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Update on Nutrient Stress

# Metabolic Adaptations of Plant Respiration to Nutritional Phosphate Deprivation[1]

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**Plants respond adaptively to orthophosphate (Pi) deprivation through the induction of alternative pathways of glycolysis and mitochondrial electron transport. These respiratory bypasses allow respiration to proceed in Pi-deficient plant cells because they negate the necessity for adenylates and Pi, both pools of which are severely depressed following nutritional Pi starvation.**

Plants exhibit numerous morphological, physiological, and metabolic adaptations to Pi deprivation. At the metabolic level, much attention has been devoted to the effect of Pi status on the secretion of acid phosphatases, Pi uptake rates, and other mechanisms that may serve to increase the availability of Pi to Pi-deprived cells (Goldstein et al., 1989; Lefebvre et al., 1990; Duff et al., 1991a, 1991b). The effects of Pi nutrition on the rates of photosynthesis and photosynthate partitioning in leaves have also been well documented (Thorsteinsson and Tillberg, 1987; Lauer et al., 1989; Fredeen et al., 1990). By contrast, investigations of the metabolic adaptations of plant respiratory pathways to Pi deficiency have been limited. In this review, we summarize recent findings that indicate that plants utilize alternative pathways of glycolysis and mitochondrial electron transport during prolonged Pi starvation.

## EFFECT OF Pi NUTRITION ON THE Pi, NUCLEOTIDE-P, AND PPi POOLS OF PLANT CELLS

$^{31}$P-NMR studies have revealed that plant cells selectively distribute Pi between cytoplasmic ("metabolic") and vacuolar ("storage") pools, with cytoplasmic Pi being maintained essentially constant at the expense of large fluctuations in vacuolar Pi (Lauer et al., 1989). With prolonged Pi-deprivation, however, vacuolar Pi stores become exhausted, and this is followed by considerable reductions in cytoplasmic Pi (Lauer et al., 1989). Although $^{31}$P-NMR is capable of distinguishing vacuolar and cytoplasmic pools of Pi, it cannot yet discriminate between Pi sequestered in cytoplasmic organelles versus the Pi contained in the cytosol (as cited by Mimura et al., 1990). Investigations of intracellular Pi compartmentation have also been performed using organelles isolated by the nonaqueous fractionation technique (Stitt et al., 1985; Mimura et al., 1990). However, Pi may leak from organelles, and cross-contamination of organelles (particularly by vacuolar material) may result in an overestimation of Pi when nonaqueous fractionation techniques are used (as cited by Mimura et al., 1990). For these reasons, there are few studies providing accurate measurements of cytosolic Pi concentrations in plant cells. Estimates of cytosolic Pi from nutrient-sufficient plants range from to 5 to 10 mM (Stitt et al., 1985). Lauer et al. (1989) utilized $^{31}$P-NMR to examine the effect of Pi nutrition on the levels of vacuolar and cytoplasmic Pi in leaves from soybean plants at various stages of reproductive development. The estimated cytoplasmic concentrations of Pi in Pi-deficient leaves ranged from <0.01 to 0.23 mM, compared to 5 to 8 mM for Pi-sufficient leaves (Lauer et al., 1989). Because the cytosol constitutes a large proportion of the cytoplasm, it is probable that the cytosolic concentration of Pi is dramatically reduced following Pi starvation.

As a consequence of the significant decline in cytoplasmic Pi, large decreases in intracellular concentrations of nucleotide-Ps also occur following nutritional Pi starvation. For example, Ashihara and coworkers (1988) reported that levels of ATP, CTP, GTP, and UTP in Pi-deficient *Catharanthus roseus* suspension cells were only 20 to 30% of the levels found in nutrient-sufficient cells and that levels of all other nucleotide-Ps were also reduced. In Pi-starved *Brassica nigra* suspension cells, ATP and ADP levels decreased to 26 and 9% of the nutrient-sufficient values, respectively (Duff et al., 1989b). Parallel findings were reported for *Glycine max* (Fredeen et al., 1990) and *Selenastrum minutum* (Theodorou et al., 1991) but differ from those obtained with *Chenopodium rubrum* suspension cells (Dancer et al., 1990) and *Scenedesmus obtusiusculus* (Tillberg and Rowley, 1989), in which the reduction in total adenylates with Pi deprivation was attributed to a larger decrease in ATP relative to ADP.

The dramatic reductions in the metabolic Pi and adenylate pools that accompany long-term Pi stress have important implications with respect to respiratory metabolism. These arise from the adenylate and/or Pi substrate dependence of

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Abbreviations: Fru-2,6-$P_2$, fructose-2,6 bisphosphate; GDH, glutamate dehydrogenase; MPF, M-phase-promoting factor; NAD(P)-G3PDH, NAD(P) dependent 3-P-glyceraldehyde dehydrogenase; PEP, phospho*enol*pyruvate; PEPC, phospho*enol*pyruvate carboxylase; PFP and PFK, PPi:- and ATP:Fru-6-P 1-phosphotransferase, respectively; 3-PGA, 3-phosphoglycerate; PK, pyruvate kinase; $PK_c$, cytosolic pyruvate kinase.

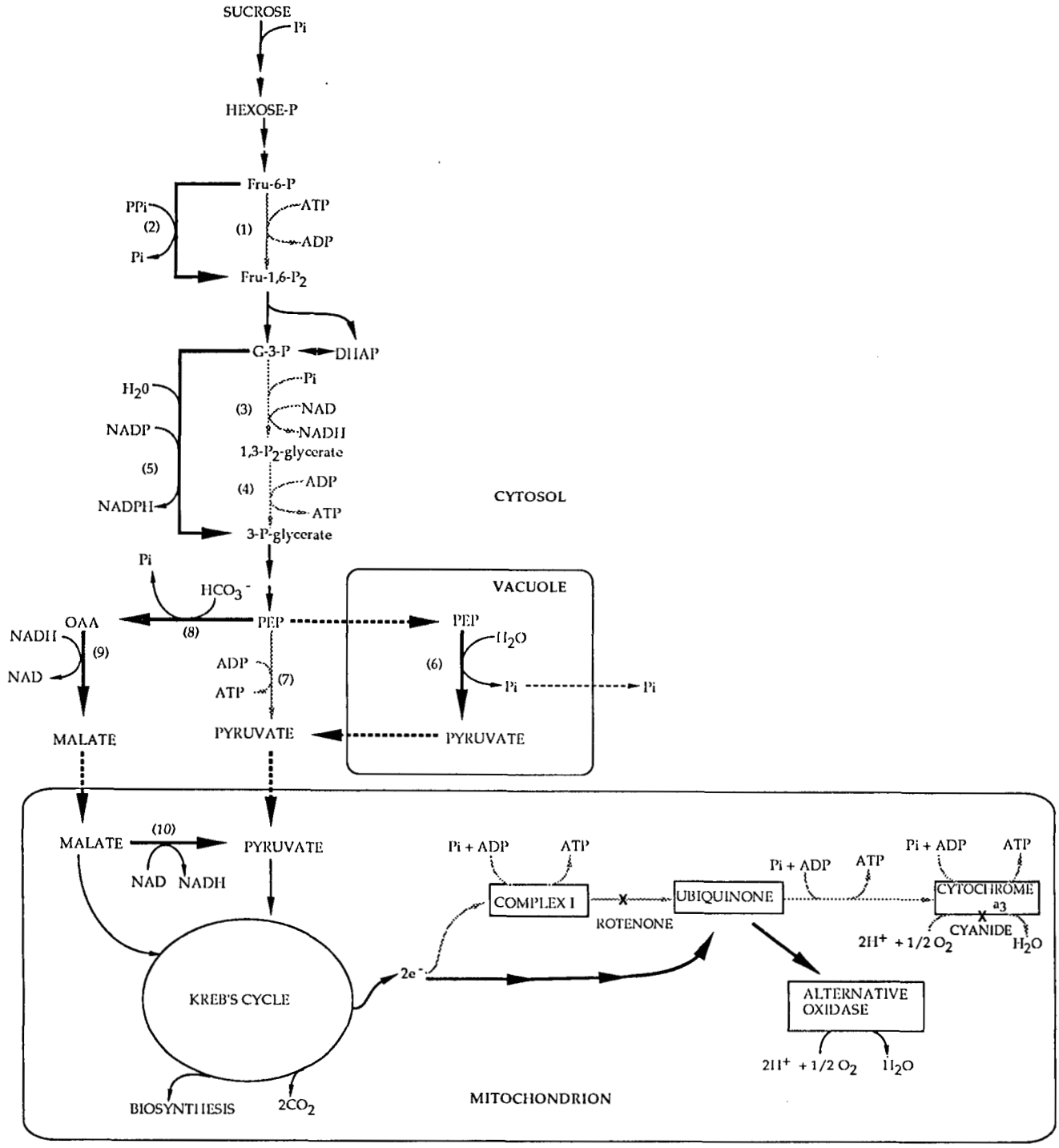


**Figure 1.** A model depicting alternative pathways of glycolytic carbon flow and mitochondrial respiration (indicated by heavy arrows) during nutritional Pi deprivation of higher plants. This metabolic flexibility permits plants to circumvent the adenylate- and Pi-dependent reactions of respiration (indicated by stippled arrows), thus allowing respiratory carbon flux to proceed in Pi-deficient plants. The designations are: (1), PFK; (2), PFP; (3), phosphorylating NAD-G3PDH; (4), 3-PGA kinase; (5), nonphosphorylating NADP-G3PDH; (6), PEP phosphatase; (7), $PK_c$; (8), PEPC; (9), malate dehydrogenase; (10), NAD malic enzyme. DHAP, dihydroxyacetone phosphate; OAA, oxaloacetate.

several enzymes of cytosolic glycolysis (i.e. PFK, NAD-G3PDH, 3-PGA kinase, and PK) and the phosphorylating (Cyt) pathway of mitochondrial electron transport (Fig. 1). Moreover, because Pi relieves the potent allosteric inhibition of plant PFKs by PEP, any significant depletion of metabolic Pi could further attenuate cytosolic PFK activity owing to a lowered cytosolic ratio of Pi:PEP.

It has been suggested (Dancer et al., 1990) that three different energy donor systems may operate in the cytosol of higher plants: adenine nucleotides, uridine nucleotides, and PPi. As described above, adenine and uridine nucleotide levels are extremely responsive to Pi nutrition. By contrast, the PPi level of plant cells appears to be unaffected by Pi deprivation (Duff et al., 1989b; Dancer et al., 1990; Stitt, 1990). These results demonstrate the importance of PPi as an autonomous energy donor in the cytosol of Pi-starved higher plants. PPi could be utilized as an energy donor in three different cytosolic reactions: (a) the PPi-dependent phosphorylation of Fru-6-P catalyzed by PFP (Fig. 1), (b) the conversion of UDP-Glc to UTP and Glc-1-P catalyzed by UDP-Glc pyrophosphorylase, and (c) the PPi-dependent proton pump of the tonoplast (Dancer et al., 1990).

## REMARKABLE FLEXIBILITY OF PLANT PEP METABOLISM

In 1989, Duff and coworkers (1989a) reported the purification and characterization of a PEP phosphatase from heterotrophic *B. nigra* suspension cells. Although its substrate specificity was not absolute, this acid phosphatase was designated a PEP phosphatase owing to a specificity constant ($V_{max}/K_m$) for PEP that was at least 6-fold greater than the value obtained for any other of 14 nonsynthetic substrates that were examined (Duff et al., 1989a). Furthermore, the $K_m$(PEP) of the enzyme was well within estimated physiological concentrations of this compound and was equivalent to $K_m$(PEP) values reported for various plant $PK_c$s. These data suggested that PEP phosphatase could compete with $PK_c$ for a common intracellular pool of PEP. Notably, *B. nigra* PEP phosphatase also demonstrated potent feedback inhibition by Pi (Duff et al., 1989a), and its specific activity was increased more than 10-fold following Pi deprivation (Duff et al., 1989b). Similar results have been reported for the PEP phosphatase of a Pi-limited green alga, *S. minutum* (Theodorou et al., 1991). It was postulated (Duff et al., 1989b; Theodorou et al., 1991) that (a) the ADP-dependent $PK_c$ is functionally eliminated from cellular metabolism during severe Pi stress (owing to >10-fold reductions in intracellular ADP levels) and (b) PEP phosphatase functions as a Pi starvation-inducible bypass to the $PK_c$ of *B. nigra* and *S. minutum* (Fig. 1).

Although energetically wasteful, this adaptation would allow Pi-deficient plants and algae to utilize the Pi provided by adenylate catabolism without impairing the conversion of PEP to pyruvate. Immunoquantification studies have revealed that the significant induction of *B. nigra* PEP phosphatase activity during Pi stress arises from de novo synthesis of PEP phosphatase protein (Duff et al., 1991b). *B. nigra* PEP phosphatase has been shown to be localized in the cell vacuole (Duff et al., 1991a), a location consistent with the enzyme's acidic pH optimum (Duff et al., 1989a). The "glycolytic bypass" theory for PEP phosphatase, therefore, requires that Pi-depleted *B. nigra* suspension cells transport PEP into, and pyruvate out of, the cell vacuole (Fig. 1). Because no data are presently available concerning this possibility, future studies must evaluate whether the tonoplast of Pi-starved plant cells is permeable to PEP and pyruvate.

Another route of primary PEP catabolism is through the enzyme PEPC (Fig. 1). When compared to nutrient-sufficient controls, PEPC-specific activity was found to be 5-fold greater in extracts of Pi-deficient *B. nigra* (Duff et al., 1989b) and 30% greater in extracts of Pi-limited *S. minutum* (Theodorou et al., 1991). The combined activities of PEPC, malate dehydrogenase, and NAD-malic enzyme may function as an alternative route of pyruvate supply to the mitochondrion during Pi limitation (Fig. 1). In *S. minutum*, the dark $CO_2$ fixation rate (i.e. representing in vivo PEPC activity) was found to increase with Pi limitation, whereas the rate of dark respiration declined (Theodorou et al., 1991). This suggested that the in vitro and in vivo elevation of PEPC activity caused by Pi limitation was in response to increased demands for pyruvate and/or Pi recycling rather than strictly for the anaplerotic replenishment of Krebs cycle intermediates.

The presence of pathways that can potentially bypass the reaction catalyzed by $PK_c$ allows plants to be remarkably flexible at the level of PEP metabolism. Nevertheless, the absence of $PK_c$ could be expected to have a major impact on plant metabolism. For example, PK-deficient mutants of yeast and *Escherichia coli* are unable to respire Glc (as cited by McHugh-Gottlob et al., 1992). Thus, it was somewhat unexpected when two morphologically normal transgenic tobacco plants that completely lacked $PK_c$ in their leaves were recovered (McHugh-Gottlob et al., 1992). The leaves had a photosynthetic rate similar to that of wild-type leaves. Also, the dark respiration rate and concentration of pyruvate were unchanged in the transgenic leaves, indicating that the supply of substrates to the mitochondrion was not significantly impeded by the physical elimination of $PK_c$ (McHugh-Gottlob et al., 1992). These findings confirm that plants have alternative pathways to $PK_c$. Although the activities of several potential $PK_c$ bypass enzymes were not altered in the $PK_c$-deficient leaves, elevated levels of PEP (McHugh-Gottlob et al., 1992) may have initiated the flow of carbon through the alternative pathways.

## THE GLYCOLYTIC NETWORK

As described above, a considerable induction of PEP phosphatase and PEPC occurred when suspension-cell cultures of *B. nigra* were transferred to media lacking Pi. Subsequent analysis of the adenylate-dependent reactions of glycolysis revealed that the activities of PFK, 3-PGA kinase, and PK were not altered following nutritional Pi deprivation of *B. nigra* suspension cells (Duff et al., 1989b). Selective maintenance of these glycolytic kinases during Pi stress may ensure that the respiratory machinery of the plant is in place should favorable nutritional conditions be restored. By contrast, following Pi starvation the specific activities of two other cytosolic enzymes, PFP and nonphosphorylating (irreversible) NADP-G3PDH, were elevated at least 20-fold, whereas that

of the Pi-dependent (reversible) NAD-G3PDH was reduced by about 6-fold (Duff et al., 1989b). PFP and nonphosphorylating NADP-G3PDH catalyze parallel reactions to PFK and 3-PGA kinase and NAD-G3PDH, respectively, but do not require adenylates or Pi as substrates (Fig. 1). Together with PEP phosphatase and/or PEPC these reactions circumvent the adenylate- and Pi-dependent reactions of glycolysis, thus providing an alternate route for the conversion of hexose-Ps to pyruvate in Pi-deprived plant cells (Fig. 1). Three of these bypass reactions (PFP, PEP phosphatase, and PEPC) may also fulfill an additional role as a Pi-recycling system that converts esterified phosphate to Pi that would be rapidly reassimilated into the metabolism of the Pi-deficient cells.

The effect of Pi starvation on activities of several glycolytic enzymes has also been examined in *C. roseus* suspension cells. Li and Ashihara (1990) reported that the activities of several glycolytic enzymes (including hexokinase, fructokinase, Glc-6-P dehydrogenase, PFK, PFP, and PK) were unaltered or slightly decreased in *C. roseus* cells undergoing transition from Pi sufficiency to Pi deficiency. These findings contrast with the large induction of PFP activity observed in Pi-starved *B. nigra* (Duff et al., 1989b; Theodorou et al., 1992). This discrepancy may have been due to differences in plant material or the method of cell culture used.

Although there was no change in PFP activity upon Pi starvation of *C. roseus* suspension cells, the activity of PFP from Pi-starved *C. roseus* cells was at least 6-fold that of PFK (Li and Ashihara, 1990). Also, ATP levels in Pi-starved *C. roseus* suspension cells were reduced to only 14% of control levels (Li and Ashihara, 1990). Both of these observations support the possible role of PFP as a glycolytic bypass to PFK in Pi-deprived *C. roseus* suspension cells.

In their reassessment of glycolysis and gluconeogenesis, Sung and coworkers (1988) concluded that these two processes are networks, rather than single pathways, and that plants can direct the flow of carbon through alternative enzymes. Furthermore, they proposed that plants regulate some enzymes adaptively in response to developmental or environmental changes, whereas other enzymes exist primarily as maintenance enzymes whose activity is invariable. Therefore, according to the terminology of Sung et al. (1988), the induction of alternate glycolytic enzymes such as PFP and PEP phosphatase during Pi stress in *B. nigra* is an adaptive response to a nutritional deficiency. By contrast, the enzymes of "classical" glycolysis, such as PFK and PK, which in *B. nigra* display uniform activities regardless of cellular Pi status, are of the maintenance type.

## PFP: AN ADAPTIVE ENZYME

PFP is a strictly cytosolic enzyme that occurs in plants and some microorganisms. PFP appears to be an adaptive enzyme whose activity is responsive to environmental stresses, such as Pi nutrition and anaerobiosis, and to developmental or tissue-specific cues (Duff et al., 1989b; Stitt, 1990; Mertens, 1991; Theodorou et al., 1992). Unlike plant PFK, most plant PFPs display potent activation by nanomolar concentrations of the regulatory metabolite Fru-2,6-$P_2$ (Stitt, 1990). The PFP of many plants consists of two pairs of different subunits of approximately 66 ($\alpha$-subunit) and 60 ($\beta$-subunit) kD (Stitt, 1990; Theodorou et al., 1992). Various evidence indicates that the $\beta$-subunit contains the catalytic site, whereas the $\alpha$-subunit may be involved in regulation of catalytic activity by Fru-2,6-$P_2$ (Stitt, 1990; Theodorou et al., 1992, and refs. cited therein).

The proposal that PFP functions as a glycolytic bypass to the ATP-dependent PFK during periods of Pi stress is supported by (a) the potent product inhibition of PFP by Pi (Stitt, 1990), (b) the striking induction of PFP activity in Pi-deprived *B. nigra* suspension cells (Duff et al., 1989b; Theodorou et al., 1992), and (c) the selective maintenance of PPi and Fru-2,6-$P_2$ pools upon Pi starvation (Dancer et al., 1990; Stitt, 1990). The PFP of several heterotrophic plant tissues has also been proposed to operate as a glycolytic bypass to PFK during periods of anaerobiosis (Mertens, 1991). The use of PPi rather than ATP could confer a significant energetic advantage to plants subjected to environmental stresses, such as Pi deprivation or anoxia.

Recent work has demonstrated that synthesis of the $\alpha$-subunit of PFP is tightly regulated in suspension cells of *B. nigra* and that this regulation is dependent on cellular Pi status. By contrast, the $\beta$-subunit of PFP from *B. nigra* is constitutively expressed under all nutrient regimens (Theodorou et al., 1992). Quantification of immunoblots indicated that, in *B. nigra* cells experiencing a transition from Pi sufficiency to deficiency or vice versa, the relative amount of immunoreactive $\alpha$-subunit correlated with the degree of activation of PFP by Fru-2,6-$P_2$ (Theodorou et al., 1992). Hence, the large induction of PFP activity by Pi starvation of *B. nigra* suspension cells appears to be based upon de novo synthesis of the enzyme's $\alpha$-subunit leading to a significant enhancement in sensitivity of the enzyme to its activator, Fru-2,6-$P_2$ (Theodorou et al., 1992).

Similarly, the large reduction in PFP activity that occurs when Pi-deprived *B. nigra* cells become Pi sufficient appears to arise from an inhibition of synthesis and/or enhanced degradation of the $\alpha$-subunit (Theodorou et al., 1992). It is evident that some form of proteolytic specificity toward the $\alpha$-subunit must exist to facilitate the selective disappearance of this polypeptide upon Pi refeeding. Overall, these findings provide additional evidence that the $\alpha$-subunit of PFP may function as a regulatory protein in controlling the catalytic activity of the $\beta$-subunit and its regulation by Fru-2,6-$P_2$. The concentration of Fru-2,6-$P_2$ in the cytosol of Pi-deprived *B. nigra* was previously estimated to be about 0.5 $\mu$M, a level that almost fully saturates the PFP from Pi-starved cells (Theodorou et al., 1992).

Few precedents exist in the literature that demonstrate regulation of an enzyme's activity by selective synthesis/degradation of one subunit while another subunit is constitutively expressed. However, Loulakakis and Roubelakis-Angelakis (1992) recently reported an increased aminating activity of GDH upon transfer of *Vitis vinifera* callus into ammonium-containing medium. This ammonium-induced increase in GDH activity was attributed to de novo synthesis of the enzyme's $\alpha$-subunit, although there was no detectable alteration in $\beta$-subunit synthesis (Loulakakis and Roubelakis-Angelakis, 1992). Likewise, in *Schizosaccharomyces pombe*, mitosis is induced by the activation of the enzyme MPF (Glotzer et al., 1991). MPF is composed of a catalytic subunit,

$p34^{cdc2}$, which is constitutively expressed, and a regulatory subunit, cyclin, whose abundance fluctuates throughout the cell cycle. Rapid degradation of cyclin signals the end of mitosis, whereas accumulation of cyclin during interphase leads to the activation of MPF, and another round of mitosis ensues (Glotzer et al., 1991).

Phosphate starvation-inducible synthesis of the $\alpha$-subunit of PFP in suspension cells of *B. nigra* is coincident with de novo synthesis of cytosolic PEPC (F.A. Cornel and W.C. Plaxton, unpublished results), vacuolar PEP phosphatase, and a cell wall-localized nonspecific acid phosphatase (Duff et al., 1991b). Parallel induction of these enzymes points to the existence of a higher plant "Pi stimulon" (i.e. a set of genes that are coregulated by Pi), as has been demonstrated to exist in a variety of microorganisms (Goldstein et al., 1989).

## MITOCHONDRIAL RESPIRATION

Studies on the effect of Pi deficiency on the overall rate of mitochondrial respiration have produced conflicting results. In heterotrophic suspension-cultured *C. roseus* cells, rates of respiratory $O_2$ uptake and $CO_2$ release were always lower in cells grown in Pi-deficient versus nutrient-sufficient medium (Li and Ashihara, 1990). Similarly, the respiration rates of *S. minutum* and *Lemna gibba* declined with decreasing nutritional Pi in a manner closely correlated to a declining relative growth rate (Thorsteinsson and Tillberg, 1987; Theodorou et al., 1991). Conversely, in *S. obtusiusculus*, respiratory $O_2$ consumption was stimulated at least 2- to 5-fold within the first 96 h of Pi starvation (Tillberg and Rowley, 1989), whereas in sycamore cell cultures and pea roots the rate of respiration was not affected by Pi starvation (see refs. cited by Rychter and Mikulska, 1990; Rychter et al., 1992). These discrepancies suggest that the overall rate of respiration may not be as important an indicator of mitochondrial adaptations to Pi limitation as is the pathway of mitochondrial electron transport by which $O_2$ is consumed (see below).

From the limited number of studies of the mechanisms of regulatory control in plant mitochondria, it appears that the absolute concentrations of ADP and Pi are the most important factors in the regulation of mitochondrial respiration. Reduced levels of ADP or large increases in the ATP/ADP ratio result in the well-known phenomenon of adenylate control of respiration (Bryce et al., 1990). When ADP or Pi is limiting, the proton motive force increases and the rate of coupled electron transport via the Cyt pathway is restricted (Bryce et al., 1990). Consequently, the significant reductions in cellular ADP and Pi levels that ensue nutritional Pi limitation could restrict electron flow through the Cyt pathway. However, plants contain two nonphosphorylating pathways of mitochondrial electron transport: the cyanide-resistant pathway and the rotenone-insensitive bypass to complex I (Fig. 1).

Rychter and coworkers (Rychter and Mikulska, 1990; Rychter et al., 1992) have examined the effect of Pi deficiency on mitochondrial electron transport in isolated bean root mitochondria as well as in intact roots. In both systems, Pi nutrition had no effect on the overall rate of $O_2$ consumption (Rychter and Mikulska, 1990; Rychter et al., 1992). However, an increased participation of the cyanide-resistant pathway and a decreased involvement of the Cyt pathway was observed during prolonged Pi deficiency (Rychter and Mikulska, 1990; Rychter et al., 1992). Cyt oxidase activity was 20% lower in the Pi-deficient bean roots, which may partially explain the lowered involvement of the Cyt pathway (Rychter et al., 1992). Reduced Cyt pathway activity was also observed in barley roots deprived of inorganic nutrients (as cited by Rychter et al., 1992). $O_2$ consumption rates of mitochondria isolated from Pi-deficient bean roots were also found to be less sensitive to rotenone inhibition, suggesting an increased participation of the rotenone-insensitive bypass (Rychter et al., 1992). This possibility is supported by the finding that mitochondria undergoing ADP-limited $O_2$ consumption (state 4 respiration) are particularly resistant to rotenone inhibition (Bryce et al., 1990). Increased involvement of the rotenone-insensitive bypass and/or the cyanide-resistant pathway could significantly alter the extent to which adenylates or Pi control the rate of mitochondrial electron transport. The operation of these alternative, nonphosphorylative pathways when the Cyt pathway activity is suppressed might allow functioning of the Krebs cycle and limited ATP production and may thereby contribute to the survival of plants during prolonged periods of Pi deprivation (Rychter and Mikulska, 1990; Rychter et al., 1992).

## CONCLUDING REMARKS

Figure 1 presents a model suggesting the major pathways of respiration that exist in Pi-deficient plant cells. The alternative pathways of glycolytic carbon flow and mitochondrial electron transport that are depicted in Figure 1 may represent key facets to the survival of Pi-limited plants and exemplify the extraordinary flexibility of plant versus nonplant metabolism. Metabolic redundancy represents an essential component of the biochemical adaptations of plants and allows them to respond dynamically and appropriately to their ever-changing and frequently stressful environment.

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