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REVIEW

Cyclic electron flow around photosystem I in unicellular green algae

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Abstract Cyclic electron flow around PSI, or cyclic photophosphorylation, is the photosynthetic process which recycles the reducing equivalents produced by photosystem I in the stroma towards the plastoquinone pool. Through the activity of cytochrome b_6f, which also transfers protons across the membrane, it promotes the synthesis of ATP. The literature dealing with cyclic electron flow in unicellular algae is far less abundant than it is for plants. However, in the chloroplast of algae such as Chlorella or Chlamydomonas, an efficient carbohydrate catabolism renders the redox poise much more reducing than in plant chloroplasts. It is therefore worthwhile highlighting the specific properties of unicellular algae because cyclic electron flow is highly dependent upon the accumulation of these stromal reducing equivalents. Such an increase of reducing power in the stroma stimulates the reduction of plastoquinones, which is the limiting step of cyclic electron flow. In anaerobic conditions in the dark, this reaction can lead to a fully reduced plastoquinone pool and induce state transitions, the migration of 80% of light harvesting complexes II and 20% of cytochrome b_6f complex from the PSII-enriched grana to the PSI-enriched lamella. These ultrastructural changes have been proposed to further enhance cyclic electron flow by increasing PSI antenna size, and forming PSI-cyt b_6f supercomplexes. These hypotheses are discussed in light of recently published data.

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Abbreviations

PSII	Photosystem II
PSI	Photosystem I
P_{700}	Primary electron donor of PSI (reduced form)
P_{700}^+	Primary electron donor of PSI (oxidized form)
PC	Plastocyanine
PQ	Plastoquinone
PQH_2	Plastoquinol
Fd	Ferredoxine
DCMU	3(3,4-Dichlorophenyl)-1,1-dimethylurea
HA	Hydroxylamine
MV	Methylviologen

Introduction

Contrary to plants, which are photoautotrophs, green algae such as Chlamydomonas are facultative phototrophs or photoheterotrophs. They can use a reduced carbon source present in their environment, to grow even in the absence of light. *Chlamydomonas reinhardtii* is a good model organism for photosynthesis research because it stays green in the dark and assimilates acetate through the ATP-dependent activity of acetate kinase which produces acetyl-CoA, the substrate of Krebs and glyoxylate cycles. In both cycles, an abundance of malate leads to the reduction and protonation of NAD^+ into NADH (malate is conversely oxidized and deprotonated into oxaloacetate). This NADH is used in the mitochondrion for oxidative phosphorylation (ATP production) and allows the cells to grow under respiratory conditions, see Fig. 1.

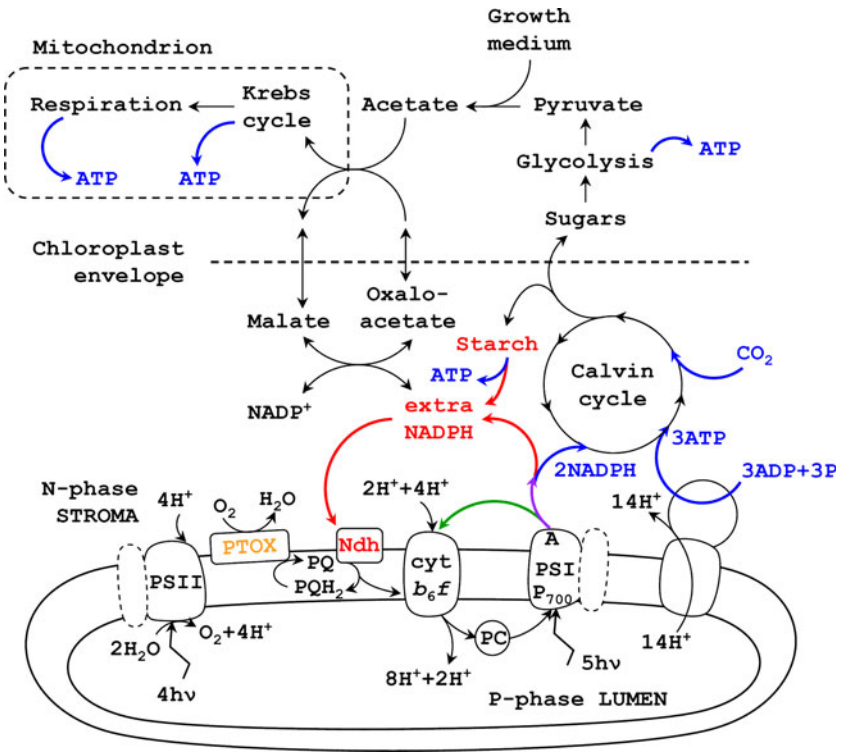

Fig. 1 Schematic representation of the photosynthetic chain of green algae and its interaction with the carbohydrate catabolism (reprinted from (Alric et al. 2010)). Acetate is incorporated via acetyl-CoA into the Krebs cycle. If respiration is halted, malate could accumulate in the mitochondrion, and this reducing power could be transferred to the chloroplast by the reversible malate/oxaloacetate shuttles. Starch breakdown and glycolysis can occur in the cytosol and in the stroma of chloroplast and yield to NAD(P)H (*red*) and ATP (*blue*) production. Part of this reducing power can be transferred to the quinone pool via NADPH dehydrogenase (Ndh, *red*) and dissipated through the activity of the plastid terminal oxidase (PTOX, *orange*). The light-induced reactions also produce ATP and NADPH (see next section for the stoichiometries). Ferredoxin-mediated cyclic electron flow is drawn in green (see last section)

The envelopes of both mitochondria and chloroplasts possess malate and oxaloacetate transporters, which shuttle these metabolites in and out of these organelles so that the catabolism of carbohydrates and the Krebs cycle, which produces NADH used for respiration, also leads to an input of reducing power (NADPH) in the chloroplast (for a review about the interdependence of chloroplasts and mitochondria, see (Hoefnagel et al. 1998)). The photosynthetic chain is capable of counterbalancing this electron flow from carbohydrate catabolism by the net production of NADPH in the light. Together with ATP, produced as well by photosynthesis, NADPH is used for the reduction of inorganic carbon (atmospheric CO_2) by the Calvin–Benson–Bassham cycle (hereafter called Calvin cycle).

This cycle, as many other cellular processes, requires high-energy bonds and reducing power in a precise stoichiometry. This stoichiometry is 3 ATP per 2 NADPH molecules for the Calvin cycle. An overabundance of reducing power at the expense of ATP is therefore detrimental for the cell, as would be an accumulation of ATP without any available reducing equivalents. Cyclic electron flow around PSI has been proposed to balance ATP and NADPH, recycling the excess NADPH (see below), increasing the proton gradient through cytochrome b_6f activity, and therefore promoting ATP synthesis.

Although cyclic electron flow is far less studied in green algae than it is in plants (for reviews, see (Shikanai et al. 2002; Allen 2003; Kramer et al. 2004; Joliot et al. 2006; Rumeau et al. 2007)), the additional input of NAD(P)H in photoheterotrophically grown algae could be considered to further imbalance the ATP to NADPH ratio.

Stoichiometric requirements

As described above, the stoichiometric requirements of the Calvin cycle are 3 ATP per 2 NADPH molecules. It differs from the production of linear electron flow. Indeed, one could consider that the net production of the photosynthetic complexes is, on average: one proton per PSII turnover, one electron per PSI turnover and two protons per electron for the cytochrome b_6f. If 8 photons are equally distributed on PSI and PSII, the 4 electrons produced at the PSI acceptor side form 2 NADPH molecules and the 4 protons released from PSII plus the 8 protons from the cyt b_6f gives 12 protons. If these 12 protons could rotate the ATPase and

synthesize 3 ATP molecules, the Calvin cycle stoichiometry would be respected, but it is not the case: the minimum number of protons required to synthetize 3 ATP molecules depends upon the number of c-subunits in the CF0 ring of the ATPase, which carry one proton each. This number is 14 in higher plants (Seelert et al. 2000) and may be 13 in Chlamydomonas (Meyer Zu Tittingdorf et al. 2004) and 15 in the cyanobacterium *Spirulina platensis* (Pogoryelov et al. 2007). The transfer of 14 protons can be easily obtained considering an additional cyclic turnover of PSI (the electron does not go to NADPH, but is redirected into the quinone pool), which gives one more cytochrome b_6f turnover and the two extra protons required (see Fig. 1). The values 13 and 15 lead to non-integer results of 0.5 or 1.5 additional cyclic turnovers (respectively).

These stoichiometric calculations allow us to estimate the balance between linear (8 turnovers of photosystems) and cyclic electron flow rate (0.5 $\ll$ 1.5 PSI turnovers). A cyclic flow rate in the range of $\sim$6 to $\sim$16% of the linear flow rate (PSI + PSII) or $\sim$11 to $\sim$27% (PSI only) would be enough to supplement ATP synthesis. These kinetics constraints can be tested experimentally if we use the experimental methods described in the next section. Indeed, these methods allow the measurement of the number of electrons transferred per photosystem per time unit.

Spectroscopic methods

Under continuous illumination, a steady state is reached where the concentrations of reduced and oxidized species in the photosynthetic chain do not change with time. It means that for a given electron carrier, its oxidation flow rate is equal to its reduction flow rate. Spectrophotometric techniques allow us to monitor several of these parameters (the relative concentration of reduced or oxidized species in the light, and the rate of their dark recovery). In order to discriminate between linear and cyclic electron flow, the latter is usually estimated in the absence of PSII activity from the kinetics of P_{700} reduction after a continuous illumination of a few seconds. It means that two experimental conditions have to be fulfilled: redox changes of P_{700} must be monitored, and specific PSI excitation must be provided. The formation of P_{700}^+ is usually detected as a positive absorbance signal at 830 nm, and corrected from oxidized plastocyanin, contributing positively at 870 nm (this kind of measurement can be performed with a Dual-PAM, Walz, Germany). The illumination used to accumulate P_{700}^+ in the light is usually provided by a continuous far red light (>700 nm). Whereas this method works nicely for plants and green algae as Chlorella, it does not for *Chlamydomonas reinhardtii*. There are two reasons for this: first, we found that the broad-band peak of P_{700}^+ at 830 nm is smaller in Chlamydomonas than it is in plants (relatively to the 870 nm wavelength); and second, the lack of far red absorbance bands in Chlamydomonas PSI antenna (Tapie et al. 1984) renders far red light (>700 nm) less specific to PSI and thus less efficient for the accumulation of P_{700}^+. We have circumvented these experimental difficulties on the one hand by monitoring absorbance at a wavelength of 700 nm where P_{700}^+ is bleached and yields absorbance changes 10-fold larger than at 830 nm, and on the other hand by the addition of DCMU, a specific and efficient inhibitor of PSII. Such measurements can be done with a JTS-10 spectrophotometer (BioLogic, France).

Figure 2 represents the light-induced spectra of absorbance changes after 1 s of saturating illumination, obtained with a suspension of Chlamydomonas whole cells treated with DCMU. In aerobic cells, a clear accumulation of P_{700}^+ is observed as troughs at around 680 and 702 nm. In the meantime, cytochrome *f* is also oxidized (downward peak at 554 nm) and the membrane potential is dissipated through the activity of ATPase (only a very weak electrochromic chlorophyll bandshift around 650–660 nm persists at 1 s). In anaerobic cells in the dark, ATP is depleted in the cell and results in an inactivation of ATPase. This is observable as a membrane potential persisting at 1 s, and detectable as a chlorophyll bandshift (negative band at 649 nm and positive band at 658 nm). The oxidation of cytochrome *f* is also observable, in this case superimposed to the oxidation of the heme b_H detectable at 563 nm (heme b_H is reduced in anaerobic conditions in the dark). Even though the light excitation is saturating, no P_{700}^+ is accumulated under anaerobic conditions. This is due to a block of electron transfer at the acceptor side of PSI, as further discussed below.

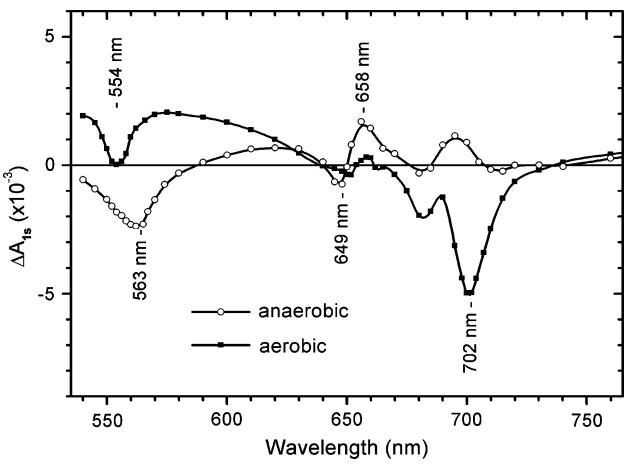

Fig. 2 spectra of photoinduced absorbance changes in a suspension of *Chlamydomonas reinhardtii* whole cells treated with 10 μM DCMU and placed under aerobic (■) or anaerobic (○) conditions

Analysis of P_{700} kinetics

From our point of view, Peter C. Maxwell and John Biggins produced a short and comprehensive digest (Maxwell and Biggins 1976) of the sparse data available at that time on cyclic photo-phosphorylation. In their article, they studied a wide range of photosynthetic algae from Chlorophyta (Chlorophyceae, Trebouxiophyceae, and Ulvophyceae) to Rhodophyta, Euglenophyta, Diatoms and even cyanobacteria and moss. In addition, in terms of in vivo spectroscopic methods, their article is a model of its kind because, by following P_{700} absorbance changes, they determined the cyclic electron flow rate in vivo. They used two actinic lights, one broad-band light for PSII and PSI excitation, and one specific to PSI (>695 nm), they tested the effect of inhibitors DCMU and DBMIB, and they studied a few mutants devoid of PSI, PSII, plastocyanine or cyt *f*. This allowed them to conclude that the limiting step of cyclic electron transfer must reside between a reduced PSI electron acceptor (at that time called P_{430}^-) and the plastoquinone pool.

One can summarize their results by the following: (i) a strong continuous light allows an accumulation of a fraction of P_{700}^+, quickly re-reduced in the dark ($t_{1/2} \sim 5$ ms, i.e. $k \sim 150\ s^{-1}$) if PSII is active (this is a way to measure the linear electron flow), (ii) if PSII is inactive (either in far red light, with DCMU addition or in a mutant inactivated for PSII) the fraction of P_{700}^+ is greater, and its re-reduction rate in the dark is considerably slowed down ($t_{1/2} \sim 150$ ms, i.e. $k \sim 5\ s^{-1}$), which gives an estimation of cyclic electron flow and (iii) when cytochrome b_6f activity is blocked (either by the absence of cyt *f* or plastocyanine, or DBMIB addition) the P_{700}^+ reduction rate (originating from a back-reaction in PSI) is even slower ($t_{1/2} \sim 575$ ms, *i.e.* $k < 1\ s^{-1}$), which demonstrates that cyclic electron flow proceeds through the plastoquinol/plastocyanin oxido-reductase activity of the cyt b_6f. These results have been confirmed in the unicellular green algae *Chlamydomonas reinhardtii* (Alric et al. 2010). However, owing to the large difference between their estimates of linear (150 $e^-\ s^{-1}$) and cyclic (5 $e^-\ s^{-1}$) electron flow rates per photosystem, Maxwell and Biggins concluded that the cyclic pathway does not contribute significantly to the energetic demands of carbon fixation. In fact, one can calculate, from the measured linear flow rate of 150 $e^-\ s^{-1}$, and from the estimate of 1 electron recycled in 9 (see the section above "stoichiometric requirements"), an expected cyclic flow rate of 150/9 $\sim$ 16.7 $e^-\ s^{-1}$, significantly faster than their measured flow rate of 5 $e^-\ s^{-1}$.

A question of redox poise

At this stage, we would like to emphasize the problem of the determination of the "maximum cyclic electron flow rate". As described above, the limiting step of cyclic electron flow is the transfer from PSI electron acceptors to the plastoquinones. Under aerobic conditions and a light excitation specific to PSI (DCMU or far red light), electrons are likely transferred to $NADP^+$ leaving ferredoxin oxidized (see discussion below). Thus, a maximum flow rate is expected when NADPH is accumulated and the plastoquinone pool is oxidized. However, such a non-equilibrium steady state is not easily reached in the light for several reasons: first, if one uses a light excitation specific to PSI which oxidizes the plastoquinone pool, the lack of PSII activity circumvents the accumulation of a large amount of reducing equivalents in the stroma; and secondly, even if a strong broad-band light (PSI + PSII) can significantly produce NADPH, it also reduces the quinone pool (a strong rise of fluorescence is observed in this case). Besides, an overabundance of NADPH could block PSI at the acceptor side (electron backflow, or charge recombination).

In spite of this complexity, it is very likely that the figure of 5 $e^-\ s^{-1}$ (Maxwell and Biggins 1976) underestimates the potential activity of cyclic flow. Indeed, the presence of an active Calvin cycle in the wild-type strains could exhaust, little by little in the light, with the ATP produced by the cyclic pathway, the reducing equivalents transferred to the stroma. This consumption of reducing power should therefore decrease the reinjection of electrons into the quinone pool. The cyclic electron flow measured in the presence of DCMU and in the absence of Calvin cycle (in a mutant devoid of Rubisco) is about 15 $e^-\ s^{-1}$ (Alric et al. 2010), i.e. 3 times faster than the value reported in (Maxwell and Biggins 1976). These new data, obtained in the presence of DCMU, are not far from the value of 16.7 $e^-\ s^{-1}$ (expected to match a linear flow of 150 $e^-\ s^{-1}$) and makes the cyclic pathway competent for the supply of the extra-ATP required for carbon fixation in the wild type. One can even expect that, in normal conditions when PSII is active (absence of DCMU or broad-band light), its contribution will readily increase the reducing power in the stroma and further enhance the cyclic flow.

Relation to chlororespiration

In this section, we would like to describe how we can relate the flow rates of plastoquinone oxidation and reduction, to glean some interesting information on the redox poise in vivo in the stroma of chloroplasts (NADPH concentration).

In the dark, i.e. at steady state, the redox state of the plastoquinone pool can be calculated from the identity relation between the flow rate of plastoquinol oxidation and the flow rate of plastoquinone reduction: $k_{ox}\ [PQH_2] = k_{red}\ [PQ]$, where k_{ox} is dependent upon oxygen concentration,

and k_{red} is dependent upon the availability of NADPH. Normalizing the pool size of quinones to 1 and substituting $[PQ] = 1 - [PQH_2]$ into the equation above, one obtains $[PQH_2] = k_{red}/(k_{ox} + k_{red})$. Under anaerobic conditions $k_{ox} = 0$ and $[PQH_2] = 1$, the quinone pool is fully reduced, independently of the value of k_{red}. But if we consider the case of ambient oxygen tension (k_{ox} = constant), the redox state of the quinone pool depends only upon k_{red}, and therefore on the availability of NADPH.

On the one hand, Pierre Bennoun has determined, in the absence of PSI or cyt b_6f activity, that the half-time of plastoquinol oxidation is about 3–4 s (Bennoun 1982; Bennoun 2001) for a pool size of nearly 10 electrons (Bennoun 1983). This gives a flow rate $F_{ox} = k_{ox}$ $[PQH_2] \sim 2\ e^-\ s^{-1}$ per PSII. This flow rate was determined when the quinone pool was fully reduced in the light ($[PQH_2] = 1$) which gives $k_{ox} \sim 2\ s^{-1}$.

On the other hand, we can determine the flow rate of plastoquinone reduction because it is the limiting step for P_{700}^+ reduction. In the presence of DCMU and methylviologen (MV), which is an efficient PSI electron acceptor and which bypasses electron transfer towards NADPH, the reducing flow rate is about $F_{red} = k_{red}$ $[PQ] \sim 2\ e^-\ s^{-1}$ per PSI (Alric et al. 2010). Here, DCMU and MV discard linear and cyclic electron flows, and the remaining flow rate is limited by the breakdown of starch, which produces NADPH. The plastoquinone pool being fully oxidized in such conditions ($[PQ] = 1$), one obtains $k_{red} \sim 2\ s^{-1}$. Although F_{red} is probed with PSI and F_{ox} with PSII, since the PSI:PSII stoichiometry is 1:1, we can directly use these values of k_{ox} and k_{red} to calculate $[PQH_2] = k_{red}/(k_{ox} + k_{red}) \sim 0.5$. This prediction of an approximately half-reduced quinone pool in the dark nicely matches the estimated concentration of PSII secondary electron acceptors in Chlorella (Joliot 1965), and validates our very basic model. Due to MV addition, electron transfer to P_{700}^+ via the quinone pool is limited by carbohydrate catabolism and therefore NADPH concentration in the stroma is very low.

In the absence of MV, and for the mutant devoid of Rubisco, the reducing flow rate measured towards P_{700}^+ is about $F_{red} = k_{red}$ $[PQ] \sim 15\ e^-\ s^{-1}$ per PSI (Alric et al. 2010). In this case, we can estimate the reducing power present in the stroma considering that, if this reducing flux was observed in the dark, it would lead to a significant reduction of the quinone pool $[PQH_2] = k_{red}/(k_{ox} + k_{red}) \sim 0.9$. Such a pronounced reduction of the PQ pool in the dark cannot be attained with the sole addition of the mitochondrial inhibitors SHAM and azide (see Table 1) but necessitates the further addition of 1 mM propyl gallate, an inhibitor of the chloroplast oxidase, which is expected to also decrease k_{ox} and further enhance the ratio $k_{red}/(k_{ox} + k_{red})$.

This latter estimation allows us to draw several conclusions: (i) the concentration of NADPH corresponding to a reducing flux of 15 $e^-\ s^{-1}$ towards P_{700}^+ is likely very high, (ii) the observed cyclic electron flow of 15 $e^-\ s^{-1}$ transferred per PSI may approach the maximum electron flow expected from NADPH to the PQ pool (see section above), and (iii) inferred from (i) if it is possible to significantly reduce NADPH in the light in the presence of DCMU (i.e. these reducing equivalents originating only from the few electrons present in plastocyanin, cyt *f*, Rieske and a few quinols), then there is not much difference between the pool size of PSI secondary electron acceptors and secondary electron donors. This last point is further discussed in the section entitled "*A picture of the redox poise at steady-state*".

Cyclic electron flow and state transitions

Unicellular algae such as *Chlamydomonas reinhardtii* have been reported to carry out massive state transitions. In simplistic terms, it is the reversible migration of light harvesting complexes (LHC2) from PSII (state 1) to PSI (state 2) in response to an over-reduction of intersystem electron carriers (PQ pool). The function of state transition is thus thought to optimize the function of the photosynthetic chain by poising the light excitation between PSI and PSII. One may reason that such a redistribution of energy between photosystems also optimizes linear (involving PSII and PSI) and cyclic (PSI only) electron flow.

If the PQ pool becomes reduced (PQH_2), thereby decreasing PSII activity, an increase of the absorption cross-section of PSI enhances its turnover and results in PQH_2 oxidation if enough PSI electron acceptors are still oxidized ($NADP^+$). If NADPH was accumulated at the same time as PQH_2, the increase in PSI turnover would steadily enhance ATP synthesis via cyclic electron flow, this extra-ATP being used in the Calvin cycle for NADPH reoxidation, and consequently PQH_2 reoxidation.

The functional consequences of the changes in photosystems absorption cross-section on the redox state of intersystem electron carriers (cyt *f*) have been extensively studied (Finazzi et al. 1999, 2001, 2002; Forti et al. 2003; Finazzi and Forti 2004; Finazzi 2005; Finazzi et al. 2006). Under a continuous broad-band illumination, a significant amount of oxidized cyt *f* is accumulated. The block of PSII activity (by addition of DCMU) increases the amount of oxidized cyt *f*. Expectedly, the effect of DCMU addition was more pronounced in state 1, when the PSII absorption cross-section is greater. It is difficult however to give a straightforward interpretation of the amplitudes of cyt *f* oxidation because of the limitations of electron transfer at PSI acceptor side, especially in anoxia (see discussion

Table 1 Compilation of data available on the dark reduction of the plastoquinone pool in vivo in green algae

Sample	Treatment	Effect on the PQ pool	References
Chlamydomonas F14 (strain devoid of PSI)	$-O_2$	Significant reduction	Bennoun (1982)
	+CO	Significant reduction	Bennoun (1982)
	+NO	Significant reduction	Bennoun (1982)
	4 mM SHAM for 2 min	No effect	Bennoun (1982)
	10 mM cyanide for 3 min	Slight reduction	Bennoun (1982)
	10 mM azide for 3 min	Significant reduction	Bennoun (1982)
Chlorella pyrenoidosa	+CO	No effect	Bennoun (1982)
	10 mM SHAM for 3 min	Significant reduction	Bennoun (1982)
	10 mM cyanide for 3 min	No effect	Bennoun (1982)
	1 mM azide	Significant reduction	Maison-Peteri et al. (1977)
Chlamydomonas 137 c	1 μM antimycin A	Slight reduction	Gans and Rebeille (1990)
	1 μM antimycin A + 1 mM SHAM	Significant reduction	Gans and Rebeille (1990)
Chlamydomonas F14 (strain devoid of PSI)	1 mM CROWN for 1 min	70% reduction	Bennoun (1983)
	for 2 min	>90% reduction	Bennoun (1983)
Chlamydomonas FUD6 (strain devoid of cyt b_6f)	5 μM FCCP	$F_{max} = 56\%$	Bulté et al. (1990)
	1 mM CROWN	$F_{max} = 63\%$	Bulté et al. (1990)
	2 μM TBT	$F_{max} = 50\%$	Bulté et al. (1990)
	5 μM DCCD	$F_{max} = 59\%$	Bulté et al. (1990)
	1 μM antimycin A + 1 mM SHAM	$F_{max} = 54\%$	Bulté et al. (1990)
Chlamydomonas DUM-1 (strain devoid of mitochondrial cyt *b*)	No add	$F_{max} = 55\%$	Bulté et al. (1990)
	1 mM SHAM	$F_{max} = 45\%$	Bulté et al. (1990)
Chlamydomonas F15 (strain devoid of PSI)	4 μM myxothiazol for 10–15 min	50% reduction	Bennoun (1990)
Chlamydomonas FUD6 (strain devoid of cyt b_6f)	1 mM azide for 2 min	25% reduction	Bennoun (2001)
	1 mM PG for 2 min	No effect	Bennoun (2001)
	1 mM PG + 1 mM azide for 2 min	90% reduction	Bennoun (2001)
	1 mM SHAM for 2 min	No effect	Bennoun (2001)
	1 mM SHAM + 1 mM azide for 2 min	75% reduction	Bennoun (2001)
	1 mM PG for 10 min	No effect	Bennoun (2001)
	1.5 mM PG for 10 min	70% reduction	Bennoun (2001)
	2 mM PG for 3 min	80% reduction	Bennoun (2001)
	1 mM SHAM for 2 min	No effect on reoxidation following a preillumination	Bennoun (2001)
	1 mM PG for 2 min	Three-times slower reoxidation following a preillumination	Bennoun (2001)
Chlamydomonas Nda2-RNAi (strain devoid of chloroplast NADPH dehydrogenase)	10 μM myxothiazol for 30 min	No effect	Jans et al. (1990)

Azide is for sodium azide (NaN_3), cyanide is for potassium cyanide (KCN), SHAM is for salicylhydroxamic acid, PG is for n-propyl gallate, CROWN is for dicyclohexyl-*crown*, FCCP is carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, DCCD dicyclohexylcarbodiimide, TBT tri-*n*-butylin. The fluorescence data of Bulté et al. (1990) are representative of state transitions, a fluorescence quenching being induced by the reduction of the PQ pool

below and Fig. 2). In order to compare the cyclic electron flow rates in state 1 and state 2, we have measured the post-illumination recovery kinetics of P_{700} in aerobic conditions, where P_{700} could be fully oxidized after 10 s of strong light, in a mutant devoid of mitochondrial activity, *dum*22, and the double mutant *dum*22*stt*9 unable to perform state transitions (Cardol et al. 2009). In *dum*22, ATP is depleted in the dark and NADH is not consumed, even in

aerobic conditions. The reducing power accumulated in the cell, leads to the reduction of the plastoquinone pool and to the transition to state 2 in the dark. The double mutant *dum*22*stt*9 is in the same redox state but it is locked in state 1 because of the lack of the kinase implicated in the transition to state 2 (Depege et al. 2003). We have observed that, in the same aerobic conditions, the P_{700}^+ reduction rate k was virtually indistinguishable between *dum*22 (state 2) and the double mutant *dum*22*stt*9 (state 1), see Fig. 3 where $k \sim 10\ s^{-1}$.

These P_{700} measurements allow a better quantification of the cyclic electron flow rate, but the same similarity between state 1 and state 2 in aerobic conditions could be observed in the data reported for the WT in (Finazzi et al. 1999). After a transition to state 2 was first induced by anoxia, and the limitation at the acceptor side of PSI was then suppressed by the rapid addition of oxygen, cyt *f* is fully oxidized in the light, and in the presence of DCMU its rereduction rate is similar in state 1 and state 2 (Finazzi et al. 1999). Very similar cyclic flow rates are also observed in the photoacoustic data obtained in aerobic conditions in the WT (state 1) and *dum*22 (state 2) (Cardol et al. 2003). In these measurements, only a small stimulation (<20%) of cyclic electron flow is observed in *dum*22 under far red light (>715 nm, specific PSI excitation), i.e. independently of the modification of PSI absorption cross-section.

All of these data show that, in aerobic conditions, the intrinsic rate of cyclic electron transfer k does not significantly depend upon state transitions. The migration of 15–20% of the cytochrome b_6f complex from grana to stroma lamellae (Vallon et al. 1991) does not significantly enhance the rate. This conclusion is consistent with the observation that the rate limiting step of cyclic flow is not between cyt b_6f and PSI, but rather upstream on the entry of electrons into the quinone pool (Maxwell and Biggins 1976).

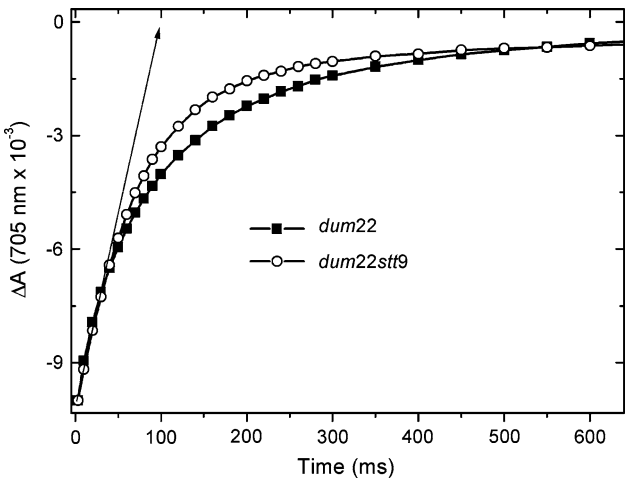

Fig. 3 Post-illumination recovery kinetics of P_{700} in *dum*22 (state 2) and *dum*22*stt*9 (state 1), after 10 s of saturating light (raw data used in (Cardol et al. 2009) to calculate fluxes reported in Table 1 therein)

Although the intrinsic rate k of cyclic electron transfer under aerobic conditions is not modified by state transitions, the PSI antenna size has to be taken into account in non-saturating light (below $\sim 100\ \mu E\ m^{-2}\ s^{-1}$), where a difference in the growth phenotype is observed between *dum*22 (state 2) and *dum*22*stt*9 (state 1) (Cardol et al. 2009). In such low light conditions, cyclic electron flow becomes limited by the PSI light capture. PSI turnover is $F = k\ [P_{700}^+]$, where $[P_{700}^+]$ is the steady-state concentration of oxidized P_{700} accumulated in the light. In saturating light $[P_{700}^+] = 1$, but in low light $[P_{700}^+]$ is significantly decreased, especially if the PSI antenna is small. In a genetic background where the production of ATP in the mitochondrion is blocked (*dum22*), and under light-limiting conditions, the inability to enhance PSI antenna size (*stt*9) is detrimental for growth (Cardol et al. 2009).

A picture of the redox poise at steady state

One could tentatively draw a schematic representation of the redox poise of the cofactors in the photosynthetic chain (see Fig. 4), in the dark (left panels A and C) and when PSI is specifically excited by light (right panels B and D), in either aerobic (top panels A and B) or anaerobic (bottom panels C and D) conditions. Although we do not know the precise stoichiometries of these components, particularly the number of PSI electron acceptors, we could make reasonable estimates because the size of the acceptor pool ($NADP^+$) must be close to the size of the donor pool (PQH_2, see above the chlororespiration section, an additional justification is given below). The filled areas correspond to the Nernst redox buffering at a given E_h.

In a dark aerobic sample of Chlamydomonas (Fig. 4a), Q_A from PSII is fully oxidized and the PQ pool significantly reduced, as well as cyt *f*, the Rieske protein and P_{700} (confirmed by absorption changes, see Fig. 2). The amount of NADPH (not determined) must be low because of its consumption through chlororespiration. In the presence of DCMU, the oxidase is only active in the dark aerobic state: in the light its activity is bypassed by the PSI + b_6f turnover (Cournac et al. 2002), and under anaerobic conditions its substrate is absent. In anaerobic conditions (Fig. 4c), the inhibition of mitochondrial and chloroplastic oxidases results in a significant reduction of Q_A in the dark, which corresponds to a very large reduction of the PQ pool. Under such conditions, a significant amount of NADPH might also accumulate, but the PSI electron acceptors are still mostly oxidized (PSI is still active following a single turnover flash).

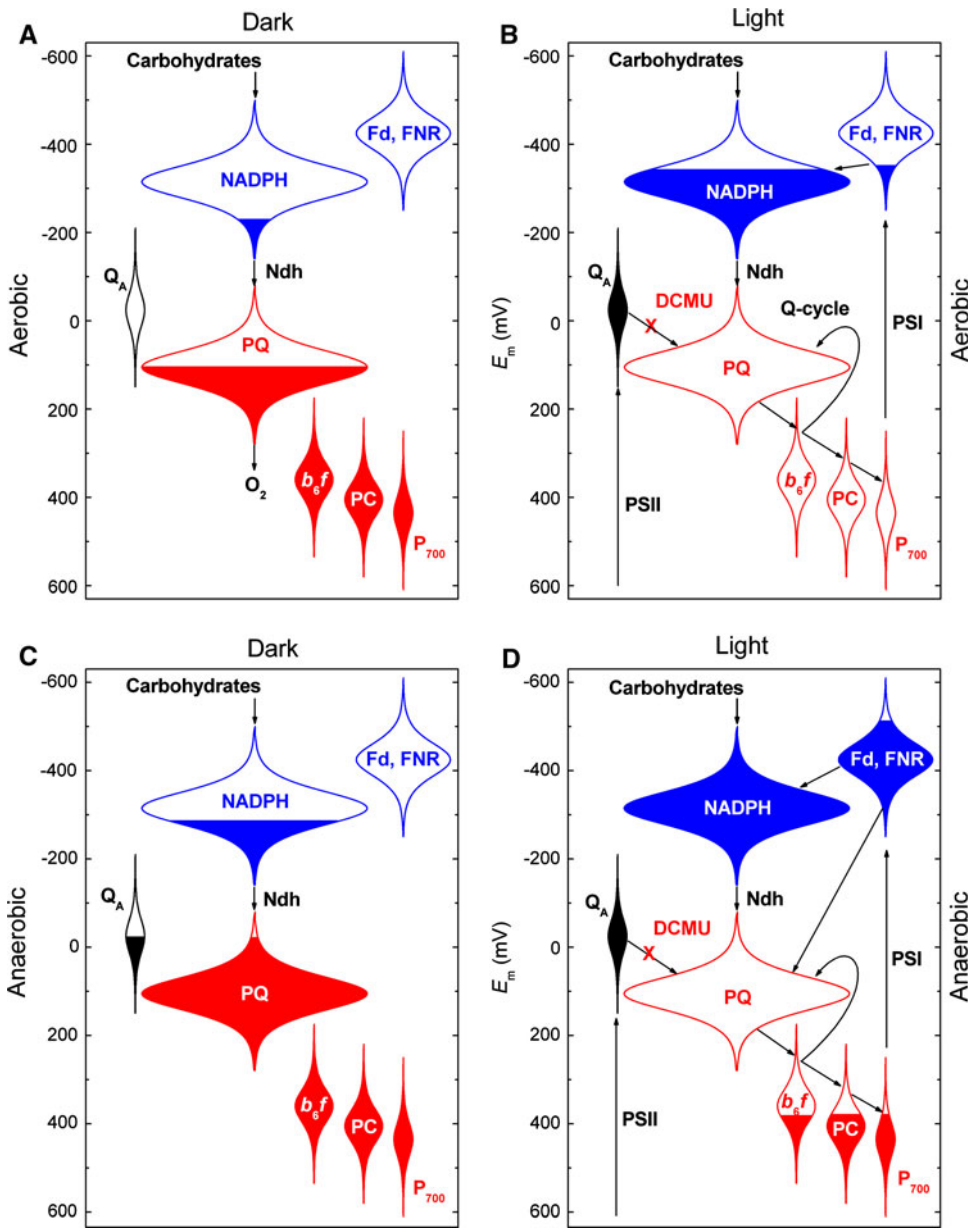

Fig. 4 A representation of the Z-scheme with flared reservoirs, where areas reflect the nernstian redox buffering of the redox components. The overall area of Q_A and P_{700} correspond to one electron. We have considered that one photosynthetic unit containing 1 PSII and 1 PSI also contained approximately the same number of electron carriers at the donor and acceptor side of PSI: PSI secondary electron donors (2 plastocyanins; 1 cytochrome b_6f complex, 1 cyt f + 1 Rieske; about 6 quinones, 12 electrons), and PSI secondary electron acceptors (5 electrons on ferredoxin and ferredoxin $NADP^+$ reductase; and 12 on $NADP^+$/NADPH). See text for details

In the light Q_A is reduced, its re-oxidation being prevented by DCMU. Under aerobic conditions (Fig. 4b), P_{700} is largely oxidized, as well as cyt f (see Fig. 2), and the electrons present in the dark in PSI secondary donors are transferred to the acceptor side, likely to $NADP^+$. Under anaerobic conditions (Fig. 4D), the amount of $P_{700}{}^+$ accumulated in the light is very low (see Fig. 2). It reveals a limitation of electron transfer at the acceptor side of PSI and the accumulation of reduced FNR and ferredoxin. Owing to this limitation in PSI turnover, P_{700}, plastocyanine and cyt f are in thermodynamic equilibrium. And despite such a lower PSI turnover, the quinone pool is oxidized, as suggested by the promotion of state 1 upon illumination in the presence of DCMU (Delepelaire and Wollman 1985).

In the absence of PSII activity, it is therefore possible to draw a relatively simple picture of the redox poise of the photosynthetic cofactors under steady state. Figure 4 depicts pools of similar sizes. It is supported by an experimental estimation in plants of a pool size of 9 PSI electron acceptors for 6 plastoquinones (12 electrons) per PSI (Joliot and Joliot 2002). And it also accounts for the conclusion drawn above at the end of the chlororespiration section: that sustained cyclic electron flow observed after several seconds of illumination shows NADPH is significantly formed under aerobic conditions (Fig. 4b). It reveals

that the influx of NADPH in the stroma from carbohydrate catabolism compensates for its consumption in the Calvin cycle. On the other hand it also illustrates how electron flow is limited at the acceptor side of PSI in anaerobic conditions (Fig. 4d) preventing the accumulation of P_{700}^+ (Fig. 2). Panel 4D also illustrates how the oxidation state of cytochrome *f* under anaerobic conditions is not just influenced by cyclic electron flow, but rather it equilibrates with P_{700} blocked in its reduced state.

Ferredoxin-mediated electron flow

Finally, we would like to raise the question of ferredoxin (Fd)-mediated electron flow. Since Arnon's early experiments (Arnon et al. 1954; Arnon et al. 1955; Tagawa et al. 1963), Fd has been thought to transfer electrons to the quinone pool, probably through cytochrome b_6f activity like in the Mitchell's original conception of the Q-cycle (Mitchell 1975a, b). To our knowledge and from our experience with green algae, there is no experimental evidence for such a Fd-mediated cyclic flow in aerobic conditions under light excitation specific to PSI, so that we have not taken into account this pathway in panel 4B where a small fraction of reduced Fd directly transfers its electrons to $NADP^+$. But under anaerobic conditions (see panel 4D) a large amount of reduced Fd should be accumulated in the light, which is, together with the presence of an oxidized quinone pool, a favourable condition for observing Fd-mediated cyclic electron flow. Unfortunately, this hypothesis could not be investigated using P_{700} measurements because no P_{700}^+ is accumulated under such conditions. Therefore, providing experimental evidence for the involvement of a Fd-mediated cyclic flow in vivo still remains a challenging issue.

During the completion of this study, an article appeared where the biochemical isolation of a supercomplex containing cytochrome b_6f, PSI, FNR, PETO, PGRL1, LHCI and LHCII is reported (Iwai et al. 2010). This supercomplex, isolated from Chlamydomonas cells in state 2, is capable of a slow ($\sim$2–3 s^{-1}, estimated from (Iwai et al. 2010) Fig. 3c) cyclic photoreduction of cyt *b* when 1 μM Fd is added. Again, this result shows that the recycling of electrons at the stromal side is the limiting step of cyclic flow (for comparison, the addition of 1 μM PC to this complex results in cyt *f* oxidation at a much higher rate of $\sim$100 s^{-1}, estimated from (Iwai et al. 2010) Fig. 3f). Contrary to that shown in (Arnon et al. 1954, 1955) cyt *b* reduction is not inhibited by Antimycin A, and the presence of PGRL1 in the supercomplex suggests that it could play the role of a catalyst in cyclic electron flow. Such a catalytic role for PGRL1 has been demonstrated in plants (DalCorso et al. 2008) as well as in Chlamydomonas (Tolleter et al. in preparation; Petroutsos et al. 2009). However, the general principles of this reaction remain unknown, and many questions are still open: does Fd-mediated cyclic electron flow exist in cyanobacteria devoid of Pgrl1? Could PGRL1 be active in state 1 conditions?

Conclusion

We have described how the measurement of P_{700} oxidation–reduction kinetics under aerobic conditions allows the determination of the cyclic electron flow rate in cells of green algae such as *Chlamydomonas reinhardtii*. As described in (Alric et al. 2010), this flow rate depends upon the reducing power present in the stroma, and the ability of the cell to use it. Despite this variability, observable when the light, redox or ATP conditions are modified, the cyclic flow rate does not change much under aerobic conditions, where it is about 10–15 e^- s^{-1} PSI^{-1}. This flow rate is within the range of 6 to 16 % of the linear electron flow (maximum 150 e^- s^{-1} PSI + $PSII^{-1}$) expected to provide the extra-ATP needed for carbon fixation. We have given a description that also takes into account carbohydrate and acetate metabolism. The utilization of reserves participates in the flux of electrons towards P_{700}^+, and makes P_{700}^+ oxidation–reduction kinetics very rich observables for the analysis of electron fluxes in the chloroplast in relation to the general cell metabolism.

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