Chapter 16
Regulation of Electron Transport in Photosynthesis

Giles N. Johnson, Pierre Cardol, Jun Minagawa and Giovanni Finazzi

Abstract Photosynthetic organisms display a remarkable flexibility in their capacity to adjust photosynthetic performances in response to changes in their environment. This flexibility arises from the interplay of a range of different responses, including fast changes in light harvesting, changes in the pathways of electron flow and slower changes in the protein composition of the photosynthetic machinery. An array of possible adaptative responses is available to most photosynthetic organisms, which have in general selected from amongst these during their evolution to cope with the environmental circumstances of their specific environment. In this chapter we describe some representative strategies employed by eukaryotic photosynthetic organisms to adapt electron transfer capacity. We discuss processes in well characterised organisms from the green lineage (*Arabidopsis thaliana* and *Chlamydomonas reinhardtii*), and then focus on some peculiar strategies that have emerged in other organisms, in particular in marine phytoplankton.

Keywords Photosynthesis · Linear electron flow · Cyclic electron flow · Water-water cycle · Mitochondria-chloroplast metabolic interactions

G. Finazzi (✉)
Centre National Recherche Scientifique, Unité Mixte Recherche 5168, Laboratoire Physiologie Cellulaire et Végétale, 38054 Grenoble, France
e-mail: giovanni.finazzi@ceaf.fr

Commissariat à l'Energie Atomique et Energies Alternatives, l'Institut de Recherches en Technologies et Sciences pour le Vivant, 38054 Grenoble, France

Université Grenoble Alpes, 38041 Grenoble, France

Institut National Recherche Agronomique, UMR1200, 38054 Grenoble, France

G. N. Johnson
Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK

P. Cardol
Laboratoire de Génétique des Microorganismes, Institut de Botanique, Université de Liège, 27 bld du rectorat, 4000 Liège, Belgium

J. Minagawa
National Institute for Basic Biology (NIBB), Nishigonaka 38, Myodaiji, Okazaki 444-8585, Japan

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Abbreviations

AOX	Mitochondrial alternative oxidase
APX	Ascorbate peroxidase
CBB	Calvin Benson Bassham cycle
CEF	Cyclic electron flow
Cyt	Cytochrome
Cyt b_6f	The cytochrome b_6f complex
Fd	Ferredoxin
FNR	Ferredoxin-$NADP^+$ oxidoreductase
FQR	Ferredoxin quinone reductase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
LEF	Linear electron flow
MDA	Monodehydroascorbate radical
NDH	NAD(P)H dehydrogenase
PC	Plastocyanin
PQ	Plastoquinone
PQH_2	Plastoquinol
PS	Photosystem
PTOX	Plastoquinone terminal oxidase
P_{700}	Primary electron donor to PSI
SOD	Superoxide dismutase

16.1 Introduction

Eukaryotic photosynthesis first evolved probably more than 1.8 billion years ago [49] and revolutionised life on this planet. It allowed different groups of photosynthetic eukaryotes to inhabit freshwater and marine environments, and ultimately led to the colonisation of land. For this purpose, organisms developed different chloroplast structures, light-harvesting apparatuses and photosynthetic metabolism. However, the basic mechanisms of photosynthesis, as described by the Z scheme proposed by Hill and Bendall [62] are practically unchanged in all photosynthetic eukaryotes so far studied. This process starts with light absorption by dedicated pigment-containing complexes (the light harvesting or "antenna" complexes), which provide energy for charge separation by two photosystems (PSII and PSI). These photosystems are linked by a series of electron carriers, eventually leading to NADPH synthesis and the net movement of protons into the thylakoid lumen. The electrochemical proton gradient (the $\Delta\mu_H^+$) generated in this way is consumed for ATP synthesis by a CF_0-F_1 ATP synthase (ATPase) complex. ATP and NADPH fuel the fixation of CO_2 in the Calvin Benson Bassham (CBB) cycle.

In PSII, photochemical conversion of absorbed light leads to water oxidation by a Mn_4-Ca cluster by a sequential process known as the Joliot-Kok clock (or the "S states" mechanism, Joliot and Kok [74]), the molecular principles of which are

now being elucidated thanks to the high resolution structures of this complex [101, 141]. Altough the H^+/e^- are variable during the operation of the clock [82], on the average, 1 proton is released into the lumen per electron subtracted from water. Electrons withdrawn from water are injected into the plastoquinone (PQ) pool, producing plastoquinol (PQH_2), which is the substrate of the cytochrome b_6f complex (Cyt b_6f) (as well as of PQ terminal oxidase (PTOX), see Sect. 16.4). As a member of the *bc*-type proteins family, Cyt b_6f couples proton translocation across the membrane to electron transfer from this lipophilic quinone to a hydrophilic one-electron acceptor protein (plastocyanin (PC) or a *c*-type cytochrome (cyt c_6)). During this reaction, electrons originating from PQH_2 are injected into a high potential chain (also called the linear path) formed by the Rieske protein and cyt *f* and into a cyclic route that comprises the *b* hemes (Q cycle; reviewed in [30]). Owing to the less positive E_m of the cyt composing this path (when compared to cyt *f* and the Rieske) this route is referred to as the low potential chain. According to the Q cycle mechanism proposed by Mitchell [94] and modified by Crofts et al. [31], quinones are oxidized and reduced at two distinct sites in the protein, the Qo and Qi (or Qp and Qn) sites respectively, which are located on the opposite side of the membrane [30]. PQH_2 oxidation on the lumenal side is associated with the reduction of both cyt *f* and b_L [31] and the release of protons into the lumen. Oxidation of the b_6 hemes occurs through a two step reduction of a PQ molecule at the Qi site, possibly involving the recently discovered c' heme [30]. The electron transfer sequence is the reduction of cyt b_L by PQH_2, electron transfer to b_H, and then a double electron transfer from these hemes to a PQ molecule located on the stromal side, a process that is coupled to proton uptake from the stroma. Overall, the Q cycle increases the H^+/e^- ratio of photosynthetic electron transfer. Previous work has suggested that this cycle is active under physiological conditions [120], and can be bypassed only upon drastic modifications of the redox features of the low potential chain [88]. On average, each electron passes twice through Cyt b_6f, once through the low and once the high potential pathways, and releases 2 protons into the lumen. The ultimate product of this complex, reduced PC or cyt c_6 (depending on species and growth conditions), is released from the b_6f complex and binds to PSI, where it reduces the primary electron donor of this complex, P_{700}. In contrast to the PQ pool, PC and cyt c_6 are extremely mobile in plant chloroplasts in the light [78], thus probably allowing the functional connection between the grana stacks and the stroma lamellae, where PSII and PSI are mainly concentrated.

Electrons arriving on the donor side of PSI are passed to the acceptor side via a charge separation reaction and then either continue their journey towards carbon assimilation, or provide reducing power for other cellular metabolic processes (nitrogen and sulphur metabolism, lipid, amino acid, pigment biosynthesis etc). 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 probably in a ratio not sufficient to support the formation of glyceraldehyde-3-phosphate, the export product of the CBB cycle [see 3 for a discussion]. Certainly, the ATP/NADPH ratio coupled to LEF does not exceed a value of 1.5, i.e. the stoichiometry required for CO_2 fixation. This

means that LEF at best produces enough ATP to match the production of NADPH used in CO_2 fixation. Alternative electron consuming reactions, such as nitrogen metabolism, synthesis of lipids, amino acids, pigments, proteins and gene expression (all of which require reducing equivalents and ATP in variable stoichiometries) will alter the relative demands for ATP and NADPH, meaning that the synthesis of these products of the electron transport chain cannot be directly coupled in a simple way—the 'energy balance' issue (reviewed by [12], see Sect. 16.4). Besides this, various types of stress reduce the capacity of plants and algae to produce ATP and NADPH in the light, further exacerbating the difficulty of supplying different processes with the appropriate "energy stocks". It follows that other mechanisms must operate *in vivo* to control the relative production of NADPH and ATP. In plants and algae, alternative electron flow pathways exist, which can compete with the CBB cycle for reducing equivalents generated by the electron transport chain, thereby reducing the overall quantum yield of CO_2 fixation (reviewed by [105]). Amongst these, molecular oxygen can act as an acceptor for electrons, either from PSII (e.g. via PQH_2 and the so-called plastoquinone terminal oxidase PTOX), or during the Mehler reaction at the PSI reducing side (see review by [105]). Respiration can also act as a sink of photosynthetic electron flow [77] with electrons being exported from the chloroplast via the oxalate-malate shunt. Another process able to produce ATP without net NADPH generation is cyclic electron flow (CEF) around PSI [70, 124], the extent of which is extremely important in the green alga *Chlamydomonas reinhardtii* under specific metabolic conditions (see Sect. 16.3). Our understanding of the importance of all these different pathways is growing and it is becoming clear that the efficiency with which electron flow is diverted to these alternative pathways could be extremely high. Owing to their different evolutionary origin, different organisms seem to have chosen a limited number of alternative electron flow processes to cope with the energy balance issue. In this chapter, we will describe the core specifics of regulation of photosynthetic electron flow in plants and microalgae, summarising knowledge obtained developed the two best characterised model systems, flowering plants such as *Arabidopsis thaliana* and the green alga *Chlamydomonas reinhardtii*. Then, we will focus on some details on the particular strategies developed in the marine environments.

16.2 Electron Transport in Flowering Plants

Flowering plants growing in natural conditions face the challenge that they have little or no control over the concentration of the principle substrates required for photosynthesis. The light intensity varies from approx. zero to up to 2000 μmol m^{-2} s^{-1} during the course of a day but can also vary by as much as two orders of magnitude on a second to second basis as cloud cover changes or the sun moves relative to shading objects. The CO_2 concentration in the atmosphere does not fluctuate significantly but the availability of CO_2 to the chloroplast does, due both to changing demand and also changes in supply caused by stomata opening and closing.

Effects of variations in light and CO_2 may be further complicated by changes in leaf temperature from day to day and through the day, which will differentially affect different parts of the photosynthetic apparatus [28]. The result of all this is that the balance between energy supply to and demand for photosynthetic electron transport is unpredictable and highly variable. Variations in supply and demand place the leaf under stress. Specifically, excess light at any moment is liable to give rise to the production of reactive oxygen species, either through the photoreduction of oxygen to form superoxide, or through the generation of singlet excited oxygen [6]. It is increasingly recognised that such fluctuations play a major role in determining the fitness of plants [80] and that most of the regulatory processes that control the electron transport chain have evolved to mitigate against that stress.

16.2.1 Regulation of NADP(H) Redox Poise

The end product of LEF is NADPH, which is mainly used to drive sugar synthesis by the CBB cycle. When conditions arise to limit CO_2 fixation, for example when drought causes stomatal closure leading to a low leaf CO_2 concentration, we would expect that the NADPH concentration should rise. This should cause Fd and the electron acceptors in PSI to become reduced. Reduced FeS centres react readily with oxygen, producing superoxide [91]. Reduction of PSI acceptors blocks PSI charge separation. PSII turnover will then continue to produce reducing equivalents which should lead to the reduction all intermediates in the electron transport chain. In fact, apart from transiently, this does not occur. The events that prevent this from occurring are complex and intricate and still only partly understood.

Exposure of a leaf to a step change from high to low CO_2 concentration results, after a short response time, in a decrease in CO_2 fixation and a reduction of the electron acceptors of PSII (Q_A and the PQ pool). Electron carriers in Cyt b_6f, PC and P700 typically all become more oxidised. This effect results from a slowing down of the turnover of PQH_2 oxidation by the Qo site of Cyt b_6f. From this we can conclude that there must be a mechanism of feedback regulation sensing the demands of CO_2 fixation and regulation of Cyt b_6f. Two non-mutually exclusive models have been put forward to explain this regulation—pH regulation and redox control.

It has long been known, from early classic experiments on both mitochondria and thylakoid membranes, that the oxidation of quinones by Cyt *bc*-type complexes is sensitive to pH. There is evidence that protonation of the Rieske FeS subunit of Cyt b_6f is known to play an important role in the kinetics of PQH_2 oxidation [42, 67]. It is clear that the lumen pH influences the oxidation kinetics of PQH_2 and so the overall flow of electrons through the electron transport chain.

The physiological importance of lumen pH in controlling electron transport has been debated. When plants, under conditions of high CO_2 are exposed to light the pH of the thylakoid lumen drops, becoming more acidic with increasing irradiance. We cannot directly measure the ΔpH *in vivo*, although we can get an indication of its extent indirectly. Perhaps the most employed indicator is the extent of pH dependent

non-photochemical quenching (qE). This term describes the enhancement of thermal dissipation of absorbed energy that occurs in the pigment-containing proteins of PSII, whenever light absorption exceeds the maximum rate of CO_2 assimilation. Based on an extensive body of work, we know that the pH dependence of qE is itself variable, in particular depending on the concentration of zeaxanthin in the light harvesting complexes [63]. In the presence of zeaxanthin, qE is induced below about pH 7.5 (pK ~7) [115]. The wild type Cyt b_6f shows an *in vitro* pH inhibition with a pK of approx. 6–6.5 [67]. In wild type plants in high CO_2, it is possible, by increasing light, to induce qE to a high level, suggesting the induction of a substantial ΔpH. At the same time, P_{700} (the primary electron donor to PSI) becomes progressively more oxidised. Measurments of the kinetics of P_{700} reduction following a light-dark transition under such experimental conditions, an indicator of flux through Cyt b_6f, indicate that no inhibition of PQH_2 oxidation is occurring [58, 106]. This implies that the *in vivo* lumen pH is too high to inhibit Cyt b_6f. Interestingly a mutant of *Arabidopsis*, pgr1, has been shown to have a mutation in the Rieske protein that results in an altered pH sensitivity, with a pK of 6.5–7 [67]. This shift results in plants which are deficient in qE—the flux through Cyt b_6f is inhibited at a pH which is too high to induce significant quenching.

When a leaf is exposed to changes in internal CO_2 concentration, either due to drought or as a result of altering the external CO_2 supply, regulation of Cyt b_6f can clearly be seen. Ott et al. [106] observed that when leaves of red campion were exposed to ambient, compared to elevated, CO_2, there was a clear inhibition of P_{700} reduction kinetics, across a wide range of irradiances. Except at the lowest irradiances, there was no change in reduction kinetics of P_{700} with irradiance however. Over the same irradiance range, reversible non-photochemical quenching (an indicator of qE) varied substantially but did not differ between ambient and high CO_2. In other words, the variation seen in P_{700} reduction kinetics cannot simply be explained by lumen pH. Similar non-correlations between qE and flux through Cyt b_6f were observed by Clarke and Johnson [28] and Golding and Johnson [47] in experiments examining responses to temperature and drought, respectively.

The alternative hypothesis for regulation of Cyt b_6f was proposed by Johnson [69]. In isolated spinach thylakoids, the flow through Cyt b_6f was shown to be sensitive to the presence of the thiol reducing agent dithiothreitol. Titration of this inhibition indicated the reduction with a pH sensitive midpoint potential in the region of −300 to −400 mV depending on the pH, i.e. between the midpoint potentials of Fd and NADPH. This led to the suggestion that thioredoxin might be responsible for feedback regulation of Cyt b_6f. This would give the redox poise of the PSI acceptor pool a direct role in regulating electron transport. To test this hypothesis, Hald et al. [54] examined plants in which electron flux away from PSI was inhibited due to reduced levels of either Fd-$NADP^+$ reductase (FNR) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively the last enzyme in electron transfer and the first in the CBB cycle. A lack of FNR is predicted to result in reduction of Fd but in oxidation of NADP. Lack of GAPDH might be expected to give rise to reduction of both Fd and NADP. Plants lacking FNR were found to be very stressed, with elevated lipid peroxidation and loss of chlorophyll. The electron transport chain in

such plants was substantially reduced, indicating a failure of feedback regulation. In contrast, plants lacking GAPDH, whilst inhibited in growth, showed no signs of stress and had an electron transport chain that was more oxidised under any set of conditions. These results were taken as evidence that it is the redox poise of the NADP/NADPH pool, rather than Fd and thioredoxin, that regulates the electron transport chain.

The above experiments provide evidence that control of Cyt b_6f is not a simple function of lumen pH. This does not exclude a role for pH in that control however. The *in vitro* redox sensitivity observed by Johnson [69] was seen to be pH sensitive in a way that would increase the sensitivity of PQH_2 oxidation to redox control at low pH. More recently, Joliot and Johnson [71] demonstrated that partial uncoupling of the thylakoid membrane by infiltrating leaves with nigericin, resulted in an inhibition of NPQ, as expected if the ΔpH is inhibited, and also in a net reduction of the electron transport chain. This is consistent with the lumen pH playing a role in regulating the activity of Cyt b_6f. In summary, both redox and pH signals can be shown *in vitro* to affect the flow through electron transport and it is likely that both play a role, possibly through a common mechanism, in the regulation of flow *in vivo*.

16.2.2 Regulation of the Trans-Thylakoid pH Gradient

The formation of a ΔpH across the thylakoid membrane is essential for the synthesis of ATP. It also plays an essential role in the regulation of light harvesting, through the process of high energy state quenching [63], and also in the regulation of LEF [71]. The generation and the regulation of this ΔpH is therefore essential to the proper function of the photosynthetic apparatus. ΔpH is generated during electron flux, however the balance between generation and utilisation of this gradient is a topic that has long been debated (see 2]). This discussion centred to a large extent on two points: is the Q-cycle in Cyt b_6f an obligate reaction; and what is the stoichiometry of ATP synthesis? Assuming that the Q-cycle is obligate [120], then each electron that is removed from water results in 3 protons being released into the thylakoid lumen (or 12 protons per O_2 evolved). The stoichiometry of ATP synthesis is related to the stoichiometry of subunits in the ATP synthase and specifically of the c subunit of the Fo part of the enzyme. In flowering plants, there are 14 c subunits, so 14 protons are required to synthesise 3 ATP molecules (discussed in [36]). If there were a strict coupling of LEF and ATP synthesis to CO_2 fixation in the CBB cycle, this would mean that there was a small deficit in ATP production.

To some extent this discussion was futile. Alternative pathways for consumption of reducing power and of ATP mean that there is no simple fixed requirement for a particular ratio of ATP to NADPH. Furthermore it cannot be assumed that the coupling of proton transfer to ATP synthesis is perfect—almost certainly, a proportion of protons will leak through the membrane. Nevertheless, what is certain is that the cell needs to maintain the balance of ATP and NADPH to ensure that the fixation

of CO_2 and other metabolic processes can proceed in a balanced way and that the chloroplast does not become over-reduced. The latter is of particular concern, as reducing conditions can result in damage to the photosynthetic apparatus itself and also to cell in general, through the excess production of reactive oxygen species [6]. Increasingly therefore we must recognise the need for regulation of different electron transport pathways in a way that ensures that ATP and NADPH concentrations are controlled.

If we accept that LEF from water to CO_2 does not give rise to a net generation of ΔpH, then how are plants able to generate, and more importantly control, the pH gradient? In terms of generation of pH gradient, it follows that there must be some flexibility in the coupling between electron flow and ATP consumption. This could result from alternative electron flow, other than to CO_2 fixation, occurring from the import of ATP of respiratory origin into the chloroplast or possibly from changes in the ATP/H^+ coupling ratio of the ATPase. Owing to the complex regulation of the pH gradient, especially given the simultaneous need for ΔpH to generate ATP and regulate light harvesting, all these hypotheses have been previously considered as possible (e.g. [79]). In a perfectly coupled system (i.e. where the movement of protons out of the lumen is strictly linked to ATP synthesis) only a small number of protons need to be pumped into the lumen over and above those used for ATP synthesis. Once there, the ΔpH would be maintained. Kramer and co-workers have discussed that regulation of the pH gradient could then be achieved through regulation of the conductance of the ATPase, such that the potential gradient required to drive ATP synthesis might vary. This is suggested to occur through changes in chloroplast phosphate concentration [75], which in turn will reflect changes in the metabolic status of the ATP pool and CBB cycle intermediates. In this way, the pH gradient required to give a particular rate of ATP synthesis might vary. An alternative view is that different electron flows, especially CEF, generate additional ΔpH and it is the relative extent of these flows compared to LEF that controls the steady state ΔpH. It is now widely accepted that CEF occurs in flowering plants and that the rate of this can be substantial (see Sect. 16.2.4).

Obviously the above models are not mutually exclusive. However, there is growing experimental evidence for the occurrence of alternative electron flows, in addition to a simple LEF to CO_2 fixation *in vivo*. Different alternative flows are discussed in the following sections.

16.2.3 Sinks for Electron Flow Not Linked to ATP Consumption

A number of pathways exist that accept reducing equivalents from PSI but do not consume ATP. Reduction of inorganic nitrogen, via nitrate reductase and nitrite reductase, and assimilation of sulphate all consume reducing equivalents from photosynthetic electron transport [57, 59]. Reducing equivalents can also be exported from the chloroplast via the malate-oxalate cycle [77]. The latter pathway in particular is known to be regulated in direct response to chloroplast stroma redox poise, via thioredoxin-linked activation of the enzyme malate dehydrogenase [93]. Activa-

tion of malate dehydrogenase occurs at a substantially more negative redox potential than that required for activation of enzymes involved in CO_2 fixation, similar to the potential seen to inhibit Cyt b_6f [69]. Recent data have provided a molecular platform to understand the dynamics of the malate shuttle and suggest that the exchange of ATP and reducing power between the chloroplast and the mitochondrion is important for the optimization of carbon assimilation in vascular plants [77].

Electron flux to oxygen in the Mehler reaction will also generate ΔpH. Oxygen is reduced by iron sulphur centres on the acceptor side of PSI, generating superoxide which is detoxified by a series of reactions referred to collectively as the Mehler-Ascorbate Peroxidase pathway (see Sect. 16.3.4). There is probably always a basal flux through this pathway, possibly as high as 5–10 % of total electron flux, and this will generate ΔpH. It has been suggested that this pathway might play an important role in regulating the ΔpH [105, 114]. However, when leaves are illuminated at low O_2 concentrations, expected to lower the rate of Mehler reaction, they are still able to generate a ΔpH to drive high energy state quenching (see e.g. [28]). Since superoxide detoxification and oxidative stress impose a significant metabolic load on the leaf, it seems unlikely that flux to the Mehler reaction is actively used to regulate ΔpH. Nevertheless, it is probable that this flux plays an important role in overall chloroplast behaviour.

16.2.4 Cyclic Electron Flow in Flowering Plants

Fluxes through the electron transport chain to these alternative electron sinks will all support proton pumping into the thylakoid lumen without being strictly coupled to ATP consumption. Hence such fluxes will generate net ΔpH. It is not however clear that they can be regulated in a way that would allow for a responsive regulation of ΔpH. Rather, regulation of these flows probably occurs in ways that are intended to limit over-reduction of the chloroplast stroma, rather than specifically to generate ΔpH. CEF, involving just PSI, almost certainly acts as the major process that allows plants to control their ΔpH independently of redox potential. CEF in flowering plants has been the subject of a number of reviews in recent years (e.g. [70, 124]) and we do not intend here to review in detail the evidence for its occurrence. Rather the focus will be on our growing understanding of how this pathway is regulated and the role it plays in flowering plants.

The detailed functioning of CEF in plants is almost certainly somewhat different to that in green algae (see Sect. 16.3.1), although the basic pathways involved are almost certainly the same. Reduced Fd, produced by PSI, is oxidised in a way that results in the reduction of PQ, feeding electrons back into the electron transport chain between the two photosystems. That reaction may be catalysed by either an NAD(P)H dehydrogenase (NDH) complex homologous to Complex I in mitochondrial electron transport [118, 124], or via an alternative reaction presumed to involve Cyt b_6f. Our understanding of the structure and function of the former complex has grown significantly in recent years [111], although the precise role of this reaction remains to be elucidated. Plants deficient in the NDH complex appear

to perform well across a wide range of conditions, though there is some evidence that they have increased sensitivity to drought stress [83, 118]. It seems however, that NDH is not essential for plant growth.

In addition to the NDH pathway, electron transfer can take place via a pathway often referred to as the FQR (ferredoxin quinone reductase) pathway [13]. In early studies, the FQR pathway was proposed to involve Cyt b_6f [132]. However, later studies hypothesized a distinct enzyme for FQR that bypasses Cyt b_6f and directly interfaces with PQ pool reduction by Fd [13]. No one, however, was able to identify or purify the FQR specific enzyme. On the other hand, physical association of PSI, Cyt b_6f, and FNR has long been suggested as a platform for the electron transfer in the FQR pathway [5, 26]. Mathematical modelling of the electron transfer [81] and *in vivo* observation of its high efficiency [73] suggest that FQR activity might operate in a complex. Indeed, Cramer and his co-workers reported that Cyt b_6f was co-purified with FNR and was reduced by Fd [149]. More recently, physical interactions between an integral membrane protein PGRL1, which was essential for FQR activity in *Arabidopsis*, and PsaD (subunit of PSI), PetB (cyt b_6), FNR, and PGR5 were shown by a yeast two-hybrid assay using the corresponding genes from *Arabidopsis* [32]. A *c*-type cyt found close to the stromal face of Cyt b_6f is suggested to be required to mediate the transfer of electrons from Fd/FNR to the Qi (quinone reducing) pocket in Cyt b_6f. [128] This model of CEF has in recent years gained an acceptance that probably exceeds the experimental evidence that supports it and several groups worldwide are working to establish a more solid mechanistic understanding of the events that are involved.

A key point in our understanding of CEF is the question of how the relative fluxes through LEF and CEF are regulated. Important points will be the steps where the two pathways converge or diverge—i.e. what is the fate of reduced Fd and how do PSII and CEF compete for PQ reduction. The need to regulate relative CEF and LEF fluxes is central to controlling ΔpH and it is widely thought that there needs to be some form of separation of the two pathways to achieve this regulation. For example, under conditions of high light or low CO_2, the PQ pool associated with PSII is known to be highly reduced. Under such conditions, CEF needs to be able to complete effectively with electron flow from PSII; indeed these are exactly the conditions that promote CEF [47, 95]. So how is it possible to ensure that the cyclic pathway has access to oxidised PQ?

In green algae there is strong evidence, both functional and structural, that supercomplexes of PSI and Cyt b_6f exist that are necessary for CEF to occur (see Sect. 16.3.2). The evidence for such complexes in flowering plants is however absent [21], and indeed, it has been argued that no such complexes are required [70, 71]. In particular, the presence in flowering plants of distinct membrane regions—the granal stacks and the stromal lamellae—provide the potential for regulating CEF and LEF in a way that is less possible in, for example, *Chlamydomonas*, where membrane stacking is much less prominent.

Key to our understanding of the structure of flowering plant chloroplasts is the observation that photosynthetic complexes are not evenly distributed in the thylakoid membrane [1] (c.f. Chap. 5 in this Volume). Functional PSII is localised in the

membrane stacks. PSI is distributed between the grana margins (~70%) and the stromal lamellae. Cyt b_6f is evenly distributed in the membrane. It has been shown that the diffusion of PQ/PQH_2 in the thylakoid membrane is highly restricted, probably due to the very high protein concentrations that characterise this membrane [139]. This means that PQ localised in the granal stacks will primarily act in mediating electron transport between PSII and those Cyt b_6f localised in the stacked regions. For reasons of access, it is unlikely that electrons will be transferred from Fd to Cyt b_6f complexes localised in the grana regions. Rather, a separate pool of Cyt b_6f, with associated PQ will be found in the stromal membranes. The physical distance between the different domains and the limitations on PQ diffusion mean that these different pools could be quite separate.

Beyond Cyt b_6f, electrons are passed in flowering plants to PC. PC is soluble in the thylakoid lumen and, in flowering plants at least, much more mobile than PQ [78]. Therefore, there is the potential for the LEF and CEF pathways to mix at this point. This is however not such a problem. Once electrons have been injected from Fd or PSII into the high potential chain, their fate, as either "cyclic" or "linear" is determined. Thus, the presence of distinct Cyt b_6f and PQ pools is sufficient to allow for the co-existence of CEF and LEF pathways.

According to this model, the point at which the relative fluxes through CEF and LEF flows is regulated will be through competition for the oxidation of reduced Fd. Insights into the regulation of this step can be obtained from examination of various mutants altered in proteins required for CEF and LEF flows. Plants deficient in PSI complex have been shown to be impaired in LEF but are still able to maintain a significant proportion of CEF, giving a higher steady state pH gradient (as indicated by non-photochemical fluorescence quenching) [55]. In other words, CO_2 fixation does not simply extract reducing equivalents at a rate determined by its own capacity. Tobacco plants over-expressing Fd from *Arabidopsis* were shown to have increased CEF, even though their capacity for CO_2 assimilation was unaltered [147]. This led to the suggestion that Fd represents a limitation on CEF. This is perhaps a surprising conclusion, since the observation that the acceptor side of PSI is maintained in an oxidised state over most conditions suggests that Fd is not functionally limiting for electron flow, but it does suggest that the behaviour of Fd plays a major role in controlling electron partitioning. Plants deficient in Fd do show an inability to generate a high ΔpH, implying that at low Fd concentrations, CEF is limited [144].

The predominant reaction oxidising reduced Fd is via FNR. There is evidence that FNR exists in multiple forms [20, 53, 96]. In *Arabidopsis* there are two genes encoding this protein. Maize has three, including one that is thought to be specific to bundle sheath cells, where CEF is thought to be the predominant form of electron transfer [104]. These genes encode proteins with different pK values and with different tendencies to attach to the thylakoid membrane. These different isoforms can undergo different post translational modifications, including N-terminal truncation and possibly phosphorylation. FNR is known to be free in solution but also to bind to the acceptor side of PSI and the stromal side of Cyt b_6f. As such, it makes a good candidate as a point of regulation of CEF. Plants lacking FNR are found to

be significantly impaired in the regulation of electron transport but are still able to perform CEF and to generate a significant ΔpH, at least under some conditions, although this is not greater than in wild type plants [54, 71]. This is consistent with a role for FNR in CEF, but there is little direct evidence for control of this step. Measurements of the distribution of FNR between different membrane-bound pools under different conditions provided no evidence of a regulatory re-distribution of FNR [21].

A protein-complex that has captured the imagination in terms of its role in CEF is the one formed by PGR5 and PRGL1 [32]. The nature of that involvement remains somewhat unclear. It was first suggested that it may form a Fd quinone oxidoreductase (FQR) [98], however there is no direct evidence for a catalytic role of either PGR5 or PGRL1 and no evidence of redox active cofactors. Nandha et al. [100] presented evidence that plants lacking PGR5 are in fact still capable of CEF at rates similar that those of wild type plants, but that they are impaired in CEF under most conditions, due to impairment of the redox poising of the chloroplast electron transport chain. Notably, the high potential portion of the electron transport chain is maintained in a reduced state in the light in PGR5 mutants. PGR5 mutants are capable of generating a significant ΔpH under conditions where photosynthesis is sink-limited, due to removal of CO_2 from the atmosphere, however the high potential is still reduced. This supports a model where PGR5 is required for feedback regulation of Cyt b_6f and it is that failure that prevents CEF from competing with LEF under most conditions. Whatever the precise role of PGR5, it is clear that it plays a crucial role in the regulation of photosynthesis. Plants lacking PGR5 are unable to survive in fluctuating growth conditions [136].

16.2.5 Alternative Electron Flow from PSII—the Plastid Terminal Oxidase

In addition to LEF through both PSII and PSI and CEF only involving PSI, there is strong evidence for electron transport reactions in flowering plants that involve PSII alone. There have been various indications that oxygen may act as an electron acceptor, from PSII directly [19] or from PQH_2 either at PSII, free in the membrane or at Cyt b_6f. [29, 76] These reactions are thermodynamically plausible. Direct reaction in the membrane between O_2 and PQH_2 is likely to be kinetically limited but reactions at quinone binding sites in either protein complex may occur, if a semiquinone exists for any time. Such reactions may occur significantly in some plants under some conditions, especially under stress, however their significance remains to be established.

Less controversial is the observation of a plastid terminal oxidase (Ptox) as a significant sink for electron transport from PSII. The PTOX protein was first identified in plants showing a phenotype where leaves developed with mottled white patches—the IMMUTANS phenotype seen in *Arabidopsis* and tobacco [25]. The protein is a di-iron non-haem protein showing homology with the alternative oxidase found

in mitochondria. PTOX is thought to act as a PQH_2 water oxidoreductase. The IMMUTANS phenotype is explained as being due to an involvement of Ptox in carotenoid biosynthesis, providing a mechanism for the oxidation of phytoene. This role is probably however only required at crucial stages in development and, given the right conditions, IMMUTANS mutants can be grown that develop normal green leaves [117]. Recently a link was established between PGR5 and leaf variegation in immutans plants [103], leading to the hypothesis that when leaves are exposed to an excitation pressure that overcomes a threshold level (e.g. in some leaf patches of *immutans* plants), pigment bleaching occurs [90].

The existence of PTOX led to the suggestion that this may act as a sink for electron transport from PSII and therefore may have a direct role in photosynthesis. Phenotypes relating to small transient changes in chlorophyll fluorescence following light-dark transitions have been used as evidence that PTOX may be able to oxidise the PQ pool associated with PSII [85]. However there is no evidence in *Arabidopsis* tobacco or tomato plants that PTOX acts as a significant electron sink [61, 116, 140]

Such evidence has however been found in some less widely studied plant species. Streb et al. [127] studied electron transport in the alpine species *Ranunculus glacialis* and found evidence both for significant levels of Ptox and for significant electron transport from PSII to oxygen. Perez Torres et al. [112] found evidence for electron flow to oxygen in the arctic grass *Deschampsia Antarctica*, although there was no direct evidence found for PTOX protein. Stepien and Johnson [126] presented evidence for a substantial level of PSII to PTOX electron flow in the salt tolerant model species *Thellungiella halophilla.* This flow accounted for up to ~30% of total PSII electron flow and was especially prominent at high light. There was no evidence that PTOX was competing with LEF at low light, suggesting that this flux is somehow regulated, such that it only occurs when LEF is saturated. Electron flow to PTOX resulted in the PQ pool being significantly more oxidised, consistent with a role in protecting PSII from stress. Surprisingly, inhibition of PTOX activity by lowering O_2 concentration did not affect the generation of non-photochemical quenching, suggesting that PTOX is not important in generating ΔpH, in contrast to the conclusion drawn in other systems (see Sect. 16.4).

The idea that Ptox might act as a significant sink for electron transport has encouraged the notion that it might be over-expressed to increase stress tolerance. To date, attempts to induce PTOX activity have met with little success. *Arabidopsis* plants over-expressing PTOX do not show any significant increase in O_2-sensitive electron transport [116], and tobacco over-expressers show elevated reactive oxygen production and increased stress sensitivity [61]. It is possible that there are differences between Ptox in *Arabidopsis* and in species where PTOX acts as a substantial electron sink, however the recent publication of the *Thellungiella* genome sequence provides no evidence for significant differences in sequence. Rather, it is likely that the known PTOX polypeptide is not acting alone but needs to be expressed alongside other peptides.

16.3 Chlamydomonas reinhardtii

Microalgae represent another ideal system to study photosynthetic electron transfer because, while they share most of the basic features of this process with plants, their growth rates are in general higher than those of plants. It is easy to get uniform cell cultures by controlling the microenvironment around the cells and to prepare a large number of samples for biochemical characterizations. Moreover, microalgae are extremely amenable to molecular and genetic manipulation. Their study has allowed us to elucidate the mechanisms of water oxidation (the Joliot-Kok clock, [72]), state transitions [18], and chlororespiration [14, 64, 68]. Recently there has been a large growth in interest in microalgae in general, and the freshwater alga *Chlamydomonas reinhardtii* in particular, as possible sources of biofuels, and an understanding of their photosynthesisis is central to this.

Chlamydomonas has been widely used as a model system to study photosynthesis in eukaryotic algae due to the fact that it can grow in the complete absence of photosynthesis (allowing mutants devoid in some of the major functions of this process to be easily characterized), has a well characterized sexual cycle (allowing mutations to be dissected and double mutants to be generated), and is suitable for molecular manipulation. This organism has recently been employed to study the functional organization of the photosynthetic apparatus (e.g. the epistatic control of the assembly of different complexes within the thylakoid membranes [27]), and the molecular mechanisms of photoprotection (state transitions and NPQ [84, 86] respectively). More recently, *Chlamydomonas* has allowed the establishment, for the first time, of a molecular basis for the regulation of alternative electron flow in photosynthesis [66] (see below).

16.3.1 Cyclic Electron Flow around PSI

CEF in *Chlamydomonas* is subjected to the same general rules that govern this process in plants, however the conditions giving rise to this flow may be quite different. Conditions leading to the reduction of the soluble electron carriers (e.g. anaerobic conditions) trigger the appearance of CEF [43], but in contrast to plants, it is the redox state of the PQ pool (instead of the redox state of PSI soluble acceptors) which primarly affects the partitioning between LEF and CEF. The mechanism linking changes in the chloroplast redox poise to the appearance of CEF is also different in this alga. In anaerobic conditions, reduction of the PQ promotes a transition to State 2, a condition in which most of the PSII antenna complexes are functionally connected to PSI, as a consequence of their phosphorylation by a specific kinase, Stt7 [34]. Enhancement of CEF is observed during the State 1 to State 2 transition, as shown by the diminished sensitivity of electron flow through Cyt b_6f [43] and PSI [24, 66] to the addition of the PSII inhibitor DCMU. Still, it is not known whether a true causal relation exists between the transition to State 2 and the onset of CEF. Indeed PSI absorption is increased to ~4 times that of PSII in *Chlamydomonas*

cells acclimated to State 2, thus largely limiting the capacity of this complex to drive electron flow. A recent study however has reported that mutants with a largely impaired CEF capacity can undergo state transitions with the same efficiency as the wild type strain [135]. This clearly challenges the existence of a strict cause and effect relationship between state transtions and cyclic flow capacity

16.3.2 Mechanisms of CEF in Chlamydomonas

As discussed above, two major routes for CEF have been proposed in oxygenic photosynthesis. The first one involves the activity of a chloroplast NDH complex. In *Chlamydomonas*, as well as in most of the microalgae studied so far, this complex is not a homologue to the respiratory Complex I, but rather is represented by a monomeric complex [35, 68]. The Nad2 protein of *Chlamydomonas*, which is the only one studied in details, is extremely active in reducing the PQ pool at the expenses of stromal reducing power [35, 68]. However, the real contribution of the NDH pathway to CEF has not yet been tested experimentally. As an alternative to the NDH pathway, the FQR is defined as the complex that catalyzes the reduction of the PQ pool using Fd as a substrate (see Sect. 16.2.4).

While the nature of any complexes involved in FQR activity is still under debate in plants, recent analysis in *Chlamydomonas* has likely provided the first molecular information concerning the machinery in charge of the FQR pathway in this alga. Iwai et al. [66] used solubilized thylakoid membranes from *Chlamydomonas* cells under State 2 conditions. Using sucrose density gradient, they purified a super-supercomplex composed of the PSI-LHCI supercomplex with LHCIIs, Cyt b_6f, FNR, and PGRL1 in a fraction heavier than the PSI-LHCI supercomplex. Spectroscopic analyses of this super-supercomplex indicated that, upon illumination, reducing equivalents downstream of PSI were transferred to Cyt b_6f, while the oxidized PSI was re-reduced by reducing equivalents from Cyt b_6f [66]. When *Chalmdyomonas* cells are in State 2, where more LHCIIs are associated with PSI, CEF can operate in this supercomplex involving PSI, Cyt b_6f, and FNR. Since CEF and LEF share several redox carriers (e.g., PQ, Cyt b_6f, PC, PSI, Fd, and FNR), they are potentially in competition with one another. Furthermore, the redox poise of the CEF components could be disturbed if reduced components for LEF coexist [2]. By localizing the mobile electron carriers (PQ, Fd, and PC) within a restricted space, the super-supercomplex could compartmentalise the CEF components, generating a functional pool. In doing so, super-supercomplex formation would play a similar role to that suggested for the segregation of PSI and PSII in the grana and stroma lamellae in plants (see Sect. 16.2.4), although providing a total physical compartmentation of the two pathways. In line with this conclusion, limitation of the overall rate of electron flow by PC diffusion between PSI and Cyt b_6f has been observed in *Arabidopsis* under some conditions [122], while, no such limitation is seen in *Chlamydomonas*, where PC release from the PSI is the limiting step of the electron flow between Cyt b_6f and PSI [44]. This could reflect the fact that, while

PC is freely diffusing (in an almost bidimensional space) in plants in the light [48, 77], the presence of a supercomplex of supercomplexes in the alga would reduce the distance to be travelled by PC to being essentially negligible, making this process non-limiting for photosynthesis. It is of note that the association of LHCII and Cyt b_6f with PSI is in line with their behaviors in the membrane domains during a State 1-to-2 transition. During this transition, LHCII and Cyt b_6f migrate from the appressed region in the thylakoid membranes, where PSII resides, to the non-appressed region, where PSI resides [142].

16.3.3 Physiological Consequences of CEF in Chlamydomonas

In contrast to plants, large state transitions are induced in *Chlamydomonas* upon oxygen deprivation. This treatment rapidly leads to a decrease in the cellular ATP content, which in turn triggers an increase in the cellular NADH/NADPH concentration, due to the well known enhancement of glycolysis by a reduced ATP content (the reverse Pasteur effect [22]. PSI absorption cross section is substantially increased in State 2 [33] due to the extensive association of LHCII proteins (including the minor monomeric LHCIIs) with PSI [133, 137]. Moreover, the generation of tightly bound super-supercomplexes allows a complete thermodynamic segregation of the soluble electron carriers from the PSII-driven LEF pathway, a process that is not observed in plants, where CEF requires a small electron input from PSII [73], to compensate for the losses of electrons towards the CBB cycle (see Sect. 16.2.4).

What then is the rationale for such major rearrangements of the photosynthetic machinery in *Chlamydomonas* upon a State 2 transition? In general, a switch to State 2 is observed upon nutrient starvation in *Chlamydomonas* (see e.g. [60, 146]). Phosphorous and sulfur deficiencies, which decrease the rate of oxygen evolution induce a systematic transition to State 2 and a loss of the ability to perform LEF [146], thus giving rise to an increased CEF capacity. Nitrogen starvation also induces a systematic transition to State 2 [110], which correlates with overreduction of the PQ pool and a loss of LEF activity [60]. Overall, the rationale for these observations would be to maintain a good capacity for ATP synthesis via CEF for housekeeping purposes every time that photosynthetic performance is limited by nutrient availability (see also [36]). Indeed, from an energetic point of view, state transitions in *Chlamydomonas* mimic a shift from an oxygenic type of photosynthesis (that generates both reducing power and ATP, State 1) to an anoxygenic bacterial one, where only ATP is synthesised (State 2). The generation of the PSI-Cyt b_6f supercomplex probably provides the molecular platform for this major metabolic change in *Chlamydomonas*. This switch may provide an advantage in different environmental conditions. First of all it would increase the capacity to survive under oxygen deprivation. This situation is probably often encountered by this alga, which is normally found in eutrophic shallow ponds, rich in biomass and therefore possibly subjected to periods of anoxia. By maintaining a high quantum yield of ATP synthesis in State 2, cells might be able to maintain vital processes and therefore to cope successfully with these unfavorable conditions, and to rapidly recover photosynthetic carbon

fixation once O_2 is resupplied. Even under oxygen replete conditions, CEF would provide a benefit, but providing ATP for carbon assimilation. However, in contrast to plants [99], no growth phenotype is observed in mutants with an altered CEF capacity in *Chlamydomonas* [24, 44, 138]. This is only observed in mutants where both respiration (dums, for dark uniparental minus) and CEF (via state transitions) are down regulated [24]. In these lines, a drastic decrease in growth is seen [24], due to a diminished photosynthetic activity. It appears that in *Chlamydomonas* (and possibly in other green algae) lack of CEF is compensated by an efficient exchange of reducing equivalents (and/or of ATP) between the mitochondrion and the chloroplast. The two metabolisms are intimately linked in this alga, owing to the interplay between respiration, reduction of the PQ pool, state transitions and CEF [36], and it is therefore reasonable to assume that they may both contribute in maintaining the cellular energy charge in this alga through optimum light utilization. This hypothesis is consistent with the finding that mutants with decreased PGRL1 (and therefore possibly impaired in their CEF capacity) avoid degrading PSI in Fe-starved cultures of *Chlamydomonas* to maintain a given CEF capacity [113], despite the very high cellular pressure to mobilise Fe for other metabolic processes. Moreover, these strains do not reduce their respiratory capacity under Fe starvation despite the high Fe requirement of the mitochondrial electron transport chain, possibly to maintain a high energetic metabolism. Recent (unpublished) data, indicate that exposure to high light in the absence of an external carbon source (as required to promote the induction of the LHCSR protein, and therefore NPQ onset in *Chlamydomonas*) also results in a transtion to State 2. Again, this could reflect a change in the cellular energy metabolism to enhance PSII protection from photoinhibition. Indeed, reducing the PSII antenna size (via state transtions) and increasing the capacity to generate a ΔpH (via CEF) could reduce the risk of photodamage in this complex.

16.3.4 Other Alternative Electron Flow Processes in Chlamydomonas

The observation that O_2 depletion triggers a reduction of the PQ pool, leading to state transitions, suggests that O_2 may act as a significant sink for electron transport in Chlamydomonas, either via a PTOX-type activity or through the Mehler reaction. This process would allow oxidation of PQH_2 under conditions where CO_2 assimilation is impaired, and by so doing, maintain the cell in State 1. As discussed above, the Mehler reaction results in the production of superoxide, which is rapidly converted into H_2O_2 by the activity of the superoxide dismutase (SOD) enzyme. H_2O_2can be efficiently scavenged by a chloroplast-associated ascorbate peroxidase (APX), leading to the production of a monodehydroascorbate radical (MDA) from ascorbate and H_2O_2 [6]. In *Chlamydomonas*, the existence of an ascorbate peroxidase similar to that of plants has been reported [134]. In this alga, the ascorbate concentration is very low and the ascorbate peroxidase enzyme has an extremely high affinity for this metabolite [134].

In *Chlamydomonas*, an additional role for these ROS has been recently proposed. 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 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 [123]. 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 signalling pathway within the cell.

16.4 Photosynthesis in the Oceans

Photosynthesis by marine algae has been the subject of studies for about 80 years (e.g. [9, 89, 119]). The ocean is the place where about half of the global carbon assimilation occurs [11, 41] thanks to the photosynthetic activity of phytoplankton communities composed of prokaryotes (mainly cyanobacteria *Prochlorococcus* and *Synechococcus*) and eukaryotes (mainly diatoms, dinoflagellates chlorophyta and haptophyta). This performance is astonishing *per se*, since marine phytoplankton probably contribute ~1% of the total photosynthetic biomass on Earth [38, 41]. This high efficiency of carbon assimilation in the oceans arises from the very fast growth rates and the high photosynthetic performances shown by phytoplankton in some regions of the oceans. Far from being homogeneous, the marine world offers a variety of environments ranging from warm nutrient-rich coastal areas to vast cold and oligotrophic oceanic areas. The oligotrophic sections of the oceans represents about 70% of the marine environment, are generally distant from costal zones, and are thus characterized by very low iron (Fe) and nitrogen (N) content. These environments have provided microalgae with strong selection pressures leading to the emergence of multiple adaptation or acclimation strategies in the photosynthetic apparatus.

The analysis of the response of marine microalgae to changes in the light intensity has revealed two opposite and paradigmatic strategies for light acclimation [39]: the modification of number of reaction centers, a strategy termed n-type photoacclimation, or of the antenna cross-section, a strategy termed σ-type acclimation. These strategies are exemplified in the diatom *Skeletonema costatum* that responds to decreasing irradiance by increasing its antenna size, and in the chlorophyte *Dunaliella tertiolecta* which withstands changes in irradiance by modifying the number of reaction centers [40, 131].

Analysis of the chlorophyll distribution between the nutrient-rich coastal regions and the vast oligotrophic parts of the oceans has also shown the cost of nutrient limitation on photosynthetic activity *in situ* [10]. In a laboratory environment, nutrient limitation usually leads to a decrease in photosynthesis, due to changes in the activity and stoichiometry of the photosynthetic complexes [92]. Acclimation to nutrient accessibility is particularly well characterized upon iron starvation, which consists of modifications of both the electron flow and light absorption capacities

of the cells. Iron is of primary importance in biological systems, notably because it is a central constituent of prosthetic groups such as hemes and iron-sulfur clusters bound to enzyme complexes involved in energy-conversion processes. Iron mobilization is thus critical for microalgae in the iron-limited oceanic areas, as it is for terrestrial plants growing on neutral and alkaline soils, where iron availability is limited, as a result of the prominent form of iron in the presence of oxygen being the poorly soluble Fe^{3+}.

Despite the fact that several mechanisms exist to ensure iron homeostasis in plants and algae [55, 102, 107], when deprivation of this metal occurs, the most prominent effects on photosynthetic apparatus are at the level of PSI. Cyanobacteria respond to iron-deprivation by lowering the relative abundance of PSI and by forming an additional light harvesting antenna around the remaining PSI. *Prochlorococcus sp*., which dominates certain regions of the oligotrophic oceans, synthesizes Pcb proteins, while fresh water species express a chlorophyll binding protein similar to CP43 (IsiA) [15–17, 125]. Modifications in PSI antenna are also observed in various eukaryotic algae. In *Chlamydomonas reinhardtii*, the LHCI-PSI association and stoichiometry are altered [97] and in the halotolerant eukaryotic alga *Dunalliela salina* a light-harvesting chlorophyll a/b-binding protein, Tidi, accumulates [143]. But the most dramatic effect of iron limitation is a marked drop in the amount of PSI centers relative to other molecular constituents of the photosynthesic apparatus. PSI has the highest iron content (12 Fe per reaction centre) among the photosynthetic complexes and requires Fe for stable assembly [46]. Up to a four-fold decrease in the PSI/PSII ratio has been observed in Fe-depleted cyanobacteria [e.g. 52, 121] or eukaryotic algae, including *Chlamydomonas reinhardtii* [97, 113] and the diatom *Phaeodactylum tricornutum* [3]. In the cyanobacterium *Synechococcus* WH8102, the prasinophyte *Ostreococcus* RCC809 and the central diatom *Thalasiossira oceanica,* three species isolated from oligotrophic regions, a markedly lower content of PSI and Cyt b_6f (which contains 6 Fe atoms) relative to PSII has been measured, even if algae are cultivated in Fe-replete medium ([8, 23], Cardol and Finazzi, unpublished, [129]). The constitutively low Cyt b_6f and PSI contents seen in oligotrophic species contrasts with the almost equimolar ratios between major photosynthetic complexes observed in closely related coastal species (*Ostreococcus tauri* and *Thalassiosira weissflogii*) [23, 129]. The PSI decrease relative to PSII also raises the question of how cells respond to a reduced capacity to reoxidize the PQ pool in the light. In *Synechococcus* WH8102 and in *Ostreococcus RCC809*, it has been shown that an enhanced PTOX activity allows rerouting of PSII-generated electrons into a water-to-water cycle to an extent of about 50 % [7, 23]. A similar observation has been made *in* situ on open ocean picophytoplankton communities dominated by the *Prochlorococcus* genus [87]. Though this efficient electron flow to oxygen might take place at the expense of CO_2 fixation, it alleviates the redox pressure on the PSII acceptor side and allows maintainance of an electrochemical proton gradient in the light, despite the very strong limitation of electron flow by Cyt b_6f and PSI (in contrast to plants, see Sect. 16.2.4). This proton gradient may in turn serve the purpose of maintaining efficient ATP synthesis and/or developing photoprotective responses (NPQ), which

are triggered by lumen acidification and protect PSII from photoinhibition. This strategy could be relevant in the overall economy of photosynthesis in iron-limited marine environments [50, 148].

Interestingly, light-stimulated oxygen uptake by phytoplankton has been described for several other species (e.g. the diatom *T. weissflogii* [145], the cryptophyte *Storeatula major*, the prasinophyte *Pycnococcus provasolii*, and the dinophyte *Prorocentrum minimum* [130]. In the case of *Nannochloropsis sp*. and *Emiliania huxleyi* (Chromalveolates), the light-stimulated O_2 uptake probably occurs close to PSII [37], but we don't know yet if it involves a PTOX-dependent mechanism, the PSI-associated Mehler reaction or even the cytochromes and alternative oxidases in the mitochondrion.

In the diatom *Phaeodactylum tricornutum,* under Fe starvation, electrons could be redirected to the respiratory chain through the alternative oxidase [3]. Recent proteomic analysis [51] has shown that the chlororespiratory complex NDH, which is an essential component of the photosynthetic machinery (see Sect. 16.2) is absent in this organism. Following the model established in *Chlamydomonas* where both CEF and respiration in the light contribute to the generation of "extra" ATP for carbon assimilation [24], it has been postulated that the strong interaction between the two energetic metabolisms observed upon Fe starvation would stem from the necessity to compensate for the diminished CEF capacity [45].

Beside water-to-water cycle strategies, another strategy to adapt the photosynthetic apparatus under iron-limitation has been described in marine algae. Surprisingly, oceanic diatom species have a greater need for copper (Cu) compared to coastal strains [4, 108]. In photosynthetic organisms, a common strategy to partially deal with Cu availability consists of employing the Fe-containing cyt c_6 as the soluble electron carrier between the Cyt b_6f and PSI complexes, instead of the Cu-containing PC [92]. In the case of the oceanic diatom *Thalassiosira oceanica*, it has been shown that this Cu requirement is due to the single Cu-containing protein, PC, which is absolutely needed for photosynthetic electron transport [109]. In contrast, the costal species *T. weissflogii* expresses a classical cyt c_6 as electron carrier [65]. These observations suggested that the selection pressure imposed by Fe limitation in oligotrophic marine areas has resulted in the use of PC which reduces the need for Fe, Cu being relatively more abundant in the open sea [109].

The analysis of photosynthesis in the ocean has provided an experimental basis for the notion that photosynthesis cannot fuel carbon assimilation and the cellular anabolic processes at the same time, (the so called 'energetic management' issue [12]). In *Prochlorococcus* PCC 9511 cells, whilst a constant photosynthetic activity can be measured during a day (i.e. the entire life span of this prokaryote), the light generated ATP and reducing power are employed to supply different metabolic pathways during this period (carbohydrate vs aminoacid synthesis) as required to complete all the steps of the life cycle of this organism. So far, no such information has been provided in the case of photosynthetic eukaryotes, but it is tempting to propose that a similar phenomenon may take place, because the same rules governing the efficiency of ATP and NADPH synthesis and consumption exist in both kingdoms.

16.5 Conclusion

Regulating electron flow in photosynthetic organisms has two purposes: adjusting the generation of ATP and reducing power, and allowing a proper response to environmental changes. Because it is well established that the ATP/NADPH ratio coupled to photosynthetic electron transport *in vivo* (although probably variable in different conditions) is not going to exceed 1.5, clearly photosynthesis cannot supply carbon assimilation and other metabolic pathways simultaneously. Alternative electron transport pathways could overcome this limitation, although they have to be tightly regulated to avoid an excessive reduction of the quantum yield of CO_2 assimilation. A survey of the literature indicates that CEF, a water-water cycle (via either PTOX or the ascorbate-Mehler reaction), and the malate shunt have the capacity to solve the "energy balance" and "ATP shortage" issues, and can operate with high efficicncies (reviewed in [36]). Obviously, if they were all operating at the same time and at their maximum capacity, the overall yield of carbon assimilation would certainly be too low to allow photosynthetic growth in a natural environment. Evolution has therefore provided plants and microalgae with the capacity to choose the most appropriate alternative elctron flow pathway among the array of processes available. Although some relevant progress has been made in the elucidation of the molecular mechanisms allowing plants and algae to regulate their electron flow capacity, future effort is still required to explore the mechanisms allowing photosynthetic organisms to cope with the energy requirements of carbon assimilation.

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