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Review

Interdependence between chloroplasts and mitochondria in the light and the dark

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Abbreviations: CR, chlororespiration; DHAP, dihydroxyacetone phosphate; ETC, electron transport chain; Fd, ferredoxin; G6PDH, glucose-6-P dehydrogenase; GAPDH, glyceraldehyde-3-P dehydrogenase; LEDR, light enhanced dark respiration; LHC, light harvesting complex; Mal, malate; MDH, malate dehydrogenase; ME, malic enzyme; NR, nitrate reductase; OAA, oxaloacetate; 2-OG, 2-oxoglutarate; PDC, pyruvate dehydrogenase complex; PEP, phosphoenol pyruvate; PEPC, PEP carboxylase; 3-PGA, 3-phosphoglycerate; PGK, phosphoglycerate kinase; PIB, post-illumination burst; PK, pyruvate kinase; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; RuBP, ribulose 1,5-bisphosphate; SHAM, salicylhydroxamic acid; TCA, tricarboxylic acid; Td, thioredoxin; TP, triose phosphate

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1. Introduction

Plants grow using light energy to photosynthetically convert atmospheric CO_2 into carbon-rich compounds (e.g. carbohydrates) in the chloroplasts. These compounds are then respired in the cytosol and mitochondria to generate the energy and carbon intermediates necessary for biosynthesis. The two processes are interdependent, with respiration relying on photosynthesis for substrate whereas cellular photosynthesis depends on respiration for a range of compounds (e.g. ATP; see later sections). Surprisingly, however, most researchers study the two processes independently. In this review, we discuss the interdependence of chloroplasts and mitochondria. The mechanisms by which common metabolites are exchanged between chloroplasts and mitochondria via the cytosol are first discussed. The review then assesses the role of mitochondria in the light. Finally, it discusses the interaction between mitochondria and chloroplasts in darkness and the phenomenon of chlororespiration.

2. Interactions between organelles depends on metabolite exchange

Interactions between chloroplasts and mitochondria depend on exchange of metabolites such as ATP (energy), NAD(P)H (reducing equivalents) and carbon skeletons. Some metabolites are transported across membranes of the organelles by specific translocators, whereas others are transported by metabolite shuttles because they cannot be translocated directly. Metabolite shuttles may also serve multiple functions such as transferring both ATP and/or reducing equivalents or carbon skeletons. In this section we outline the ways in which metabolites are transported across organelle membranes.

2.1. ATP exchange

The highly active mitochondrial ATP/ADP translocator rapidly exports ATP from the matrix to the cytosol in exchange for ADP [1] (Fig. 1). In contrast, the activity and affinity of the chloroplast translocator are very low [2,3] and possibly only active in young chloroplasts to import ATP [4].

Chloroplast ATP exchange can also occur via the dihydroxyacetone 3-phosphate (DHAP)/3-phosphoglycerate (3-PGA) shuttle, using the phosphate translocator of the chloroplast membrane [5] (Fig. 1). The conversion of 3-PGA to DHAP in the chloroplast consumes ATP and NADPH, which are regenerated in the cytosol by the NAD^+-dependent, phosphorylating GAPDH/PGA-kinase (Fig. 1). However, no ATP is exported when cytosolic DHAP is converted to 3-PGA by the $NADP^+$-dependent non-phosphorylating GAPDH/PGK, which produces NADPH only (Fig. 1).

Although these shuttles are capable of transporting *both* NADPH and ATP, they do not appear to export significant quantities of ATP under physiological conditions, as the non-phosphorylating system predominates [6]. The DHAP/3-PGA shuttle therefore utilises chloroplastic ATP and exports reducing equivalents from the chloroplast [6].

Import of ATP by this shuttle is probably more efficient, because DHAP can be converted to PGA via only one route (Fig. 1) which yields both NADPH and ATP. In chloroplasts, isolated from a mutant of *Chlamydomonas* deficient in the chloroplast ATP synthase, the DHAP/3-PGA shuttle had a much larger capacity for ATP import than the ATP translocator [7]. In these illuminated chloroplasts protein synthesis was highly stimulated by DHAP and GAP (5-fold) but less so by ATP (2-fold). On the other hand, 3-PGA strongly inhibited protein synthesis. Protein synthesis in the wild-type chloroplasts was not affected by these metabolites.

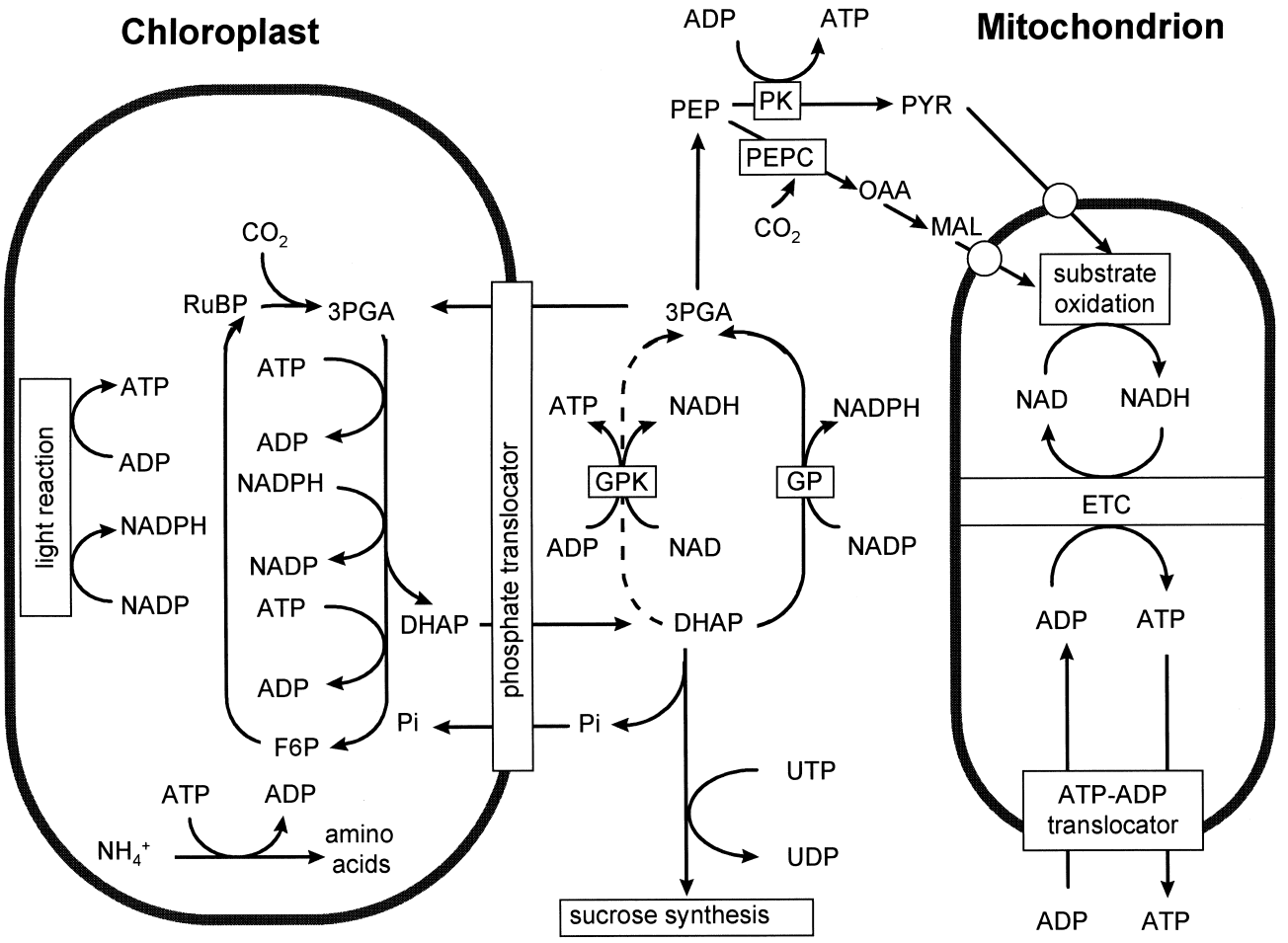

Fig. 1. ATP exchanges among chloroplasts, the cytosol and mitochondria. ETC, electron transport chain; GP, $NADP^+$-GAPDH (glyceraldehyde-3-P dehydrogenase); GPK, PGK (phosphoglycerate kinase) and NAD^+GAPDH (glyceraldehyde-3-P dehydrogenase); PEPC, PEP carboxylase; PK, pyruvate kinase.

In summary, chloroplasts exhibit a far lower capacity for ATP export than mitochondria.

2.2. *Transport of reducing equivalents across membranes*

NAD(P)H cannot cross the membranes of organelles directly and the reducing equivalents must be transported using shuttles, such as the chloroplast DHAP/3-PGA translocator mentioned above, or via the malate/oxaloacetate (Mal/OAA) shuttle [8] (Fig. 2). In chloroplasts, malate dehydrogenase (MDH) is $NADP^+$-dependent, whereas an NAD^+-MDH operates in the cytosol and the mitochondria. Chloroplast $NADP^+$-MDH is activated in the light and converts OAA to malate when the chloroplast NADPH/$NADP^+$ ratio is high [8,9].

Mitochondria can also export reducing equivalents by exchanging citrate for cytosolic malate [1] (Fig. 3). Subsequent decarboxylation of citrate to 2-OG results in the production of NADPH. Reducing equivalents can also be exchanged across the chloroplast and mitochondrial membranes via the malate/aspartate shuttle, involving the malate/2-OG and glutamate/aspartate translocators [6]. However, the contribution of these two translocators to the transport of reducing equivalents is minor compared with the Mal/OAA shuttle [10].

Plant mitochondria can oxidise cytosolic NAD(P)H directly via the mitochondrial electron transport chain (ETC) using the externally facing NAD(P)H dehydrogenases [11] (Fig. 2). However, given the low concentrations of NADH (0.3–1.2 μM) and NADPH (150 μM) in the cytosol under

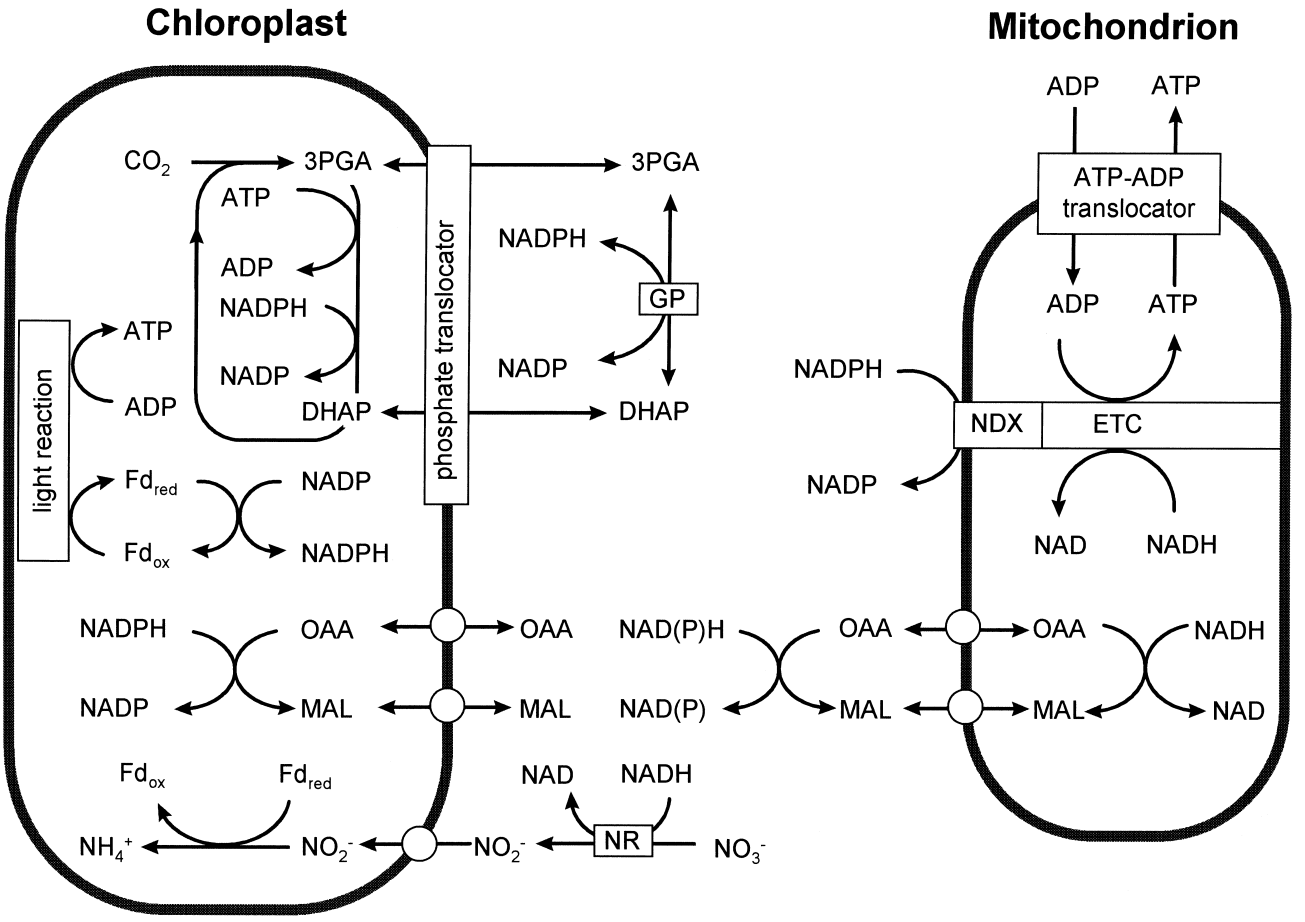

Fig. 2. Exchanges of reducing equivalents among chloroplasts, the cytosol and mitochondria. ETC, electron transport chain; GP, $NADP^+$-GAPDH (glyceraldehyde-3-P dehydrogenase); NDX, externally facing NADPH dehydrogenase; NR, nitrate reductase.

physiological conditions and the substrate affinities of the external NAD(P)H dehydrogenase (K_m; 1.4 μM for NADH and 80 μM for NADPH) [6,8,10,12], it is most likely that only NADPH is oxidised by these NAD(P)H dehydrogenases, and even then at a very low rate.

2.3. Exchange of carbon compounds

In addition to transporting ATP/ADP and reducing equivalents, mitochondria and chloroplasts also exchange carbon compounds. Chloroplasts export carbon at a high rate via the phosphate translocator [3] (Fig. 3). Cytosolic P_i concentrations determine whether DHAP remains in the chloroplast (to be converted to starch) or is exported (to serve as a substrate for sucrose, malate and/or pyruvate synthesis [13]).

Mitochondria have specific organic acid translocators for most of the TCA cycle intermediates [14]. In addition to the Mal/OAA and the malate/citrate shuttles described in Section 2.2, malate can enter mitochondria via a dicarboxylate carrier which catalyses malate/P_i exchange [15].

In addition to being a reducing equivalent exchange system, the malate/citrate shuttle also exports carbon from the mitochondria (see Section 2.2). The 2-OG produced from cytosolic citrate decarboxylation serves as a carbon skeleton for amino acid synthesis in the chloroplast (Fig. 3). Import of 2-OG into the chloroplast is via the 2-OG/dicarboxylate exchange carrier that exchanges 2-OG for OAA and malate [14]. Whenever citrate is exported from the mitochondria, OAA or malate must be imported to replace the carbon lost from the TCA cycle. Because plant mitochondria have an NAD^+-malic enzyme (NAD-ME) that converts malate to pyruvate, any TCA-cycle intermediate will suffice.

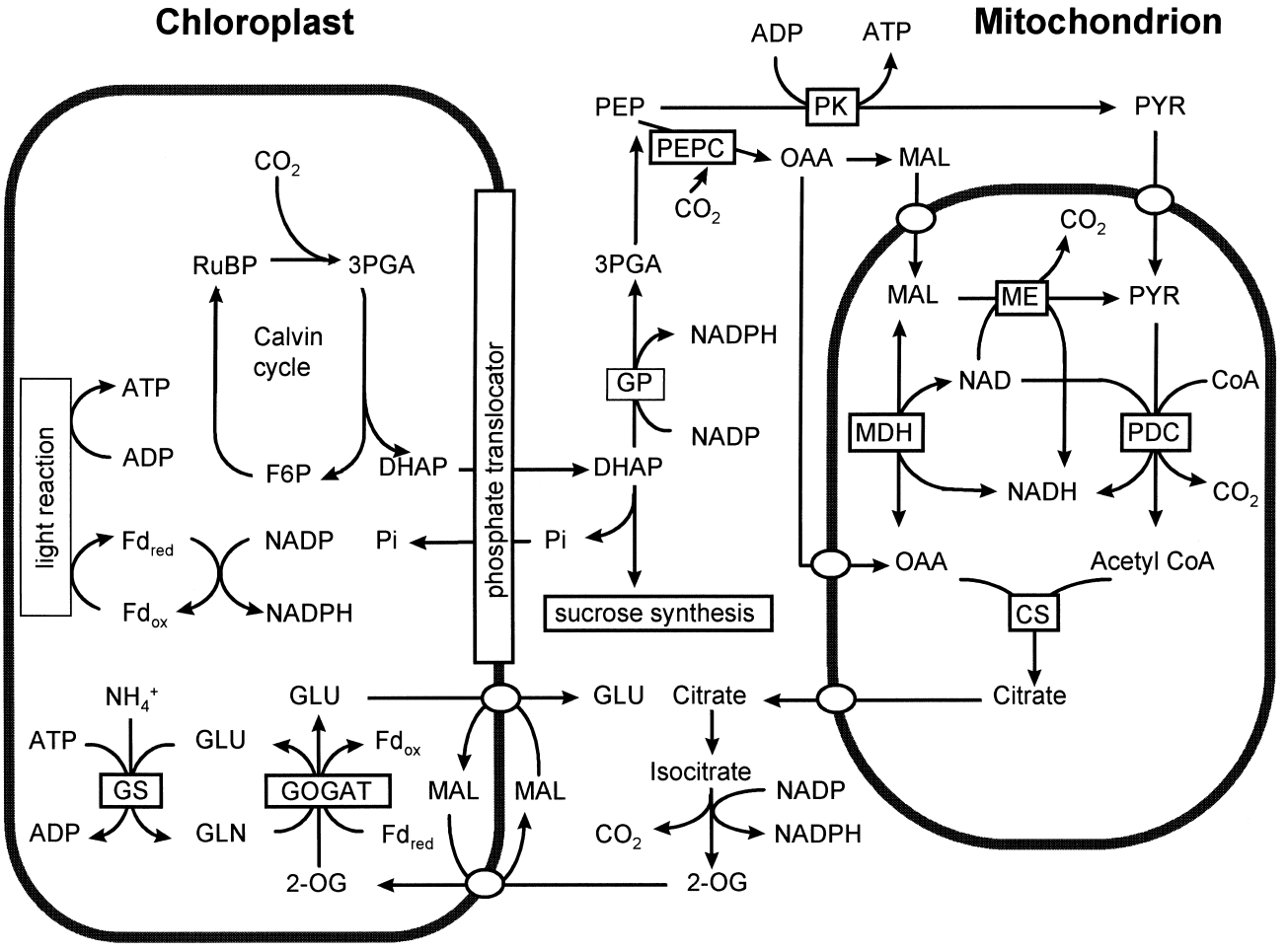

Fig. 3. Carbon exchange among chloroplasts, the cytosol and mitochondria. CS, citrate synthase; GOGAT, glutamate oxoglutarate transaminase; GP, $NADP^+$-GAPDH (glyceraldehyde-3-P dehydrogenase); GS, glutamine synthase; MDH, malate dehydrogenase; ME, malic enzyme; PDC, pyruvate dehydrogenase complex; PEPC, PEP carboxylase; PK, pyruvate kinase.

The above sections demonstrate that plant cells possess a large number of transport systems to exchange metabolites among organelles and cytosol. The large array of transport systems help provide the cell with the metabolic flexibility it needs to respond to different conditions. However, the same variety of transport systems makes it very difficult to study the effects of altered conditions within the cell.

3. Respiration in the light

3.1. Does respiration continue in the light?

A question that has stimulated considerable debate is whether respiration continues in the light in photosynthetic cells, and, if so, whether it has the same rate as it does in the dark. Respiration (i.e. oxidative degradation of stored and recently fixed carbohydrates) is the main source of ATP for photosynthetic cells in the dark. In the past it was believed that respiration was fully inhibited in the light, probably as a result of photosynthetic ATP production, via adenylate control of glycolysis and limitations in substrate supply to the mitochondria [16]. This view is now considered too simplistic and experimental data suggest that mitochondrial activity continues in the light under most conditions. Mitochondria provide the cell with TCA cycle carbon skeletons for light-dependent NH_4^+ assimilation in the chloroplast (Fig. 3) and ATP and NADH for other biosynthetic reac-

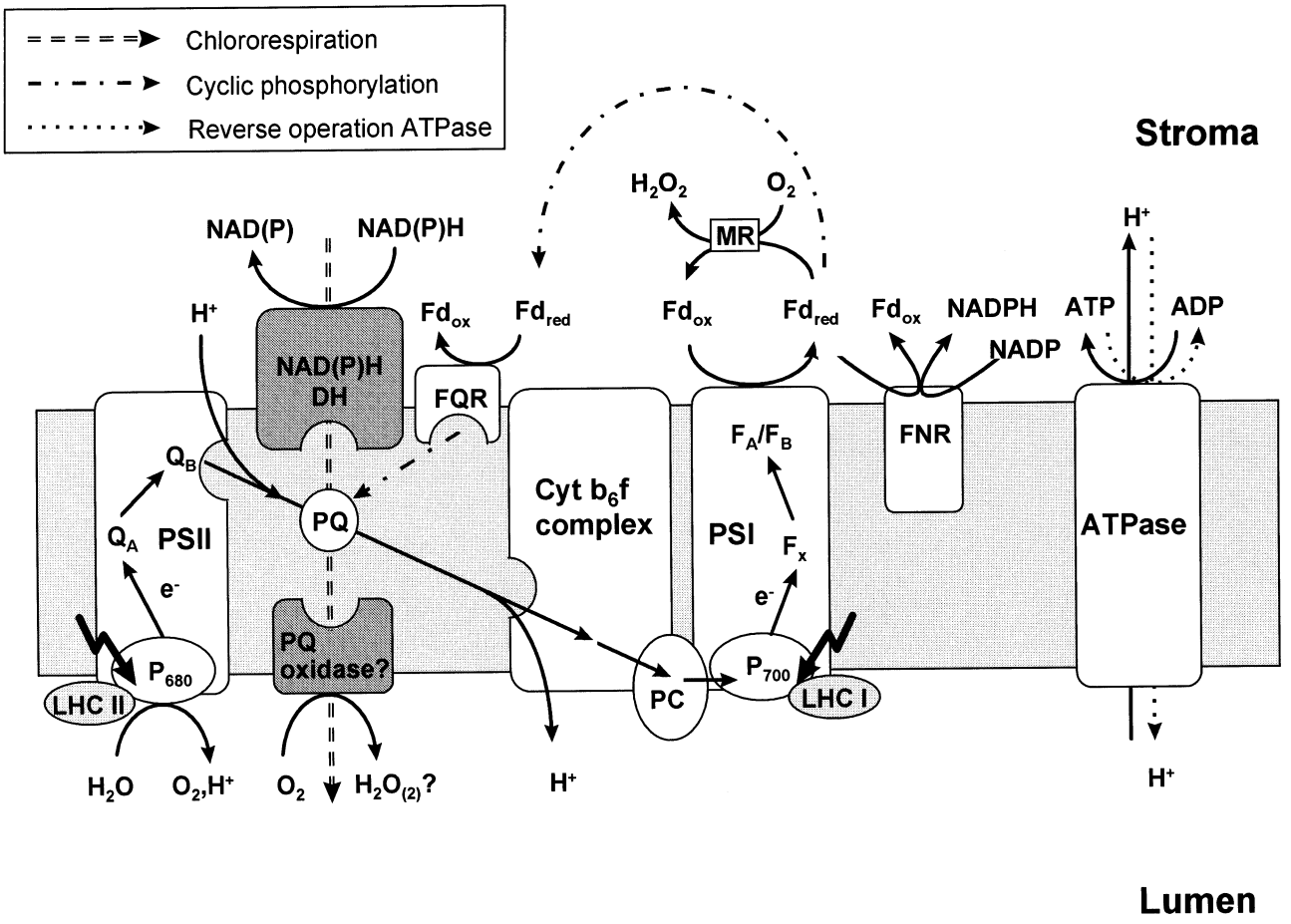

Fig. 4. Organisation of the thylakoid membrane. FNR, ferredoxin-NADP$^+$ oxidoreductase; FQR, ferredoxin-plastoquinone reductase; LHC, light harvesting complex; MR, Mehler reaction; PC, plastocyanin; NAD(P)HDH, NAD(P)H dehydrogenase; PQ, plastoquinone; PS, photosystem. The elements of the suggested chlororespiratory pathway are indicated by dark-shaded rectangles.

tions in the light (Figs. 1 and 2). Mitochondria may also oxidise excess photosynthetic reducing equivalents in the light. Respiration is therefore likely to continue in the light, with the actual role that mitochondria serve in the light being dependent on the developmental stage and the environmental conditions.

3.2. Substrates for the mitochondria in the light

Several substrates support respiration in the light, including photorespiratory glycine and products of recent photosynthetic activity, such as malate, OAA, pyruvate and NAD(P)H. Pärnik and Keerberg [17] defined these substrates as the primary products of photosynthesis. The degree to which primary products provide substrates for respiration is likely to increase under conditions where there is an excess of photosynthetic reducing equivalents (see Section 3.5.5).

Respiration of stored substrates (e.g. starch and sucrose) represents 40–50% of the total substrate oxidised by mitochondria in the light [17,18] and 100% in the dark.

3.3. ATP supply in the light: chloroplasts versus mitochondria

The degree to which mitochondrial ATP supply in the light is required for optimal photosynthesis depends on the balance of ATP production and consumption in chloroplasts. It is possible that non-cyclic photosynthetic electron transport (Fig. 4), which produces ATP and NADPH in a ratio of 2.6:2 [19],

does not satisfy the requirements of CO_2 fixation and of other ATP-demanding processes in the chloroplast. Fixation of CO_2 to yield DHAP in the chloroplast requires an ATP to NADPH ratio of 3:2 or greater. Additional ATP, if required, must therefore be provided to fix CO_2 [20,21] and for other cellular processes, such as sucrose synthesis, protein synthesis, NH_4^+ assimilation, metabolite transport and maintenance. Clearly, the demand for ATP can exceed the level of ATP synthesis by non-cyclic electron transport in the chloroplast, and additional ATP must be produced by other processes.

The degree to which mitochondria provide ATP to the chloroplast depends on the contribution from cyclic [22,23] and pseudo-cyclic [22,24] phosphorylation (Fig. 4). In cyclic phosphorylation, the acceptor of PSI (Fd or NADPH) is oxidised by PQ, which serves as a donor to PSI, yielding ATP as the sole product. Experiments with pea leaves suggested that substantial cyclic phosphorylation will only occur at high irradiances in combination with very low CO_2 concentrations [25]. In pseudo-cyclic phosphorylation oxidation of the PSI acceptor produces H_2O_2 (Fig. 4), which is rapidly removed by catalase, and depends on both PSI and PSII. Although these two processes have sufficient capacity to meet the demand for extra ATP [23,24], they probably play a minor role in vivo [6]. However, little additional ATP synthesis may be needed to balance the NADPH:ATP ratio, allowing the Calvin cycle to operate.

If the chloroplast is unable to meet its ATP requirements, additional ATP must be imported from other compartments of the cell. The most likely source of additional ATP is mitochondrial phosphorylation. Mitochondria have a greater capacity for ATP synthesis than chloroplasts, producing up to 3 ATP per NAD(P)H compared to the 1.5–2 ATP per NAD(P)H in the chloroplast [26]. Indeed, mitochondrial oxidative phosphorylation maintains most of the cytosolic ATP pool [6] and is essential for maximal rates of tissue photosynthesis in some instances [27–30]. Experiments with barley leaf protoplasts showed that photosynthetic O_2 evolution was 30–40% lower when mitochondrial ATP production was inhibited by oligomycin at a concentration that did not affect the process of photosynthesis directly [30]. Subsequent rupturing of the protoplasts that left the chloroplasts intact restored the photosynthetic rate [30]. These experiments suggest that under these conditions mitochondrial ATP production was essential for optimal photosynthesis and may reflect the energy demands of sucrose synthesis, which utilises UTP [6,27] (Fig. 1).

The degree to which mitochondrial ATP production is necessary for cell function in the light is likely to vary among tissues. For example, the amount of ATP produced in non-cyclic electron transport in the chloroplasts appears to be sufficient to account for CO_2 uptake in photoautotrophic carnation cell cultures, without involving cyclic phosphorylation or mitochondrial ATP production [31]. However, mitochondria may still contribute to cellular ATP synthesis in such cells, for other energy demanding processes that occur in the light (e.g. N-assimilation). Environmental factors can also affect the need for mitochondria to supply ATP. For example, Hurry et al. [32] reported that mitochondria contribute to ATP pools in illuminated non-hardened leaves of winter rye, but not in cold-hardened leaves.

3.4. Adenylate control of respiration in the light

Adenylates can restrict respiration in various ways [33]. Firstly, in isolated mitochondria an ATP/ADP ratio higher than 20 will restrict oxidative phosphorylation [34], a ratio reported to occur in vivo [35]. Secondly, phosphorylation can be restricted if the concentration of ADP is too low (below 20–50 μM; depending on the ATP/ADP ratio [34]). About 40–50% of cellular ADP is bound to proteins and in maize root tips the concentration of free ADP was estimated to be about 50 μM [36], within the concentration range where it restricts phosphorylation. Low concentrations of free ADP (as distinct from ATP/ADP ratios) may be more important in metabolic regulation than previously recognised. Thirdly, the rate of glycolysis is regulated by the concentrations of ATP and ADP in the cytosol: an increase in the ATP concentration will decrease the activity of key enzymes of glycolysis. Small increases in the ATP/ADP ratio in the cytosol are sufficient to modify the rate of glycolysis [37]. Moreover, low ADP concentrations can restrict the rate of substrate level phosphorylation, especially at pyruvate kinase (PK, Fig. 1) [38].

Cytosolic ATP/ADP ratios in the light are similar

or lower than in darkness [39–41], suggesting that respiration is not completely inhibited by adenylates in the light. However, the lower ATP/ADP ratios in the light than in darkness [41] may actually reflect a faster turnover of ATP in the light, rather than a lower ATP level per se. It is likely therefore, that respiration is restricted by adenylates in the light *and* the dark. Despite this, respiration generally continues in the light (see Section 3.8): the degree of adenylate control is insufficient to fully inhibit respiration in the light. Plant cells also have mechanisms for avoiding adenylate control; their mitochondria have non-phosphorylating pathways, allowing respiration without ATP production (see Section 3.5.6). Similarly, glycolytic adenylate control can be avoided if PEP is converted to malate by PEP carboxylase (PEPC), bypassing PK which can be limited by low ADP [42] (Fig. 3). Thus, plant metabolism need not be as strongly controlled by adenylates as animal metabolism, giving the plant cells greater flexibility.

3.5. *Mechanisms to avoid over-reduction of the chloroplast*

Another role for mitochondria in the light may be the removal of excess photosynthetic reducing equivalents, which can lead to damage of the photosynthetic electron transport system. It is therefore essential that chloroplasts export excess reducing equivalents to be either stored (e.g. as malate) or to be oxidised by respiration.

3.5.1. *Over-reduction and photoinhibition*

When the chloroplast NADPH/NADP ratio becomes too high, photosynthetic electron transport components will become highly reduced, resulting in photoinhibition [43], which reduces photosynthetic efficiency [44] and occurs when the ability of the photosynthetic ETC to readily dissipate absorbed energy, either photochemically (e.g. fluorescence, ATP and NADPH synthesis) or non-photochemically (e.g. dissipation of energy as heat), is reduced. This results in a change or damage to the photosynthetic apparatus (mostly likely to the D_1 protein of PSII [43]). Therefore, other pathways for dissipation of energy in PSII need to exist, e.g. fluorescence, state transitions and the xanthophyll cycle.

3.5.2. *State transitions and the xanthophyll cycle*

In state transitions, light harvesting complexes (LHCs) move from one reaction centre to the reaction centre of the other photosystem (for a review see [45]). A highly reduced PQ pool induces a transition to state II, when LHCs of PSII are phosphorylated and move to PSI. A return to state I requires ATP and a highly oxidised PQ pool [46]. These transitions modulate flux through PSI and the rate of PQ oxidation, balancing the energy distribution between the two photosystems and avoiding over-reduction of the ETC components, especially of PQ. Over-reduction of quinone pools in mitochondria or chloroplasts can lead to the production of active oxygen species, which can damage the cell [47–49]. State transitions affect the degree of cyclic and non-cyclic phosphorylation and change the ratio of NADPH:ATP production.

State transitions have a limited capacity to protect photosystems against photoinhibition, because they only re-distribute the photochemical energy between the photosystems and also PSI can become photoinhibited [50]. The xanthophyll cycle, on the other hand, protects both photosystems [50], allowing LHCs to dissipate energy as heat and reducing photo-efficiency [50–52]. The heat dissipation capacity of the xanthophyll cycle only increases when the plant is exposed to high light for a long time [52].

The above systems do not provide complete protection against photoinhibition. They are also only invoked when the ETC is already highly reduced (e.g. state transitions) or when the plant has been exposed to photoinhibitory light for an extended periods (e.g. xanthophyll cycle). The xanthophyll cycle cannot dissipate all excess photochemical energy under stress conditions [50]. Further, these protective mechanisms reduce photosynthetic efficiency. It would therefore be beneficial to have other systems to deal with dissipation of excess chloroplast energy, especially for short-term transient imbalances.

3.5.3. *Avoiding over-reduction: sinks for NADPH and ATP*

Imbalances leading to over-reduction of the ETC typically occur when the supply of NADPH and ATP exceeds the demand for these metabolites. The electron flow in the chloroplast ETC can be limited by a low availability of $NADP^+$ (terminal acceptor) or ADP. Because electron transport is coupled to

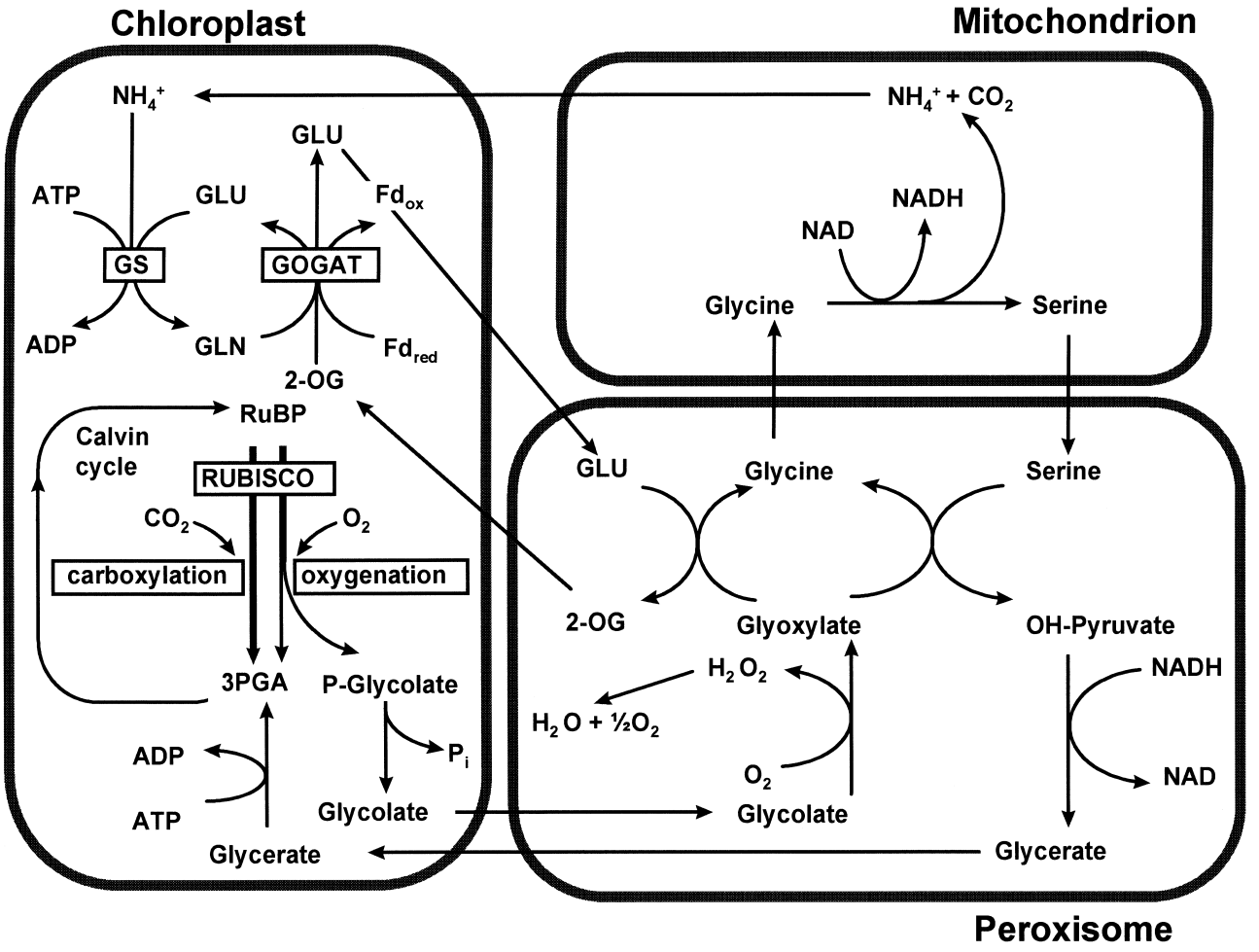

Fig. 5. Photorespiration or glycolate cycle. GOGAT, glutamate oxoglutarate transaminase; GS, glutamine synthase.

ATP synthesis it is restricted in the absence of ADP (similar to the 'state 4' of mitochondria) [53]. Therefore, regeneration of ADP is also important for unobstructed photosynthetic electron flow. While chloroplasts have mechanisms to produce ATP without NADPH, there is no photosynthetic system to produce NADPH without ATP. However, given the ratio in which NADPH and ATP are produced, NADPH is generally in excess (see Section 3.5.1). Over-reduction can be avoided if the rate of NADPH and ATP production is matched or exceeded by the potential consumption of these metabolites and/or if excess metabolites are exported from the chloroplast.

Photosynthetic CO_2 fixation and photorespiration (Fig. 5) require substantial amounts of NADPH and ATP. CO_2 and O_2 compete for binding sites on Rubisco, with 20–35% of the net photosynthetic activity occurring by the oxygenase reaction (photorespiration) under normal conditions [54,55]. In the Calvin cycle two 3-PGA are produced for each RuBP, whereas photorespiration results in the conversion of RuBP to 3-PGA and 2-P-glycolate (Fig. 5). The carbon lost to glycolate is salvaged in the photorespiratory cycle with the evolution of CO_2 and NH_3 (Fig. 5; for details see [56]). 2-P-Glycolate is converted to glycolate and exported to the peroxisome, where the glycolate is converted to glycine and then metabolised in the mitochondria as a respiratory substrate. The photorespiratory glycolate cycle provides a substantial sink for NADPH and ATP (2 NADPH and 3.5 ATP per glycolate; totalling 4 NADPH and 6.5 ATP per oxygenation if the re-fixation of lost CO_2 is included), especially under conditions when the carboxylation reaction is limited by low intercellular CO_2 concentrations (e.g. following stomatal closure).

Because CO_2 fixation is such an important sink for chloroplast NADPH and ATP, it must be active or rapidly activated whenever photons are absorbed if the chloroplast is to avoid over-reduction. In darkness, chloroplastic enzymes that use NADPH (e.g. Calvin cycle) are typically inactive. The redox state of the chloroplast increases dramatically during dark to light transitions, with Fd, Td and NADPH levels increasing as a result of photosynthetic electron flow [57,58]. This could result in a build up of excess NADPH and over-reduction of the ETC and rapid activation of the processes that use photosynthetic NADPH is therefore needed. Indeed, enzymes of the Calvin cycle (e.g. $NADP^+$-GAPDH, fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase and phosphoribulokinase (PRK)) are rapidly activated in the light, by the increased Td levels [9,57], which reduce a sulphydryl group oxidised by O_2. The light-regulated Calvin cycle enzymes are continually reduced and oxidised ensuring that their activity is tightly controlled, with overall regulation being controlled by the redox state of the chloroplast.

In addition to carbon fixation and photorespiration, another important sink for NADPH and ATP is nitrogen assimilation. NADPH exported from the chloroplast can be used for the cytosolic reduction of NO_3^- to NO_2^- by nitrate reductase (NR; Fig. 2), which is inactivated within minutes in the dark [59]. In the chloroplast, NO_2^- is converted to NH_4^+ using reduced Fd. The rate of NO_3^- assimilation is typically about 4% of CO_2 fixation and uses 10% of the reducing equivalents used for CO_2 fixation [60]. However, this value will vary substantially between species, developmental stages and environmental conditions. Limitations in NO_3^- supply, in particular, will influence the rate of nitrogen assimilation and thus the demand for reducing equivalents in illuminated leaves. Similarly, the demands for ATP associated with nitrogen assimilation will vary as a function of the rate of nitrogen assimilation: substantial amounts of ATP are needed for NH_4^+ assimilation and amino acid synthesis [61]. Clearly, nitrogen assimilation provides a major sink for chloroplast NADPH and ATP. High rates of nitrogen assimilation should, therefore, reduce the potential for over-reduction of the photosynthetic ETC.

The effects of nitrogen assimilation on photosynthesis and respiration have been studied extensively in green algae (for a review see [61]). Addition of NO_3^- or NH_4^+ to nitrogen-starved algae diverts the flow of photosynthetic electrons away from CO_2 fixation to nitrogen assimilation [62], lowering the level of reduction of the chloroplast, and reducing the activity of the CO_2 fixing enzymes (e.g. phosphoribulose kinase and G6P-dehydrogenase [63,64]). When NH_4^+ is added instead of NO_3^- (thus lowering the demand for reducing equivalents for nitrogen assimilation), PRK is not inhibited, demonstrating the strong redox regulation of this process. The slowdown of PRK upon NO_3^- addition inhibits the regeneration phase of the reductive pentose phosphate pathway and leads to an increase in RuBP and a decrease in photosynthesis. Experiments with isolated spinach chloroplasts have also demonstrated that NO_3^- reduction lowers the rate of photosynthesis, due to the diversion of reductant from CO_2 fixation to nitrogen assimilation [65]. However, photosynthesis is unlikely to be limited by the NADPH demand of nitrogen assimilation very often, as electron flow in the chloroplasts is frequently in excess of that required for CO_2 fixation and photorespiration [66,67].

3.5.4. Avoiding over-reduction: export of excess NADPH via the Mal/OAA shuttle

Over-reduction of the chloroplast can also be avoided via export of excess reducing equivalents to other cell compartments. The primary export mechanism appears to be the Mal/OAA shuttle mechanism described in Section 2.2: NADPH reduces OAA to malate (via $NADP^+$-MDH), which is exported from the chloroplast (Fig. 3). $NADP^+$-MDH is activated by high NADPH levels in the chloroplast and this activation is inhibited by O_2 and $NADP^+$ [9].

In the absence of OAA, NADPH to can also be re-oxidised in the chloroplast by the Mehler reaction, consuming O_2 (Fig. 4), which has been suggested to be an alternative Hill oxidant acting as a fail/safe system [24]. However, the Mal/OAA shuttle appears to be preferred, because H_2O_2 production stops when OAA is added to illuminated chloroplasts [68]. Experiments with spinach and sunflower leaves showed that the Mehler reaction is not sufficient to protect against photoinactivation [69].

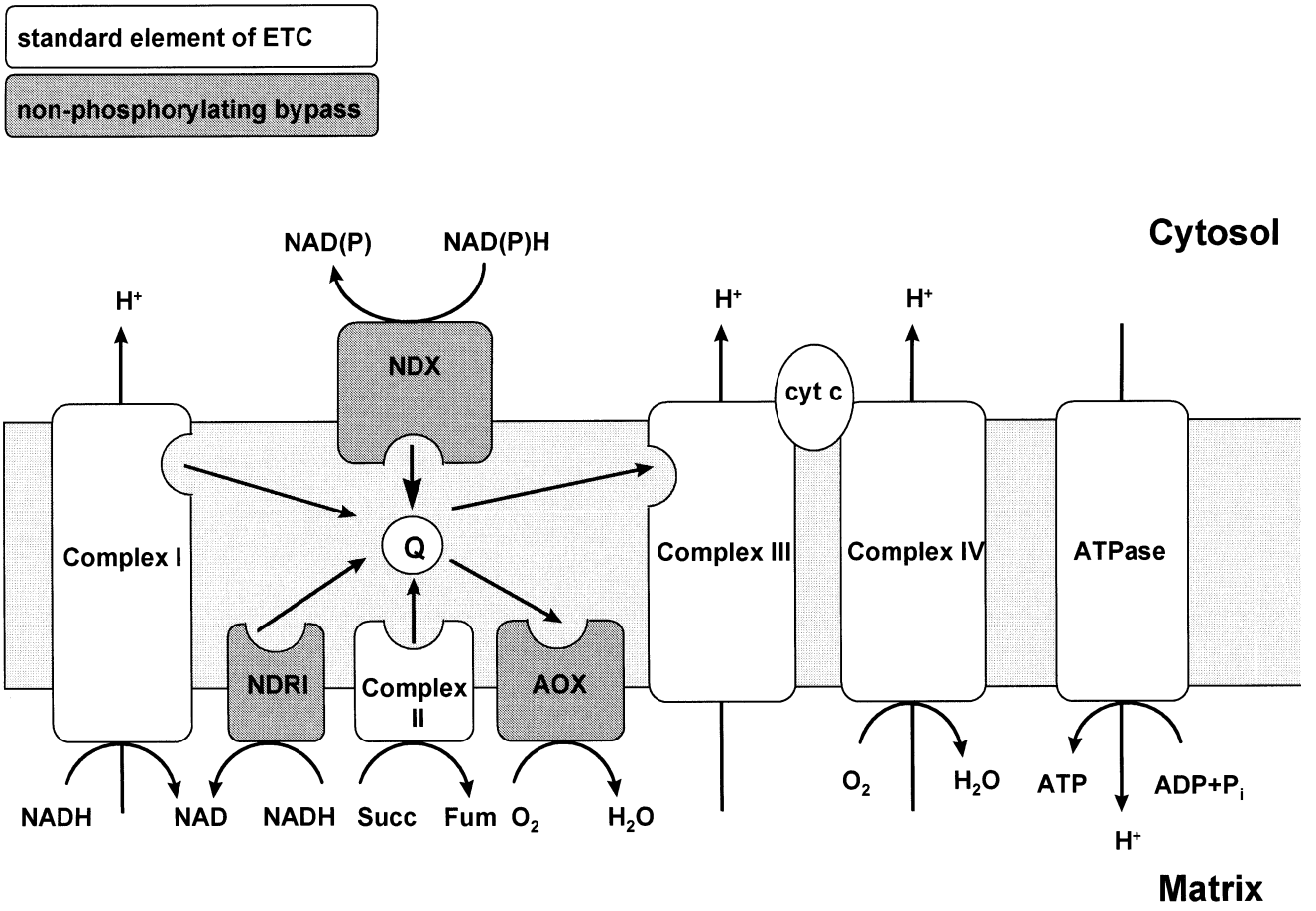

Fig. 6. Organisation of the plant mitochondrial membrane. AOX, alternative oxidase; NDRI, matrix-side rotenone insensitive NADH dehydrogenase; NDX, external NAD(P)H dehydrogenase(s); SDH, succinate dehydrogenase. The non-phosphorylating bypasses are dark-shaded.

3.5.5. *Oxidation of excess photosynthetic reductant outside the chloroplast*

For the Mal/OAA shuttle to operate as an effective NADPH export system, the exported malate must be oxidised to regenerate OAA for transport back to the chloroplast (Fig. 3). Malate can be oxidised in the cytosol, peroxisomes or the mitochondria, using the reducing equivalents for various reactions, such as NO_3^- reduction in the cytosol or reduction of hydroxypyruvate in the peroxisomes. Under conditions where more reductant is produced than is required for cytosolic and peroxisome processes, malate can be imported into the mitochondria for oxidation. Experimental evidence indicates that mitochondrial activity in the light can reduce photoinhibition and that this protection is probably related to the removal of excess photosynthetic reducing equivalents [32,70–73].

A recent study has suggested that proline synthesis may be another way of re-oxidising excess NAD(P)H in the cell [74]. Proline has long been recognised as a metabolite that accumulates during stress and is rapidly oxidised once the stress is removed. It may be that the ATP produced during its oxidation is important in the recovery from stress.

3.5.6. *Role of non-phosphorylating pathways in avoiding over-reduction*

Oxidation of reducing equivalents in the mitochondria can be coupled to the production of ATP. Under conditions where ATP demand is low, the recycling of ADP would limit the rate of oxidation. However, the existence of non-phosphorylating bypasses in the ETC of plant mitochondria allows electron flow to continue even when the demand for

ATP is limited and the ADP availability is low [75]. These include the alternative oxidase (AOX) [76], a quinol oxidase with O_2 as its acceptor that bypasses complexes III and IV in the mitochondrial ETC (Fig. 6). Plant mitochondria also have non-phosphorylating NADH dehydrogenases that bypass complex I [11] and can oxidise internal and external NADH without any ATP production and without any requirement for ADP (Fig. 6).

Taken together, the above discussion demonstrates that photosynthetic cells have a diverse range of systems to deal with excess reducing equivalents that gives them flexibility to respond to various conditions.

3.6. Environmental factors and excess NADPH

The imbalance between the production and consumption of NADPH and ATP will be accentuated under adverse environmental conditions, such as when the demand for NADPH and ATP for biosynthesis is limited (e.g. by low temperatures or nutrient limitations) or when the ability to use these metabolites for CO_2 fixation is restricted by low internal CO_2 concentrations (e.g. during drought). Under those conditions the rate of processes involved in removing excess NAD(P)H will increase.

3.6.1. Excess photosynthetic reductant: low temperatures and high irradiance

It is well known that low temperatures increase the susceptibility of plants to photoinhibition. At low temperatures (e.g. less than 10°C for plants growing in moderate climates) sucrose synthesis is severely limited. This restricts the recycling of P_i and the export of DHAP, inhibiting the Calvin cycle and the use of photosynthetic NADPH [77] (Fig. 1). Therefore, plants are much more susceptible to photoinhibition under cold conditions even at moderate light intensities. The deleterious effects of bright light and cold temperatures may, however, be ameliorated by the oxidation of excess photosynthetic reducing equivalents by the mitochondria [32,71,72]. Respiratory rates at a given temperature also increase in plants that are exposed to cold temperatures for extended periods [32,78]: the increase in respiratory capacity may represent an increased capacity to oxidise excess photosynthetic reducing equivalents. Plants also acclimate to low temperatures by increasing photosynthetic and sucrose synthesis activity [32,79], and reducing the P_i-mediated feedback inhibition of photosynthesis [77].

3.6.2. Excess photosynthetic reductant: low intercellular CO_2 and drought

Severe inhibition of photosynthesis can be expected when intercellular CO_2 concentrations (c_i) are low (as occurs when stomata close), as CO_2 fixation provides the largest sink for photosynthetic NADPH and ATP. Photoinhibition is enhanced in *Phaseolus vulgaris* leaves when c_i is reduced [80]. Increases in c_i also result in an increased rate of CO_2 fixation and a decrease in the ATP/ADP ratio in spinach leaves [81]. Low c_i values therefore reduce the demand for NADPH and ATP, and increase the potential for photoinhibition.

Although stomatal closure and low c_i values decrease CO_2 fixation rates, they do not reduce the rate of photorespiration. In fact, it is slightly increased at low c_i values [82] and with this the demand for NADPH and ATP for photorespiration is maintained or increased. Photorespiration thus helps avoid over-reduction of the ETC and long-term damage to the photosystem under conditions where CO_2 fixation is limited by low c_i values [20,43]. This is likely to be particularly important under drought conditions when stomata are closed. Various studies have shown that photorespiration increases during drought and offers protection against photoinhibition [20,80,82–84]. In *Digitalis lanata* water stress reduces net photosynthesis by 70%; however, the metabolic demand for energy decreases only 40% due to continued demand for NADPH and ATP by photorespiration and because much of the CO_2 released by mitochondrial glycine decarboxylation is reassimilated by Rubisco in the chloroplast [82]. By maintaining the demand for these metabolites, *D. lanata* is able to avoid over-reduction of the chloroplast and recover quickly from water stress [82]. Similarly, a mutant tobacco plant with a higher photorespiratory capacity (higher glutamine synthase activity) was less susceptible to photoinhibition at 25°C than wild-type plants, whereas a mutant with a lower photorespiratory capacity was more sensitive than the wild-type plants [85].

The fact that mitochondrial activity is essential for

the glycine metabolism during photorespiration may be partly why inhibition of the mitochondrial ETC results in increased photoinhibition [32,70–73]. In vivo, much of the NADH produced by glycine decarboxylation may be exported to the cytosol via the Mal/OAA shuttle and oxidised in the peroxisome. However, decarboxylation of glycine can contribute to the mitochondrial ETC if the peroxisome requirements for NADH are partly met by glycolysis or the chloroplast. The latter would be likely whenever there was an excess of NADPH in the chloroplast (e.g. when low c_i values limit CO_2 fixation rates). Krömer and Heldt [86] suggested that only 25–50% of the NADH produced from glycine oxidation in the mitochondria is exported to the peroxisomes. Therefore, 50–75% of the reducing equivalents needed to support the peroxisome requirements for NADH has to come from the chloroplasts.

In wheat leaves in vitro $NADP^+$-MDH activity increases following drought treatment [87]. Although this does not necessarily reflect actual changes in the in vivo activity, it does suggest that drought increases use of the Mal/OAA shuttle mechanism to export excess photosynthetic reducing equivalents. These reducing equivalents have to be oxidised elsewhere and in another study on wheat leaves it was found that drought induced an increase in O_2 uptake related to the oxidation of photosynthetic reductant [83].

3.6.3. Mitochondrial activity and protection against photoinhibition

There is evidence to suggest that the protective mechanisms against photoinhibition may be different at different temperatures. In the cold, the most important mechanism to prevent photoinhibition appears to be the ability to keep Q_A relatively oxidised and to avoid damage to the D_1 protein of PSII [73]. In addition to the mechanism described in Section 3.5.1, over-reduction of Q_A can also be avoided via mitochondrial oxidation of excess photosynthetic reducing equivalents [32,71,72].

At high temperatures and high irradiance, photoinhibition is less dependent on the rate of damage to the D_1 protein. Rather, photoinhibition at high temperatures is more dependent on the rate of D_1 protein repair [20,72,88]. The fact that D_1 protein repair is ATP-dependent means that mitochondrial ATP production may contribute to the prevention or minimisation of photoinhibition at high temperatures [70,71]. The D_1 protein is continually repaired and as long as repair can keep up with damage no net photoinhibition will be observed [88]. In cyanobacteria, inhibition of either dark respiration (using azide) or uncoupling of mitochondrial phosphorylation results in an increase in photoinhibition [70], suggesting that prevention of photoinhibition is dependent on mitochondrial ATP synthesis.

3.6.4. Nutrient limitations and excess photosynthetic reductant

The imbalance between the production and consumption of NADPH and ATP will be increased under nutrient limiting conditions which may restrict biosynthesis [89,90]. An excess of NADPH production can therefore occur under conditions of nutrient stress [91,92]. The fact that the demand for ATP is also low under low nutrient supply may also mean that the processes that oxidise reductant without ATP production might increase in activity (e.g. non-phosphorylating pathways of mitochondrial electron transport) as suggested by several authors [93–98]. The in vivo involvement of the non-phosphorylating mitochondrial pathways in the light under nutrient limitations or their effect on the redox state of the chloroplast in leaf tissue has not yet been confirmed. On the other hand, an increase in energy dissipation by the xanthophyll cycle under nitrogen limitation has been demonstrated [50].

3.7. Role of mitochondria in providing carbon skeletons in the light

In addition to producing ATP and oxidising excess photosynthetic reducing equivalents, mitochondria serve another important role in the light: production of carbon intermediates for biosynthesis (e.g. the production of 2-OG and/or citrate). Most researchers prior to the 1990s assumed that mitochondria exported 2-OG. However, more recent work suggests that citrate is the primary carbon skeleton exported [10]. For example, when spinach leaf mitochondria are incubated in a medium with a composition similar to the cytosol in the light, the main product of malate oxidation is citrate [10]. Citrate can be converted in the cytosol to 2-OG [6,99] (see Section 2.3;

Fig. 3) as the precursor for glutamate and glutamine formation [10].

Many processes require carbon skeletons from the mitochondria, of which nitrogen assimilation is the most important [61,100]. The different pathways by which carbon can enter and leave the mitochondria enable the mitochondria to be flexible in their supply of carbon skeletons.

3.8. Rates of O_2 uptake and CO_2 release in light versus darkness

In darkness, there are several phases to respiration including glycolysis, the TCA cycle and the oxidation of NADH and $FADH_2$. Gas exchange occurs in two of these processes: CO_2 release from decarboxylation reactions in the TCA cycle and O_2 uptake related to oxidation of NAD(P)H and $FADH_2$ in the mitochondrial ETC. Measurements of respiration (O_2 uptake or CO_2 release) in photosynthetic tissues in the dark are relatively straight forward, with the ratio of O_2 uptake to CO_2 release (respiratory quotient, RQ) typically being between 0.8 and 1.6 ([101] and refs. therein).

Measurements of respiratory gas exchange in the light are not so straightforward, because photosynthetic, photorespiratory and respiratory processes occur at the same time. Photorespiratory and non-photorespiratory reactions result in mitochondrial O_2 consumption, while O_2 is produced by photosynthesis. O_2 is also consumed in the chloroplast as a result of photorespiration and the Mehler reactions [102]. Photosynthesis and PEP carboxylase result in CO_2 uptake at the same time that CO_2 is released in the mitochondria by photorespiration and the TCA cycle, in addition to CO_2 released by the oxidative pentose phosphate pathway. If the TCA cycle is differently affected by light than is mitochondrial electron transport, the effect of light on CO_2 release will differ from that on O_2 uptake. For example, oxidation of excess photosynthetic reducing equivalents by the mitochondria may be coupled to O_2 uptake but not to CO_2 release.

Despite the problems in determining respiratory gas exchange in the light, numerous studies have used gas exchange and mass spectrometry techniques to measure respiration in the light. In all studies, respiration continued in the light. However, the degree to which it continued depended strongly on whether CO_2 release or O_2 uptake was measured. Variations in experimental conditions and plant species also contribute to the variability in the estimates of respiration in the light.

The effects of light on mitochondrial O_2 uptake are not uniform, varying from partial inhibition [103,104], no change [31,105] to a substantial increase [106]. The variability in mitochondrial O_2 consumption in the light may reflect variability in the supply of substrate to the mitochondria (e.g. glycolytic products and excess photosynthetic reducing equivalents) and the degree to which photorespiratory NADH is oxidised in the mitochondria (see Section 3.6.2). It may also reflect variability in the demand for respiratory ATP by cellular processes in the light.

The effect of light on CO_2 release is more clear. Under photorespiratory conditions, total mitochondrial CO_2 release is higher in the light than in darkness due to the combined release of CO_2 by glycine decarboxylation and non-photorespiratory processes (e.g. TCA cycle [17]). However, non-photorespiratory CO_2 release is lower in the light than in darkness in most species investigated, with the degree of inhibition by light ranging from 25 to 75% in studies using mass spectrometry [31,72,107,108] and gas exchange techniques [108–114].

3.9. Mechanisms responsible for inhibition of CO_2 release in the light

The mechanism responsible for light inhibition of non-photorespiratory CO_2 release is unresolved. However, Atkin et al. [114] recently suggested that light inhibition of respiration may be the result of the inactivation of PDC and NAD^+-ME in the light [115–119]. PDC and NAD^+-ME determine the flux of carbon into the TCA cycle [119] (Fig. 3). While the mechanism responsible for the light inhibition of NAD^+-ME is not known, the inhibition of PDC is clearly the result of phosphorylation [115,116]. The inhibition of PDC activity mainly occurs under photorespiratory conditions [117,120]. The photorespiration-dependent inhibition of PDC may be enhanced by NH_3 (produced during glycine decarboxylation) stimulating the protein kinase that phosphorylates PDC [6,115]. Increased ATP synthesis due to increased electron transport during glycine

oxidation could also contribute to PDC inactivation [118].

The apparent light inhibition of non-photorespiratory CO_2 release may also be partly the result of reduced flux through glycolysis in the light. For example, pyruvate kinase and PEPC activities are lower in the light than in darkness in the green alga *Chlamydomonas* [106]. PDC activity was 25% lower in the light than in darkness in this species.

Another factor that may be partly responsible for light inhibition of non-photorespiratory CO_2 release are enhanced rates of export of TCA cycle carbon intermediates to the cytosol to support light-dependent nitrogen assimilation [121]. TCA cycle CO_2 release could be reduced under conditions where 2-OG and/or citrate are exported from the mitochondrion to support amino acid synthesis. Removal of these would eliminate one site of TCA cycle CO_2 release. This hypothesis, which remains to be tested, is supported by the fact that the CO_2 compensation point of barley leaves increases when plants are transferred from NO_3^- to NH_4^+ nutrient [122]. NH_4^+ is not transported from roots to shoots but rather is assimilated in the roots. This eliminates leaf nitrogen assimilation, thereby decreasing the demand for TCA cycle intermediates in the leaves (and increase the rate of CO_2 release and CO_2 compensation point).

3.10. Effect of light-to-dark transitions on respiration

The fact that non-photorespiratory CO_2 release is lower in the light than in darkness suggests that light-to-dark transitions might result in a direct increase in CO_2 release until steady state dark respiration values are achieved. This is, however, rarely the case. When first exposed to darkness following a period in the light, leaves often exhibit transient increases in dark respiration before steady state values are achieved. The first transient increase (after approx. 15–20 s of darkness [114]) is the photorespiratory post-illumination burst (PIB), while the second (180–250 s [114]) has been defined as light enhanced dark respiration (LEDR [123]).

PIB occurs because of a difference in time that the RuBP and glycine pools remain in the cell following the transition to darkness. CO_2 fixation by Rubisco consumes the RuBP within 30 s [122] while the glycine pool initially remains stable (for 15–20 s) before declining. The continued decarboxylation of glycine is observed as a burst of CO_2 release.

LEDR has been reported as increased O_2 consumption [106,119,123–126] and CO_2 evolution [78,112,127]. It takes about 3–5 min for LEDR to reach its maximum rate in darkness. It appears to reflect the initially high concentration of photosynthetic metabolites immediately available to the mitochondria (e.g. pyruvate or malate) in darkness after a period of illumination [72]. LEDR also appears to be associated with reversal of light inhibition of key enzymes (e.g. pyruvate dehydrogenase complex, PDC and NAD^+-ME) that control entry of carbon into the mitochondrial TCA cycle [119]. The magnitude of LEDR is dependent on the size of the substrate pool at the end of the light period. This pool size reflects two things: firstly, the rate and duration of photosynthesis in the preceding period and, secondly, the rate of substrate consumption (e.g. by respiration) during the light period, which will be affected by the degree of light inhibition of the key enzymes of pathways that use photosynthetic products (e.g. PDC and NAD-ME). This hypothesis is supported by recent work that shows that the degree of inhibition of leaf respiration by light closely matches the magnitude of LEDR, and that LEDR and light inhibition of leaf respiration are equally sensitive to increasing irradiances in the light period [114]. Moreover, both parameters are insensitive to light quality and are tightly correlated [106,114].

4. Interactions between chloroplasts and mitochondria in the dark

Interactions between mitochondria and chloroplasts in photosynthetic cells also occur in the dark, as demonstrated by the fact that inhibition of mitochondrial activity in the dark affects the PQ redox state and the thylakoid electrochemical gradient [128,129]. In the dark, mitochondria are the main source of ATP for cell processes, including those in the chloroplasts, which, although not photosynthesising, are still metabolically active, e.g. starch that has been accumulated in the light needs to be converted to hexose-P and TP and exported to the cytosol [130]. In the dark, mitochondrial ATP, and sometimes reductant, might also be necessary to prepare

the chloroplast for optimal photosynthetic activity when the light returns, by maintaining a proton gradient across the thylakoid membrane or by poising the PQ pool [131].

4.1. Mitochondrial ATP maintains the thylakoid proton gradient

In *Chlorella* in the dark the electrochemical gradient across the thylakoid membrane can be sustained or restored by ATP (supplied by the mitochondria) through reverse operation of the ATPase [131] (Fig. 4). Similarly, in higher plants ATP hydrolysis can maintain a proton gradient across the thylakoid membrane in the dark [132]. Although chloroplast proton gradients are not maintained in the dark under favourable growth conditions, they are maintained in the dark following exposure to photoinhibitory cold, bright conditions [132]. This maintenance of a dark proton gradient may be important to allow non-radiative dissipation by the xanthophyll cycle, offering photoprotection by non-radiative dissipation upon re-illumination. The degree to which the ATPase remains active in the dark is dependent on the levels of zeaxanthin and violaxanthin in the lumen and the temperature [132]. At high temperatures the ATPase is inactivated within minutes in the dark to avoid wasteful ATP hydrolysis [132]. In contrast, the ATPase can remain active for hours in the dark at low temperatures, even overnight [132]. Although the ATP requirement for maintaining the proton gradient is low, maintenance of ATPase activity may partly explain why cold hardening of plants results in higher respiration rates at a given temperature [32].

4.2. Reduction of PQ by NAD(P)H

For chloroplasts to function in the light, it is important that PQ remains partly reduced in the dark to provide electrons for PSI upon re-illumination [133]. The reducing equivalents needed for this are supplied by the mitochondria and/or by starch degradation in the chloroplast [46,134]. Reduction of the PQ pool in the dark has been reported for both higher plants and algae [135–139] and active re-reduction of PQ is observed in the dark after oxidation by far red light [137,140]. Reduction of PQ by NAD(P)H may be mediated by a NAD(P)H-PQ oxidoreductase located in the thylakoid membrane [128]. Several lines of evidence indicate the existence of an NAD(P)H-PQ oxidoreductase in the chloroplast of both green algae [129,141–143] and higher plants [140,144]. In addition, 11 open reading frames showing great similarity with parts of complex I (a mitochondrial NADH-Q oxidoreductase) have been found in the chloroplast genome [134,145]. Isolated thylakoid membranes have also been shown to oxidise NAD(P)H in the presence of several electron acceptors, such as ferricyanide and benzoquinone [134]. Although an enzyme with demonstrated NADPH-PQ activity has not been purified or isolated thus far, a large protein complex with NAD(P)H to nitrotetrazolium blue oxidoreductase activity was isolated from barley thylakoids [146]. Also the reduction of PQ has been shown to be inhibited by rotenone, an inhibitor of complex I [142,147].

4.3. Interaction between mitochondrial activity and PQ redox state

The redox level of the chloroplast PQ pool in the dark responds strongly to mitochondrial activity. Inhibition of mitochondrial phosphorylation (via uncoupling, anaerobiosis or by inhibition of mitochondrial electron transport or ATPase activity) in the dark often results in an increase in the oxidation state of the chloroplast PQ pool [128,136,148]. For *Chlamydomonas* strong evidence was presented to suggest that the reduction of PQ was mediated by an increase in glycolysis [128,148]. Inhibition of mitochondrial phosphorylation lowers cellular ATP levels, resulting in an increase in glycolytic activity (via the Pasteur effect), leading to increased NADPH production in the chloroplast. In *Chlamydomonas*, oxidation of hexose-P to 3-PGA (the initial stage of glycolysis) occurs in the chloroplast [149]. In higher plants glycolysis occurs in the cytosol and redox equivalents are transported into the chloroplast by the Mal/OAA or DHAP/3PGA shuttles. However, the reduction of PQ in higher plants is likely to occur in a manner similar to that in *Chlamydomonas*, especially since PQ reduction also responds to lowering of intracellular ATP. PQ reduction in tobacco protoplasts was stimulated when respiration was inhib-

ited by KCN, probably increasing the rate of glycolysis by the Pasteur effect [140]. In spinach leaves it was found that PQ reduction in the dark was dependent on a reductant from the cytosol [136].

The movement of LHCs from one photosystem to the other (i.e. state transitions; Section 3.5.2) also occur in the dark [46] and respond to mitochondrial activity. For example, lowering of the chloroplast ATP concentration by inhibiting ATP mitochondrial synthesis in the dark (by uncoupling or inhibiting respiration) can result in a transition from state I to state II [46]. A decrease in the level of ATP in the cell is usually accompanied by a reduction of the ETC between the photosystems. The transition from state I to state II is regulated by the redox state of PQ [45] and is probably a consequence of the reduction of PQ. For a return to state I both oxidation of the ETC and a high ATP level are essential [46]. The state transition is suggested to prepare the chloroplast for cyclic phosphorylation [46] or to prevent over-reduction of the ETC between the photosystems when the light returns [150].

Taken together, the above studies demonstrate the strong interaction between mitochondria and chloroplasts in the dark.

4.4. Chlororespiration

4.4.1. Overall characteristics of chlororespiration

The reduction PQ in the dark may represent the first step of chlororespiration (CR [141]). The term chlororespiration was introduced for a proposed electron transport pathway consuming O_2 in the thylakoid membrane. CR is thought to represent the oxidation of NAD(P)H, involving an NAD(P)H-PQ oxido-reductase and a PQ oxidase (Fig. 4) and could explain the reduction of PQ in the dark and its increased reduction upon anaerobiosis [141]. Although considerable evidence for an NAD(P)H-PQ oxidoreductase has been found [129,140–143], evidence for a PQ oxidase is lacking [129,134,147]. In *Chlamydomonas* it was shown that O_2 uptake was related to reduction of the PQ pool [151,152]. Most evidence for CR has been found in *Chlamydomonas*, although the existence of CR in higher plants has also been suggested [135,140,147,153,154]. However, the evidence for CR in higher plant chloroplasts is limited to reduction of PQ in the dark, rather than O_2 uptake in association with CR. The existence of a PQ oxidase in higher plants has been suggested [147] but based only on PQ reduction data and using inhibitors, which can lead to ambiguous results (see Section 4.4.2).

Other components of the chloroplast ETC, such as cytochrome b_6f complex and plastocyanin (PC, Fig. 4) are not believed to be involved in chlororespiration, because CR is not sensitive to DBMIB (2-nonyl-4-hydroxyquinoline *N*-oxide) an inhibitor of electron transport between PQ and cytochrome b_6f complex. Moreover, CR still occurs in mutants of *Chlamydomonas* deficient in cytochrome b_6f complex or photosystem I [151]. On the other hand, electrons flowing from PSII to PQ can be used in CR as shown by the fact that DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea) inhibits the PSII dependent O_2 uptake in a mutant deficient in PSI [151]. So, the only components involved in CR appear to be a NAD(P)H dehydrogenase (or NAD(P)H-PQ reductase), PQ and a putative PQ-oxidase [129] (Fig. 4).

Apart from the NAD(P)H-dehydrogenase and the PQ oxidase, it has been suggested that CR activity depends on a proton gradient across the thylakoid membrane [129,141,155]. This has been used to explain the inhibition of CR by the ionophore, dicyclohexyl-18-crown-6, an uncoupler of photophosphorylation [141,155].

4.4.2. Inhibitors of chlororespiration

Experimental testing of the model of chlororespiration is not straightforward. One problem is that chlororespiration does not have an unique feature that can be measured, e.g. it shares O_2 uptake with Mehler reactions, mitochondrial respiration and photorespiration. Furthermore, a change in the PQ redox state does not necessarily reflect changes in chlororespiration activity. Also, mitochondrial and chlororespiratory enzymes are often sensitive to the same class of inhibitors and their use can lead to ambiguous results [129]. For example, although myxothiazol was thought to inhibit CR [156], CR was found to be insensitive to this inhibitor in a mutant of *Chlamydomonas* in which the mitochondrial cytochrome bc_1 complex was resistant to myxothiazol [129].

In addition to myxothiazol, various inhibitors of mitochondrial respiration such as antimycin A [156],

KCN [141,157], CO [141,147] and SHAM [141] have been suggested to inhibit CR. If correct, KCN would be expected to inhibit CR in all chloroplasts. However, while KCN inhibits CR in *Chlamydomonas*, no such inhibition is seen in *Chlorella* [141]. KCN (and other inhibitors of cytochrome oxidase) itself can induce CR via increases in the redox state of PQ [128,136,148]. The efficacy of KCN as a CR inhibitor, therefore, remains in doubt. Similar doubts exist for the other inhibitors and conclusions based on their effects must be considered with care. At the moment there is no single compound which has been shown to be an undisputed inhibitor of CR.

4.4.3. Role of chlororespiration and in vivo activity

With the model of CR still unconfirmed one can only speculate on the role of chlororespiration. CR has been suggested to be an adaptation to N-limitation in *Chlamydomonas*, because CR-dependent O_2 consumption increased under N-limitation, concomitantly NADPH-PQ oxido-reductase increased 7-fold [158]. Chlororespiration can facilitate NADPH oxidation to dissipate photosynthetic reducing equivalents and thus minimise photoinhibition or prevent the production of active oxygen species [158]. Such a role would be comparable to that suggested for the mitochondrial alternative oxidase [159]. Another role that has been suggested is the recycling of $NADP^+$ for starch degradation [129,147].

The in vivo activity of CR is also unclear. Maximum activity of CR (i.e. when PQ is completely reduced after inhibition of mitochondrial respiration) is 10–20% of total respiration [141,157]. It is possible that the small O_2 uptake by CR is the result of non-enzymatic oxidation of PQ without any in vivo significance. For experimental data to be conclusive about the activity of a PQ oxidase measurements will need to include rates of O_2 uptake, because PQ reduction levels can be affected by many factors. It seems essential that the components, and especially the oxidase, are isolated and characterised, before the model of CR can be accepted.

5. Concluding remarks

The above discussion demonstrates the interdependence of chloroplasts and mitochondria and the importance of respiration to photosynthesis. The role of mitochondria in the light can vary strongly depending on the conditions. Mitochondrial ATP production may be important for maximum photosynthesis, but an important question is whether this occurs only under conditions favourable for biosynthesis or is more general.

Under adverse conditions, such as drought, high light and/or low temperatures, mitochondria may allow the photosynthetic activity to continue without a net gain of carbon or energy for the cell. This would help a plant to avoid photoinhibition and structural damage (e.g. chlorophyll bleaching) to the photosynthetic apparatus via dissipation of light energy. High leaf respiration rates may thus be a feature of plants exposed to adverse conditions. Indeed inherently slower growing species, characteristic of harsh environments, exhibit relatively high respiration rates compared with fast-growing species characteristic of favourable sites [78]. Cold hardening of plants also increases respiratory capacity [32]. The importance of respiration under stress conditions has thus far only been based on circumstantial (albeit strong) evidence and future research should be directed to obtain more direct evidence. Especially the role of the non-phosphorylating pathways, under those conditions, needs to be established.

If citrate and not 2-OG is the main organic acid exported by the mitochondria (see Section 3.7), extra reducing equivalents (which may be needed for nitrate reduction) are produced in the cytosol. This would change our understanding of mitochondrial metabolism and emphasise the importance of cytosolic $NADP^+$-dependent isocitrate dehydrogenase. Further evidence, possibly involving transgenic plants, is required to establish this; e.g. transgenic plants without cytosolic $NADP^+$-dependent isocitrate dehydrogenase were shown to have elevated levels of citrate and isocitrate. However, they showed no phenotype and the levels of 2-OG were not lower than in wild-type plants [160].

A strong interaction between mitochondria and chloroplasts also occurs in the dark, which is demonstrated by the strong response of the reduction state of the dark-adapted PQ pool to respiratory activity. Mitochondrial ATP and reductant are necessary for chloroplast functioning in the dark and to prepare the chloroplast for optimal photosynthetic

activity upon re-illumination. This is most obvious under conditions of high light and low temperatures, where ATP is used to maintain the proton gradient across the thylakoid membrane in the dark [132]. Such a condition allows the xanthophyll cycle to offer immediate protection against photoinhibition on re-illumination. Before speculating on the in vivo importance of CR it is essential that the components and especially the PQ-oxidase are isolated and characterised. At this stage the existence and significance of CR remain elusive.

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