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Essentiality of Mitochondrial Oxidative Metabolism for Photosynthesis: Optimization of Carbon Assimilation and Protection Against Photoinhibition

*K. Padmasree, L. Padmavathi, and A.S. Raghavendra**

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad - 500 046, India

Referee: Christine Foyer, Dept. of Biochemistry and Physiology, IACR - Rothamsted, Harpemden, Herts AL5 2JQ, England, United Kingdom

* Author for correspondence.

** Name and address of corresponding author: Professor A.S. Raghavendra, Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India. Tel: +91-40-3010630. Fax: +91-40-3010145 E-mail: asrsl@uohyd.ernet.in

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ABSTRACT: The review emphasizes the essentiality of mitochondrial oxidative metabolism for photosynthetic carbon assimilation. Photosynthetic activity in chloroplasts and oxidative metabolism in mitochondria interact with each other and stimulate their activities. During light, the partially modified TCA cycle supplies oxoglutarate to cytosol and chloroplasts. The marked stimulation of O_2 uptake after few minutes of photosynthetic activity, termed as light enhanced dark respiration (LEDR), is now a well-known phenomenon. Both the cytochrome and alternative pathways of mitochondrial electron transport are important in such interactions. 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, maintenance of photorespiratory activity, and supply of ATP for sucrose biosynthesis as well as other cytosolic needs. Further, the mitochondrial oxidative electron transport and phosphorylation also protects chloroplasts against photoinhibition. Besides mitochondrial respiration, reducing equivalents (and ATP) are used for other metabolic phenomena, such as sulfur or nitrogen metabolism and photorespiration. These reactions often involve peroxisomes and

cytosol. The beneficial interaction between chloroplasts and mitochondria therefore extends invariably to also peroxisomes and cytosol. While the interorganelle exchange of metabolites is the known basis of such interaction, further experiments are warranted to identify other biochemical signals between them. The uses of techniques such as on-line mass spectrometric measurement, novel mutants/transgenics, and variability in metabolism by growth conditions hold a high promise to help the plant biologist to understand this interesting topic.

KEY WORDS: alternative pathway, chloroplasts, cytochrome pathway, interorganelle interaction, mitochondria, peroxisomes.

I. INTRODUCTION TO THE TOPIC

Photosynthesis, the primary source of energy for the living world, consists of two distinct phases. The first phase of photochemical reactions, involves the conversion of radiant solar energy into chemical forms like ATP and NADPH (or reduced ferredoxin) with concomitant evolution of oxygen. In the second biochemical phase, the ATP and NADPH (or reduced ferredoxin) are utilized to reduce carbon dioxide (or other compounds like NO_2 or SO_2) into energy rich carbon (or nitrogen or sulfur) compounds. Respiration on the other hand involves the oxidation of carbon compounds and production of NADH or FADH with the simultaneous release of CO_2. The reductants (NADH or FADH) are oxidized through the electron transport (and oxidative phosphorylation) to produce ATP, involving consumption of O_2 and release of water.

Thus, photosynthesis is a process of reduction and respiration is a process of oxidation. Both processes provide ATP for cellular needs. The nature of these two metabolic pathways implies that they complement each other. The major 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.

Besides the carbon metabolism, the reduced equivalents are consumed in metabolic reactions of photorespiration, nitrate assimilation, and sulfur metabolism. For example, the requirement of NADH for hydroxypyruvate reduction in peroxisomes is met by chloroplasts as well as mitochondria. Naturally, the cytoplasm is a common medium for the flux of all related metabolites. Thus the interaction of chloroplasts and mitochondria is not exclusive but extends to cytoplasm and peroxisomes.

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 both glycine and malate as respiratory substrates). Under both situations, nitrogen metabolism and recycling of ammonia/keto acids are always integrated with the functioning of chloroplasts, mitochondria, peroxisomes, and cytoplasm. Any modulation of respiration leads to changes in the patterns of photosynthesis and photorespiration and subsequently modification of nitrogen as well as sulfur metabolism.

The current review attempts to critically assess and emphasize the physiological and biochemical features of interorganelle interaction: chloroplasts, mitochondria, peroxisomes, and cytoplasm. However, emphasis is given to the essentiality of mitochondrial respiration for photosynthetic carbon metabolism. The mitochondrial oxidative metabolism not only helps to optimize photosynthesis in varied environmental conditions but also protects the chloroplasts against the photoinhibition of photosynthesis.

A. Scope of the Present Review

Due to the intriguing but interesting nature of the topic, considerable effort has been made in the last decade to study the occurrence of mitochondrial respiration in light and its importance for photosynthesis, particularly in mesophyll protoplasts and leaves. In view of the limited space, all the original articles are not referred to in this review. Readers interested in the extensive literature in this and related areas may consult the previous reviews (Azcón-Bieto, 1992; Raghavendra et al., 1994; Krömer, 1995; Gardeström and Lernmark, 1995; Gardeström, 1996; Hoefnagel et al., 1998; Padmasree and Raghavendra, 1998, 2000; Atkin et al., 2000b; Gardeström et al., 2002).

Most of the work on the interaction of mitochondrial respiration and chloroplast photosynthesis is based on the use of metabolic inhibitors that inhibit specific reactions at low concentrations. An inherent disadvantage, however, is that these inhibitors cause nonspecific effects particularly at higher concentrations (see Section VI). Among the mitochondrial inhibitors referred frequently in this review are rotenone (an inhibitor of complex I in the mitochondrial respiratory chain); antimycin A (an inhibitor of complex III); KCN/NaN_3 (inhibitors of complex IV); oligomycin (an inhibitor of complex V); salicylhydroxamic acid (SHAM)/propyl gallate (inhibitors of alternative oxidase); and aminoacetonitrile (AAN, an inhibitor of glycine decarboxylase).

B. Differential Effects on CO_2 Efflux/O_2 Uptake

The occurrence of mitochondrial respiration in light has been a matter of debate, for a long time, because of ambiguous reports on the extent and pattern of dark respiration in light (Graham, 1980; Raghavendra et al., 1994; Krömer, 1995; Villar et al., 1995; Atkin et al., 1997; Hoefnagel et al., 1998; Atkin et al., 2000a). Some studies have indicated that dark respiration was either unaffected or stimulated, while others found that respiration was inhibited (Table 1). Such a large variation in these reports appears to be due to a combination of factors: the component of dark respiration being monitored (CO_2 efflux/O_2 uptake), the experimental technique being used, and the subject of experimental system (leaves, algal cells, or cell cultures).

It is difficult to monitor precisely CO_2/O_2 exchange in light by conventional methods because the measurements are compromised by the occurrence of related phenomena besides dark respiration, for example, photorespiration, photosynthesis, Mehler reaction, chlororespiration. Each one of the above processes contributes significantly to the net CO_2 or O_2 exchange.

A promising solution was provided by the technique of mass spectrometry, which could distinguish between the uptake/efflux of O_2 or CO_2 occurring simultaneously during respiration, photosynthesis, and related

TABLE 1
The Effect of Light on Dark Respiration in Plant Tissues, Determined by Different Techniques, Indicating a Large Variation in the Extent of Inhibition

Plant	Experimental System	Parameter of Respiratory activity	Effect of light on respiration: Stimulation (+) or inhibition (-)*	Reference
Zea mays	Leaves	CO_2 release	-96	Heichel (1971)
Eucalyptus pauciflora	Leaves	CO_2 release	-60	Atkin et al., (2000a)
Triticum aestivum	Leaves	CO_2 release	-14	Pärnik and Keerberg (1995)
Eucalyptus pauciflora	Leaves	CO_2 release	-03	Kirschbaum and Farquhar (1987)
Nicotiana tabacum	Leaves	CO_2 release	+32	Heichel (1971)
Phaseolus vulgaris	Leaves	Metabolism of $^{14}CO_2$	-81	Mangat et al., (1974)
Vicia sepium	Leaves	Assimilation of ^{14}C labelled succinate	-45	Graham and Walker (1962)
Triticum aestivum	Leaf slices	Metabolism of ^{14}C labelled TCA cycle compounds	-25	McCashin et al., (1988)
Pisum sativum	Leaf discs	O_2 uptake	-42	Diethelm et al., (1990)
Glycine max	Leaf discs	O_2 uptake	-40**	Diethelm et al., (1990)
Spinacea oleracea	Leaf discs	O_2 uptake	-39**	Diethelm et al., (1990)

*% over the control (steady state rate in darkness).
**Determined at very low light intensity of 10 $\mu E\ m^{-2}\ s^{-1}$.

cellular processes. Mass spectrometric studies using $^{13/12}CO_2$ and $^{18/16}O_2$ have revealed that light has a differential effect on CO_2 efflux and O_2 uptake (Avelange et al., 1991; Raghavendra et al., 1994; Xue et al., 1996; Atkin et al., 2000a). On illumination, CO_2 efflux is suppressed by almost 81%, while O_2 consumption is either unaffected or stimulated up to 3.5-fold (Table 2). A major observation from these mass spectrometric experiments is that mitochondrial oxidative electron transport continues to be active, irrespective of illumination. The sustenance of active mitochondrial oxidative electron transport is essential for optimal photosynthesis.

C. Light-Enhanced Dark Respiration (LEDR)

Although the extent of respiration in light is often debated, the stimulation of dark res-

TABLE 2
Selected Examples Showing the Differential Effect of Light on Respiratory CO_2 Release and O_2 Uptake, as Determined by Mass Spectrometry. On Illumination, the Decarboxylation Reactions Are Usually Inhibited Resulting in a Decrease of CO_2 Release, While the Process of Oxidative Electron Transport (Indicated by O_2 Uptake) Is Either Unaffected or Even Enhanced

Plant species (Experimental material)	Respiratory reaction % stimulation (+) or inhibition (-)*		Reference
	CO_2 release	O_2 uptake	
Dianthus caryophyllus (Photoautotrophic cells)	-56	-06	Avelange et al., 1991
Commelina communis (Mesophyll protoplasts)	-31	+62	Gautier et al., 1991
Selenastrum minutum (Wildtype cells)	-59	-15	Weger et al., 1988
Chlamydomonas reinhardtii (Wildtype cells)	-81	+347	Xue et al., 1996
Chlamydomonas reinhardtii (mutant devoid of Rubisco)	-73	+157	Gans and Rébeillé 1988

*% over the control (steady state rate in darkness).

piration due to illumination, particularly in green tissues, is now well established. The respiratory O_2 uptake in dark increases quite significantly, soon after illumination (Figure 1). This phenomenon termed as 'light enhanced dark respiration' (LEDR) occurs after even short periods of exposure to light (Padmasree and Raghavendra, 1998). The phenomenon of LEDR has been recorded in different experimental systems and the extent of stimulation by light, varied from 1.2- to 7-fold (Table 3).

The extent of LEDR is positively correlated with the intensity and duration of the preceeding period of illumination (Raghavendra et al., 1994; Xue et al., 1996). The sensitivity of LEDR to DCMU (an inhibitor of photosystem II electron transport) and D,L-glyceraldehyde (inhibitor of Calvin cycle) establishes that LEDR is dependent on products of photosynthetic carbon assimilation and electron transport (Reddy et al., 1991). Exposure of *Euglena gracilis*, a flagellate to UV radiation decreased both the rate of photosynthesis and LEDR, especially at higher light intensities (Ekelund, 2000).

LEDR is different from photorespiratory post-illumination burst (PIB). The phenomenon of PIB results due to CO_2 released during decarboxylation of photorespiratory glycine. In tobacco leaf PIB occurs within 20 s after the light is switched off, while LEDR occurs between 180 to 250 s after stopping illumination (Atkin et al., 1998). Further at 2% O_2 (where photorespiration is minimized) PIB is not seen, whereas LEDR is still ob-

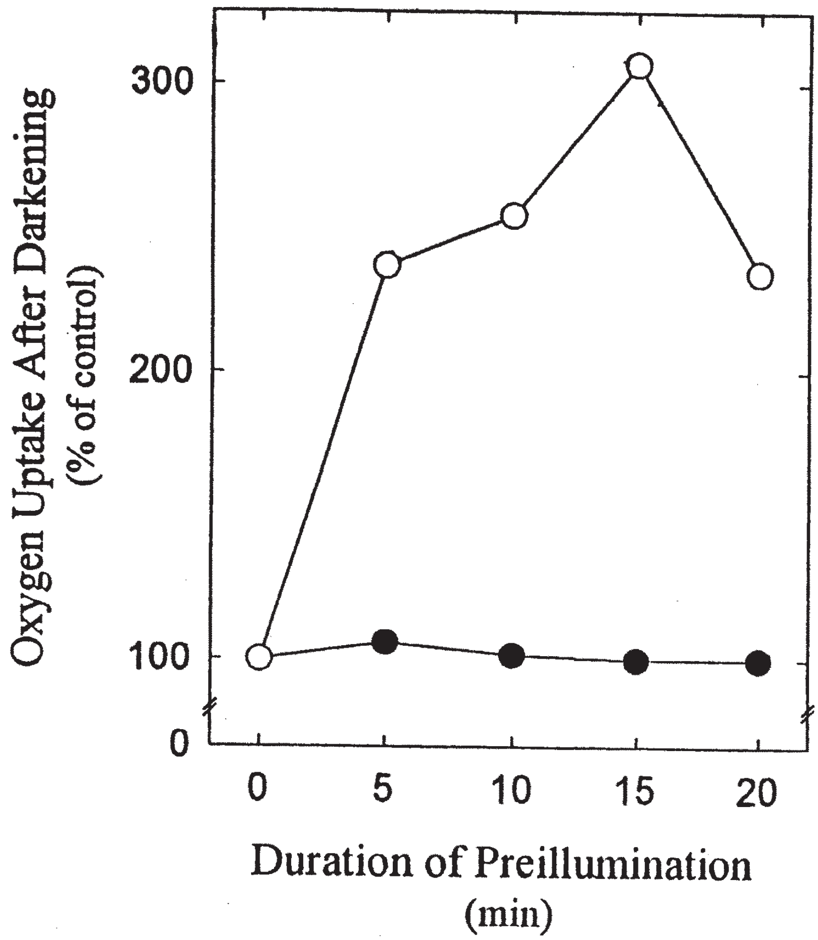

FIGURE 1. LEDR in mesophyll protoplasts of pea. The rate of respiration was stimulated by three-fold after 15 min of illumination, but not when protoplasts were kept in darkness for similar periods. (Modified from Reddy et al., 1991.)

served (Atkin et al., 1998; Atkin et al., 2000a). LEDR is also insensitive to AAN (an inhibitor of mitochondrial glycine metabolism) demonstrating that LEDR is not directly related to photorespiration (Gardeström et al., 1992; Raghavendra et al., 1994).

The occurrence of LEDR within a few minutes suggests that the interaction between photosynthesis and respiration is quite rapid and involves primary photosynthetic products, especially malate (Raghavendra et al., 1994; Padmasree and Raghavendra, 1998). The malate concentration is high at the end of illumination, and it is rapidly metabolized during the subsequent dark period (Hill and Bryce, 1992). In contrast, the levels of sucrose, glucose, and fructose did not change significantly during LEDR in the mesophyll protoplasts of barley (Hill and Bryce, 1992).

Plant mitochondrial pyruvate dehydrogenase complex (PDC) and NAD-malic enzyme are reversibly inhibited in light (see Section I.D). On switching over to darkness, these two enzymes are reactivated. Photosynthetically generated malate is oxidized via both malate dehydrogenase (MDH) and NAD-malic enzyme, resulting in the formation of oxaloacetate (OAA) or pyruvate and CO_2. Pyruvate is decarboxylated by PDC and converted into acetyl CoA. Both OAA and acetyl CoA

TABLE 3
The Occurrence and Extent of Light Enhanced Dark Respiration (LEDR) in Plant Tissues. The Rate of Respiration Increases Markedly After a Few Minutes of Photosynthetic Carbon Assimilation and LEDR Is Represented as the % Increase in Respiration Just After Illumination Over the Rate of Respiration (Steady State) in Continuous Darkness

Plant	Material	LEDR*	Reference
Pea	Mesophyll protoplasts	190	Reddy et al., 1991
Barley	Mesophyll protoplasts	30	Gardeström et al.,1992
Barley	Mesophyll protoplasts	700	Hill and Bryce, 1992
Barley	Mesophyll protoplasts	43	Igamberdiev et al.,1997
Dianthus caryophyllus	Cells	20	Avelange et al., 1991
Chlamydomonas reinhardtii	Algal Cells	180	Xue et al., 1996
Euglena gracilis	Cells	160	Ekelund, 2000
Spinach	Leaf discs	600	Stokes et al., 1990
Tobacco	Leaves	33**	Atkin et al., 1998

* % increase in O_2 uptake over the steady state rate in dark.
** % increase in CO_2 release.

enter TCA cycle and enhance the process of respiratory CO_2 release and oxidative electron transport. All these result in an upsurge in CO_2 release or O_2 uptake termed LEDR (Raghavendra et al., 1994; Padmasree and Raghavendra, 1998; Atkin et al., 2000a). Thus, malate could be the substrate and signal for LEDR, which could accomplish the rapid conversion of photosynthetically generated reducing power into ATP. Experiments designed to alter chloroplast malate production or the import of malate into mitochondria, for instance, by altering the function of chloroplastic MDH or the overexpression/supression of mitochondrial OAA/malate translocator would help throw more light on the cause of LEDR.

D. Mitochondrial Respiration in Light: Modified TCA Cycle

Dark respiration consists of three steps: (1) glycolysis in the cytosol, (2) the TCA cycle, consisting of decarboxylation of carbon compounds resulting in the production of NADH/FADH and CO_2, (3) the electron transport chain involving NADH/FADH oxidation to produce ATP, and O_2 consumption.

The processes of CO_2 production and O_2 uptake are not as tightly coupled in light as in darkness. As discussed in the previous section, there is usually a reduction in the extent of respiratory CO_2 efflux, while oxygen uptake is either unaffected or even stimulated (Table 2). The main reason for such decrease in CO_2 release is the partial inhibition/modification of TCA cycle activity occurring in light. Two of the possible causes that downregulate/modify TCA cycle activity are (1) reversible inactivation of mitochondrial pyruvate dehydrogenase complex in light (Budde and Randall, 1990), (2) rapid export of oxoglutarate out of mitochondria (Hanning and Heldt, 1993), thus resulting in a short-circuit of Kreb's cycle.

PDC is phosphorylated (inactivated) in light by a PDC-protein kinase and dephosphorylated (activated) in darkness (Leuthy et al., 1996; Randall et al. 1996). The inactivation of PDC is linked to photosynthetic activity as indicated by its sensitivity to DCMU (inhibitor of PSII) and the absence of the phenomenon in etiolated seedlings. Further, the products of glycine decarboxylation, NADH and NH_4^+, also enhance the phosphorylation of PDC. Conditions that reduce photorespiration (high CO_2 and/or low O_2) limit the extent of PDC inactivation. Taken together these reports indicate that under conditions of high rates of photosynthesis or photorespiration, PDC is inactivated, but oxidation of glycine or malate continues.

The second possible reason for reduced CO_2 efflux in light is the export of TCA cycle compounds from mitochondria to chloroplast (mainly for NH_4^+ assimilation in light), thus limiting the substrates available for further steps of the TCA cycle. Mitochondria export TCA cycle intermediates in the form of citrate, which is converted into oxoglutarate in cytosol and sent into chloroplast (Figure 2; Chen and Gadal, 1990; Gout et al., 1993; Hanning and Heldt, 1993; Krömer, 1995; Atkin et al., 2000b). This results in a partial activity of TCA cycle in light resulting in reduced CO_2 efflux (Parnik and Keerberg, 1995). Thus the main function of TCA cycle in light seems to be the supply of carbon skeletons to the chloroplasts for ammonium assimilation (Weger et al., 1988 and Weger and Turpin, 1989).

The ATP levels in cytosol during light do not appear to play any crucial role in downregulating TCA cycle. The ATP/ADP ratio required in the cytosol to decrease mitochondrial respiration is much higher than that usually occurs. In fact, the ATP/ADP ratio in cytosol is lower in light compared to darkness at saturating CO_2 levels (Krömer, 1995; Atkin et al., 2000b). If mitochondrial function in light were to be inhibited due to high ATP levels in cytosol, it would have also inhibited O_2 uptake besides CO_2 release. However, experimental evidence shows that O_2 uptake does not decrease much in light. The mitochondrial electron transport chain oxidizes not only the NADH produced by the partially active TCA cycle, but also that produced by photorespiratory glycine decarboxylation. Glycine oxidation therefore can contribute significantly to oxygen consumption by mitochondria in light.

II. ESSENTIALITY OF MITOCHONDRIAL RESPIRATION FOR PHOTOSYNTHESIS

One of the first indications about the importance of mitochondria came from the frequent observation of a positive relationship between dark respiration and photosynthesis. A strong positive correlation exists between steady state rates of photosynthesis and respiration in a wide range of species (Ceulemans and Saugier, 1991) and the respiratory rate of leaves increases significantly after the light period or hours of illumination (Raghavendra et al., 1994; Atkin et al., 1998). Mitochondrial oxidative metabolism (particularly oxidative electron transport and oxidative phosphorylation) has been shown to be essential for maintaining high rates of photo-

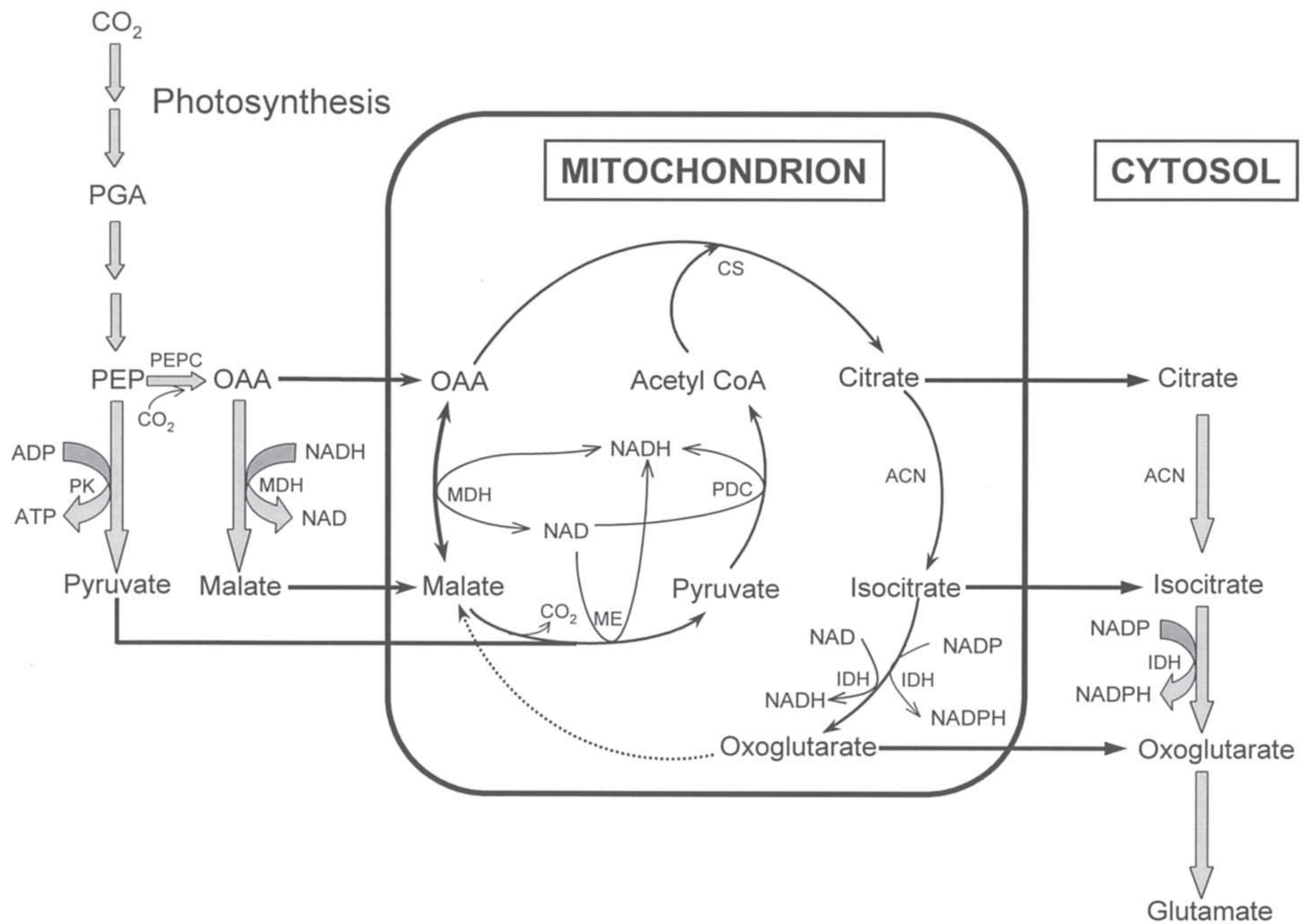

FIGURE 2. During illumination a partial or modified TCA cycle operates in mitochondria of photosynthetic cells. After entering mitochondria, a part of OAA is converted to malate, which on decarboxylation yields pyruvate. Acetyl CoA, derived from pyruvate combines with OAA to form citrate, which is exported to cytosol and converted to 2-oxoglutarate to be used for nitrogen metabolism. Isocitrate and 2-oxoglutarate may also be exported directly to cytosol. However, an NADP dependent ICDH operates when cytosolic isocitrate is converted to 2-oxoglutarate. The other reactions of TCA cycle, which are restricted in light are indicated by dashed arrow. The important enzymes involved are PEPC, phospho*enol*pyruvate carboxylase; PK, pyruvate kinase; MDH, malate dehydrogenase; ME, malic enzyme; PDC, pyruvate dehydrogenase complex; CS, citrate synthase; ACN, *cis*-aconitase; IDH, isocitrate dehydrogenase.

synthesis under a variety of conditions. The beneficial effects of mitochondria are known during not only short cycles of darkness and illumination, but also under stress environments like photoinhibitory light or low temperature (Vani et al., 1990; Saradadevi and Raghavendra 1992; Saradadevi et al., 1992; Shyam et al., 1993; Hurry et al., 1995).

The marked interaction between photosynthesis and respiration is convincingly demonstrated by experiments with leaf mesophyll protoplasts and intact leaves (Krömer et al., 1988; Vani et al., 1990; Krömer and Hedlt, 1991a; Krömer et al., 1993; Padmasree and Raghavendra, 1999a,b,c) using inhibitors of mitochondrial metabolism. The essentiality of different components of mitochondrial respiration for chloroplastic photosynthesis is documented by the use of typical mitochondrial inhibitors. For example, oligomycin was employed as inhibitor of oxidative phosphorylation (Krömer et al., 1988; Krömer and Heldt, 1991a; Krömer et al., 1993), antimycin A to inhibit the cytochrome pathway (Igamberdiev et al., 1997a,b; Padmasree and Raghavendra, 1999a,b,c), while SHAM was used to inhibit the alternative pathway (Igamberdiev et al., 1997a,b; Padmasree and Raghavendra, 1999a,b,c). On the other hand, the importance of glycolytic reactions and TCA cycle in stimulating photosynthetic O_2 evolution was assessed by the usage of sodium fluoride and sodium malonate, respectively (Vani et al., 1990). Recently, studies were carried out using mutants deficient in mitochondrial glycine decarboxylase (Igamberdiev et al., 2001).

A. Restriction of CO_2 Assimilation, but Not of Photochemical Activities

Preliminary experiments have indicated that the inhibitors employed during these studies had no direct effect on chloroplasts (Krömer et al., 1988; Padmasree and Raghavendra, 1999a). The suppression of photosynthetic activity was reversed and the full rate was restored when the protoplasts were ruptured, leaving the chloroplasts intact. These results indicate that the strong inhibition of photosynthesis observed with oligomycin or antimycin A or SHAM was due to not an effect on chloroplast photosynthesis as such, but interference of reactions between the chloroplasts, cytosol and mitochondria (Figure 3).

The statistical significance of the interaction between photosynthesis and respiration only in the presence of CO_2 (but not in its absence) suggested that carbon assimilation was a prerequisite (Vani et al., 1990). It is intriguing to note that despite the small effects of SHAM on respiration or ATP levels, the decrease in photosynthetic activity is always pronounced (Padmasree and Raghavendra, 1999a).

A recent comprehensive study reexamined the effects of mitochondrial inhibitors: oligomycin, antimycin A, and SHAM on the photosynthetic carbon assimilation and photochemical electron transport activities, monitored in intact mesophyll protoplasts (Padmasree and Raghavendra, 2001b). When mesophyll protoplasts were illuminated in presence of mitochondrial inhibitors, there was a significant decrease (>45%) in HCO_3^--dependent O_2 evolution, while the decrease in O_2 evolution was marginal (<10%) in presence of benzoquinone (BQ), [PSII mediated] and NO_2^- [dependent on PSII + PSI] as electron acceptors (Figure 4). DCMU, a typical photosynthetic inhibitor decreased drastically all the three reactions: HCO_3^- or BQ or NO_2^--dependent O_2 evolution in mesophyll protoplasts. The effect of mitochondrial inhibitors on photosynthetic reactions was similar in the presence or absence of NH_4Cl, an uncoupler, indicating that photophosphorylation also was not af-

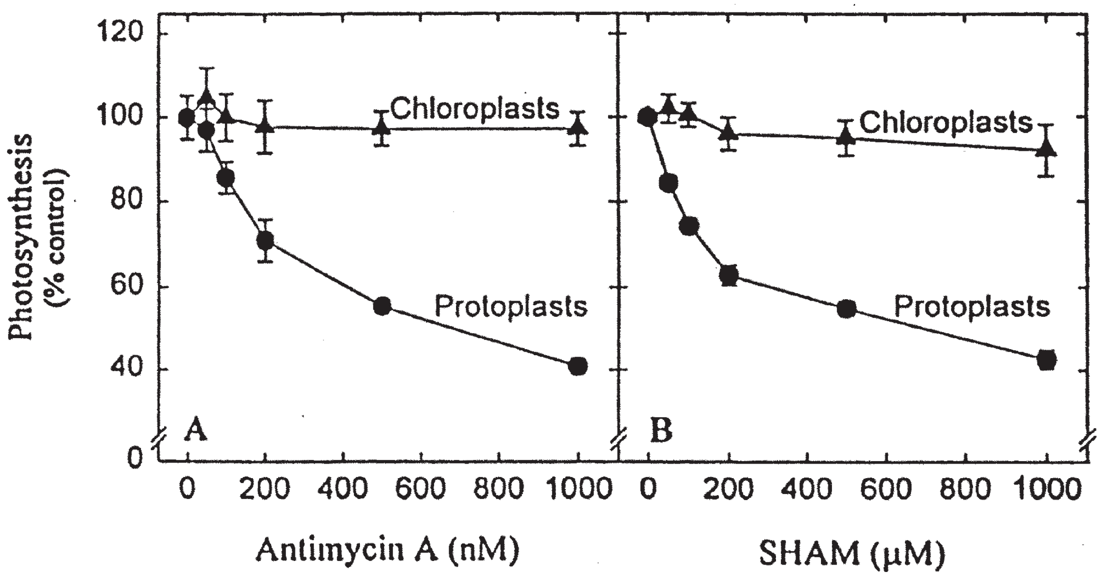

FIGURE 3. Marked suppression of bicarbonate dependent O_2 evolution (●) in pea mesophyll protoplasts by both antimycin A (inhibitor of cytochrome pathway of mitochondrial electron transport) and SHAM (inhibitor of alternative pathway). These two compounds had no direct effect on chloroplast photosynthesis (▲). (Adapted from Padmasree and Raghavendra, 1999a.)

fected. Thus, mitochondrial oxidative metabolism (through both cytochrome and alternative pathways) was essential for the maintenance of photosynthetic carbon assimilation, but had no direct effect on PSI- or PSII-dependent photochemical electron transport activities in mesophyll protoplasts of pea. However, during a long-term incubation, interference with mitochondrial metabolism can lead to disturbance in photochemical activities through feedback effects of thylakoid overenergization (Krömer et al., 1993).

B. Importance at Both Limiting and Optimal CO_2

The rate of photosynthetic O_2 evolution depends on not only light intensity but also the CO_2 concentration. At saturating CO_2 and light, the rate of photosynthesis is limited by the flux of assimilated carbon into sucrose and at limiting CO_2 and saturating light, the rate of photosynthesis is limited by rubisco activity (Krömer et al., 1993). At optimal CO_2 (nonphotorespiratory conditions), the photosynthetic demand for ATP is expected to be very high, while such a need for ATP would be lower at limiting CO_2. The decrease in the rate of photosynthesis due to mitochondrial inhibitors, oligomycin, antimycin A, or SHAM at optimal CO_2 (1.0 m*M* $NaHCO_3$) was much stronger than that at limiting CO_2 (0.1 m*M* $NaHCO_3$) under similar conditions (Figure 5). Nevertheless, the significant decrease in the rate of photosynthesis under both limiting and optimal CO_2 in the presence of these inhibitors suggests that mitochondrial oxidative metabolism is essential for maximal photosynthesis at both limiting CO_2 (photorespiratory conditions) as well as optimal CO_2 (Padmasree and Raghavendra, 1999a).

At optimal CO_2, most of the photosynthate is converted to sucrose-consuming ATP and Glc-6-P. Both oligomycin and antimycin A while causing a decrease in photosynthesis also raised the levels of Glc-6-P and triose-P at optimal CO_2 (Krömer et al., 1988; Krömer

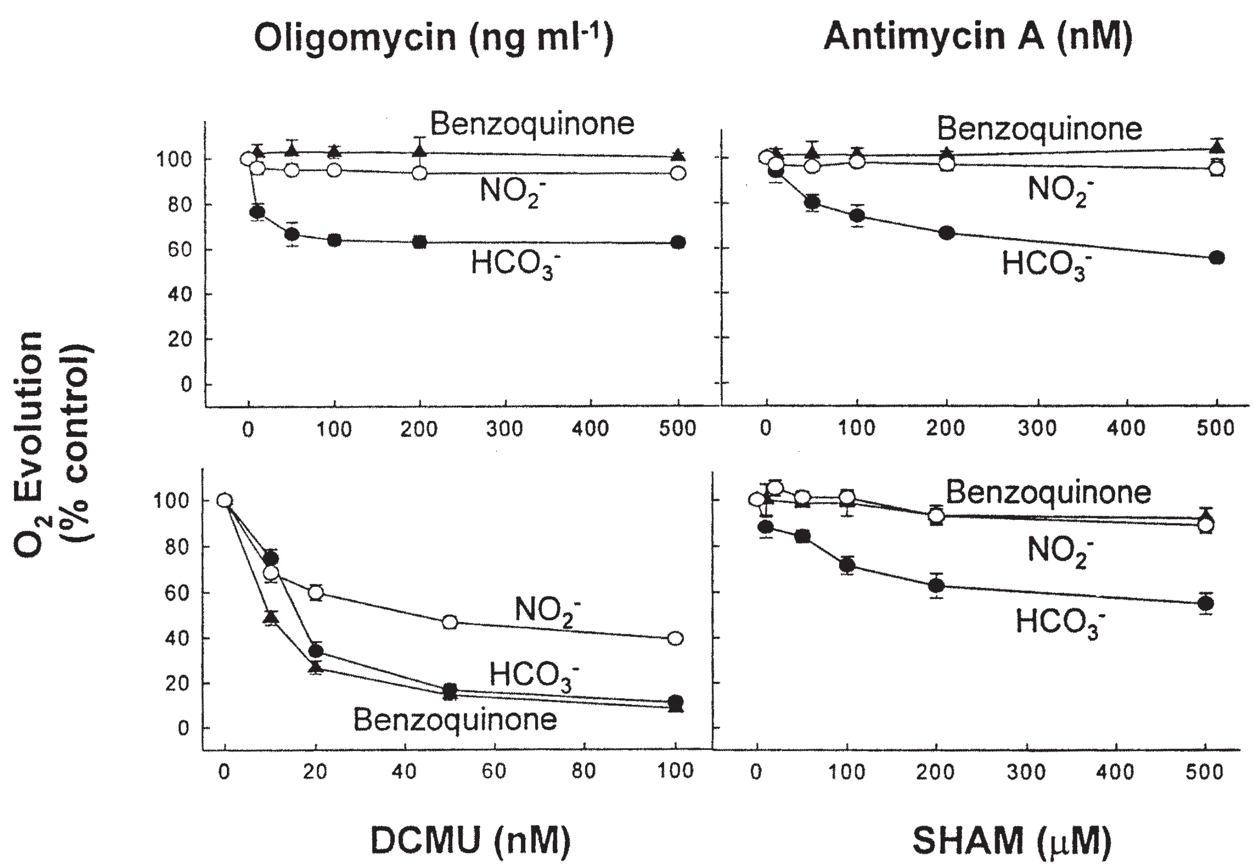

FIGURE 4. Change in rates of oxygen evolution when mesophyll protoplasts are incubated with different concentrations of oligomycin, antimycin A, DCMU or SHAM. ●: HCO_3^--dependent O_2 evolution (reflects the trend of CO_2 assimilation); ○: Rates of oxygen evolution when electrons are transferred from H_2O to NO_2 (representing PSII and PSI activity); ▲: Benzoquinone-dependent oxygen evolution (indicates PSII activity excluding PSI, H_2O, to BQ). In controls, the rates of O_2 evolution in µmol mg^{-1} Chl h^{-1} under different conditions were 154 (HCO_3^--dependent O_2 evolution); 445 (BQ-dependent O_2 evolution) or 52 (NO_2-dependent O_2 evolution). (Adapted from Padmasree and Raghavendra, 2001b.)

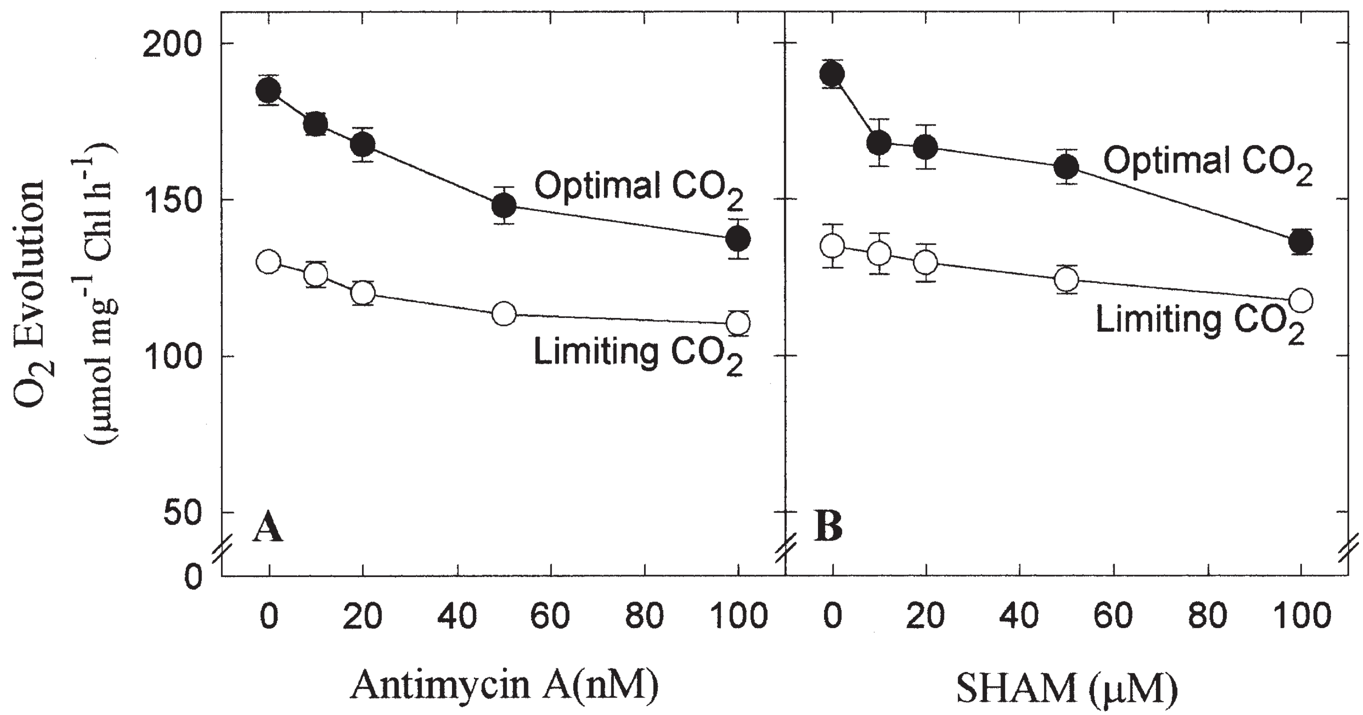

FIGURE 5. Effect of antimycin A (A) or SHAM (B) on photosynthesis at optimal (1.0 m*M* $NaHCO_3$) or limiting (0.1 m*M* $NaHCO_3$) CO_2 in mesophyll protoplasts of pea. (Modified from Padmasree and Raghavendra, 1999a.)

and Heldt, 1991a; Krömer et al., 1993; Padmasree and Raghavendra, 1999a). Obviously, mitochondrial metabolism and ATP supply are essential for the maintenance of sucrose biosynthesis. At limiting CO_2 there is either no change or only a marginal decrease in Glc-6-P in the presence of oligomycin or antimycin A (Padmasree and Raghavendra, 1999a). However, the presence of SHAM (which leads to only a limited ATP production) caused a decrease in the levels of Glc-6-P and had no effect on ATP/ADP levels, while markedly affecting photosynthesis in mesophyll protoplasts (Padmasree and Raghavendra, 1999a). Thus, the mitochondrial supply of ATP for sucrose synthesis may be only a secondary factor during the interaction with photosynthesis at limiting CO_2.

Although the marked decrease in the rate of photosynthetic O_2 evolution at both optimal and limiting CO_2 demonstrates the essentiality of mitochondrial oxidative metabolism in optimizing photosynthesis, under photorespiratory conditions mitochondrial electron transport is more crucial than oxidative phosphorylation in benefitting photosynthesis. A major function of the mitochondrion in a photosynthesizing cell, particularly under low light intensities and optimal CO_2, seems to be the supply of ATP for cytosolic carbon metabolism, that is, sucrose synthesis. In high light, mitochondria take on the additional role of oxidizing the excess reducing equivalents generated by photosynthesis, preventing overreduction of chloroplastic redox carriers and thus maintaining high rates of photosynthesis (Krömer, 1995; Padmasree and Raghavendra, 1998, 2000).

C. Role of Cytochrome and Alternative Pathways of Oxidative Electron Transport

The sensitivity of protoplast photosynthesis to mitochondrial inhibitors at limiting CO_2 (when the ATP requirement for photosynthesis is expected to be low) and the lack of correlation between the photosynthetic rates and the ratios of ATP/ADP in protoplasts (particularly in presence of SHAM) have indicated that mitochondrial electron transport activity is more important than the oxidative phosphorylation for optimal photosynthesis (Padmasree and Raghavendra, 1999a).

Plant mitochondria have the unique capability of oxidizing NADH/FADH through their electron transport by two different routes: (1) cyanide-sensitive cytochrome pathway and (2) cyanide-resistant alternative pathway (Lambers, 1985; Vanlerberghe and McIntosh, 1997; Mackenzie and McIntosh, 1999). The alternative pathway is catalyzed by alternative oxidase (AOX), which has been purified, characterized, and its gene isolated (Siedow and Umbach, 2000). While the molecular biology and regulation of AOX are studied in detail (McIntosh, 1994; Siedow and Umbach, 1995, 2000), the information on the physiological significance/metabolic function of AOX is still limited.

The relative proportion of cytochrome and alternative pathways is flexible and varies with environmental conditions such as temperature, age of the tissue, and injury/wounding. The circumstantial evidence suggests that the operation of alternative pathway is likely to increase in illuminated plant tissues. The levels of AOX increase during greening of etiolated leaves (Atkin et al., 1993). The accumulation of sugars during the illumination promotes the engagement of alternative pathway (Azcón-Bieto, 1992). A significant part of the light-enhanced dark respiration (LEDR) appears to involve alternative pathway (Igamberdiev et al., 1997a). It is not known, however, if there is any modulation by illumination of the extent of mitochondrial electron transport through the alternative pathway.

Although the essentiality of mitochondrial oxidative phosphorylation for photosynthetic carbon assimilation is well established, the role of cytochrome and alternative pathways in benefitting 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, an 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; Krömer and Heldt, 1991a; Krömer et al., 1993; Igamberdiev et al., 1997a, 1998; Padmasree and Raghavendra, 1999a,b,c). 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.

The importance of the alternative pathway during the interaction between respiration and photosynthesis is suggested by also the sensitivity of LEDR to SHAM in mesophyll protoplasts of barley (Igamberdiev et al., 1997a) and algae *Selenastum minutum*, *Chlamydomonas reinhardtii*, and *Euglena gracilis* (Lynnes and Weger, 1996; Xue et al., 1996; Ekelund, 2000).

Restriction of cytochrome pathway by antimycin A or uncoupling of cytochrome pathway from oxidative phosphorylation by oligomycin prolonged both the induction phase of photosynthesis and the activation of NADP-MDH during transition from dark to light (Igamberdiev et al., 1998; Padmasree and Raghavendra, 1999b). The exact mechanism of the optimization of photosynthesis by alternative pathway is not completely understood, but one of the reasons appears to be the effective modulation of intracellular redox state (Padmasree and Raghavendra, 1999c). A major function of AOX pathway in mesophyll cells can be the maintainance of the oxidation of malate, particularly under excess light (see Section IV.B).

A recent report suggests that the phenomenon of the Kok effect (progressive light induced inhibition of dark respiration at low light intensities) is modulated strongly by cytochrome pathway of mitochondrial electron transport. The alternative pathway appears to be less important in modulating the Kok effect (Padmavathi and Raghavendra, 2001).

D. Pronounced Interaction in Algal Mutants/Guard Cells

The interaction between respiration and photosynthesis is quite pronounced in cells that are deficient in Rubisco/Calvin cycle activity, such as stomatal guard cells and mutants of *Chlamydomonas* (Raghavendra et al., 1994).

Guard cells have high rates of respiratory activity but contain very low levels of Rubisco and consequently limited carbon metabolism through Calvin cycle (Raghavendra and Vani, 1989; Parvathi and Raghavendra, 1995). Despite the limited CO_2 fixation in guard cells, the reduced equivalents produced by their chloroplasts are exported to the cytosol through OAA-malate or PGA-DHAP shuttles (Shimazaki et al., 1989). The reduced pyridine nucleotides formed in the cytosol from the oxidation of malate and/or DHAP may act as the respiratory substrates for mitochondrial ATP production needed for K^+ uptake. A very strong interaction between respiration and photosynthesis has been shown in guard cell protoplasts of *Vicia faba* and *Brassica napus* at varying O_2 concentrations (Mawson, 1993). A strong cooperation between chloroplasts and mitochondria appears to be essential for the maintenance of guard-cell bioenergetic processes.

A similar situation appears to operate in two mutants of *Chlamydomonas reinhardtii*, one devoid of Rubisco and the other lacking functional chloroplast ATP synthase. The *C. reinhardtii* mutant FUD50 lacks the β-subunit of chloroplast ATP synthase and cannot produce ATP during photophosphorylation (Gans and Rébéille, 1988). A modified strain of this mutant FUD50su can grow under photoautotrophic conditions, although it still showed no synthesis of the β-subunit of the coupling factor. Photosynthesis in FUD50su mutant was extremely sensitive to inhibitor antimycin A, a specific inhibitor of mitochondrial electron transport. Photosynthesis in the FUD50su strain is achieved through an unusual interaction between mitochondria and chloroplasts (Lemaire et al., 1988). The export of reduced compounds, made in light, from the chloroplast to the mitochondria elicits ATP formation in the latter, and ATP is subsequently imported to the chloroplast.

III. PROTECTION AGAINST PHOTOINHIBITION

Photoinhibition can be defined as the marked decrease in the photosynthetic rate under supraoptimal light or limitation on CO_2 assimilation. Such situations develop when there is excess light or conditions limiting the biochemical reactions, for example, low temperature, water stress, limiting CO_2, limiting N_2, or any limitation on enzymes. The function of photochemical electron transport is optimized when the reductants generated in light are quickly used up for biochemical reduction of carbon (or nitrogen or sulfur). Any imbalance between the photochemical and biochemical processes leads to the phenomenon of photoinhibition (Long et al., 1994; Andersson and Barber, 1996).

Photoinhibition occurs due to the overreduction of the photosynthetic electron transport system. Reactive oxygen species generated in the light as a result of the Mehler reaction can lead to damage of the photochemical apparatus, particularly PS II. The chloroplasts have the necessary machinery to repair the damage caused to PSII (Carpentier, 1997; Critchley, 1998; Ohad et al., 2000). The recovery is accomplished by a continuous synthesis of PSII components, particularly D1 protein. However, such recovery is optimal under very low light and is rather slow at moderate light intensities (Park et al., 1996; Singh et al., 1996; Anderson, 2001). Thus, photoinhibition of photosynthesis sets in when the rate of damage exceeds that of repair (Long et al., 1994; Critchley, 1998).

Plants have evolved in different ways to cope with photoinhibition by preventive as well as repair mechanisms. Some of them are adjustment of chloroplast antennae size, xanthophyll cycle, CO_2 fixation, photorespiration, water-water cycle, PS I cyclic electron transport, scavenging reactive molecules through antioxidant enzymes, rapid turnover of D1 protein of PS II (Niyogi, 1999). However, in the past decade there has been convincing evidence to show that mitochondrial respiration, especially oxidative electron transport and phosphorylation, play a significant role in protecting the chloroplasts from photoinhibition (Saradadevi and Raghavendra, 1992; Shyam et al., 1993; Raghavendra et al., 1994; Singh et al., 1996; Padmasree and Raghavendra, 1998; Atkin et al., 2000b). The protection of chloroplast photosynthetic machinery against photoinhibition is accomplished by mitochondria through not only the oxidative electron transport/oxidative phosphorylation but also through the key photorespiratory reactions.

A. Oxidative Electron Transport and Oxidative Phosphorylation

Even at very low concentrations, antimycin A or sodium azide or oligomycin enhanced markedly the extent of photoinhibition in mesophyll protoplasts of pea (Saradadevi and Raghavendra, 1992). These inhibitiors at such low concentrations did not affect photosynthesis directly. Sodium fluoride (inhibitor of glycolysis) or sodium malonate (inhibitor of TCA cycle) did not significantly affect photoinhibition (Table 4). Apparently, oxidative electron transport and phosphorylation play a major role in protecting photosynthesis aganist photoinhibition.

After an initial increase, dark respiration decreases significantly after prolonged exposure to photoinhibitory light in pea mesophyll protoplasts (Saradadevi and Raghavendra, 1992) or algal cells of *Anacystis nidulans* and *Chlamydomonas reinhardtii* (Shyam et al., 1993; Singh et al., 1996). These observations indicate a marked correlation between chloroplast and mitochondrial activity during even photoinhibition. The initial increase might represent an enhanced oxidation of excess redox equivalents generated by chloroplast under high light. A subsequent decrease is probably due to the reduced flux of redox equivalents from chloroplasts, which are now photoinhibited.

The initial increase in respiration occurred even in the presence of KCN in

TABLE 4
A Comparison of the Effect of Five Metabolic Inhibitors on Respiration, Photosynthesis, and Photoinhibition in Mesophyll Protoplasts of Pea. The Protoplasts Were Examined for Their Photosynthetic Activity After a Preincubation (With or Without Inhibitors) for 10 min at 30°C in Either Darkness or Photoinhibitory Light (Adapted from Saradadevi and Raghavendra, 1992)

Respiratory Inhibitor	Respiratory Rate	Photosynthetic Rate after Preincubation: Dark	Photosynthetic Rate after Preincubation: Photoinhibitory light	Effect on Photosynthesis	Extent of Photo-inhibition of photosynthesis
	µmol O_2 uptake/evolution mg^{-1} Chl h^{-1}			% of control	% of respective dark treatment
None (control)	8.4	122	78	100	36
10 mM Sodium fluoride	5.9	117	70	96	40
10 mM Sodium malonate	5.0	120	69	98	42
1 µM Antimycin A	6.0	100	38	82	62
1 µM Sodium azide	4.5	113	38	93	66
1 µg mL^{-1} Oligomycin	5.1	105	48	80	54

Chlamydomonas reinhardtii (Singh et al., 1996), implying that it mostly represents the activity of the alternative pathway (Singh et al., 1996). The alternative pathway of mitochondrial oxidative electron transport is a potential channel for "overflow" and dissipation of excess photosynthetic reductants (Lambers, 1982; Millar and Day, 1997). Alternative pathway therefore is likely to play a significant part in preventing the overreduction of chloroplast photosynthetic apparatus and to alleviate photoinhibition. Further experiments are needed to confirm the role of the alternative pathway of mitochondrial oxidative electron transport in protection aganist photoinhibition.

B. Sustenance of Repair Mechanism by Mitochondrial ATP

Photoinhibition is often a result of imbalance between the synthesis and degradation of D1 protein. Supraoptimal light accelerates the degradation while slowing down the process of synthesis of D1 protein and subsequent recovery (Figure 6). In the cyanobacterium *Anacystis nidulans* and green alga *Chlamydomonas reinhardtii*, inhibition of dark respiration by NaN_3 or KCN not only increased photoinhibition but also accelerated photoinhibition (Shyam et al., 1993; Singh et al., 1996). The uncoupler FCCP also had a similar effect of intensifiyng and hastening photoinhibition in both the organisms (Shyam et al., 1993; Singh et al., 1996).

In algal cells, mitochondrial respiration may help in even the recovery of photosynthesis after photoinhibition. Treatment with sodium azide or FCCP slowed down recovery in *Anacystis nidulans* (Shyam et al., 1993). Similarly, the use of KCN and FCCP impaired the reactivation of photosynthesis in *Chlamydomonas reinhardtii* (Singh et al., 1996). The above results imply that the process of recovery that involves synthesis of D1 protein is helped by mitochondrial oxidative phosphorylation (Singh et al., 1996).

C. Photorespiratory Reactions

In C_3 plants, photorespiration helps in reducing/preventing the damage caused by supraoptimal light. A classic and convincing demonstration of such a role is provided by transgenic tobacco plants with altered GS activity. The transgenics with increased GS_2 (a key photorespiratory enzyme) activity had higher photorespiratory rates and more tolerance to supraoptimal light than the wild-type plants. On the other hand, those with reduced GS_2 were low on photorespiration and were sensitive to high light (Kozaki and Takeba, 1996).

There are two possible ways by which photorespiration could help prevent photoinhibition under excess light and limited CO_2 assimilation: (1) by using up the reducing power generated by photochemical reactions in chloroplasts and (2) maintaining the optimal Pi levels in chloroplasts. When stomata are closed (e.g., drought) or when CO_2 is limiting, disturbance in the levels of NADPH and ATP is prevented by regulatory mechanisms, which include photosynthetic control and photorespiratory glycolate metabolism (Osmond et al., 1997). Photorespiration was shown to be essential and even more important than the Mehler or Asada reactions in preventing photoinactivation of photosynthesis in *Chenopodium bonus-henricus* (Heber et al., 1996).

When protoplasts of barley mutants with reduced glycine decarboxylation were incubated in limiting CO_2, their chloroplasts had

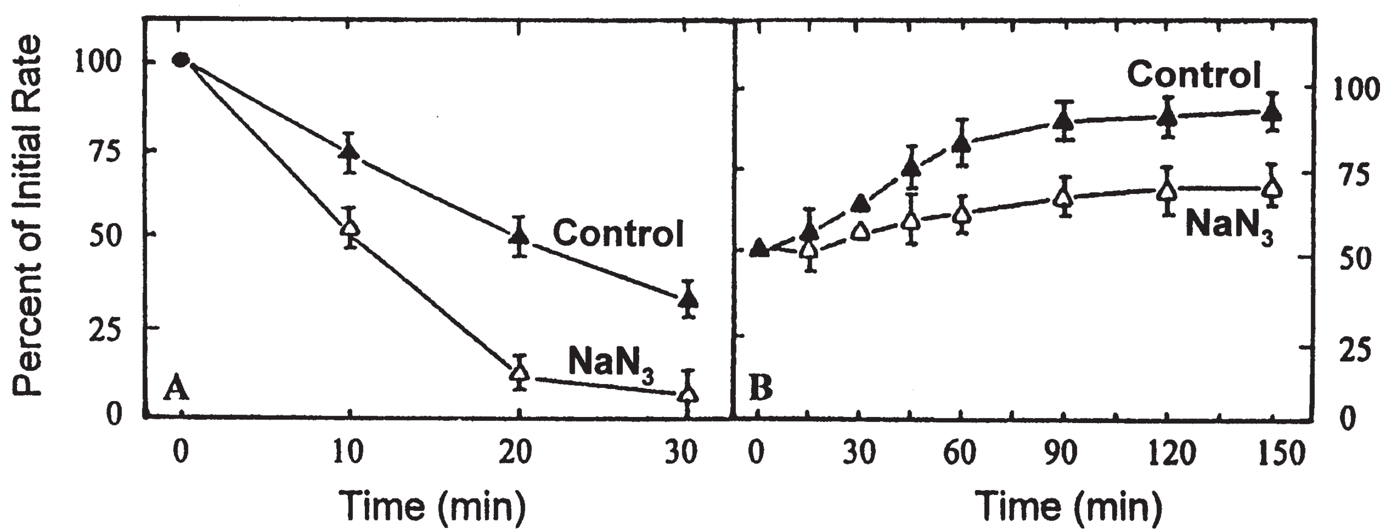

FIGURE 6. Importance of mitochondrial oxidative electron transport for photosynthesis during photoinhibition or recovery after photoinhibition in *Anacystis nidulans.* (A) Effect of photoinhibitory light (2500 µmol m^{-2} s^{-1}) on photosynthesis in the absence (▲) or presence (Δ) of 1 m*M* sodium azide. (B) Reactivation of photosynthesis from photoinhibition in the absence or presence of 1 m*M* sodium azide. 50% photoinhibited cultures were used to study the reactivation of photosynthesis from under dim light. (Modified from Shyam et al., 1993.)

high ratios of ATP/ADP and NADPH/NADP (Igamberdiev et al., 2001). This indicates that glycine decarboxylation and associated NH_4^+ recycling are sinks for excess chloroplastic reductants and help to prevent a overreduction of the chloroplast. The GDC-deficient barley mutants also showed significant increase in the activity of malate valve and chloroplastic NADP-MDH, apart from an increase in the activity of NAD-MDH of cytosol and mitochondria. Obviously, the malate shuttle compensates for the decreased glycine decarboxylation by dissipating the excess reducing equivalents (Gardeström et al., 2001).

In cotton leaves, maintained at low O_2 concentration, a nonphotorespiratory condition, photosynthesis, was severely inhibited under strong light, compared with the ones kept at normal O_2 levels. The low O_2 samples also had reduced levels of chloroplastic Pi. When Pi was fed to these leaves, the rates of photosynthesis were restored to the levels of those kept in normal air containing 21% O_2. This indicates that Pi limitation could partly be alleviated by photorespiratory recycling of Pi. Thus, photorespiration reduces photoinhibition by keeping up rates of photosynthesis through making Pi available for the process (Guo et al., 1995).

Mitochondria, being major players in the photorespiratory pathway, have to interact with chloroplasts and peroxisomes and take part in balancing the photosynthetic redox equivalents and protection against photoinhibition.

D. Significance Under Temperature or Water Stress

In addition to protection from photoinhibition, mitochondria also help to optimize photosynthesis under stress conditions like chilling temperature or osmotic stress (Table 5). After a period of cold hardening, the leaves of winter rye exhibited an increase in the rates of dark respiration in light along with those of photosynthesis (Hurry et al., 1995). Oligomycin treatment resulted in the inhibition of photosynthesis more in cold hardened leaves than that in nonhardened ones, suggesting that the increase in photosynthetic capacity following cold hardening is contributed to by mitochondria. A similar situation of increased tolerance to photoinhibition following cold hardening has been reported in the leaves of winter and spring wheat (Hurry and Huner, 1992).

Circumstantial evidence points out to the possible roles of AOX during the maintenance of photosynthesis in low temperature. The level of alternative oxidase protein in tobacco (Vanlerberghe and McIntosh, 1992) as well as the capacity of alternative respiration (Rychter et al., 1988) usually increase at low temperatures. The extent of electron partitioning to the alternative oxidase raises significantly at low temperatures in cold grown mung bean (Gonzàlez-Meler et al., 1999). These results indicate a role for alternative respiratory pathway in protecting the plant tissues from chilling and related photoinhibition and suggest a general increase in alternative respiration under stress conditions.

However, Ribas-Carbo et al. (2000) have found increased electron flow in the alternative pathway following cold treatment in a chilling sensitive cultivar of maize compared with a chilling tolerant one, indicating no specific role for the alternative pathway of respiration in conferring chilling tolerance. Similarly, no specific increase in alternative respiration ocurred following chilling in soybean cotyledons (Gonzàlez-Meler et al., 1999). Further studies and direct evidence are needed to assign any direct role of alternative pathway in the

TABLE 5
Correlation Between the Pattern of Changes in Respiration and the Extent of Photoinhibition in Different Plant Tissues in Presence of Mitochondrial Inhibitors or Stress Conditions. Any Decrease in Respiratory Activity Leads to an Increase in the Extent of Photoinhibition, While an Increase in Respiration Helps to Decrease the Photoinhibition

Plant material/ system	Treatment/ stress	Response		References
		Respiratory O_2 uptake	Photoinhibition of photosynthesis	
Mesophyll Protoplasts				
Pisum sativum	Antimycin A, NaN_3, Oligomycin	Decrease	Increase in susceptibility	Saradadevi and Raghavendra 1992
Pisum sativum	Hyperosmotic medium	Decrease	Increase in susceptibility	Saradadevi and Raghavendra, 1994
Algal cells				
Anacystis nidulans	NaN_3	Decrease	Increase in susceptibility & decrease in reactivation after photodamage	Shyam et al., 1993
Chlamydomonas reinhardtii	KCN	Decrease	Decrease in reactivation after photodamage	Singh et al., 1996
Leaves				
Cereals	Cold hardening	Increase	Decrease in susceptibility	Hurry and Huner 1992; Hurry et al., 1995

protection of photosynthesis from chilling stress in plants.

Mitochondrial respiration seems to be related to decreased photosynthesis and increased susceptability to photoinhibition under osmotic stress. Mesophyll protoplasts of pea kept in hyperosmotic medium were highly susceptable to photoinhibition when they were exposed to photoinhibitory light. On exposure to hyperosmotic medium at 0°C, both photosynthetic and respiratory rates decreased, indicating a correlation between the two processes (Saradadevi and Raghavendra, 1994). However, at 25°C, respiration increased, while photosynthesis decreased. More experiments are needed to understand the role of respiration *vis-a-vis* photosynthesis during osmotic stress under varying temperatures.

IV. OPTIMIZATION OF PHOTOSYNTHETIC CARBON ASSIMILATION

The optimization of photosynthetic carbon assimilation requires a coordination of different components: generation and use of assimilatory power (ATP and NADPH), induction of photosynthesis, activation of enzymes, and maintenance of metabolite levels. In a photosynthesizing cell the mitochondrial respiratory system may benefit different components of chloroplast photosynthesis by modulating any of the above components. However, emphasis has already been made on the significant role of mitochondria in maintaining either cytosolic redox status or ATP or both (Krömer et al., 1988; Krömer and Heldt, 1991a; Krömer et al., 1993; Raghavendra et al., 1994; Gardeström and Lernmark, 1995; Krömer, 1995; Igamberdiev et al., 1998; Padmasree and Raghavendra, 1998).

A. Sustenance of Sucrose Biosynthesis: Role of ATP

The biosynthesis of sucrose, one of the major end products of photosynthetic carbon assimilation, occurs in the cytosol of mesophyll cells. Sucrose biosynthesis in the cytosol requires a continuous supply of carbon skeletons and energy. Although it is obvious that chloroplasts play a significant role in supplying the carbon compounds for the synthesis of sucrose, the relative importance of mitochondria in meeting the cytosolic demands for ATP, particularly during sucrose formation is emphasized only during the last decade (Raghavendra et al., 1994; Gardeström and Lernmark, 1995; Krömer, 1995; Hoefnagel et al., 1998; Padmasree and Raghavendra, 1998; Atkin et al., 2000b). Studies with a starchless mutant NS 458 of *Nicotiana tabacum* (defective in plastid phosphoglucomutase) in the presence of oligomycin also suggested that the mitochondrial supply of ATP could affect assimilate partitioning into sucrose and thereby modulate photosynthesis (Hanson, 1992).

The transfer of redox equivalents generated during the oxidation of TCA cycle intermediates along the mitochondrial electron transport chain accumulate significant amounts of ATP in the mitochondrial matrix. Mitochondria have a very high capacity for ATP synthesis, in fact higher than that of chloroplasts, producing up to 3 ATP per NAD(P)H compared with 1.5 to 2.0 ATP per NAD(P)H in the chloroplast (Hoefnagel et al., 1998; Siedow and Day, 2000). It is possible that the ATP pools generated in the mitochondrial matrix are translocated to cytosol (through adenylate translocator) to be used in sucrose synthesis or even imported into chloroplasts to be used in various other biosynthetic processes like protein synthesis, NH_4^+ assimilation, metabolite transport, and maintenance (Hoefnagel et al., 1998).

It is possible in light that mitochondrial respiration is subjected to adenylate control (Hoefnagel et al., 1998). However, the ability of plant mitochondria to switch between the rotenone-sensitive and rotenone-insensitive as well as the cyanide-sensitive cytochrome pathway and cyanide-resistant alternative pathways provides for a flexible system and ATP production. However, the degree to which mitochondrial ATP supply in the light required for optimal photosynthesis depends on the balance of ATP production and consumption in chloroplasts. Two key observations indicate the primary role of mitochondria in assisting chloroplasts in meeting the cytosolic demands of ATP for sucrose synthesis: (1) An increase in cytosolic or cellular levels of glucose-6-P and other phosphates (e.g., fructose-6-P and fructose-2,6-bisphosphate) in the presence of oligomycin or antimycin A (Krömer and Heldt, 1991a; Krömer et al., 1993, Padmasree and Raghavendra, 1999c). Subcellular analysis of protoplasts revealed that the increase in hexose monophosphates was mostly in the cytosol, demonstrating the restriction of sucrose biosynthesis (Krömer et al., 1992); (2) Restriction of mitochondrial ATP synthesis by oligomycin or antimycin A or photorespiratory glycine oxidation using AAN in isolated protoplasts caused a marked reduction in ATP/ADP ratios in the cytosolic and mitochondrial compartments than that of chloroplasts (Gardeström et al., 1981; Gardeström and Wigge, 1988; Krömer and Heldt, 1991a; Krömer et al., 1993; Igamberdiev et al., 1998).

The change in the levels of intracellular ATP and ADP during illumination caused by mitochondrial inhibitors at limiting CO_2 was in contrast to that of photosynthesis. Despite the expectation that ATP demands would be low at limiting CO_2, there was a steep positive correlation between the rates of photosynthesis and ratios of ATP to ADP in protoplasts in the presence of oligomycin or antimycin A but not SHAM (Figure 7). The Glc-6-P level increased by about 19 to 30% in the presence of both oligomycin and antimycin A at optimal CO_2 conditions. The marked increase in Glc-6-P in mesophyll protoplasts in the presence of only oligomycin or antimycin A but not SHAM suggests that the cytochrome pathway of electron transport (and oxidative phosphorylation) modulates sucrose biosynthesis, while the alternative pathway may not have a significant role (Padmasree and Raghavendra, 1999a).

The restriction of mitochondrial ATP synthesis by oligomycin and antimycin A would not only limit sucrose synthesis but also cause feedback inhibition of photosynthetic activity because the phosphate translocator in the inner chloroplast membrane is regulated by the equilibrium of the triose-P concentration in the stroma and the cytosol. However, an elevated cytosolic level of DHAP or reduced flux of DHAP from chloroplast can also lead to decreased stromal PGA level and thereby decreased Calvin cycle activity (Krömer et al., 1993,

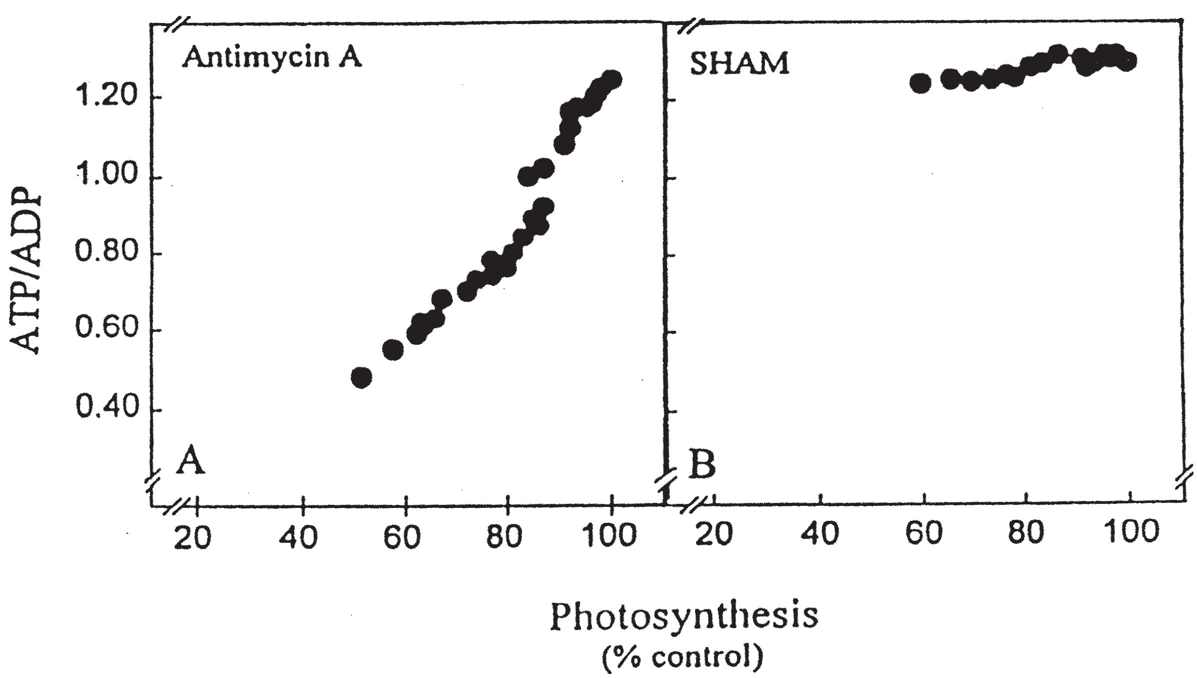

FIGURE 7. Positive correlation occurs between the ratios of ATP/ADP and the relative rates of photosynthesis in pea mesophyll protoplasts in presence of antimycin A (inhibitor of cytochrome pathway in mitochondria) but not SHAM, (inhibitor of alternative pathway). In other words, SHAM which markedly inhibits photosynthesis of protoplasts, does not alter the relative ratio of ATP/ADP. (Adapted from Padmasree and Raghavendra, 1999a.)

Padmasree and Raghavendra, 1999a; Flügge and Heldt, 1991). Thus, mitochondrial oxidative electron transport plays a significant role in optimizing photosynthetic carbon assimilation by sustaining sucrose biosynthesis. The flexibility of mitochondrial electron transport chain to meet cytosolic demands under both dark and light conditions makes it a ready source of energy to meet cellular needs supplementing the chloroplast metabolism.

B. Maintenance of Cellular Redox State: Ratios of Malate/OAA and Triose-P/PGA

Chloroplasts always have a tendency to get overreduced as the rate of photochemical reaction and utilization of reducing potential in metabolism have been estimated to differ by at least 15 orders of magnitude (Huner et al., 1998). It is essential that the excess reduced equivalents are taken out or disspiated quickly to prevent damage to the thylakoid membranes (Gillmore, 1997; Niyogi et al., 1998; Niyogi, 1999).

Mitochondria also appear to play a significant role in maintaining optimal levels of redox equivalents in the chloroplasts to keep up the Calvin cycle activity, possibly by coordinating with peroxisomes and cytosol. The reductants in excess of the requirements of the Calvin cycle are exported out of chloroplasts through the shuttling of either OAA-malate (by dicarboxylate translocator) or PGA-DHAP (Pi-translocator).

The relative levels of triose-P/PGA and malate/OAA reflect the redox state of cytosol and the cell. Mitochondrial electron transport appears to be one of the efficient processes to use up the reduced equivalents. Any limitation on the mitochondrial metabolism leads to a marked rise in the redox state of cells, as indicated by the rise in the ratios of malate/OAA or triose-P/PGA (Padmasree and Raghavendra, 1999c).

The steep gradient in redox levels between stromal compartment and cytosol is maintained by regulation at several steps such as (1) chloroplastic NADP-MDH, (2) triose-P/Pi translocator, (3) glycolate/glycerate translocator, and (4) glycine/serine translocator (Gardeström et al., 2001). Among these, NADP-MDH functions like a valve, releasing the excess reductant from chloroplasts as malate (Scheibe, 1991). Malate valve allows chloroplasts also to provide reducing equivalents either to peroxisomes for reduction of hydroxypyruvate (under photorespiratory conditions, Krömer, 1995) or mitochondria to be oxidized by the internal NADH-dehydrogenase system (under nonphotorespiratory conditions; Padmasree and Raghavendra, 1998). This would still allow some of the NADH formed during glycine decarboxylation to be retained in the mitochondria, rather than shuttling it to the peroxisome to support hydroxypyruvate reduction. As a result, NADH can be oxidized within the mitochondria to provide additional ATP for extrachloroplastic processes, such as sucrose synthesis or reduction of PGA in the cytosol (Krömer and Heldt, 1991a,b).

The operation of cyanide-resistant alternative and cyanide-sensitive cytochrome pathways of mitochondria appear to be closely integrated with the redox regulation during photosynthetic metabolism (Padmasree and Raghavendra, 1999c). Restriction of a cyanide-resistant pathway by SHAM markedly elevated the malate/OAA ratios, while the restriction of cyanide-sensitive pathway by antimycin A or oligomycin lead to a marked increase in triose-P/PGA ratios (Figure 8). Because an accumulation of malate represents an overreduction of chloroplasts (Backhausen et al., 1994), the marked increase in malate/OAA in the presence of SHAM suggests an accumulation of redox power in protoplasts when AOX pathway is

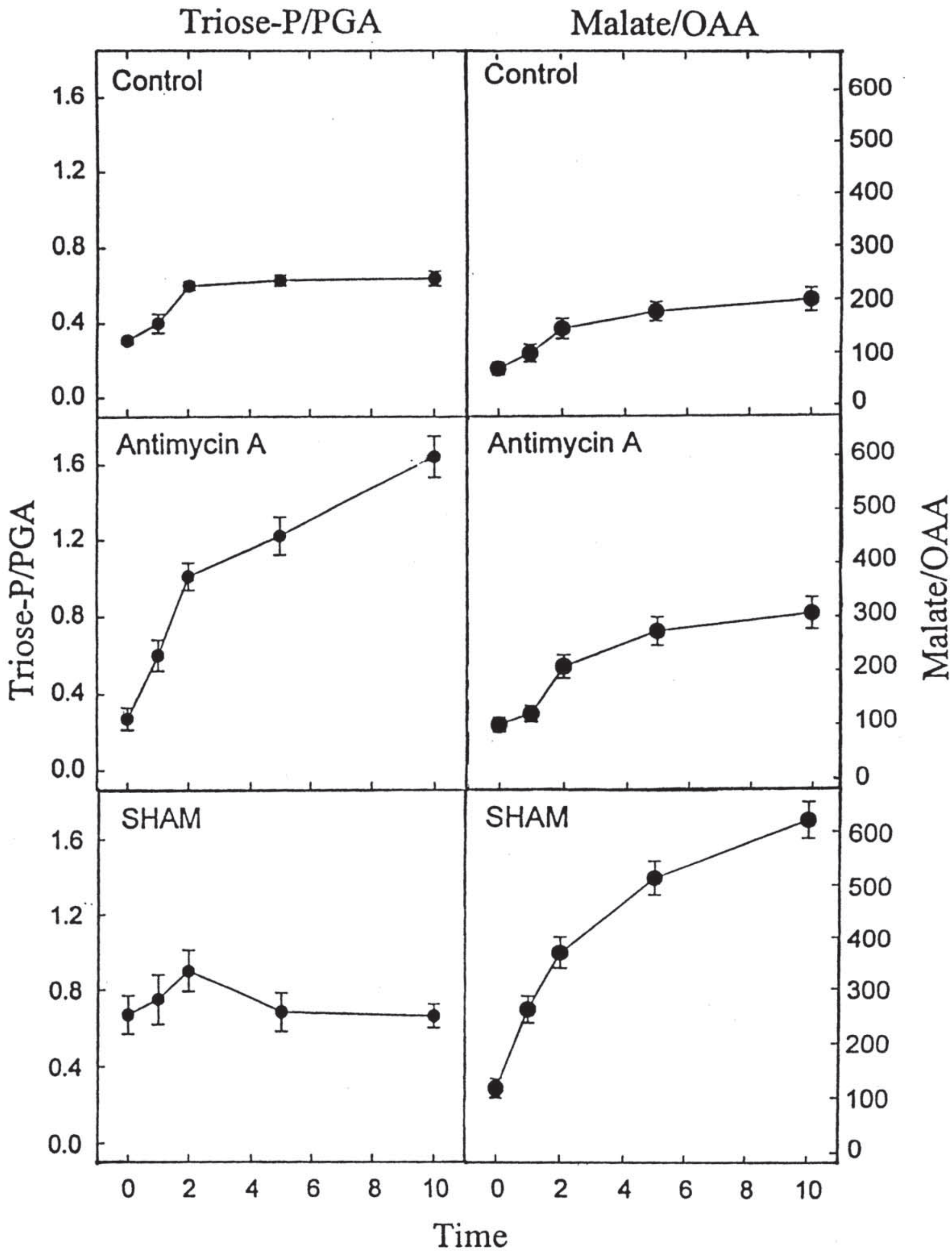

FIGURE 8. Changes in the redox state of pea mesophyll protoplasts during photosynthesis in the absence or presence of typical inhibitors of mitochondrial electron transport. On illumination in presence of 1.0 m*M* bicarbonate, the ratios of Triose-P/PGA or Malate/OAA rise with time indicating the increase in the redox state of protoplasts. The presence of 250 n*M* antimycin A (inhibitor of cytochrome pathway) results in the preferential accumulation of triose-P, while the presence of 200 μ*M* SHAM (inhibitor of alternative pathway of mitochondrial electron transport) causes the accumulation of malate. (Adapted from Padmasree and Raghavendra, 1999c.)

restricted. Thus, AOX appears to promote the consumption of malate in pea mesophyll protoplasts.

C. Shortening of Induction

The phenomenon of induction (delay in achieving maximal rates) is a common feature of photosynthesis (Edwards and Walker, 1983; Walker, 1988). Among the most important factors that cause photosynthetic induction are the activation of key chloroplastic enzymes (including NADP-malate dehydrogenase, NADP-glyceraldehyde 3-phosphate dehydrogenase, stromal FBPase, PRK) and the autocatalytic build-up of Calvin cycle metabolites, for example, RuBP (Salvucci, 1989; Scheibe, 1991; Edwards and Walker, 1983).

Mitochondrial contribution to photosynthetic metabolism during photosynthetic induction was investigated in mesophyll protoplasts from barley or pea leaves by using rotenone or oligomycin (Table 6). Both the inhibitors increased the lag phase of photosynthetic induction during the transition of protoplasts from darkness to light (Igamberdiev et al., 1998). Prolongation of photosynthetic induction period was observed also with antimycin A, SHAM, and propyl gallate (Figure 9). However, SHAM and propyl gallate (inhibitors of alternative pathway) had a negligible effect on the photosynthetic induction period (Padmasree and Raghavendra, 1999b).

TABLE 6
Prolongation of Photosynthetic Induction in Mesophyll Protoplasts as a Consequence of Restriction of Mitochondrial Metabolism. The Lag Period for Reaching the Maximum Rate of Photosynthetic Carbon Assimilation (in Presence of 1.0 m*M* Bicarbonate) is Extended by the Presence of Mitochondrial Inhibitors

Plant Material*	Mitochondrial inhibitor	Lag Period (minutes)	References
Barley	None (Control)	2	Igamberdiev et al., 1998
	0.1 μM Oligomycin	4	-do-
	20 μM Rotenone	3.25	-do-
Pea	None (Control)	3	Padmasree and Raghavendra, 1999b
	1 μg ml^{-1} Oligomycin	8	-do-
	1 μM antimycin A	5	-do-
	1 mM SHAM	3.5	-do-

* Mesophyll protoplasts .

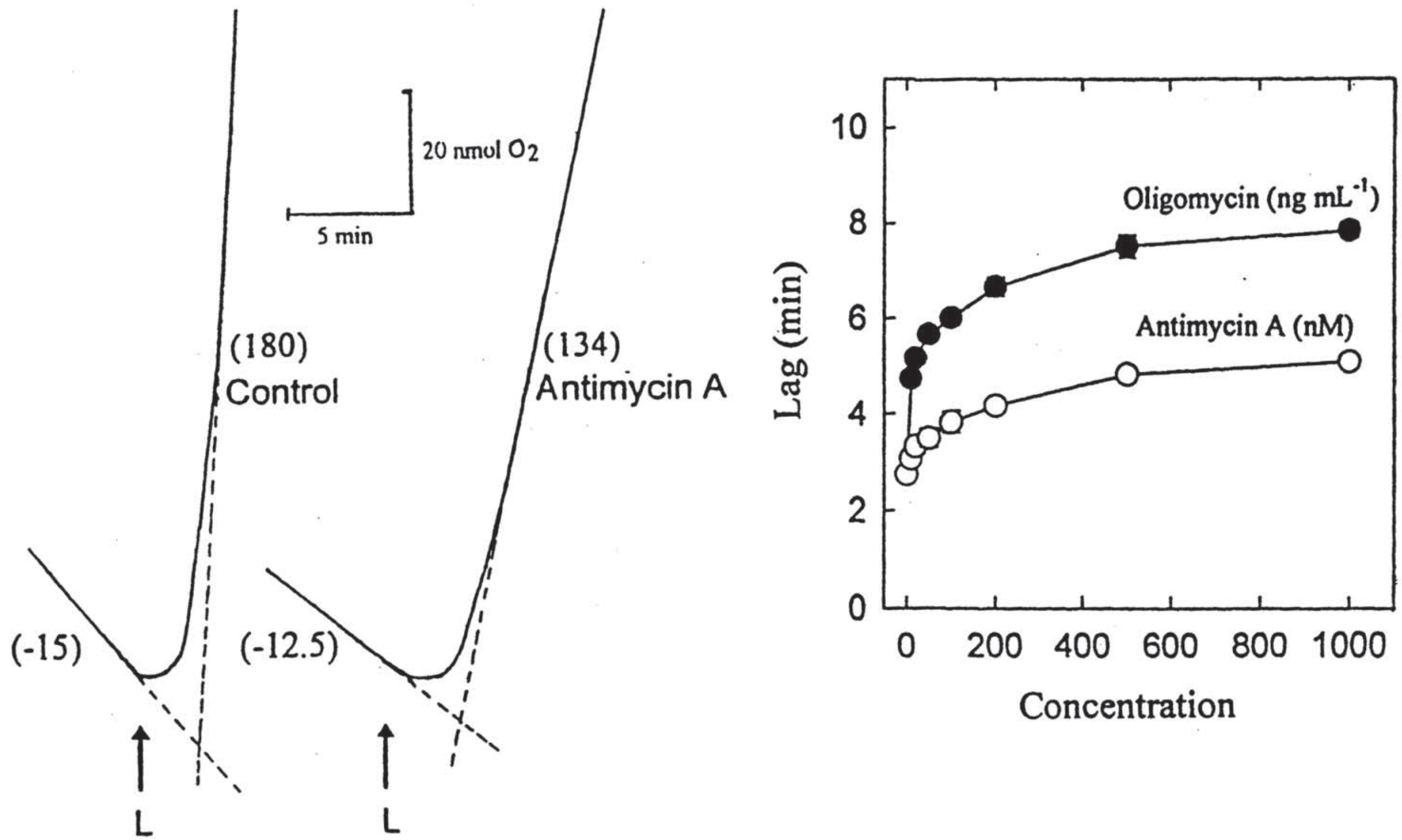

FIGURE 9. Prolongation of photosynthetic induction in presence of typical inhibitors of mitochondrial transport. The lag period for reaching the maximum rate of photosynthesis after switching on the light (indicated by ‘L’) is usually less than 3 min, while this lag increases to almost 5 or 8 min in presence of oligomycin (1 μg ml^{-1}) or antimycin A (1 μ*M*). The exact reasons for such marked increase in the photosynthetic induction period are not clear. It could be also one of the consequences of depression in carbon assimilation by mitochondrial inhibitors. (Adapted from Padmasree and Raghavendra, 1999b.)

Despite the apparent surplus of ATP in the chloroplast, the demands for ATP during the initial phase of photosynthetic induction are met by mitochondrial oxidative phosphorylation. The delay by rotenone and oligomycin in photosynthetic induction appears to be caused by a restriction on the reoxidation of redox equivalents from the chloroplasts (and associated reactions) by the mitochondrial electron transport chain (Igamberdiev et al., 1998). This hypothesis may indeed be complemented by the negligible changes in the RuBP levels associated with prolonged induction period in the presence of oligomycin and antimycin A in pea leaves (Padmasree and Raghavendra, 1999b). The marked sensitivity of photosynthetic induction period to rotenone or antimycin A suggests that the redox equivalents from chloroplasts are being oxidized by internal dehydrogenase via complex I and complex III, but not through the rotenone-insensitive dehydrogenases. One of the benefits of the mitochondrial oxidative electron transport coupled to oxidative phosphorylation is the maintenance or minimization of the induction phase of photosynthesis. The negligible effect on photosynthetic induction by SHAM or propyl gallate compared with the marked delay by antimycin A or oligomycin suggests that electron transport via alternative oxidase may not be as significant as that of cytochrome pathway during photosynthetic induction.

Thus, the restriction of mitochondrial activity leads to an increase in the photosynthetic induction period in mesophyll protoplasts of pea as well as barley (Igamberdiev et al., 1998; Padmasree and Raghavendra, 1999b). However, this correlation may be incidental because there is no direct evidence to suggest that restricted mitochondrial metabolism is the cause for the prolongation of photosynthetic induction.

D. Activation of Enzymes

When leaves are illuminated, the activation state/activity of not only chloroplastic enzymes but of several enzymes located in different compartments of the cell is stimulated. In this review, attention is drawn to the enzymes involved in coordinating the interactions between chloroplasts, mitochondria, peroxisomes, and cytosol. These enzymes located in different compartments of the cell are fine tuned and coordinated with each other by specific metabolites.

The light activation of photosynthetic enzymes located in stroma is regulated by several factors: ferredoxin-thioredoxin system, metabolite levels, pH, ionic status, and oxidation-reduction potential (Scheibe, 1990; Faske et al., 1995). Rotenone and oligomycin both delayed the activation of chloroplastic NADP-MDH during the transition from darkness to light (Igamberdiev et al., 1998). The timing of the delay in activation of NADP-MDH is very similar to the delay in photosynthetic O_2 production and the delay in build-up of nonphotochemical quenching. Restriction of mitochondrial electron transport delays alkalization of the chloroplast stroma, which in turn delays the activation of NADP-MDH and there by the export (and thus use) of redox equivalents (Igamberdiev et al., 1998). A marked decrease in light activation of not only NADP-MDH, but also FBPase, NADP-GAPDH, and PRK in presence of SHAM during steady state photosynthesis indicates that the alternative pathway may play a significant role in maintaining the activation status of chloroplastic enzymes (Padmasree and Raghavendra, 2001a). This is possible by regulating the redox equivalents through malate-OAA shuttle.

Apart from chloroplastic enzymes, cytosolic enzymes can also be regulated by light. One of such is sucrose phosphate synthase (SPS), which plays a significant role is sucrose biosynthesis. SPS is subjected to reversible phosphorylation-dephosphorylation cascade. The dephosphorylated enzyme is more active than the phosphorylated from (Huber and Huber, 1996). At high CO_2, the decrease in the mitochondrial and cytosolic ATP/ADP ratios caused by the oligomycin treatment at high and low irradiance can lead to a decrease in SPS activity (Krömer et al., 1993). Under high CO_2, this inhibition of sucrose synthesis by oligomycin apparently increased cytosolic Glc-6-P levels and caused feedback inhibition of the Calvin cycle and photosynthetic activity.

E. Integration with Photorespiration and Nitrogen Metabolism

The interaction of mitochondrial respiration, nitrogen metabolism, and photosynthesis has been the subject of two extensive reviews (Padmasree and Raghavendra, 1998; Gardestrom et al., 2001). The interplay of these three metabolic pathways involves recycling of carbon, nitrogen, and marked amounts of reduced equivalents and some ATP. The rapidity in the turnover (production and consumption) of NAD[P]H, reduced ferredoxin and ATP allows them to coordinate at least four different metabolic pathways viz., photosynthesis, respiration, photorespiration, and nitrogen metabolism. Apart from CO_2 fixation, the second largest sink for photosynthetic energy in many higher plants is nitrate. As the assimilation of nitrate in many species occurs predominantly in the leaves, this process will often be ongoing simultaneously with CO_2 fixation in photosynthetic cells (Noctor and Foyer, 1998).

Three processes related to photorespiration and nitrogen metabolism form important links between chloroplasts, mitochondria, and peroxisomes. These are (1) glycine oxidation; (2) reductive amination of oxoglutarate, and (3) hydroxypyruvate reduction. These three processes are highly coupled, and modulation of any one of them leads to a cascading effect on the other.

Glycine is formed in peroxisomes and oxidized in mitochondria. The precursor of glycine is glycolate from chloroplasts. Glycine oxidation yields considerable amounts of NADH and NH_4, besides CO_2. The resulting NADH is either used up for ATP production or exported out in the form of malate to meet the requirements of hydroxypyruvate reduction in peroxisomes (Heldt et al., 1998; Raghavendra et al., 1998).

Oxidation of photorespiratory glycine is coupled to hydroxypyruvate reduction (Hanning and Heldt, 1993; Heldt et al., 1998; Raghavendra et al., 1998). Any uncoupling of glycine oxidation and hydroxypyruvate reduction would imply a huge photorespiratory production of ATP, particularly in the mitochondria. If metabolic conditions demand, the extra NADH is used also for nitrate reduction in cytosol. In algae, Weger et al. (1988) have shown that high rates of NH_4^+ assimilation are associated with a marked increase in cyanide-sensitive O_2 uptake.

The NADH generated by the glycine decarboxylase reaction is expected to account only for 50% of the reducing power used in a subsequent reduction of the photorespiratory hydroxypyruvate in peroxisomes (Krömer and Heldt, 1991b). The remainder of the reducing power is provided by photosynthetic processes in the chloroplast (Krömer and Heldt, 1991b; Igamberdiev and Kleczkowski, 1997). Glycine oxidation can also increase the intramitochondrial and cytosolic ATP/ADP ratio (Gardeström and Wigge, 1988); therefore, the mitochondrial respiratory chain can play a role in the cellular ATP production in the light. Glycine oxidation in mitochondria of photosynthetic tissue in leaves of wheat and maize are coupled in more degree with cyanide-resistant and rotenone-resistant paths of electron transport contrary to etiolated leaves, where these pathways are involved to a much less extent (Igamberdiev and Kleczkowski, 1997c; Igamberdiev et al., 1998).

Besides CO_2, the other major sinks for reducing equivalents in chloroplasts are the metabolic reactions involving the reduction of nitrogen or sulfur. The reduction of oxoglutarate to glutamate (or nitrite to NH_4^+) occurs exclusively in chloroplasts. The major substrate for these reactions, namely, oxoglutarate is exported from mitochondria, which operate a partial TCA cycle in light (see Section I.D). A continuous supply of oxoglutarate from mitochondria is required for NH_4 assimilation into amino acids in chloroplasts. Similarly, the supply of nitrite to chloroplasts is also dependent on mitochondrial activity, which provides significant amounts of NADH for nitrate reduction in cytoplasm (Weger and Turpin, 1989; Padmasree and Raghavendra, 1998). The required NADH in mitochondria is generated from glycine coming from peroxisomes. Thus, chloroplasts, mitochondria, and peroxisomes have to work together to keep up the reduction of nitrite and reductive amination of oxoglutarate (Figure 10).

Glycine and malate, both of which are formed during active photosynthesis, form the substrates for leaf mitochondrial oxidation *in vivo*. However, the main substrate for mitochondrial respiration in the light is probably glycine, which is produced at high rates during photorespiration. At least 25% of the NADH formed during oxidation of these metabolites is used for extra-mitochondrial requirements, particularly hydroxypyruvate reduction in peroxisomes and NO_3^- reduction in cytosol. The export of reducing equivalents from mitochondria may proceed by either a malate-aspartate shuttle or a malate-OAA shuttle. Chloroplasts form alternative sources of reducing equivalents. Cytosolic nitrate reductase (NR) and peroxisomal hydroxypyruvate reductase can be served via a chloroplastic malate-OAA shuttle with reducing equivalents generated from photosynthetic electron transport (Heupel and Heldt, 1992). Thus, mitochondrial metabolism becomes a very important link among photosynthesis, photorespiration, and nitrogen assimilation in recycling NH_4^+, reduced equivalents, and carbon skeletons (Figure 10).

F. Role in C_4 photosynthesis

Mitochondria play a direct role in carbon metabolism of certain C_4 and CAM plants, particularly those utilizing NAD-malic enzyme or PEP carboxykinase for C_4-acid decarboxylation. In these plants the decarboxylation of malate or aspartate occurs in mitochondria. During this function, mitochondria supply not only the carbon skeletons but also extra ATP needed for C_4 pathway. The photosynthetic rates attained by NAD^+-malic enzyme plants suggest that carbon flux through the bundle sheath mitochondria is 10- to 20-fold greater than the standard respiratory carbon flux, and severalfold greater than the flux of glycine through the mitochondria of C_3 plants during photorespiration. Further, the NADH generated by NAD^+-malic enzyme is utilized also for ATP synthesis. The predicted stoichiometry is about two malate molecules oxidized per five molecules of PEP produced (Siedow and Day, 2000). In PEP carboxykinase type plants, the situation is more complex.

The C_4 plants do not show any photorespiration because the process is confined to bundle sheath cells and any CO_2 released out is refixed efficiently in the surrounding layer of mesophyll cells. Thus in C_4 plants, mitochondrial location of GDC and result-

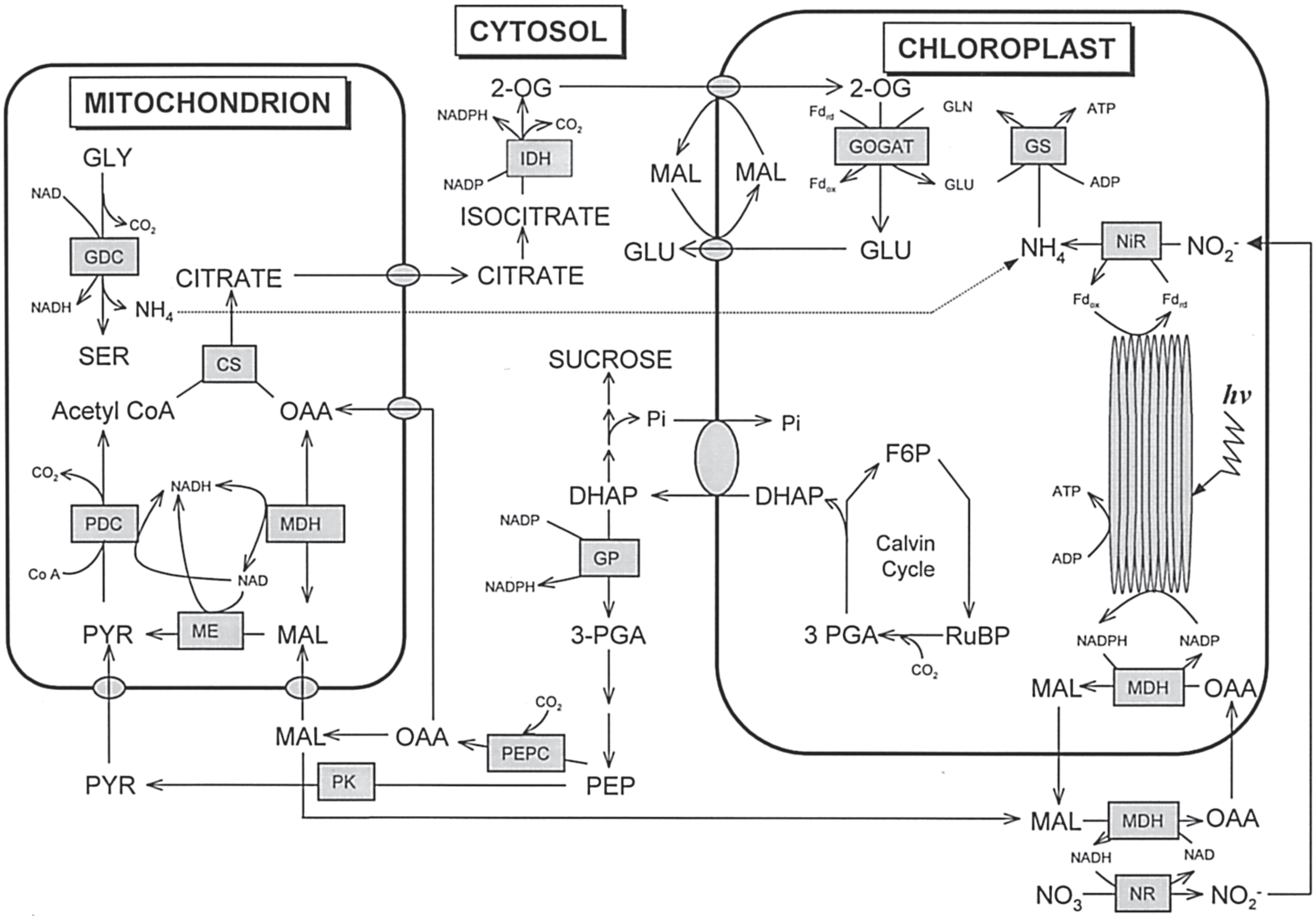
MITOCHONDRION
CYTOSOL
CHLOROPLAST
GLY
NAD
CO_2
GDC
NADH
NH_4
SER
CITRATE
CS
Acetyl CoA
OAA
PDC
Co A
NADH
NAD
MDH
ME
PYR
MAL
2-OG
NADPH
IDH
NADP
ISOCITRATE
SUCROSE
Pi
DHAP
GP
NADPH
3-PGA
PEP
PEPC
PK
GLU
GOGAT
Fd_{rd}
Fd_{ox}
GLN
GS
ATP
ADP
NiR
NO_2^-
F6P
Calvin
Cycle
3 PGA
RuBP
hν
NR
NO_3

FIGURE 10. The interrelation between nitrogen metabolism, chloroplast photosynthetic reactions, and the mitochondrial respiratory activity in plant cells. The initial step of nitrate reduction to nitrite occurs in cytoplasm. The major steps of formation and assimilation of ammonia are located in chloroplasts. The reducing power for nitrogen assimilation is supplied from both chloroplasts and cytosol. The provision of carbon skeletons for ammonia assimilation as well as the recycling of photorespiratory ammonia is facilitated by mitochondria. The key enzymes involved in these processes are CS, Citrate synthase; GDC, Glycine decarboxylase; GOGAT, Glutamate oxoglutarate amino transferase/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, Phosphoenolpyruvate carboxylase; PK, Pyruvate kinase.

ing CO_2 efflux becomes a crucial factor. Only the mitochondria located in bundle sheath cells of C_4 palnts possess GDC, but not those of mesophyll cells. The inter- and intracellular localization of GDC thus facilitates the function of not only C_4 photosynthesis but also C_3-C_4 intermediacy (Devi et al., 1995; Rawsthorne, 1998).

V. BIOCHEMICAL BASIS: INTERORGANELLE INTERACTION

Rapid movement of metabolites occurs between chloroplasts, cytoplasm, mitochondria, and peroxisomes. Such metabolite movement forms an important basis of interorganelle interaction as well as the optimization of different metabolic pathways in a plant cell. Several investigators therefore attempted to study the metabolite patterns as the biochemical basis of essentiality of mitochondrial respiration for photosynthetic carbon assimilation, under varied conditions, for example, limiting or saturating CO_2, variable light intenstity (Krömer et al., 1988; Krömer et al., 1992; Krömer et al., 1993; Igamberdiev et al., 1997a,b, 1998; Padmasree and Raghavendra, 1999a,b,c). These metabolites can be categorized into four groups:

1. Metabolites related to redox status, e.g., malate or triose-P (mainly DHAP)
2. Adenylate compounds such as ATP or ADP
3. Metabolites related to sucrose biosynthesis
4. Intermediates of the Calvin cycle

A. Major Products of Organelle Metabolism

The interaction between the chloroplasts and mitochondria often involves not only cytosol but also peroxisomes. Within the cell, there is always a high demand for ATP and reducing equivalents. The responsibility of meeting the cellular requirements of ATP and NADH is shared by both chloroplasts and mitochondria. The metabolism within chloroplasts, mitochodria, or peroxisomes is optimized only when these organelles are able to export their metabolites and keep up the interorganelle metabolite movement. The export of reduced equivalents from chloroplasts is essential to prevent overreduction of chloroplasts.

1. Chloroplasts

The major products exported from chloroplasts of C_3 plants in light are glycolate, triose-P, and malate. The pattern of export depends on the carboxylase vs. oxygenase activity of Rubisco, which in turn depends on the ambient CO_2. While the carboxylase activity of Rubisco results in the formation of triose-P, the oxygenase activity of Rubisco leads to the formation of glycolate. Because the ratio of oxygenation to carboxylation during photosynthesis in a leaf is 0.2 to 0.5, very high metabolic flux of glycolate occurs through the leaf peroxisomes (Heupel et al., 1991; Reumann et al., 1994). About 50 to 75% of carbon from glycolate is salvaged in a sequence of photorespiratory reactions that involves cooperation between the chloroplasts, the mitochondria, and the peroxisomes.

Part of the triose-P formed by the reduction of 3-PGA in chloroplasts is used up to regenerate RuBP, while the majority is exported out to be utilized for either sucrose synthesis or respiratory glycolytic pathway. Triose-phosphates are exported in exchange for Pi through triose-P-Pi translocator located on the chloroplast inner membrane (Flügge, 1999). Triose-P exported mostly in the form of DHAP to cytosol serves two major purposes: (1) form sucrose, (2) oxidation to PGA releasing ATP and NADH to meet the needs of cytosol.

In light, the export of malate from chloroplasts plays a significant role in the transfer of reducing equivalents formed in excess of those required to operate Calvin cycle. When the NADPH/NADP+ ratio in the chloroplast is high, OAA is converted to malate and exported via the dicarboxylate translocator in the inner envelope membrane of the chloroplasts (Heineke et al., 1991). For the malate/OAA shuttle to operate as an effective NADPH export system, the exported malate must be oxidized to regenerate OAA for transport back to the chloroplast. Thus, malate released into the cytosol is either oxidized in cytosol to support nitrate reduction or transferred to peroxisomes to support hydyroxypyruvate reduction (Atkin et al., 2000b). Under conditions where more reductant is produced than is required for cytosolic and peroxisomal processes, malate can be imported into mitochodria for oxidation and allow ATP synthesis (Hoefnagel et al., 1998).

Among the products of chloroplast metabolism, glycolate is the substrate for photorespiratory metabolism, which helps in the dissipation of excess energy as well as protection against photoinhibition (see Section III.C). Triose-P and malate facilitate export of the reducing power and ATP from chloroplasts and thus act as sinks. At limiting CO_2 malate is the major carrier of reducing equivalents sent out of chloroplasts, while at optimal CO_2, triose-P becomes the dominant carrier of reducing equivalents and leads to the formation of sucrose.

2. Peroxisomes

Glycine and glycerate are the major products exported from peroxisomes. In the peroxisomes, glycolate is oxidized to glyoxylate and then to glycine using glutamate as the amino donor.

Glycine is exported from peroxisomes to mitochondria. On the other hand, peroxisomes import serine from mitochondria and convert it to hydroxypyruvate. The reduction of hydroxypyruvate leads to the formation of glycerate, which is exported to chloroplasts, facilitating the salvage of carbon. The reduction of hydroxypyruvate to glycerate places a high demand for reducing equivalents. This demand is met in the form of malate exported to peroxisomes from both chloroplasts and mitochondria (Heldt et al., 1998; Raghavendra et al., 1998).

The metabolites within the peroxisomes are channelled through multienzyme complexes located in the matrix of peroxisomes. The metabolite movement into and out of peroxisomes occurs through specific pores called ‘porins’ (Reumann et al., 1995).

3. Mitochondria

The three major compounds exported from mitochondria are serine (participates in photorespiratory cycle), oxoglutarate (to supply carbon compounds for N_2 metabolism), and malate (to transfer reducing equivalents to peroxisomes).

The glycine formed in the peroxisomes is transported into the mitochondria, where it is oxidized by glycine decarboxylase-serine hydroxymethyl transferase complex to yield serine, CO_2, NH_4^+, and NADH (Oliver, 1998; Douce and Neuberger, 1999). Serine leaves the mitochondria via a specific translocator, possibly the same translocator by which glycine is taken up.

Carbon intermediates, particularly 2-oxoglutarate, are exported from the TCA cycle to support GOGAT activity for glutamate synthesis in the chloroplasts (Figure 10). While the major route of

2-oxoglutarate production involves partial operation of the TCA cycle in the mitochondrion, 2-oxoglutarate synthesis may also occur via a cytosolic isocitrate dehydrogenase (see Section I.D., Figure 2). In mature leaves, the most important input of C_4 acids for 2-oxoglutarate synthesis appears to be as oxaloacetate, generated by cytosolic phosphoenolpyruvate carboxylase. Approximately half of the PEP available in cytosol is carboxylated by PEPC to oxaloacetate, which is converted to 2-oxoglutarate through reactions catalyzed by citrate synthase, aconitase and isocitrate dehydrogenase (Foyer et al., 2000).

In contrast to mitochondria from animal tissues, whose inner membrane is impermeable to oxaloacetate, the plant mitochondrial inner envelope membrane has a malate-oxaloacetate translocator that facilitates the exchange of malate and oxaloacetate (Ebbighausen et al., 1985; Douce and Neuburger, 1990). The high activity of malate dehydrogenase in the mitochondrial matrix ensures an efficient reduction of oxaloacetate to malate. Thus, the NADH formed during glycine oxidation is incorporated into malate and is exported by the malate-oxaloacetate shuttle. This shuttle has a high capacity to transfer reducing equivalents from mitochondria. Although the amount of NADH generated in the mitochondria from glycine oxidation is quite high, mitochondria deliver only about half the reducing equivalents required for peroxisomal hydroxypyruvate reduction, while the rest is provided by the chloroplasts (Heldt et al., 1998; Padmasree and Raghavendra, 2000).

B. Metabolite Exchange between Chloroplasts, Mitochondria, Peroxisomes, and Cytosol

ATP and NAD(P)H are required in several steps of metabolic reaction occurring in different cellular compartments. However, ATP and NAD(P)H being not permeable across the membrane have to be transported indirectly through different metabolite shuttles. The rapid exchange of metabolites between chloroplasts, mitochondria, peroxisomes, and cytosol according to the cellular needs of energy demand is the biochemical basis as well as essential component of interorganelle interaction (Figure 11).

During illumination, the difference in redox potentials between the stromal compartment (NADPH/NADP) and cytosol (NADH/NAD) is quite large, leading to the transfer of redox equivalents from the chloroplast stroma to the cytosol (Heineke et al., 1991). The transfer of reducing equivalents from chloroplasts is mediated by two different metabolite shuttles: the triose-P-PGA shuttle mediated by the phosphate translocator and the malate-OAA shuttle facilitated by the dicarboxylate translocator. The triose-P-PGA shuttle is regulated by Pi availability for counter-exchange by the phosphate translocator, as well as PGA reduction in chloroplasts and triose-P oxidation in cytosol. On the other hand, the malate-OAA shuttle is regulated by stromal NADP-MDH and the [NADPH]/[NADP], and also by the translocating step across the innerchloroplast envelope membrane. In addition, metabolite shuttles of malate and OAA between mitochondria and the cytosol as well as cytosol and peroxisomes facilitate further the exchange of reducing equivalents between mitochondria, cytosol, and peroxisomes (Heldt, 1997).

A photosynthetic cell has two different systems to produce and meet cytosolic demands of ATP: photophosphorylation and oxidative phosphorylation. The ATP produced during photophosphorylation is transferred from chloroplast to cytosol through the exchange of triose-P and PGA mediated by triose-P-Pi translocator. An NAD-dependent glyceraldehyde phosphate dehydroge-

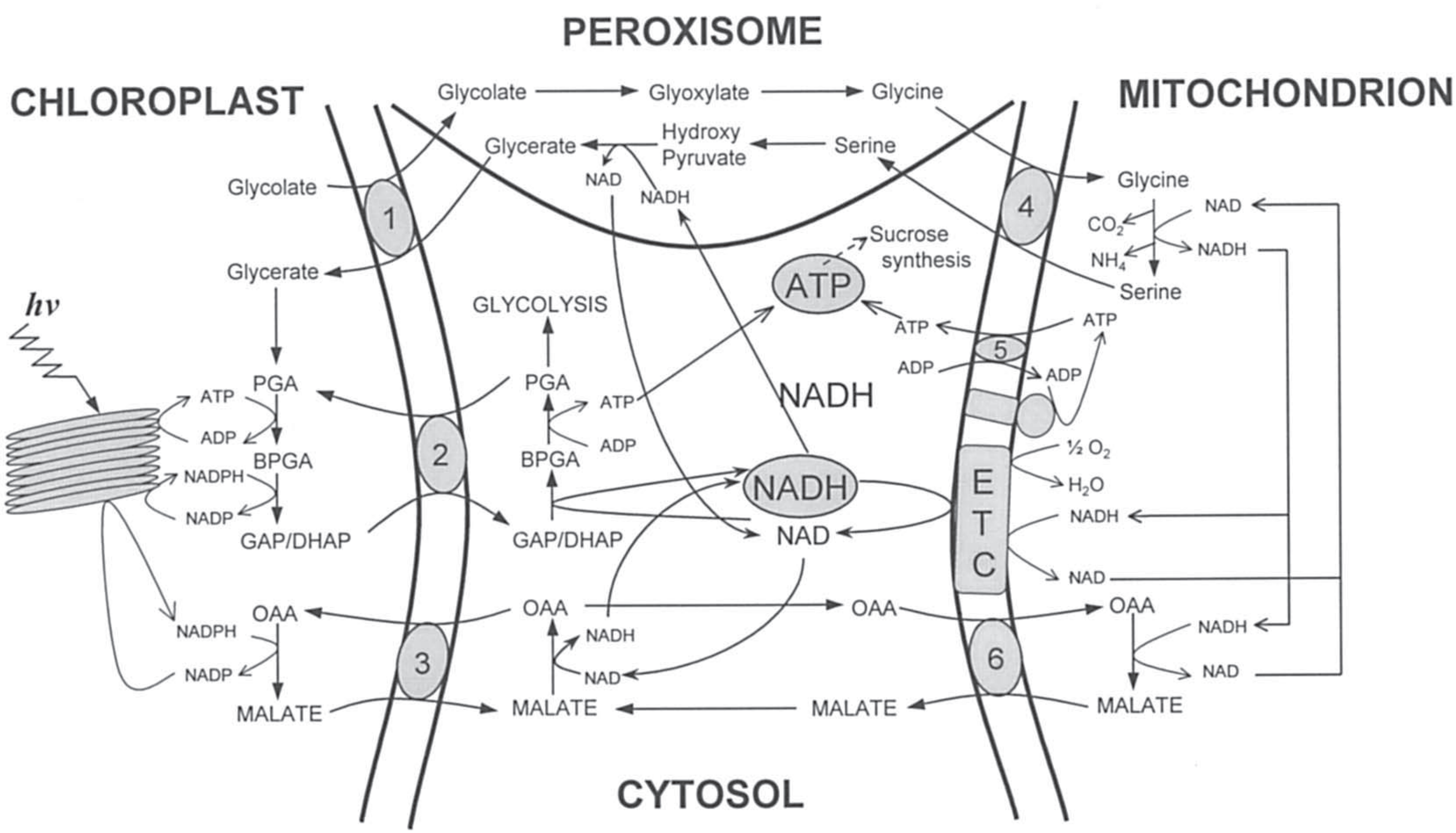

FIGURE 11. The biochemical basis of interorganelle interaction between chloroplasts, cytosol, mitochondria, and peroxisomes. The rapid movement of metabolites between these organelles facilitates the export of reduced equivalents as well as ATP from chloroplasts and mitochondria. Peroxisomes form a major sink for reduced equivalents, while ATP is needed for several activities (including the sucrose biosynthesis) in cytosol. The metabolite shuttle is facilitated by specific carrier proteins on the membranes, called translocators, which are indicated by numbers 1 to 6. The glycolate/glycerate translocator (1) located on inner chloroplast membrane and glycine/serine translocator (2) located on inner mitochondrial membrane coordinate the metabolite traffic involving major photorespiratory metabolites, between chloroplasts, peroxisomes, and mitochondria. The other two major gateways involved are the Pi translocator (2) and dicarboxylate translocator (3) in chloroplasts. In mitochondria, the other channels are the adenylate translocator (5) and oxaloacetate translocator (6). The mitochondrial electron transport (ETC) system can oxidize external as well as internal NADH and generate ATP. (Adapted from Padmasree and Raghavendra, 1998.)

nase plays a significant role in facilitating availability of the chloroplastic ATP to cytosolic demands (Krömer, 1995). On the other hand, ATP produced during oxidative phosphorylation in mitochondria can be transferred directly to cytosol through adenylate translocator.

Illuminated chloroplasts usually have excess NADPH or related metabolites because their electron transport activity is in much excess of the capacity of carbon fixation (Huner et al., 1998). The excess reducing equivalents are transported from the chloroplasts (in the form of DHAP and malate) to the cytosol to generate NADH. Mitochondria are capable of oxidizing external NADH. However, the oxidation could also be indirect through the shuttles of related metabolites formed during photosynthesis (Gardeström et al., 2001). For example, glycolate/glycerate translocator of chloroplasts and glycine/serine translocator of mitochondria can channel large amounts of glycine into mitochondria. As glycine is the prefered mitochondrial substrate over malate, under photorespiratory conditions NADH generated during glycine oxidation can be succesfully oxidized through the nonphosphorylating pathways of mitochondrial electron transport even when ADP is limited. Half of the reducing equivalents produced in the mitochondrial matrix are transferred to peroxisomes in the form of malate to support hydroxypyruvate reduction, while the rest is supplemented by malate derived from chloroplasts.

On the basis of the metabolite movements described above, the photosynthetic and respiratory activity in chloroplasts and mitochondria, respectively, appears to be modulated by one or more of the following factors: (1) the redox state due to the relative levels of NAD(P) or NAD(P)H (b) interorganelle movement of metabolites such as PGA, DHAP, malate, and OAA and (c) adenine nucleotides (ATP, ADP). Peroxisomes and cytoplasm naturally and closely linked to these processes and form an active and integral components of metabolite movements and subsequent interorganelle interaction.

VI. FUTURE PERSPECTIVES

Most of the experiments on the interaction between mitochondria and chloroplasts during photosynthesis have been made with protoplasts (e.g., Gardeström et al., 1992; Krömer et al., 1988, 1993; Igamberdiev et al., 1998; Padmasree and Raghavendra, 1999a,b,c). Only a few experiments were conducted with intact leaves (Krömer and Heldt, 1991a; Hanson, 1992; Hanning and Heldt, 1993; Hurry et al., 1995; Atkin et al., 1998). However, more experiments are needed using intact tissues or leaf discs so as to understand and extrapolate the situation in leaves because the isolated protoplasts lack the cell wall typical of plant cells and may deviate in their metabolism.

An inherent limitation of metabolic inhibitors is the possibility of their unspecific and multiple effects on different processes in the cell. For example, SHAM, used extensively to inhibit alternative oxidase pathway, may also affect chlororespiration (Singh et al., 1992), chloroplastic glycolate-quinone oxidoreductase (Goyal and Tolbert, 1996), besides stimulating peroxidase (Lambers, 1985; Møller et al., 1988). Similarly, antimycin A used to suppress cytochrome pathway may also affect chlororespiration (Singh et al., 1992) as well as photosynthetic O_2 evolution (Cornic et al., 2000) due to the interference with ferredoxin-dependent reduction of cyt b-559 particularly in intact chloroplasts (Scheller, 1996; Endo et al., 1998; Ivanov et al., 1998) besides stimulation of carbon fixation (Schacter and Bassham, 1972). Therefore, the use of inhibitors to examine the role and importance of the cytochrome and alternative pathways has been questioned frequently. Neverthless, these metabolic inhibitors were used in mitochondrial studies by choosing carefully the concentrations that affect only mitochondrial respiration but not chloroplast reactions (Igamberdiev et al., 1997a,b; Padmasree and Raghavendra, 1999a,b,c).

The respiratory measurements often utilize the Clark-type oxygen electrode, which monitors only the net changes in the O_2 levels (caused by both consumption of O_2 in respiration and evolution of O_2 in photosynthesis). It is desirable that these two processes be monitored seperately, so as to make precise measurements of photosynthesis or respiration. Mass spectrophotometer, which can monitor $^{18/16}O_2$ or $^{13/12}CO_2$, has been extremely useful for not only distinguishing between photosynthesis and respiration (Avelange et al., 1991) but also to make quantitative measurements of alternative pathway activity (Robinson et al., 1995).

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. Extensive studies are made on transgenic plants with overexpression or (antisense) depression of enzymes such as triose-P dehydrogenase, rubisco, activase, rubisco or proteins such as triose-P-phosphate-translocator (Vivekanandan and Saralabai, 1997; Heineke, 1998; Sharkey, 1998; Huber, 1998; Flügge, 2000; Häusler et al., 2000). Similarly, mutants or transgenics with altered levels of invertase or sucrose synthase or ADP-glucose pyrophosphorylase or PRK or FBPase or glutamine synthetase or glutamate synthase are available (Häusler et al., 1994; Heineke, 1998; Paul et al., 2000). In contrast, only a few studies are made on mutants/transgenics with altered respiratory characteristics (Vanlerberghe et al., 1994; Hiser et al., 1996; Gutierres et al., 1997; Igamberdiev et al., 2001). In an interesting recent study, Sabar et al. (2000) used the male sterile mutants of *Nicotiana sylvestris* to study some aspects of respiration and photosynthesis. So far, no studies have been reported on the chloroplast-mitochondria interactions in suitable transgenic plants.

The plant mitochondria have an unique system of two different types of oxidative electron transport-cytochrome pathway and the alternative pathway. Being a major route for ATP formation in mitochondria, the importance of cytochrome pathway is unquestionable and is obvious. However, the physiological importance of alternative pathway is not completely understood. Oxidative electron transport in mitochondria occurs predominantly through alternative pathway during glycine oxidation in mitochondria or LEDR in barley protoplasts (Igamberdiev et al., 1997a,b). This phenomenon has to be analyzed further preferably by employing tools other than the metabolic inhibitors. Further, the role of alternative pathway in optimizing chloroplast function also has to be studied under varied environmental conditions, such as light intensity, temperature, and stress conditions. The results would be quite exciting and would help us understand not only interorganelle interaction but also the alternative pathway itself, which is unique to plant mitochondria.

The strong interaction between chloroplasts, mitochondria, peroxisomes, and cytosol is possible only when there is an effecient cross-talk between these organelles. Obviously, metabolite movement is an important factor or signal. However, it is possible that there are other signals. Further work is necessary to identify and establish the importance of different signals between the organelles. Among the possibilities are cytosolic pH, phosphate status, and even the superoxide radicals.

The major advantage of the interorganelle interaction appears to be optimization of their function and protection from any damage due to the unfavorable factors. For example, the chloroplasts are protected from getting overreduced. It is quite likely that mitochondria are also prevented from getting overoxidized. However, not much information is available pertaining to protection of mitochondria.

On exposure to supra-optimal light and likely photoinhibition, the mitochondria (along with peroxisomes) rescue the chloroplasts by dissipating their excess reduced equivalents (Saradadevi and Raghavendra, 1992; Hurry et al., 1998; Padmasree and Raghavendra, 2000; Gardeström et al., 2001). It would be of great interest and exciting to examine the interaction between the different organelles, particularly chloroplasts, mitochondria, and peroxisomes and the consequences on metabolic regulation when the plant is subjected to other stress conditions such as temperature or water.

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