Beneficial interactions of mitochondrial metabolism with photosynthetic carbon assimilation

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Chloroplasts and mitochondria are traditionally considered to be autonomous organelles but they are not as independent as they were once thought to be. Mitochondrial metabolism, particularly the bioenergetic reactions of oxidative electron transport and phosphorylation, continue to be active in the light and are essential for sustaining photosynthetic carbon assimilation. The marked and mutually beneficial interaction between mitochondria and chloroplasts is intriguing. The key compartments within plant cells, including not only mitochondria and chloroplasts but also the peroxisomes and cytosol, appear to be in a delicate metabolic equilibrium. Disturbance of any of these compartments perturbs the metabolism of whole cell. Nevertheless, mitochondria appear to be the key players because they function during both photorespiration and dark respiration.

Photosynthesis and respiration are among the most important metabolic processes in the plant cell. Mitochondrial metabolism, particularly oxidative electron transport and phosphorylation, are essential for sustaining photosynthetic carbon assimilation [1–7]. The interaction between chloroplasts and mitochondria is not surprising, but it is intriguing. The concept of the dependence of photosynthesis on mitochondrial metabolism has therefore evoked considerable interest. A strong debate persisted for a long time about the nature and even occurrence of dark respiration in leaves under light, with some reports suggesting a complete suppression and others indicating continuation of respiration in light [4,8]. It is now clear that some of the reactions of tricarboxylic acid (TCA) cycle are inhibited, whereas oxidative electron transport and phosphorylation continue to be active in light and benefit chloroplast metabolism [5,7].

In spite of being the subject of frequent criticism, the use of inhibitors such as oligomycin (inhibitor of oxidative phosphorylation), antimycin A [inhibitor of cytochrome oxidase (COX)] and salicylhydroxamic acid [SHAM, an inhibitor of alternative oxidase (AOX)], demonstrated the essentiality of mitochondrial metabolism for photosynthesis (Box 1). At low concentrations, these mitochondrial inhibitors had no direct effect on photosynthesis in chloroplasts and restricted only the carbon assimilation, not the photochemical activities of protoplasts [7,9]. New insights into this exciting aspect are being provided from studies involving novel mutants [10–12].

Here, we focus on three important benefits of mitochondria to photosynthesis: (i) dissipation of excess reduced equivalents for chloroplasts; (ii) optimization of photosynthetic carbon assimilation; and (iii) protection of chloroplasts against photoinhibition. Photorespiratory reactions form an integral and essential component of such interaction between mitochondria and chloroplasts. The beneficial interaction between mitochondria and chloroplasts is strongly expressed in certain specialized cells and mutants (Box 2).

Dissipation of redox equivalents from chloroplasts

Photosynthesis in chloroplasts is composed of photochemical reactions (to produce ATP and NADPH) and carbon assimilation (which uses ATP and NADPH). There is a difference of several orders of magnitude between the generation and use of reductants through photochemical reactions and the Calvin cycle, respectively [13]. It is therefore essential to dissipate excess redox equivalents to prevent over-reduction of electron transport components and thereby to avoid damage to thylakoid membranes [14,15].

There are four major possibilities for export of NADPH (and consumption of ATP in some cases) and dissipation of excess redox equivalents from chloroplasts: (i) export of glycolate and import of glycerate involving photorespiratory reactions; (ii) export of malate and operation of the 'malate valve'; (iii) import of oxoglutarate to form glutamate; and (iv) export to the cytosol of triose phosphate and its conversion to sucrose. Mitochondria are involved in all these processes (Figure 1).

Pyruvate, glycine and malate are all substrates for mitochondrial respiration in plant cells [6]. The relative importance of each substrate varies according to the environmental conditions, reflecting the unique flexibility of plant mitochondrial respiration. Nevertheless, in green tissues, pyruvate and glycine appear to be the major substrates for mitochondria. The role of malate as the major substrate for mitochondrial respiration is questionable because most of the experiments on mitochondrial oxidation of malate have been done *in vitro*. Nuclear magnetic resonance (NMR) studies have indicated that

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Box 1. Cytochrome oxidase and alternative oxidase pathways of mitochondrial electron transport

The oxidative electron transport in mitochondria of higher plants uses two different pathways (Figure I) – the cyanide-sensitive cytochrome oxidase (COX) pathway and the cyanide-insensitive alternative oxidase (AOX) pathway [23,74,75]. The COX pathway accomplishes most of the ATP production in plant mitochondria, whereas energy tends to be dissipated as heat through the AOX pathway [76]. The operation of the AOX pathway is dynamic and depends on the environmental conditions [74]. AOX plays an important role in integrating the processes of carbon metabolism and mitochondrial electron transport, particularly when there is an accumulation of reduced equivalents and pyruvate (e.g. under phosphate deficiency) [23]. However, the physiological function of the AOX pathway in green tissues is not clear, although it is known to increase under stress conditions [23,74].

Experiments with inhibitors suggested that the mitochondrial pathway through COX and AOX is essential for photosynthesis [9,31]. The AOX pathway can play an important role in protecting chloroplasts against photoinhibition, by dissipating the excess redox equivalents from chloroplasts. Interestingly, the extent and engagement of AOX seems to increase when the cytosol and mitochondria are over-reduced, as happens in the light [44]. Furthermore, several pieces of evidence suggest the pronounced operation of the AOX pathway in light in green tissues: (i) increased electron flow through the AOX pathway during glycine oxidation [10,42]; (ii) synthesis of AOX protein during greening [77]; (iii) increased electron flux through the AOX pathway in light and its decrease on transition to dark [78]. Most of the experiments that suggest the importance of AOX in maintaining the redox state and optimizing photosynthesis have been conducted using salicylhydroxamic acid, whose reliability is frequently questioned [6]. However, experiments with a stable oxygen isotope revealed that ~60% of mitochondrial respiration in light occurred through AOX [78]. The concept of the participation of the AOX pathway in modulating photosynthesis or photoinhibition needs to be re-examined using other approaches, such as mutants or transgenics with altered AOX protein levels [23] and factors that modulate AOX, such as temperature [46].

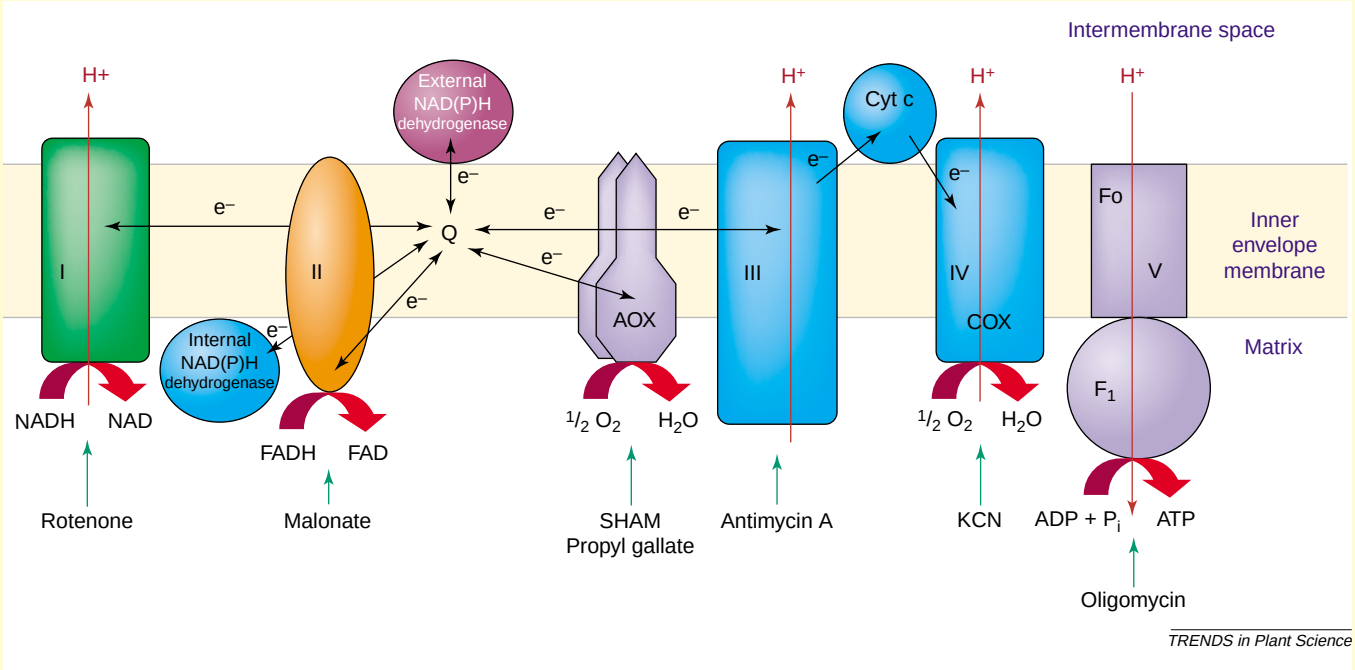

Figure I. Mitochondrial oxidative electron transport and its typical inhibitors. Abbreviations: AOX, alternative oxidase; COX, cytochrome oxidase; Cyt c, cytochrome *c*; SHAM, salicylhydroxamic acid.

very little malate is metabolized through malic enzyme in maize root tips under hypoxia [16]. Further experiments are therefore needed to establish the role of malate as the substrate for mitochondrial respiration *in vivo*. However, when photorespiration is restricted because of either metabolic control or mutations, malate oxidation increases owing to enhanced operation of the malate valve [6,10,17]. The malate valve in chloroplasts is an efficient system to dissipate the excess redox equivalents from chloroplasts [18,19].

Mitochondria operate a modified TCA cycle in the light and export isocitrate to the cytosol, which is converted to oxoglutarate and sent to chloroplasts [5,7]. The reduction of oxoglutarate to glutamate facilitates the consumption of not only ammonia but also the reduced ferredoxin within the chloroplasts. The function of mitochondrial TCA cycle to supply carbon skeletons to chloroplasts for ammonium assimilation in light is important to prevent overexcitation of chloroplasts, and this phenomenon is pronounced in algal cells [20,21]. Thus, mitochondrial oxidative metabolism can dissipate the redox equivalents from chloroplasts during both carbon and ammonia assimilation.

The NADH oxidation in mitochondria is not always linked to ATP production. The mitochondria use a non-phosphorylating AOX pathway in addition to the conventional phosphorylating COX pathway [22,23]. The AOX in mitochondria is an important component for dissipating the redox equivalents from chloroplasts (Box 1). Plant mitochondria also have a rotenone-insensitive bypass to complex I mediated by external/internal NAD(P)H dehydrogenases [24]. The light enhanced expression of these dehydrogenase proteins and the high rates of NADH oxidation [25] suggest a significant role for this pathway in leaves. Further experiments are needed to characterize the nature and the role of the rotenone-insensitive pathway in mitochondrial as well as in chloroplast metabolism.

Box 2. Pronounced interactions in mutants and specialized cells

The interaction between mitochondria and chloroplasts becomes pronounced in cells that are deficient in any component of either mitochondrial respiration or photosynthesis. The tobacco *CMSII* mutant is deficient in functional complex I and has normal photosynthetic capacity, but shows great perturbation of photosynthesis at atmospheric levels of CO_2 owing to the decreased contribution of mitochondrial electron transport to glycine oxidation. Mitochondrial complex I is therefore required for optimal photosynthesis as well as dissipation of redox equivalents through malate and glycine metabolism [11,65]. Transgenic plants of tobacco with altered levels of alternative oxidase (AOX) protein [23] could be interesting systems in which to study the role of AOX during photosynthesis and/or related processes.

Stomatal guard cells are unique, having high respiratory activity and normal photochemical activities but the Calvin cycle has decreased capacity. However, the guard cell chloroplasts export reduced equivalents efficiently. A strong interaction has been reported between photosynthesis and respiration in guard cell protoplasts of *Vicia faba* and *Brassica napus* [79]. Bundle sheath cells of NAD–malic enzyme and phosphoenolpyruvate carboxykinase of type C_4 plants are other examples of the extreme dependence of photosynthesis on mitochondria [6,70]. In a starchless mutant NS458 of *Nicotiana tabacum*, a mitochondrial supply of ATP was needed for carbon partitioning into sucrose and thereby feed-forward regulation of photosynthesis [80]. The FUD50Su mutant of *Chlamydomonas reinhardtii* lacks the β-unit of chloroplast ATP synthetase but can grow under phototrophic conditions. The high sensitivity of photosynthesis in this mutant to antimycin A suggests that mitochondria supplement the ATP needs of chloroplasts [81]. Studies of transgenics with altered levels of NADP–malate dehydrogenase [68] would be rewarding to understand further the role of the malate valve in the interorganelle interaction between chloroplasts and mitochondria, and also the interaction between the cytosol and peroxisomes.

Similarly, the role of cytosolic glycolysis in plant cells cannot be ignored even in the light and ATP produced in some of these reactions is an important source of energy for cellular metabolism. Triose-P continues to be metabolized to phosphoenolpyruvate (PEP) and pyruvate. PEP carboxylase and pyruvate kinase are also crucial for the production of carbon skeletons needed by glutamine synthetase and glutamine-2-oxoglutarate aminotransferase in chloroplasts [1,6,26].

Optimization of photosynthetic carbon assimilation

The optimization of photosynthetic carbon assimilation requires the coordination of different components, such as the generation and use of assimilatory power (ATP and NADPH), the induction of photosynthesis, the activation of enzymes and the maintenance of metabolite levels. In a photosynthesizing cell, the mitochondrial respiratory system can benefit different components of chloroplast photosynthesis by modulating any of the above components. Mitochondrial oxidative metabolism through both COX and AOX is essential for the maintenance of photosynthetic carbon assimilation [7,9] (Box 1).

The export of triose phosphate and its conversion to sucrose in the cytosol can facilitate high rates of carbon assimilation. Mitochondria have a higher capacity for ATP synthesis than chloroplasts do [3,27]. ATP generated in the mitochondrial matrix can be translocated to the cytosol (through the adenylate translocator) to be used in sucrose synthesis or even imported into chloroplasts for various biosynthetic processes [3,28,29]. However, the direct import of ATP by chloroplasts is yet to be demonstrated in higher plants.

The photosynthetic induction period is prolonged when the respiration is restricted in mesophyll protoplasts of pea or barley [30,31]. AOX of mitochondrial electron transport is important during the activation of key chloroplastic enzymes, as suggested by the marked decrease in light activation of NADP–malate dehydrogenase, NADP–glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase in presence of SHAM [30,32]. However, it is not clear whether this correlation is incidental or consequential. Further evidence is needed to conclude that restricted mitochondrial metabolism is the cause for either prolonging photosynthetic induction or decreasing enzyme activation.

The optimization of photosynthesis is further tuned by the associated reactions of nitrogen metabolism. Chloroplasts, mitochondria and cytosol have to work together to keep up the reduction of nitrite and the reductive amination of oxoglutarate [4,6]. Mitochondrial metabolism becomes an important link between photosynthesis, photorespiration and nitrogen assimilation, so as to maintain recycling not only of carbon skeletons and reduced equivalents but also of ammonia and amino acids. Furthermore, the mitochondrial ATP is an important source of energy for several processes in the cell (e.g. ion uptake) and this would modulate the metabolic state of the cell, including photosynthesis and respiration.

Protection against photoinhibition

Under conditions of supraoptimal light or suboptimal CO_2, photoinhibition of photosynthesis occurs owing to overexcitation of chloroplast membranes, generation of excess active oxygen species (AOS) and damage of photosystem II (PSII) and sometimes photosystem I (PSI) [33,34]. Mitochondrial respiration has been shown to protect photosynthesis against photoinhibition in pea mesophyll protoplasts and algal cells of *Anacystis nidulans* and *Chlamydomonas reinhardtii* [35–37]. As well as being able to dissipate redox equivalents exported out of chloroplasts, mitochondria could also help to sustain the repair and recovery of PSII by providing the ATP required for the protein biosynthesis [7,37]. Photorespiration is an important protective mechanism to prevent photoinhibition [10,38]. Being the hosts of glycine oxidation, ATP production and ammonia recycling, mitochondria play a crucial role in protecting chloroplasts through photorespiration.

Photoinhibition is a consequence of the build-up of excess redox equivalents in chloroplasts under various conditions – suboptimal CO_2 or O_2, excess light levels, or stress conditions leading to any of these [15]. Plants have different outlets to release the pressure of excess redox equivalents of chloroplasts and to minimize the damage caused by photoinhibition, including thermal dissipation capacity, the xanthophyll cycle, adjustment of chlorophyll antennae size, the water–water cycle, PSI cyclic electron transport and rapid turnover of the D1 protein of PSII [14,33,39]. Mitochondrial oxidative metabolism and

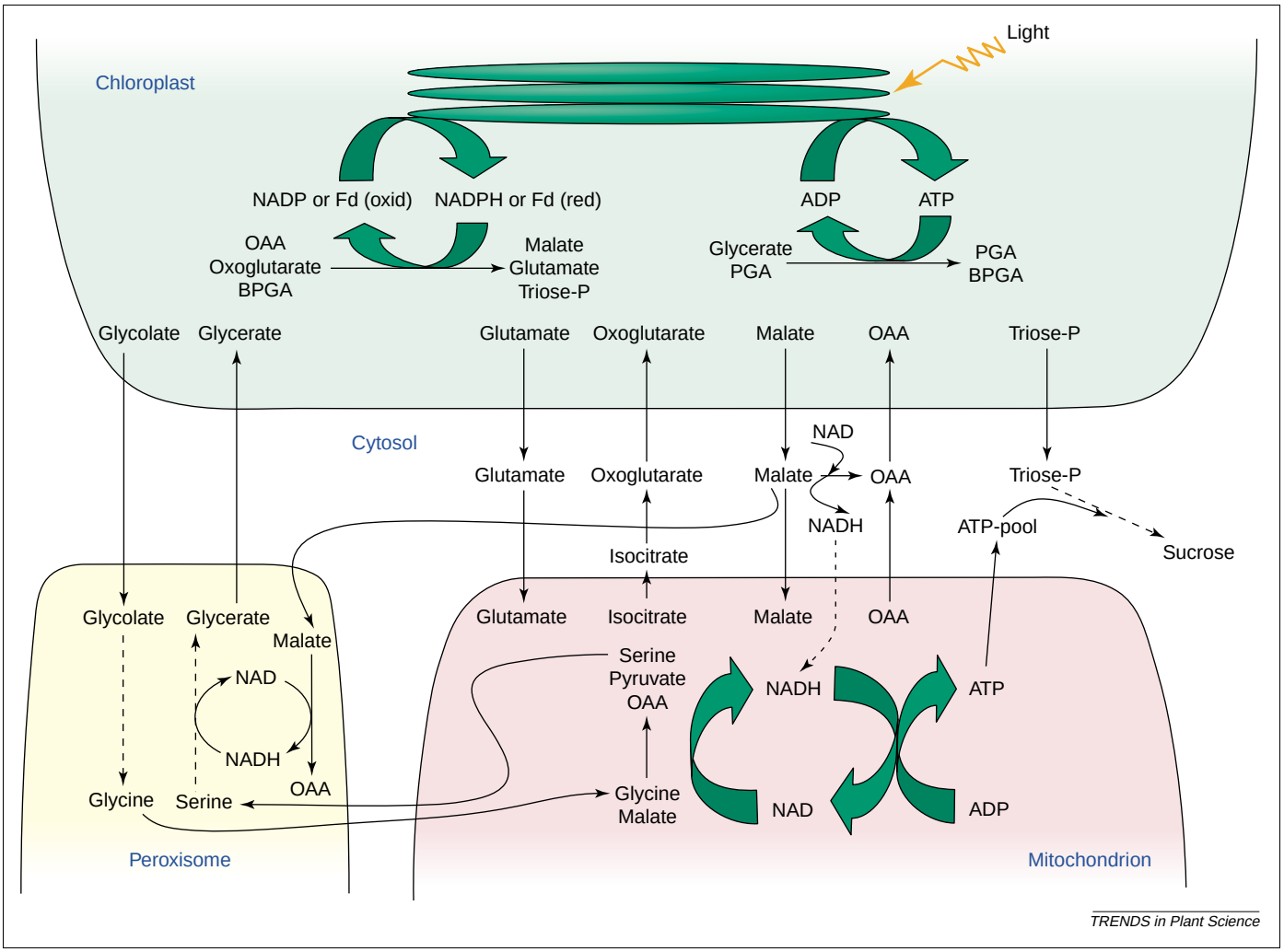

Figure 1. Use of reduced equivalents [NADPH or ferredoxin (Fd)] from chloroplasts. Mitochondria, cytosol and peroxisomes are common sinks for reduced equivalents. The dissipation of excess redox equivalents from chloroplasts is ensured by the export of glycolate, malate, glutamate and triose phosphate (triose-P). The arrows indicate the metabolite movement. The recycling of these four compounds involves mitochondria. Furthermore, mitochondrial ATP synthesis helps to sustain the formation of sucrose, an important end product of carbon assimilation. Abbreviations: BPGA, 1,3-bisphosphoglycerate; Fd(oxid), oxidized Fd; Fd(red), reduced Fd; OAA, oxaloacetate; PGA, 3-phosphoglycerate. The electron transport system of mitochondria is indicated in Box 1.

photorespiration offer additional, effective protection to chloroplasts against photoinhibitory conditions (Figure 2). Such protection by mitochondrial metabolism against photoinhibition was pronounced when the plants were under osmotic or chilling stress [40].

Photorespiratory metabolism: an indispensable link

The beneficial interaction of mitochondria with chloroplasts is observed under conditions of both limiting and optimal CO_2 concentrations [1,41]. Under limiting CO_2 conditions, the mitochondrial oxidation of glycine ensures not only the dissipation of excess redox equivalents from chloroplasts but also the generation of significant amounts of glycerate for re-entry to the Calvin cycle [6]. The importance of mitochondria to prevent overexcitation of chloroplasts in limiting CO_2 intensifies under high light. At optimal CO_2 concentrations, mitochondria help to sustain the biosynthesis of sucrose [1,7]. The enhanced ATP requirement is met from glycine decarboxylation and NADH oxidation, and other non-photorespiratory reactions, in mitochondria. Thus, mitochondrial photorespiratory metabolism provides strong support for chloroplast photosynthesis at both limiting and optimal CO_2 concentrations.

Photorespiratory reactions facilitate an efficient outlet for dissipating excess redox equivalents from chloroplasts. Furthermore, photorespiratory reactions of mitochondria produce large amounts of ATP through glycine oxidation and maintain a high degree of recycling of carbon, nitrogen and phosphorus [42–44]. Naturally, peroxisomes and cytosol form an essential link with chloroplasts and mitochondria to constitute an extremely effective multiorganelle interaction [6,7,43]. Photorespiration can also provide a significant source of internal CO_2, particularly under limiting CO_2 conditions, but a direct assessment of this phenomenon has yet to be made.

Modulation by chloroplasts of mitochondrial respiration

The interaction between chloroplasts and mitochondria is mutual. However, studies of the effects of chloroplast metabolism on mitochondrial respiration in plant cells are limited. On illumination, the pyruvate dehydrogenase complex is inactivated to a significant extent and the TCA cycle is modified to function with partial reactions.

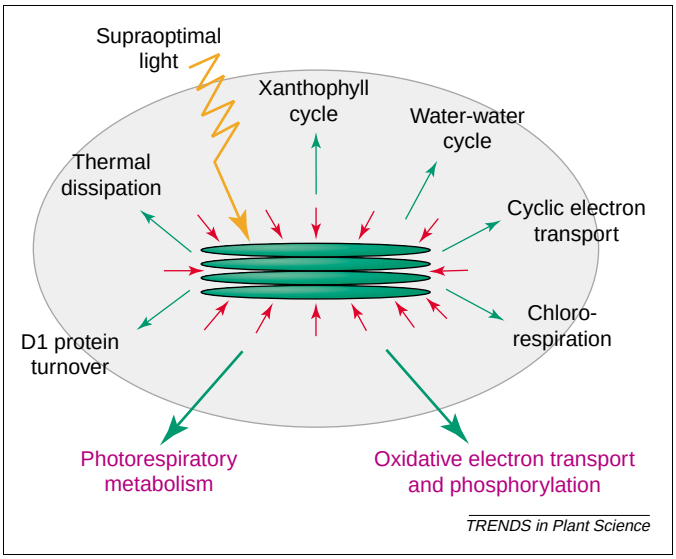

Figure 2. Protection of chloroplast thylakoid membranes against photoinhibition by different processes. The chloroplasts are vulnerable to photoinhibition under supraoptimal light levels or suboptimal CO_2 concentrations. The red arrows indicate the stress on chloroplast membranes. There are several mechanisms within the chloroplasts that protect them from overexcitation (green arrows): thermal dissipation, scavenging active oxygen species, cycling of electrons and repair of damaged proteins. Marked protection against photoinhibition is provided by photorespiration and oxidative electron transport, both of which are mediated by mitochondria. Thus, mitochondria play a significant role in protecting chloroplasts against photoinhibition.

However, the mitochondrial electron transport functions normally and is even stimulated, as indicated by an increase in the respiratory O_2 consumption. Such light-enhanced dark respiration (LEDR) has been demonstrated in several plant systems, including leaves, cell suspension, protoplasts and algal cells [6,7,45–47]. Malate appears to be the substrate during LEDR [7]. The high sensitivity of LEDR to SHAM suggests that AOX plays an important role during LEDR, as shown in barley mesophyll protoplasts and several algal species [6].

Photosynthetic carbon assimilation promoted LEDR, as indicated by the stimulation in the presence of bicarbonate. Restriction of photosynthesis by DL-glyceraldehyde or DCMU (3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea) decreased not only LEDR but also mitochondrial dark respiration [45]. Such downregulation of mitochondrial respiration consequent to restriction on photosynthesis is highly interesting and needs to be studied further. On illumination, the redox state of cytoplasm increases, as reflected by the marked increase in the ratios of malate/oxaloacetate (OAA) or triose-phosphate/3-phosphoglycerate (PGA) [48,49]. The elevated redox status within the cell could in turn affect processes in mitochondria, for example stimulating AOX activity and scavenging AOS [6]. Further experiments are needed to analyse and identify the influence of photosynthetic carbon assimilation on different mitochondrial reactions.

Biochemical signals between organelles

The rapid exchange of metabolites between chloroplasts, cytosol, peroxisomes and mitochondria is well known and forms an important channel of communication (Figure 3). The metabolite exchange is mediated by the translocators, located on the inner envelope membranes of mitochondria and chloroplasts. The most important are glycolate–glycerate, phosphate translocators on chloroplasts and adenylate, pyruvate, OAA, dicarboxylate and glycine–serine (putative) translocators on mitochondria [6,7,50]. The balance between compounds such as triose-phosphate/PGA or malate/OAA reflects the redox status of the compartment and plant cell, and can modulate respiration as well as photosynthesis.

There are other possible signals between mitochondria, chloroplasts, peroxisomes and cytosol, including ascorbate, nitric oxide (NO) and the cytosolic pH (Figure 3). Mitochondria can produce NO [51] and AOS [52], both of which can mediate signal transduction pathways. NO is a powerful modulator of both nitrogen metabolism and mitochondrial respiration [53]. The recent demonstration [54] that the NO-synthesizing enzyme is a variant form of the P protein of glycine decarboxylase suggests a direct and active involvement of mitochondria in NO signalling.

Under high redox conditions, AOX in plant mitochondria can help to decrease the levels of AOS [55]. In *CMSII* mutants of tobacco, which lack a functional mitochondrial complex I, whole cell redox balance was maintained by effective antioxidant cross talk and acclimation between the mitochondria and other organelles [12]. The production and scavenging of AOS can send signals to other compartments of the plant cell to modulate their metabolism.

Plant mitochondria are the major site for the biosynthesis of ascorbate, an important antioxidant and a key component of the AOS scavenging enzyme system in chloroplasts [56,57]. Further experiments are necessary to establish the role of AOS or ascorbate during the interaction of mitochondria and chloroplasts. On illumination, the cytosol of mesophyll cells in the leaves of both C_3 and C_4 plants is alkalinized [58,59] by the activity of chloroplasts. The change in cytosolic pH could be an important factor modulating the metabolism not only in the cytosol and chloroplasts but also in mitochondria. The role of cytosolic pH during the cross talk between chloroplasts and mitochondria needs to be studied further.

Perspectives

Several studies on the modulation of photosynthesis and respiration have been made using light as one of the regulatory tools [7,35]. The effects of mitochondria on chloroplasts need to be studied by modulating other factors such as temperature and osmotic or mineral stress [5,40,60]. For example, cadmium or iron exerted interesting effects on respiration and photosynthesis [61,62]. Phosphate is a strong regulator of metabolism not only during photosynthesis in chloroplasts [63] but also during respiratory reactions, particularly glycolysis [26]. The interaction between mitochondria and chloroplasts after varying the nutritional status should therefore be interesting.

The interaction between mitochondria and chloroplasts increases in tissues deficient in one or other components of metabolism (Box 2). Mutants and transgenics are now available that are either deficient or sufficient in key components of chloroplasts and mitochondria. For example, there are mutants of tobacco deficient in mitochondrial complex I [64,65] and of barley deficient in glycine decarboxylase or glutamine

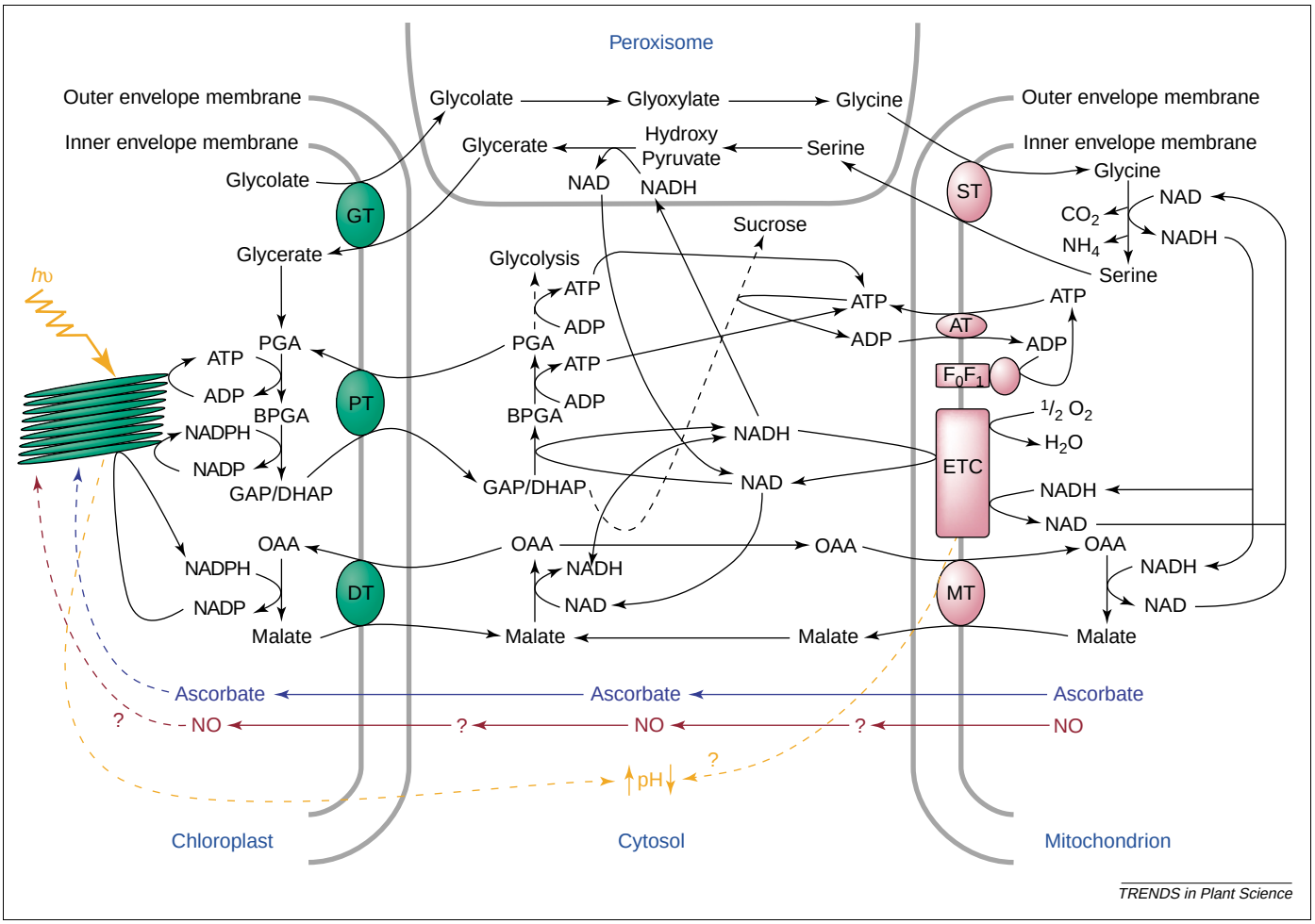

Figure 3. Biochemical cross talk between chloroplasts, mitochondria, cytosol and peroxisomes mediated by metabolites or other signals. Metabolite movement (indicated by arrows) is facilitated by the translocators (on the inner envelope membranes of chloroplasts and mitochondria) or porins (of peroxisomes). Such metabolite flux ensures the maintenance of the redox state and allows an efficient recycling of carbon and nitrogen across cellular compartments. The mitochondria are the most active organelles because they oxidize glycine, malate and even NADH through their electron transport chain (ETC) and synthesize ATP by the help of ATPase complex (F_0F_1). Only mitochondria can export ATP to cytosol using the adenylate translocator (AT). The photorespiratory reactions, which help chloroplasts to dissipate energy and NADPH, are also backed up by mitochondria. The translocators shown on chloroplast membrane are the dicarboxylate translocator (DT), glycolate–glycerate translocator (GT) and phosphate translocator (PT), whereas those on the mitochondrial membrane are the malate translocator (MT) and a putative glycine–serine translocator (ST). The other possible signals between the organelles include ascorbate, nitric oxide (NO) and cytosolic pH, but these phenomena are yet to be studied in detail. Abbreviations: BPGA, 1,3-bisphosphoglycerate; DHAP, dihydroxyacetone-3-phosphate; GAP, glyceraldehyde-3-phosphate; OAA, oxaloacetate; PGA, 3-phosphoglycerate.

synthetase/glutamine 2-oxoglutarate aminotransferase [10,17,66,67]. Transgenic plants have been produced with altered levels of key proteins such as NADP malate dehydrogenase [68] or mitochondrial AOX [69]. These plants offer a promising system to study the interaction of mitochondria and chloroplasts.

The beneficial effect of mitochondria on photosynthesis extends beyond the immediate implication of helping to dissipate excess redox equivalents from chloroplasts. The CO_2 produced during photorespiratory reactions of mitochondria can be a significant, crucial source of CO_2 for chloroplasts, particularly under limiting CO_2 levels (such as that of the atmosphere). Mitochondrial respiration and CO_2 recycling are important in CAM plants and C_4 plants [70,71]. Further studies are needed to assess the recycling of photorespiratory CO_2 in mesophyll cells of C_3 plants and the role of mitochondria in such phenomena.

Novel experimental techniques need to be used to characterize the interorganelle interaction *in vitro* as well as *in vivo*. Peroxisomes and mitochondria from spinach leaves were isolated, reconstituted and their interaction during photorespiration has been demonstrated [72]. A reconstituted system such as this can be an interesting approach to study the interaction between chloroplasts and other organelles *in vitro*. The powerful combination of mass spectrometry, NMR and stable isotopes of carbon and oxygen has proved to be a versatile way to analyse the metabolic fluxes *in vivo* [73]. These techniques can provide further insight into the multiorganelle interaction within the plant cell. Similarly, emerging concepts in plant biochemistry (such as metabolons, metabolomics and proteomics) would be needed to understand the complex mechanism of mitochondrial influence on chloroplasts.

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