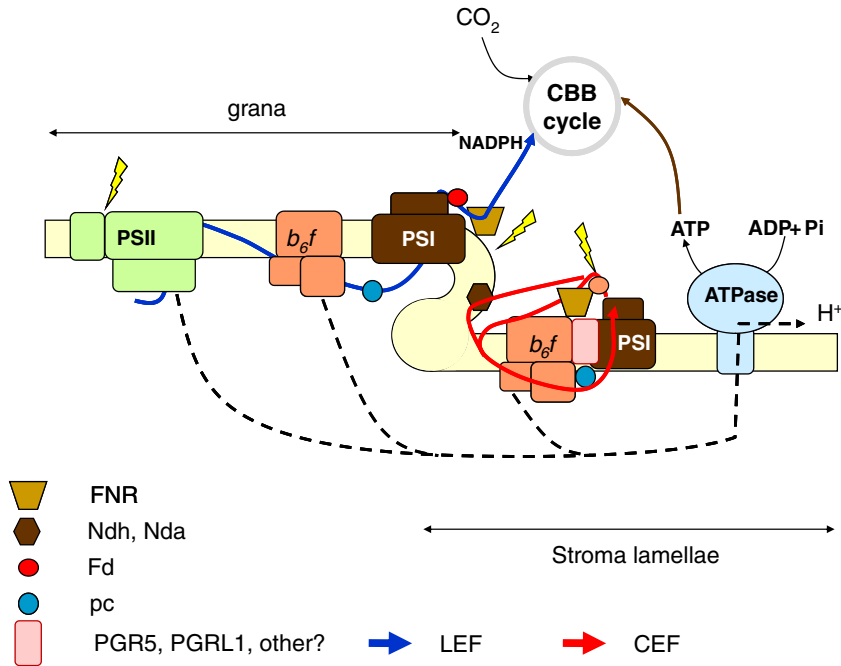

Fig. 3. Cyclic electron flow around PSI. Electrons generated by PSI photochemistry can aliment reduction of its electron donor pools, in a process called cyclic electron flow (CEF). This process involves either a direct re-injection of electrons from the PSI acceptor side into the plastoquinone pool (FQR, possibly linked to PGR5 and PGRL1), or an activity of the chlororespiratory enzyme Ndh. It has been proposed that CEF can occur in a freely diffusing system or through a compartmentalization of the photosynthetic complexes and electron carriers mediated by protein–protein interactions within the two compartments, the grana and the stroma lamellae. Recent data indicate that these interactions result in the formation of a supercomplex capable of performing CEF in Chlamydomonas [23].

complex containing PSI, LHCI, cyt b₆f, FNR and LHCII [23]. This complex has the capacity to perform CEF in vitro.

Besides the mechanistic issues, what is the physiological role of CEF in photosynthetic eukaryotes? In plants, the severe growth phenotype observed in mutants deficient in both the Ndh and the FQR/PGR5 complexes has led to the suggestion that CEF is essential for plant growth [42]. On the other hand, the lack of a growth phenotype associated with the single mutants has been interpreted as an evidence for both CEF pathways to be active and capable of compensating each other in the chloroplast [42]. In Chlamydomonas, despite the high CEF capacity observed, no clear evidence for a significant contribution of this process to the photosynthetic metabolism was obtained (e.g. reference [15] for a review), until the growth capacity of mutants altered in both respiration (*dums*, for dark uniparental minus [56]) and CEF (via state transitions) was tested. These mutants show a decrease in photosynthetic growth and therefore in biomass production [57], suggesting that both the energetic interaction between the chloroplast and the mitochondria and CEF can provide extra ATP for photosynthetic activity. These processes are intimately linked in Chlamydomonas, owing to the interplay between respiration, reduction of the plastoquinone pool, state transitions and cyclic electron flow [54]. It is therefore conceivable that CEF and metabolic exchanges between respiration and photosynthesis may be active in living cells and adjust each other to ensure optimum light utilization.

2.3. Metabolic interactions between organelles

Reducing equivalents generated by photosynthesis can be consumed in the mitochondria (Fig. 2) by the respiratory chain, owing to metabolic exchanges between the two organelles. This process can be mediated by the activity of a malate–oxaloacetate shuttle or by the aspartate oxaloacetate shuttle [58]. Metabolic interactions between the chloroplast and the mitochondrion may have multiple physiological consequences in plants (reviewed by Noctor et al. [59]), where recent estimates indicate that this process may utilize up to 10% of the total PSII-driven electron flow. In algae, a malate shuttle has been identified in Chlamydomonas [50], where the photosynthetic characterization of *dums* mutants [60] has pinpointed a significant role for the metabolic interactions between the chloroplast and the mitochondrion. Up to a 40% decrease in the light saturated capacity of oxygen evolution was found in the case of *dum19/25*, a double mutant lacking the respiratory complexes I and IV activities. An independent demonstration of the tight link between respiration and photosynthesis in unicellular algae has been recently obtained by a global analysis of the energetic metabolism in Fe-starved cells of the diatom *Phaeodactylum tricornutum*. Diatoms are very well adapted to feast-or-famine regimes of Fe availability. Typically, nutrient (Fe) limitation imposes a strong constraint on the amount of Fe that can be incorporated into the photosynthetic complexes. Thus, Fe starvation results in a dramatic unbalance between the amount of PSII and of PSI, the latter being reduced owing to its high Fe requirement for assembly (reviewed by Merchant et al. [4]). *Phaeodactylum* cells respond to Fe starvation by down-regulating genes encoding enzymes supplying substrate for RuBisCO, by enhancing nitrogen metabolism and by increasing protein degradation to aliment housekeeping functions [61]. In this alga, which also possess the machinery to perform metabolic exchanges between the chloroplast and the mitochondrion, an up-regulation of the mitochondrial alternative oxidase (AOX) activity has been observed in Fe deplete conditions [61], opening a possible way to reroute photosynthetic electrons towards respiration (Fig. 1).

Most interestingly, the AOX enzyme of *Phaeodactylum* contains a putative Ca^{2+} binding domain [61]. A Ca^{2+} binding domain is also seen in the Nda2 enzyme of Chlamydomonas [45], which likely participates in the establishment of cyclic electron flow in this alga. It is tempting

therefore to speculate that calcium binding could be involved in modulating electron diversion in algae, in response to environmental stimuli.

Other examples exist that underline the possible interplay between calcium signaling and changes in photosynthetic activity, in response to environmental cues. In aquatic habitats where plants and/or algae are growing thickly, the water pH changes extensively. This value is about 7 at night, but it can increase to about 10 in the daytime (e.g. reference [62]). The pH change is closely related to the photosynthetic fixation of inorganic carbon. *Characean* cells have been instrumental to study the link between photosynthesis and pH changes because of the large size of the internodal and leaflet cells. In *Chara corallina*, alkaline bands are formed on the surface of these cells [63] having a pH of 8.5–9.5, in contrast to acid regions (pH about 5.5) which are more uniform. In the dark, heterogeneity rapidly disappears, the pH of the cell surface reaching a value of ~6.0. It has been proposed that alkalinization results from HCO_3^- uptake, CO_2 fixation and subsequent OH^- efflux. A relationship between the appearance of pH banding phenomena, the presence of membrane electrical signals and calcium has been also proposed because action potentials change the cytosolic Ca^{2+} composition, while affecting proton fluxes. Recent data have shown that *Chara* cells exposed to illumination arrange plasma-membrane H^+ fluxes and photosynthesis in a coordinated spatial pattern. In the alkaline bands, the quantum yield of PSII-driven electron flow is lower than in acid regions, reflecting the lower CO_2 concentration. The periodic profile of extracellular pH follows the profile of the photoprotective response, while PSII-driven electron flow presents an opposite trend [64]. After triggering the action potential, the pH banding temporarily disappears while the differences in the PSII-driven electron flow and photoprotection remain. Based on these data, Krupenina et al. [[64]] suggested that transient changes in pH banding, photosynthesis and photoprotection reflect alterations in intracellular Ca^{2+} and H^+ concentrations during and after the action potential.

3. Alternative electron flow processes around PSII

3.1. The PTOX-based water to water cycle

While electron diversion at the PSI acceptor side clearly represents a major tool to adjust the rate of electron flow to the rate of CO_2 assimilation in plants and freshwater algae, recent findings suggest that other regulatory processes may become prominent in particular photosynthetic environments. Due to limited content of PSI, Fe-starved marine organisms have to face an over-reduction of the PSII acceptors, opening the way to photoinhibition (e.g. reference [34]). In order to counterbalance this phenomenon, some marine cyanobacteria [65,66] and some ecotypes of the green alga *Ostreococcus* [67] reroute part of the PSII-generated electrons into a water-to-water cycle, catalyzed by the plastoquinone terminal oxidase (PTOX [68]) (Fig. 2). This alternate electron flow, which can involve a significant fraction of the PSII-driven electron flow at the cost of CO_2 fixation, has some clear advantages when compared to electron diversion downstream of PSI, when the amount of this complex is drastically reduced [14]. Indeed, by shunting the bottleneck step of photosynthesis, PTOX can sustain a ΔpH to fuel ATP synthesis (solving the "ATP shortage" issue) and to trigger photoprotective responses in Fe limited cells.

3.2. Cyclic flow around PSII

Besides electron donation to PTOX, lines of evidence exist for electrons generated at the PSII acceptor side to be recycled around this complex in order to decrease its light sensitivity when the electron flow carriers are over-reduced. Experimental evidence for this phenomenon comes from measurements of the yield of O_2 production

in algae, when exposed to a series of 'single turnover' flashes, i.e. flashes that are short and strong enough to induce only one charge separation in every photo-center. In *C. pyrenoidosa*, pre-illumination by saturating light decreases the yield of O_2 evolution, without reducing the PSII charge separation capacity [69]. This effect has been attributed to the onset of cyclic electron flow around PSII following reduction of the plastoquinone pool. According to the authors, the latter would act as a switch to disconnect O_2 evolution and PSII photochemistry in some conditions. More recently, a similar effect has been observed in *Phaeodactylum* cells exposed to a few minutes of saturating light (e.g. reference [70]). The lower oxygen yield observed in a flash series recovered after several flashes, suggesting that few (up to 3) charge separations per PSII cannot contribute to H_2O oxidation but rather oxidize another compound. The electron transfer cycle in PSII observed in these conditions should involve its quinone acceptor Q_B, the cytochrome b_{559} and the Chl_Z (D2)/carotene components, as earlier proposed [71]. This process could provide an additional layer of photoprotection to *Phaeodactylum* cells, accounting for the extreme resistance of their PSII towards photodamage.

4. Other regulatory mechanisms

In physiological conditions, photosynthetic electron flow is controlled at the level of plastoquinol oxidation at the lumenal site of the cytochrome b_6f complex. Indeed, lumen acidification typically decreases the rate of plastoquinol oxidation by a factor of 3–4, suggesting that this step is the bottleneck of photosynthesis. This kinetic effect, known as "photosynthetic control," is the consequence of the tight coupling between electron and proton transfer during oxidation of PQH_2 [72]. When photosynthesis is limited by CO_2, all the electron donors upstream of PSI should be reduced. Conversely, onset of the photosynthetic control leads to the oxidation of the electron carriers located downstream of the cytochrome b_6f—i.e. plastocyanin and P_{700} (the primary electron donor to PSI). It is thought that this mechanism could protect PSI against photodamage, which is seen upon over-reduction of the electron flow chain (e.g. reference [73]). Indeed, by slowing down the rate of electron flow to PSI, photosynthetic control would decrease the accumulation of reducing equivalents in this complex. Recent data in plants have shown that accumulation of oxidized P_{700} (the primary electron donor to PSI) in the light only occurs when the PGR5/PGRL1 complex is present in the thylakoids. Mutants lacking this complex totally fail to accumulate P_{700}^+ even in high light [48,49]. The finding that P_{700}^+ still accumulates upon exposure to high light in a knockdown mutant of PGRL1 in Chlamydomonas [51] suggests that the PGR5 protein (rather than PGRL1) could be responsible for this observation. Consistent with this, a recent biochemical and mass spectrometry analysis of Chlamydomonas cells has failed to detect PGR5 in the thylakoids [23]. The role of the PGR5 protein in modulating the oxidation extent of PSI is still obscure. It could play a direct role in the modulation of the redox state of PSI, as suggested in reference [74]. Alternatively, its role could be indirect, linked to the contribution of the PGR5/PGRL1 complex to the generation of a ΔpH in the light (via cyclic electron flow [48]). Further experiments are required to answer this important question.

5. Conclusion

The regulation of electron transport in photosynthesis has two main roles: the balance between the generation of ATP and NADPH and the response to environmental stimuli, the most critical being light intensity and nutrient availability, including CO_2. While it is well established that 3 ATP and 2 NADPH are required for CO_2 assimilation in C3 plants, the actual value of the ATP/NADPH ratio coupled to photosynthetic electron transport in vivo is controversial, probably variable in different conditions but likely lower than 1.5. Contribution of alternative electron transport pathways is therefore expected, but a

tight regulation of these pathways is also needed in order to avoid waste of absorbed quanta. Extra ATP needed could be provided by either CEF or water–water cycle mediated by PTOX and the ascorbate–Mehler reaction (two LEF processes). Contribution of CEF to photosynthetic electron transport has been shown in Chlamydomonas under physiological conditions [75,76]. The Mehler reaction has been shown [30] to be coupled to ATP formation with the same ATP/2e ratio as NADP reduction and to utilize the same electron transport machinery [29]. Its occurrence in steady state photosynthesis is supported by a variety of observations in vivo as well as in isolated chloroplasts [9]. The PTOX activity has also been shown to support the proton gradient generation under PSI-deficiency [67]. Finally, respiration may also be important during steady state photosynthesis and photoautotrophic growth. In summary, it appears that all the alternative electron flow pathways have the capacity to solve the "energy balance" and "ATP shortage" issues in specific conditions by operating, either alone or in synergy with other processes. Further work combining genomic, genetic, functional and biochemical approaches is needed to reveal how microalgae employ the most appropriate electron flow path in order to optimize carbon assimilation in different environments.

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