S29-001

Optimization by mitochondrial metabolism of photosynthetic carbon assimilation

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Keywords: alternative pathway, chloroplasts, cytochrome pathway, interorganelle interaction, respiration

Introduction

Photosynthesis is a process of reduction and respiration is a process of oxidation. Both the processes provide ATP for cellular needs. The nature of these two metabolic pathways implies that they complement each other. The sites of photosynthesis and respiration are chloroplasts and mitochondria. Although chloroplasts and mitochondria are traditionally considered to be autonomous organelles, recent literature has established that these two organelles are not only interdependent in their functions but also are mutually beneficial in their interaction. There are reviews that examine in detail the different aspects of mitochondrial respiration in the light and its interaction with photosynthesis (Raghavendra et al. 1994, Krömer 1995, Gardeström and Lernmark 1995, Hoefnagel et al. 1998, Padmasree and Raghavendra 1998, 2000, Atkin et al. 2000, Gardeström et al. 2001, Padmasree et al. 2001).

Mutually beneficial interaction between chloroplasts, mitochondria, peroxisomes and cytoplasm

Several biosynthetic processes within the photosynthetic tissues of higher plants, including photorespiration or nitrogen metabolism are highly demanding in terms of energy (ATP), reducing power and carbon skeletons. The requirements of ATP, NADH and NADPH are met by the products exported from both chloroplasts and mitochondria (Fig. 1). The cytoplasm is common medium for the flux of all related metabolites. The import of reduced equivalents by peroxisomes from both chloroplasts and mitochondria demonstrates the flexibility of interorganelle dependence within the plant cells. Thus the interaction of chloroplasts and mitochondria is not exclusive but extends to cytoplasm and peroxisomes.

The plant cells seem to have developed a strategy to distribute the demands of energy (ATP) and the reducing equivalents (NADH/NADPH) to different compartments. The supply and demand patterns would be dynamic depending on the microenvironment of the cell. Under limiting CO_2, photorespiration is highly active and becomes a major link between chloroplasts, peroxisomes, cytoplasm and mitochondria. Glycine is the major substrate of mitochondrial respiration under limiting CO_2 and can contribute significant amounts of ATP to cell. At high CO_2, the enhanced requirement of ATP in cytosol (for sustenance of sucrose biosynthesis) is met again from mitochondria (which can use either glycine or malate as respiratory substrates). Under both situations, nitrogen metabolism and recycling of ammonia/keto acids are always integrated with the functioning of chloroplasts,

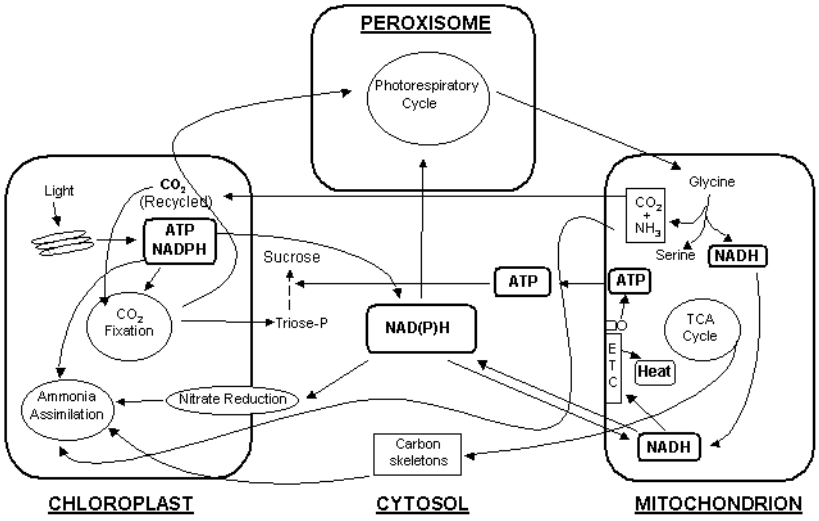

Fig. 1. An overview of metabolic processes and circulation of energy and redox equivalents between chloroplast, mitochondrion, peroxisome and cytosol as the basis of interorganelle interaction.

mitochondria, peoxisomes and cytoplasm. Any modulation of respiration leads to changes in the patterns of photosynthesis and photorespiration and subsequently modification of nitrogen as well as sulphur metabolism. Therefore the processes of photorespiration and nitrogen assimilation are linked closely to chloroplast function as well as to mitochondrial oxidative metabolism (Champigny 1995, Padmasree and Raghavendra 1998).

Although the extent of respiration in light is often debated, the stimulation of dark respiration due to illumination, particularly in green tissues, is now well established. The respiratory O_2 uptake in dark, increases quite significantly (1.2 to 7 fold), soon after illumination. This phenomenon termed as 'light enhanced dark respiration' (LEDR), occurs after even short periods of exposure to light and has been recorded in a variety of organisms (Padmasree and Raghavendra 1998, Padmasree et al. 2001). The sensitivity of LEDR to DCMU (an inhibitor of photosystem II electron transport) and D,L-glyceraldehyde (inhibitor of Calvin cycle) established that LEDR is dependent on products of photosynthetic carbon assimilation and electron transport (Reddy et al. 1991).

Although the essentiality of mitochondrial oxidative phosphorylation for photosynthetic carbon assimilation is well established, the role of cytochrome and alternative pathways in benefiting photosynthetic metabolism is examined only to a limited extent. The importance of cytochrome and alternative pathways during photosynthesis was studied in mesophyll protoplasts of pea and barley, using low concentrations of mitochondrial inhibitors: oligomycin (inhibitor of oxidative phosphorylation), antimycin A (inhibitor of cytochrome pathway) and salicylhydroxamic acid (SHAM, inhibitor of alternative pathway). All the three compounds decreased the rate of photosynthetic O_2 evolution in mesophyll protoplasts, but did not affect chloroplast photosynthesis (Krömer et al. 1988, Padmasree and Raghavendra 1999a,b,c, 2001). The marked sensitivity of photosynthesis to both SHAM and antimycin A suggests that the alternative pathway

is as essential as the cytochrome pathway for optimal photosynthesis. These results also demonstrate an important role of the alternative pathway in plant cells: essentiality for chloroplast photosynthesis.

Optimization of photosynthetic carbon assimilation

The optimization of photosynthetic carbon assimilation requires the coordination of different components: generation and use of assimilatory power (ATP and NADPH), induction of photosynthesis and maintenance of metabolite levels. The function of chloroplast is optimized by the complementary nature of mitochondrial metabolism in multiple ways: facilitation of export of excess reduced equivalents from chloroplasts, shortening of photosynthetic induction, activation of enzymes, maintenance of photorespiratory activity and supply of ATP for sucrose biosynthesis as well as other cytosolic needs (Padmasree et al. 2001). Emphasis has been made on the significant role of mitochondria in maintaining either cytosolic redox status or ATP or both (Raghavendra et al. 1994, Gardeström and Lernmark 1995, Krömer 1995, Padmasree and Raghavendra 1998, Gardeström et al. 2001, Padmasree et al. 2001).

While mitochondria provide ATP for sucrose synthesis (Krömer 1995) and oxidize excess chloroplastic reductants in the light (Hoefnagel et al. 1998, Padmasree and Raghavendra 1999c), peroxisomes play an important role during photorespiration and provide glycine as a major substrate to mitochondria (Gardeström et al. 2001). Similarly cytosol and mitochondria coordinate with chloroplasts for N_2 assimilation, by providing a site for nitrate reduction (cytosol) and carbon skeletons for amino acid synthesis (mitochondria). These beneficial interactions are achieved by the well-known metabolite shuttles across the envelope membranes of chloroplasts, mitochondria and peroxisomes, with cytosol as the connecting medium (Fig. 2). Thus the quadrangular interaction of chloroplasts, peroxisomes, mitochondria and cytosol forms an integral component during the optimization of photosynthesis by the combined processes of dark respiration and photorespiration.

Mitochondrial respiration can prevent overreduction of photosynthetic electron transport chain by oxidizing the excess reductants generated in chloroplast (Raghavendra et al. 1994, Hoefnagel et al. 1998, Gardeström et al. 2001). Chloroplasts export reductants to cytosol through PGA-DHAP shuttle or malate/OAA shuttle (Fig. 2). Malate possibly enters mitochondria through mitochondrial OAA translocator, where it will be oxidized to OAA by mitochondrial malate dehydrogenase. The NADH thus released in mitochondria could then be used for production of ATP or dissipated through alternative pathway of electron transport. In fact inhibition of alternative pathway by SHAM led to a drastic increase in the cellular malate/OAA levels (Padmasree and Raghavendra 1999c), indicating an important role for the alternative pathway in dissipating excess photosynthetic reductants through malate/OAA shuttle. The importance of these dissipating mechanisms increases under CO_2 limitation.

Nitrogen assimilation and photorespiration form major sinks for chloroplastic reducing equivalents during light. The reduction of NO_3^- to NO_2^- in the cytosol and NO_2^- to NH_4^+ in chloroplast require high amounts of ATP and NAD(P)H, which could be partly met by chloroplasts, while the main source is the mitochondria (Fig. 2). Thus nitrogen metabolism helps in removing a part of the large excess of chloroplast

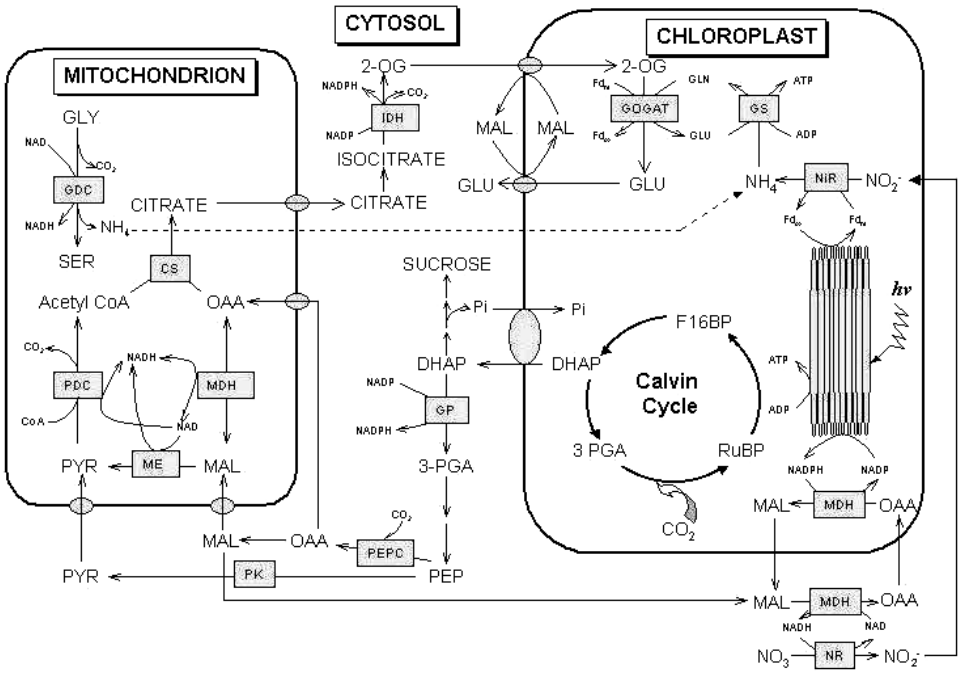

Fig. 2. Metabolite shuttles facilitate the mutually beneficial interaction between nitrogen metabolism, chloroplast photosynthetic reactions and mitochondrial respiratory activity in plant cells. The key enzymes involved are: CS-citrate synthase, GDC-glycine decarboxylase, GOGAT-glutamate oxoglutarate aminotransferase/glutamate synthase, GP-phosphoglyceraldehyde dehydrogenase, GS-glutamine synthetase, IDH-isocitrate dehydrogenase, MDH-malate dehydrogenase, ME-malic enzyme, NiR-nitrite reductase, NR-nitrate reductase, PDC-pyruvate decarboxylase, PEPC-phospho*enol*pyruvate carboxylase, PK-pyruvate kinase.

reductants, with mitochondrial participation as an essential and integral component of both photorespiration and nitrogen metabolism (Padmasree and Raghavendra 1998, 2000, Padmasree et al. 2001).

Protection against photoinhibition

Mitochondrial respiration plays an important role in not only optimizing photosynthesis but also protecting against photoinhibition. The importance of mitochondria in protection of chloroplasts against supra-optimal light doubles up in view of mitochondrial participation in dark respiration as well as photorespiration.

Plants evolved different preventive as well as repair mechanisms to adapt to the unavoidable phenomenon of photoinhibition. These include physical changes (e.g. chloroplast or leaf movement) and biophysical adaptations such as adjustment of the light harvesting chlorophyll antennae size, thermal dissipation, scanvenging reactive oxygen species (Niyogi 1999). Metabolic processes which consume ATP and NADPH (or reduced ferredoxin) help to dissipate the excess energy and thus protect against photoinhibition. Under photoinhibitory stress, photosynthetic carbon reduction, nitrogen assimilation and photorespiration play a very crucial role in removing excess ATP and NAD(P)H from chloroplasts and prevent overexcitation of the photochemical apparatus (Fig 2). Mitochondrial oxidative metabolism (and

associated interorganelle interaction) is an additional and important mechanism of protecting photosynthesis against photoinhibition.

The presence of classic mitochondrial inhibitors, e.g. antimycin A, or SHAM, or oligomycin, resulted in an increase and/or acceleration of photoinhibition in different systems: mesophyll protoplasts, leaves, cyanobacteria and algal cells.
The observation that inhibitors of both cytochrome pathway, alternative pathway apart from those of oxidative phosphorylation, enhance photoinhibition (Saradadevi and Raghavendra 1992, Shyam et al. 1993, Singh et al. 1996) implies that both these components contribute towards protection of chloroplasts against photoinhibition. Further investigations are needed to elucidate the importance of alternative pathway in protection against photoinihibition.

The degradation of D1 protein faster than its synthesis leads to photoinhibition. The continuous synthesis of D1 is necessary to prevent photodamage to PS II. Mitochondrial respiration may help even in the recovery of photosynthesis after photoinhibition. For e.g. in *A. nidulans*, treatment with sodium azide or FCCP slowed down the recovery from photoinhibition (Shyam et al. 1993). Similarly KCN and FCCP impaired the reactivation of photosynthesis in *C. reinhardtii* (Singh et al. 1996). Thus the mitochondria may also supply the energy (ATP) for the synthesis of D1 protein.

Concluding remarks

The metabolic inhibitors may exert unspecific and multiple effects on different processes in the cell. Hence the importance of cytochrome and alternative pathways proposed by experiments involving the use of inhibitors has been frequently questioned. Nevertheless, these metabolic inhibitors were used in mitochondrial studies by choosing carefully the concentrations which affect only mitochondrial respiration but not chloroplast reactions. The studies using inhibitors can be complemented by experiments involving mutants or transgenic plants, with an altered pattern of proteins/enzymes related to chloroplasts, mitochondria and peroxisomes (e.g. glycine decarboxylase, Igamberdiev et al. 2001).

Acknowledgements

LP and KP are recipients of Senior Research Fellowship and Research Associateship respectively from Council for Scientific and Industrial Research, New Delhi, India. The work in our laboratory and the preparation of this manuscript are supported by a grant (to ASR) from Department of Science and Technology (SP/SO/A-12/98), New Delhi, India.

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