Review Letter

Effect of photosynthesis on dark mitochondrial respiration in green cells

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Green plant cells can generate ATP in both chloroplasts and mitochondria. Hence the effect of photosynthesis on dark mitochondrial respiration can be considered at a variety of levels. Turnover of citric acid cycle dehydrogenases, which is essential for supply of carbon skeletons for amino acid synthesis, seems to be largely unaffected during photosynthesis. The source of carbon for the anaplerotic function of the citric acid cycle in light is however, not known with certainty. NADH generated in these reactions is probably not oxidised via the mitochondrial electron transfer chain coupled to ATP synthesis. However, it may be oxidised by the alternative cyanide-insensitive pathway, exported to the cytosol via the oxaloacetate–malate dicarboxylate shuttle or directly utilised for cytosolic nitrate reduction. Oxidation of succinate via cytochrome oxidase may also be similarly inhibited in light. Whether increase in the cytosolic ATP/ADP ratio in light is responsible for the inhibition of mitochondrial electron transfer to O_2 is not clearly established, because the ATP/ADP ratio is reported to be already quite high in the dark. Effective collaboration between photophosphorylation and oxidative phosphorylation in order to maintain the cytosolic energy charge at a preset high level is discussed.

Photosynthesis *Photophosphorylation* *Cytosolic ATP level* *Mitochondrial respiration* *Carbon source* *Citric acid cycle* *Green cell*

1. INTRODUCTION

Since mitochondrial oxidative phosphorylation is a major source of ATP in heterotrophic organisms, considerable work has been done on the regulation of this process by a variety of factors including ATP, ADP, P_i, extramitochondrial phosphate potential or adenylate energy charge. In photosynthetic cells ATP is also derived from photophosphorylation reactions and hence the problem regarding the effect of photosynthesis on mitochondrial respiration is being debated in the scientific literature. Citric acid cycle dehydrogenases in mitochondria, in addition to their role in ATP synthesis, also generate carbon skeletons in the form of 2-oxo acids for the synthesis of amino acids. The study of mitochondrial respiration in green plant cells is complicated by photorespiratory reactions, which also involve uptake of O_2 and release of CO_2. These latter reactions involve formation of phosphoglycolate in the chloroplasts by the oxygenase reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase, subsequent oxidation of glycolate in the peroxisomes to generate glyoxylate, which is transaminated to form glycine and finally mitochondrial oxidative decarboxylation of 2 molecules of glycine to generate serine, NADH, CO_2 and NH_3. Although most photorespiratory reactions are not coupled to ATP synthesis, mitochondrial oxidation of NADH generated during glycine oxidation is known to generate 3 molecules of ATP in the dark. Whether in

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the light NADH can be oxidised via the mitochondrial cytochrome oxidase pathway is not established. The complexity of dark respiration in photosynthetic cells is further increased by the Mehler reaction in which reduced ferredoxin is oxidised by O_2 to form H_2O_2 in the chloroplasts. In this brief review recent work on the relationship between photosynthesis and mitochondrial dark respiration is examined.

2. EFFECT OF LIGHT ON DARK RESPIRATION

Earlier work up to 1980 has been comprehensively reviewed by Graham [1]. He tends to conclude that while citric acid cycle dehydrogenases do turn over in light at almost the same rate as in the dark, mitochondrial oxidation of NADH via the electron transfer chain is largely inhibited. According to evidence presented by him, when leaves are exposed to light, the oxidative pentose phosphate pathway does not function in the chloroplasts. It seems unlikely that both oxidative and reductive (Calvin cycle) pentose phosphate pathways can function simultaneously in light. The key enzyme glucose-6-phosphate dehydrogenase is known to be inhibited in light by reduced thioredoxin [2–5]. As regards the effect of light on mitochondrial electron transfer reactions, earlier work reviewed by Heber [6] suggested that the light-dependent increase in the cytosolic adenylate energy charge, which is transmitted to mitochondria, inhibits electron transfer for NADH to O_2. Inhibition of mitochondrial respiration by ATP has also been observed in bean leaves [7]. Similarly it was shown in moss spores that the cytochrome oxidase pathway does not function under high light intensities as it is under the control of the ATP/ADP ratio [8]. These reports thus show that oxidation of NADH via the cytochrome oxidase pathway is inhibited in light. Since succinate and NADH share the same electron transfer pathway at the cytochrome oxidase level, it is likely that oxidation of succinate may also be similarly inhibited [9–11]. Recently, Foreman and Wilson [12] showed that in rat liver mitochondria reduction of molecular oxygen by cytochrome *c* oxidase is the only irreversible step in oxidative phosphorylation and hence may be the primary site of respiratory control.

3. TRANSPORT OF ATP FROM MITOCHONDRIA AND CHLOROPLASTS TO THE CYTOSOL

ATP generated in plant cell organelles is transported to the cytoplasm, where energy consuming reactions such as protein and sucrose synthesis etc. are located. Important differences have been observed between the mechanism of transport of ATP to the cytosol from mitochondria and chloroplasts. As in mitochondria from animal cells, transport of ATP across the membranes of plant mitochondria occurs via an adenylate translocator, which brings about a counter exchange of ATP and ADP [13]. Activity of the adenylate translocator in the chloroplast is however, extremely low, although Jordan and Givan [14] reported that ATP derived from cyclic photophosphorylation may be transported to the cytosol directly. It is known from the work of Giersch et al. [15] that this is not a major source of ATP export from the chloroplast in plant cells. Heber [6] and Giersch et al. [15] have shown that ATP derived from photophosphorylation is largely transported via the dihydroxyacetone phosphate–3-phosphoglyceric acid (DHAP/3-PGA) shuttle. Triose phosphates subsequently generate ATP in the cytoplasm via the glycolytic 3-phosphoglyceraldehyde dehydrogenase. These observations apply mainly to the envelope of spinach chloroplasts, and not necessarily to other species. For example, in pea chloroplasts it has been shown that the envelope is permeable to exogenous nucleotides [16]. Maximum rates of CO_2 fixation were obtained by uptake of ATP from the external medium. However, substantial direct export of ATP from the chloroplast to the cytosol without the participation of the DHAP/3-PGA shuttle has not yet been demonstrated. Probably, ATP generated within the chloroplast is primarily used for essential reactions of CO_2 assimilation, which are likely to suffer if the ATP supply is depleted by export.

4. EFFECT OF PHOTOPHOSPHORYLATION ON THE MITOCHONDRIAL ELECTRON TRANSFER CHAIN

Heber et al. [17] have shown that during photosynthesis the cytosolic ratio of

DHAP/3-PGA increases considerably resulting in simultaneous enhancement of the cytosolic phosphorylation potential (ATP/ADP × P_i). They have proposed that this increase is communicated to the mitochondrial adenylate system which inhibits dark respiratory activity giving rise to the Kok effect. According to Heber et al. [17] the extrachloroplast ATP/ADP ratio has been observed to increase considerably in light. Since respiration is controlled by the phosphorylation potential or ATP/ADP ratio, any light-dependent increase in the latter would be expected to decrease the rate of mitochondrial respiration. However, Dry and Wiskich [18] found that control of respiration in isolated pea leaf and cauliflower mitochondria occurred only with a very high ratio of ATP/ADP exceeding about 20. They suggested that respiration is regulated not by the external ATP/ADP ratio alone but also by the absolute concentration of ADP available for uptake into the mitochondria. Since under physiological conditions, the in vivo ATP/ADP ratio in extrachloroplast fraction of light-treated leaf tissue is usually between 4 and 8, they concluded that, if leaf mitochondrial respiration is inhibited at all in light, some mechanism other than the ATP/ADP ratio must be responsible. Recently Hampp et al. [19] used very rapid and refined techniques to measure adenylate levels in green mesophyll protoplasts of *Avena sativa* and their distribution in chloroplasts, cytosol and mitochondria. They found that the cytosolic energy state is always maintained at a high constant pre-set value in both light and dark, on account of effective collaboration between photosynthesis and oxidative phosphorylation. They concluded that there is a very tight control of mitochondrial. They concluded that there is a very tight control of mitochondrial respiration by light via the cytosolic energy state (ATP/ADP ratio) to keep it constant. According to this concept it appears that since ATP consuming reactions are mostly confined to the cytosol, photophosphorylation supplies ATP to maintain a high adenylate charge in the cytosol during active photosynthesis, while in the dark the same function is performed by mitochondrial oxidative phosphorylation. Stitt et al. [20] showed that illumination of wheat protoplasts is not accompanied by an increase in ATP/ADP ratio in the cytosol, because it is already high in the dark. Hence, according to them, this factor may not be responsible for the suppression of mitochondrial oxidative phosphorylation in light. They suggested that further experiments are required to identify the mechanism involved. As mentioned earlier a direct experimental demonstration of inhibition of the mitochondrial electron transfer chain in light is difficult because under these conditions photorespiratory and Mehler reactions are also stimulated, which results in O_2 uptake [21]. Perhaps the strongest direct evidence of light inhibition of mitochondrial respiration in leaves was obtained from in vivo experiments using $^{18}O_2$ [22]. It was demonstrated that rates of $^{18}O_2$ uptake observed in darkness are completely suppressed in light.

Recently, Azcón-Bieto and Osmond [23] studied the relationship between photosynthesis and respiration in wheat leaves. They have shown that the post-illumination rate of CO_2 production is dependent on the carbohydrate status of the leaves. Although on account of technical difficulties they could not directly demonstrate the effect of photosynthesis on mitochondrial oxidation of NADH in light, they concluded that the evidence is consistent with the suggestion made by Graham [1] to the effect that glycolysis and citric acid cycle reactions are modified in light to allow the continuous anaplerotic carbon flow for supplying 2-oxo acids which the chloroplast is unable to make. According to them the important feature of this anaplerotic flow is the probable operation of phosphoenolpyruvate carboxylase (PEP-carboxylase) in the cytosol and NAD^+-malic enzyme (decarboxylating) in the mitochondrion to replenish the carbon loss from the citric acid cycle. They have expressed doubt about the operation of the citric acid cycle in light beyond succinate oxidation [9–11]. For the operation of the mitochondrial electron transfer chain in light they have attributed a role for mostly non-phosphorylating cyanide-insensitive respiration which is insensitive to energy charge. According to them one molecule of CO_2 would be released per molecule of glutamine formed by these reactions in illuminated leaves. The presence of $NADP^+$-isocitrate dehydrogenase in chloroplasts has been reported [24] and hence formation of some 2-oxoglutarate within the chloroplasts is possible, provided isocitrate is available for the reaction.

It is thus not yet clearly established whether oxidation of NADH via the mitochondrial respiratory chain is completely or partially inhibited in light. The role of the external ATP/ADP ratio in this reaction is also controversial. In a recent critical review by Tager et al. [25] it has been shown that in state 3, control of mitochondrial respiration is distributed between a number of steps and cannot be exclusively attributed to the ATP/ADP ratio. The adenine nucleotide translocator, which is displaced from equilibrium even at rather low rates of respiration, contributes significantly to the control of respiration. Other factors including cytochrome oxidase, inorganic phosphate and hydrogen supply also play a significant role. According to the authors, distribution of control among different steps is a function of the rate of respiration and probably a similar situation is encountered in the intact cell.

5. SOURCE OF CARBON FOR THE CITRIC ACID CYCLE IN LIGHT

According to Azcón-Bieto and Osmond [23] phosphoenolpyruvate synthesised from recently produced triose phosphate is the likely source of carbon for the citric acid cycle in light. PEP-carboxylase and malate dehydrogenase would generate malate, which can serve as a potential carbon source for the citric acid cycle. However, Kent [26, 27] has shown that export of triose phosphates from the chloroplast may not be a significant source of carbon for the citric acid cycle during active photosynthesis. Since these compounds are mostly utilised for sucrose synthesis in the cytoplasm, he also suggested that serine or some unknown anaplerotic carboxylation reactions may provide carbon for the citric acid cycle, which is an important source of 2-oxo acids independent of ATP synthesis. Palmer [28,29] has suggested that a modified citric acid cycle functions in light, making use of malate as a source of both pyruvate and oxaloacetate. He argued that the mitochondrial NAD^+-malic enzyme (decarboxylating) generates pyruvate which can subsequently give rise to acetyl CoA. Oxaloacetate is also generated within mitochondria by malate dehydrogenase. Thus the citric acid cycle can function as long as malate is available. Plant cells are known to accumulate large quantities of malate. Thus, it is clear that this modified citric acid cycle can function independently of a glycolytic source of pyruvate. However, during prolonged operation of the citric acid cycle in light, de nove synthesis of malate is essential. Alternatively, some other source of carbon would be necessary to replenish the carbon skeletons consumed in amino acid synthesis. The source of this carbon is not yet clear. It is known that two carbon compounds such as glycolate and glyoxylate are produced in large quantities in C_3 plants during photorespiration, also known as the glycolate oxidation pathway. In these reactions, phosphoglycolate is synthesised in the chloroplast by the action of ribulose-1,5-bisphosphate oxygenase. Subsequent oxidative decarboxylation of glycolate in the peroxisomes or mitochondria is assumed to be the source of photorespiratory CO_2. It is suggested by Tolbert [30] that glycine synthesised in the peroxisomes by transamination of glyoxylate is transported to the mitochondria where 2 molecules of glycine generate 1 molecule each of serine, CO_2, NH_3 and NADH. Dark oxidation of this NADH in isolated spinach leaf mitochondria is known to be coupled to the synthesis of 3 molecules of ATP [13]. To what extent this reaction can proceed during active photosynthesis is not known.

Photorespiration is considered to be a wasteful process and no definite physiological role has yet been indicated. Naik and Singh [11] suggested that photorespiration could possibly function as a source of carbon for the citric acid cycle during photosynthesis.

Two mechanisms are possible. Firstly, glyoxylate and acetyl CoA can form malate by the action of malate synthetase. This could be a source of de novo synthesis of malate making use of glyoxylate derived from photorespiration. However, the presence of malate synthetase in plant mitochondria has not been reported. A second possible mechanism is the reversal of the reaction catalysed by isocitrate lyase. This enzyme is present in leaf mitochondria [32]. Although the equilibrium of this reaction favours cleavage of isocitrate to form succinate and glyoxylate, the reaction is reversible in the presence of high concentrations of succinate and glycoxylate [33]. Singh and Naik [34] have shown that in isolated leaf mitochondria reversal of the isocitrate lyase reaction as indicated above is quite feasible, when

enhanced levels of succinate and photorespiratory glyoxylate are available in the green cell. Thus it is likely that glyoxylate could be used for the synthesis of isocitrate which can be metabolised further in the citric acid cycle during active photosynthesis. Malate and isocitrate produced by condensation of glyoxylate with acetyl CoA or succinate can be oxidised by the partial reactions of the citric acid cycle from malate to acetyl CoA and from isocitrate to succinate, respectively. Thus catalytic quantities of acetyl CoA and succinate would suffice to support a continuous oxidation of glycoxylate, which is generated in large quantities during photorespiration. In these reactions 1 molecule of glyoxylate would be oxidised to 2 molecules each of CO_2 and NADH. This could be an additional source of photorespiratory CO_2 [11,34].

6. FATE OF NADH GENERATED IN MITOCHONDRIA IN LIGHT

As described above there is overwhelming evidence in favour of the operation of a partial or complete citric acid cycle in light. It is obvious that NADH generated in these reactions must be rapidly oxidised for sustained activity of the citric acid cycle dehydrogenases. Cyanide-insensitive respiration, which is not tightly coupled to ATP synthesis, could be one mechanism to achieve this. Alternatively, a dicarboxylate shuttle (oxaloacetate-in, malate-out) could export reducing equivalents from the mitochondria to be used for a variety of functions such as nitrate reduction in the cytoplasm [35–39], or reduction of hydroxypyruvate to glycerate in the peroxisomes [40]. Day and Wiskich [41] have proposed a direct export of mitochondrial NADH via a transmembrane transhydrogenase. According to them citric acid cycle dehydrogenases can reduce external NAD.

7. CONCLUSION

Metabolic interactions between chloroplasts and mitochondria function via exchange of adenylates and reducing equivalents. Since the citric acid cycle dehydrogenases have to perform anaplerotic functions in order to supply carbon skeletons for amino acid synthesis, the problem of disposal of NADH generated in these reactions is very important. Moreover, photorespiratory glycine is also oxidised in mitochondria in light to generate additional NADH. Rapid utilization of this NADH in extramitochondrial reactions such as nitrate reduction would facilitate uninterrupted turnover of the citric acid cycle in light. To what extent this NADH is also oxidised via cyanide-insensitive or other pathways within the mitochondria is not yet clearly established. However, studies under physiological conditions in leaves have shown that $^{18}O_2$ uptake via dark mitochondrial respiration is significantly inhibited in light [22]. This inhibition may not be due exclusively to an increase in the ATP/ADP ratio as was earlier postulated, but a complexity of factors including cytochrome oxidase, inorganic phosphate, adenine nucleotide translocator and hydrogen supply, as suggested by Tager et al. [25] may be responsible.

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