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Carbon metabolite feedback regulation of leaf photosynthesis and development

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Abstract

Photosynthesis is regulated as a two-way process. Light regulates the expression of genes for photosynthesis and the activity of the gene products (feedforward control). Rate of end-product use down-stream of the Calvin cycle, determined largely by nutrition and temperature, also affects photosynthetic activity and photosynthetic gene expression (feedback control). Whereas feedforward control ensures efficient light use, feedback mechanisms ensure that carbon flow is balanced through the pathways that produce and consume carbon, so that inorganic phosphate is recycled and nitrogen is distributed optimally to different processes to ensure growth and survival. Actual mechanisms are sketchy and complex, but carbon to nitrogen balance rather than carbon status per se is central to understanding carbon metabolite feedback control of photosynthesis. In addition to determining the activity of the metabolic machinery, carbon metabolite feedback mechanisms also regulate photosynthesis at the leaf level through the regulation of leaf development. This review summarizes the current sketchy, but growing, knowledge of the mechanisms through which carbon metabolite feedback mechanisms regulate leaf photosynthesis.

Key words: Feedback regulation, metabolic signalling, photosynthesis, trehalose.

Introduction

Metabolism in leaves is dominated by photosynthesis. Light affects the expression of genes for photosynthesis and the activity of the gene products. Other environmental variables such as temperature and nutrition determine the rate at which end-products from the Calvin cycle are used. The concept of photosynthesis as a two-way process subject to feedback regulatory processes, determined by the rate of consumption of end-products such as starch, began in the second half of the 19th century (Boussingault, 1868) almost a century after the demonstration of photosynthesis as a light-driven process. Metabolic feedback regulation of photosynthesis could potentially occur from any of the routes of end-product synthesis in plants, the dominant ones in leaves being sucrose, starch and amino acid biosynthesis. Definition of the precise metabolic mechanisms that give rise to feedback control has been elusive and still remains sketchy, but is necessary if photosynthesis is to be engineered to improve carbon acquisition in order to break through the yield ceilings that have developed for many crops in the late twentieth century (Mann, 1999). To achieve such a goal requires an understanding of the regulation of photosynthesis in the wider picture of the whole plant and its environment, taking into account feedforward and feedback controls of photosynthesis. This is an ambitious task, as, while an understanding of individual enzymes and pathways is reasonably advanced, understanding of the mechanisms that regulate metabolism in the context of the whole plant in its environment remains, to a large extent, a mystery.

This article deals with metabolic feedback regulation of photosynthesis within and down-stream of the Calvin cycle that encompass biochemical and molecular metabolic signalling mechanisms. The impact of metabolic signalling mechanisms on photosynthesis at the whole leaf level in terms of leaf development is also considered.

Inorganic phosphate regulates metabolism

Phosphate is used as an intermediate in the majority of coupled reactions in organisms. The excessive accumula-

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tion of Pi in phosphorylated intermediates inhibits metabolic flow (Stitt and Quick, 1989; Hohmann *et al*., 1996; Teusink *et al*., 1998) and is a potential means of feedback regulation of photosynthesis. In photosynthesis, photophosphorylation and CF1-ATPase activity is particularly sensitive to Pi concentration, which can become inhibited if the free Pi concentration falls (Quick and Mills, 1988; Pammenter *et al*., 1993). In leaves, the rate of end-product synthesis (sucrose, starch, amino acids) largely determines the rate at which Pi is recycled back to the reactions of photosynthesis. The question is, does this potential mechanism feedback inhibit photosynthesis *in vivo*? The answer is yes, seen most obviously where Pi is withdrawn from the growing medium of plants (Pieters *et al*., 2001). In these experiments, creation of extra demand for sucrose by shading treatments can offset the effects of Pi deficiency on photosynthesis by increasing the rate of sucrose synthesis which recycles more Pi back to the chloroplast (Pieters *et al*., 2001). Feedback regulation by low Pi can also be seen at low temperature which slows the rate of sucrose synthesis and photosynthesis can become limited in plants unadapted to cold due to sequestration of Pi in pools of intermediates (Labate and Leegood, 1988; Strand *et al*., 1999). Feeding Pi to leaves (Stitt and Grosse, 1988; Hurry *et al*., 1993) can readily reverse this. Pi sequestration during the early stages of acclimation to low temperature actually appears to be part of a mechanism that leads to acclimation of metabolism to winter conditions. Recently, using a combination of low temperature treatments and *Arabidopsis pho* mutants with increased and decreased shoot Pi content relative to wild type, it has been shown that low Pi can increase the expression of sucrose phosphate synthase, cytosolic fructose-1,6-*bis*phosphatase (Hurry *et al*., 2000), and UDP-glucose pyrophosphorylase (Ciereszko *et al*., 2001) important in the flow of carbon to sucrose. Further, Hurry *et al*. (2000) also demonstrated an increase in expression of enzymes in the segment of the Calvin cycle between Rubisco and fructose-1,6-*bis*phosphatase. These adaptations serve to promote the synthesis and partitioning of carbon to sucrose necessary as a cryoprotectant and liberate and recycle Pi more effectively. Thus, the short-term feedback regulation of photosynthesis mediated by low Pi can set in motion mechanisms that lead to longer-term adaptive control of photosynthesis to low temperature. Interestingly, the stimulation of photosynthetic rate in cucumber plants infected with arbuscular mycorrhizas has been shown to be due to increased leaf phosphate status (Black *et al*. 2000).

Carbon to balance nitrogen

As carbon metabolism is regulated to utilize limiting Pi resources efficiently, so it is also regulated to utilize limiting nitrogen efficiently. Photosynthetic machinery in particular accounts for a large investment of nitrogen. This machinery provides carbon skeletons for amino acid synthesis. Carbon supply from photosynthesis also directs the synthesis and mobilization of protein. An abundant supply of carbon can induce the expression of genes for enzymes involved in the utilization and storage of carbon and can repress genes for photosynthesis. Poor carbon supply has the opposite effect (Koch, 1996; Pollock and Farrar, 1996). Thus, carbon directs its own metabolism which means that nitrogen is invested in catalytic machinery where it is most needed to ensure growth and survival under fluctuating carbon and nitrogen supplies.

Whilst it is known that such sugar-signalling mechanisms exist, their mechanistic detail is sketchy. This is because of their complexity, multiplicity and cross-talk with hormone signalling and nitrogen metabolism (Paul and Foyer, 2001). With regard to feedback regulation of photosynthesis, close coupling of carbon and nitrogen metabolism and resources is most obvious (Paul and Driscoll, 1997; Martin *et al*., 2002). This may be because Rubisco, as the largest reserve of nitrogen in leaves, impacts significantly on both carbon and nitrogen availability. Both high carbon and low nitrogen inhibit photosynthesis, possibly by a convergent mechanism (Paul and Driscoll, 1997). The effects of high carbon and low nitrogen on photosynthesis are strikingly similar. Nitrogen deficiency rapidly inhibits growth, carbohydrates accumulate (Thorsteinsson *et al*., 1987; Thorsteinsson and Tillberg, 1990; Paul and Stitt, 1993) and photosynthesis subsequently becomes inhibited (Thornsteinsson *et al*., 1987; Arp, 1991; Paul and Driscoll, 1997). Rubisco protein and maximum activity fall rapidly in leaves when nitrogen is withdrawn from the growing medium (Paul and Driscoll, 1997). As Rubisco is not fully activated under most conditions except for saturating light, then loss of Rubisco protein can be compensated for by an increase in activation state (Krapp and Stitt, 1995). Thus, carbon fixation can be maintained whilst nitrogen is liberated for use elsewhere, for example, root development (Paul and Stitt, 1993) enabling growth to continue through the redirection of nitrogen resources. With continuing N deficiency, the activation of Rubisco may be lost and the whole-scale mobilization of the photosynthetic apparatus occurs (Stitt *et al*., 1995). Sugar feeding and elevated CO_2 lead to similar effects on Rubisco (Krapp and Stitt, 1995; van Oosten and Besford, 1996). Paul and Driscoll (1997), through shading treatments that altered the source/sink balance and carbohydrate content of nitrogen-deficient plants, showed that the accumulation of glucose during the development of nitrogen deficiency appeared to be a necessary part of the mobilization of the Rubisco response to low nitrogen. Glucose levels are often high in plants grown at elevated CO_2 or fed sugar where Rubsico expression is repressed (van Oosten and Besford, 1996). This potentially provides the basis for a common mech-

anism. However, in sugar feeding experiments or at elevated CO_2 giving rise to high leaf carbohydrate, no loss of photosynthetic gene expression or photosynthetic capacity is observed where nitrogen is kept high (Geiger *et al*., 1999; Martin *et al*., 2002). Photosynthetic capacity can actually be stimulated by high CO_2 under high nitrogen (Habash *et al*., 1995). Collectively, these data suggest that carbon supply is an important controlling force for photosynthetic gene expression, but that nitrogen status is the overriding determinant of repression of photosynthesis by carbon, at least when carbon is high. Under conditions of carbon starvation the impact of nitrogen may be different, as in the case of the regulation of nitrate reductase expression where low sugar completely overrides signals derived from nitrate and nitrogen metabolism (Klein *et al*., 2000). Whilst nitrogen metabolites and carbon metabolites in tandem can regulate nitrogen metabolism and organic acid metabolism in plants, for example, glutamine and 2-oxoglutarate (Klein *et al*., 2000; Ferrario-Mery *et al*., 2001), and in microorganisms (Jiang *et al*., 1998), it is not known whether analogous mechanisms feedback regulate photosynthesis, or whether carbon metabolites interact with other factors determined by nitrogen. For example, control may be exerted by nitrogen on the metabolism and transport of carbon leading to qualitative and quantitative changes in carbon metabolite signals and in the sensitivity to them (Paul and Stitt, 1993; Stitt *et al*., 1995; Martin *et al*., 2002). In a similar vein, overall growth and development are profoundly affected by nitrogen supply and further modulation and cross-talk with other factors, such as hormones, cytokinins and abscisic acid in particular, is likely (see Paul and Foyer, 2001, for a fuller discussion and references therein).

In the face on such complexity, when can one begin to look for carbon metabolite signals that regulate photosynthetic gene expression to balance carbon and nitrogen resources? Most obviously is where carbon and nitrogen metabolism converge from the provision of 3-phosphoglyceric acid (3-PGA) from photosynthesis which flows towards important amino acid precursors phosphoenol pyruvate (PEP), pyruvate and Krebs cycle intermediates, oxaloacetic acid and 2-oxoglutarate. In plants, glycolysis is regulated from the bottom up, with primary regulation exerted on the consumption of PEP by pyruvate kinase and PEP carboxylase and secondary regulation on the fructose 6-phosphate to fructose 1,6-*bis*phosphate interconversion catalysed by phosphofructokinase and pyrophosphate-dependent phosphofructokinase (Plaxton, 1996; Givan, 1999). The regulation of pyruvate kinase and PEP carboxylase, in particular, enables integration of glycolysis with nitrogen assimilation and the provision of carbon skeletons for amino acid synthesis (Huppe and Turpin, 1994). Abundant carbon and nitrogen stimulate flow in the glycolytic direction to promote amino acid synthesis. Nitrate and sugars transcriptionally up-regulate genes encoding enzymes for organic acid synthesis, such as PEP carboxylase and pyruvate kinase (Koch, 1996; Scheible *et al*., 1997). The properties of pyruvate kinase and PEP carboxylase also facilitate the integration of PEP partitioning with the generation of 2-oxoglutarate needed for ammonium assimilation by GS/GOGAT and oxaloacetate needed for asparate production (Huppe and Turpin, 1994; Smith *et al*., 2000). Protein phosphorylation allows for further co-ordination of metabolism via PEP carboxylase, nitrate reductase and sucrose phosphate synthase. Interestingly, nitrate also stimulates sucrose synthesis, probably because of the mutual interdependence of sucrose and amino acids in supporting growth (Scheible *et al*., 1997).

Owing to the regulation of organic acid synthesis by nitrate and nitrogen metabolism, nitrogen deficiency leads to a low abundance of organic acids and amino acid precursors such as malate, citrate and 2-oxoglutarate, but a tendency to increase intermediates further up glycolysis such as 3-PGA and glucose-6-phosphate (Paul and Stitt, 1993; Scheible *et al*., 1997). Excess carbon through sugar feeding or very high rates of photosynthesis, particularly when export from leaves is decreased, leads to a similar metabolite profile, at least towards the top end of glycolysis (Krapp and Stitt, 1995). This would provide potential metabolite signals responding to both high carbon and low nitrogen and hence C:N balance. Back-up through glycolysis will mean that glucose will accumulate. Glucose has been linked closely with nitrogen deficiency (Paul and Driscoll, 1997) and is almost certainly a sugar signal; its exact mode of operation is less certain. It could interact with glucose receptors and sensors such as hexokinase, associated with Rubisco repression (Jang and Sheen, 1994; van Oosten and Besford, 1996). The work of Jang *et al*. (1997) on the role of hexokinase would fit the idea of glucose rather than a glycolytic intermediate as a signal that regulates photosynthetic gene expression. In their work, *Arabidopsis* was transformed with a yeast hexokinase that increased the glucose phosphorylating capacity of cells and, hence, presumably flux through glycolysis (although this was not measured) and led to seedlings that were less sensitive to glucose. This implies that glucose interaction with hexokinase rather than flux through glycolysis or an intermediate of glycolysis is the important component of sugar repression of photosynthesis. The logic being that the yeast hexokinase would compete for interaction with glucose with the native hexokinase linked to the signal transduction pathway. Therefore seedlings would perceive a lower glucose content and have decreased sensitivity to glucose. However, there is still equivocation about the exact role of hexokinase (Halford *et al*., 1999). Herbers *et al*. (1996) have shown that hexose release into the apoplast away from the known location of hexokinase results in repres-

sion of photosynthetic genes inconsistent with the hexokinase model, unless hexokinase-mediated signalling also requires a transport step. More mechanistic detail of how hexokinases function as sensors is required, particularly with regard to the separation of signalling and catalytic functions and the role of the diversity of hexokinases in plants.

Glucose signalling independent of hexose phosphorylation can also be observed in plants (Godt *et al*., 1995; Roitsch *et al*., 1995), although it is not clear how important this is for photosynthetic regulation or the identity of the receptors and mechanisms that sense glucose. In yeast, SNF3 and RGT2 membrane proteins with homologies to hexose transporters sense low and high levels of glucose, respectively (Ozcan *et al*., 1996, 1998). Even more recently, GPR1, a G protein coupled receptor specifically required for glucose activation of the cAMP pathway has been identified (Kraakman *et al*., 1999). Glucose also affects protein interactions, for example, PRL1 with AKIN10 and AKIN11, the *Arabidopsis* homologues of yeast SNF1 protein kinase (Bhalero *et al*., 1999). PRL1 probably inhibits the phosphorylating activity of AKIN10 and 11. 14-3-3 proteins, too, interact in a phosphorylation-dependent manner with numerous important enzymes such as sucrose phosphate synthase, nitrate reductase, glyceraldehyde 3-phosphate dehydrogenase, an interaction that is dependent on sugar supply (Cotelle *et al*., 2000). Again these proteins could function in the regulation of photosynthesis by sugar, but this has not yet been established.

In the generation of hexose signals that communicate carbon status, rate of cycling of hexoses may be more important than absolute amounts of sugars. This could explain why the changes in amount of *rbcS* transcript and Rubisco amount do not correlate well with absolute levels of sugars (Moore *et al*., 1999). Correlations between acid invertase activity and feedback regulation of photosynthesis can be demonstrated, adding weight to the theory that sucrose cycling provides a conduit through which

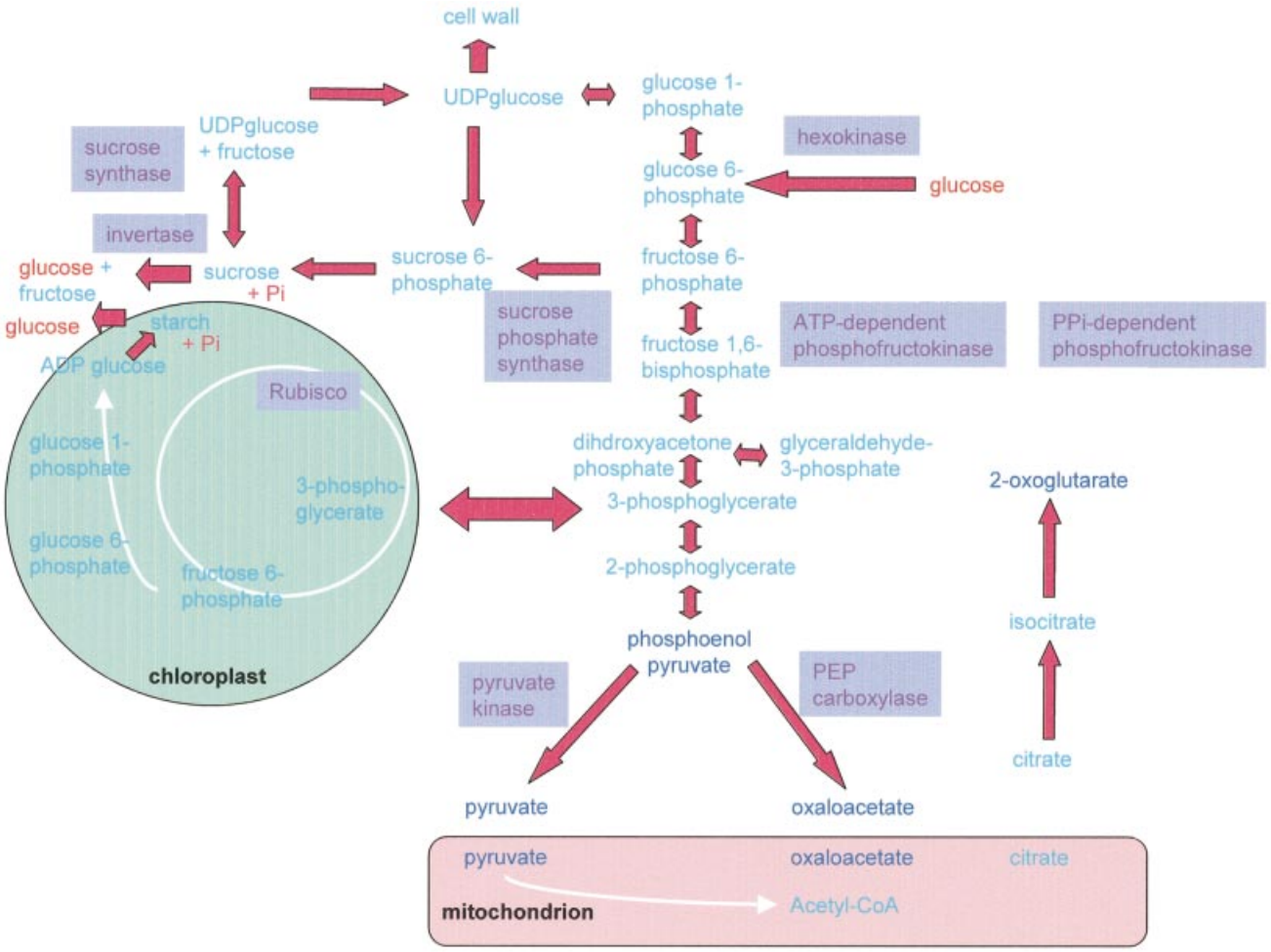

Fig. 1. Fluxes of carbon between carbohydrates, glycolysis and amino acid synthesis provide the framework for feedback regulation of photosynthesis by metabolism. Glucose and Pi known feedback regulators of photosynthesis are in red; important carbon skeletons for amino acid synthesis are in blue.

feedback regulation occurs (Goldschmidt and Huber, 1992; Moore *et al*., 1998). If glycolysis were backed up, elevated glucose-6-phosphate could potentially lead to a futile cycle of sucrose synthesis and breakdown, as glucose 6-phosphate can activate sucrose phosphate synthase through inhibition of an SNF1-related protein kinase (Toroser *et al*., 2000). Starch turnover too may contribute hexoses to signalling (Cheng *et al*., 1998). These workers showed that, in plants grown at elevated CO_2, hexoses were found to be unusually high during the early part of the night, correlating with inhibition of normal recovery of the *rbcS* transcript. Such diurnal differences in hexose content and *rbcS* abundance may explain why correlations have been difficult to establish when based on single timepoint measurements. However, a model of sugar repression of photosynthesis based on glucose is an oversimplification, because of the modulation by high nitrogen previously discussed, which can prevent repression of photosynthetic gene expression when glucose levels are high (Geiger *et al*., 1999; Martin *et al*., 2002). Combined approaches of metabolite and transcript profiling would help unravel the network of metabolite signals (summarized in Fig. 1) that may be operating to regulate photosynthetic gene expression.

Carbon metabolite regulation of leaf development

Carbon metabolite control of photosynthesis also operates at the whole leaf level by influencing leaf development and senescence. A fall in CO_2 content of the atmosphere at the end of the Devonian period, 360 million years ago, may have triggered the evolution of megaphyll leaves through increased leaf gas exchange which promoted greater transpirational cooling allowing maintenance of favourable leaf temperatures (Beerling *et al*., 2001). More recently, increasing CO_2 levels have been shown to affect leaf level processes probably through sugar-sensing mechanisms as part of a mechanism network that regulates leaf development and senescence. The role of sugar production in leaf evolution is not known; however, sugars have been shown to control the cell cycle. Both sucrose and glucose induce the expression of the cyclin genes *cycD2* and *cycD3* (Riou-Khamlichi *et al*., 2000) and there are examples of plants grown at elevated CO_2 and increased sugar content where meristem cells of grasses are stimulated to divide (Kinsman *et al*., 1997). There are also examples of larger thicker leaves in plants grown at elevated CO_2 (Radoglou and Jarvis, 1990; Maroco *et al*., 2002) and sucrose sensing has been implicated in the regulation of leaf shape (Hanson *et al*., 2001). As with regulation of metabolism by sugars, however, response at the leaf level is complex and interactive and dependent on other factors. This is particularly so for the process of leaf senescence where sugars can potentially both inhibit and stimulate the senescence process (Quirino *et al*., 2000). During the early phase of senescence, sugars have been shown to stimulate senescence (Ono and Watanabe, 1997) and senescence-associated genes (SAGs) are sugar-inducible during the early phase of senescence (*SAG21*; Xiao *et al*., 2000; Weaver *et al*., 1998). However, SAGs expressed during the later stages of senescence are sugar-repressible (*SAG12* for example, Noh and Amasino, 1999). To complement these studies and from which a consensus on the interaction between photosynthesis and leaf senescence emerges, the interaction of photosynthesis and sensescence has been examined in transgenics with genetically modified photosynthesis and in studies at elevated CO_2. Where carbon is low, leaf sensescence is delayed. This can be seen clearly in transgenics with low photosynthesis due to decreased expression of Rubisco (Miller *et al*., 2000) or Rubisco activase (He *et al*., 1997). It follows that sugar accumulation due to inadequate nitrogen supply, for example, during the early phase of senescence will promote the early phase of senescence. However, if sugar levels are high during the later stages of senescence then expression of SAGs will be repressed. Therefore, high photosynthesis may actually lead to a longer photosynthetically active life of leaves if sugars do not accumulate during the early phase of senescence and if high photosynthesis is maintained as the leaves gets older and do not become shaded by others in a canopy. Light itself has been shown to be an important modulator of leaf senescence (Wingler *et al*., 1998). Studies at high CO_2 have attributed effects on photosynthetic acclimation to a temporal shift in leaf ontogeny and senescence (Miller *et al*., 1997; Ludewig and Sonnewald, 2000). This may be particularly so where plants run into nitrogen deficiency in such experiments. Hence the carbon–nitrogen interaction is also crucial for leaf developmental processes. Low rates of photosynthesis may not necessarily delay leaf senescence. In hexokinase transgenics with low rates of photosynthesis, senescence is stimulated, although only in tomato and not *Arabidopsis* (Dai *et al*., 1999). This may be because this particular genetic modification perturbs the carbon to nitrogen balance, which may override the effects of low photosynthesis on senescence processes.

Trehalose-6-phosphate content correlates with photosynthetic capacity

Evidence is increasing of a new player in carbon metabolite regulation of photosynthesis (Paul *et al*., 2001; Pellny *et al*., 2002). Until recently, trehalose metabolism was thought to be of physiological importance in only a few plant species, such as resurrection plants, where its accumulation to high levels enables protection from desiccation. However, functional genes encoding trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) have recently been detected

in *Arabidopsis* (Blázquez *et al*., 1998; Vogel *et al*., 1998, 2001). Pellny *et al*. (2002) have demonstrated the presence of trehalose-6-phosphate (Tre6P) in tobacco leaves in the low μM range. Tre6P content can be modified in transgenics and this correlates consistently with changes in photosynthetic capacity (Pellny *et al*., 2002). In tobacco expressing the *E. coli otsA* gene encoding trehalose phosphate synthase (TPS) (Fig. 2), plants have smaller, darker green, leaves and greater photosynthetic capacity per unit leaf area than in the wild type. In plants expressing the *otsB* gene encoding trehalose phosphate phosphatase (TPP) or trehalose phosphate hydrolase (Trec) leaves are larger and paler with lower photosynthetic capacity per unit leaf area. The genetic modifications result in no measurable trehalose in leaves under the growing conditions, but do perturb Tre6P content. The enhancement of photosynthetic capacity per unit leaf area correlates with an increase in Tre6P. In TPS plants Tre6P content is up to 5-fold (low μM range) higher than in the wild type. The causal involvement of Tre6P is quite compelling, bearing in mind that trangenics expressing different transgenes that both lower Tre6P content below wild type give rise to the same phenotype of lower photosynthetic capacity per unit leaf area.

To rationalize this effect, comparison can be made with what is known from yeast where Tre6P regulates glucose influx into metabolism through interaction with hexokinase and, possibly, other glycolytic enzymes (Thevelein and Hohmann, 1995). Yeast mutants that lack Tre6P cannot grow on glucose due to the uncontrolled flow of carbon into metabolism reflected in the accumulation of phosphorylated intermediates. This parallels the phenotype of the TPP and Trec transgenics described above where Tre6P is absent, which have a higher phosphorylated intermediate content indicating that the flow of carbon into metabolism has become less tightly regulated (Pellny *et al*., 2002). Whilst the precise mechanism awaits elucidation, it appears that Tre6P may perturb glycolytic carbon flow and the carbon to nitrogen balance already implicated in the regulation of leaf photosynthesis. Recently, Eastmond *et al*. (2002) have shown an obligate requirement for one TPS gene during embryogenesis where it appears to enable embryo cells to respond to sucrose. Thus a generic role for trehalose metabolism in plants seems possible.

Conclusion

Feedback regulation of photosynthesis serves to balance the flow of carbon in order to ensure growth, survival and completion of the life cycle through the optimized allocation of nitrogen resources. The feedback mechanisms are complex and interactive probably involving the integration of numerous signals that arise from carbon and nitrogen metabolism downstream of photosynthesis. The regulation of glycolytic carbon flow by nitrogen is an obvious source of signals that signal carbon to nitrogen balance. The regulation also operates at the whole leaf level in terms of leaf development and senescence. Such complex mechanisms that underpin the regulation of metabolism provide tools with which to engineer changes in the activities of numbers of enzymes that are likely to be necessary to achieve significant changes in metabolic flux.

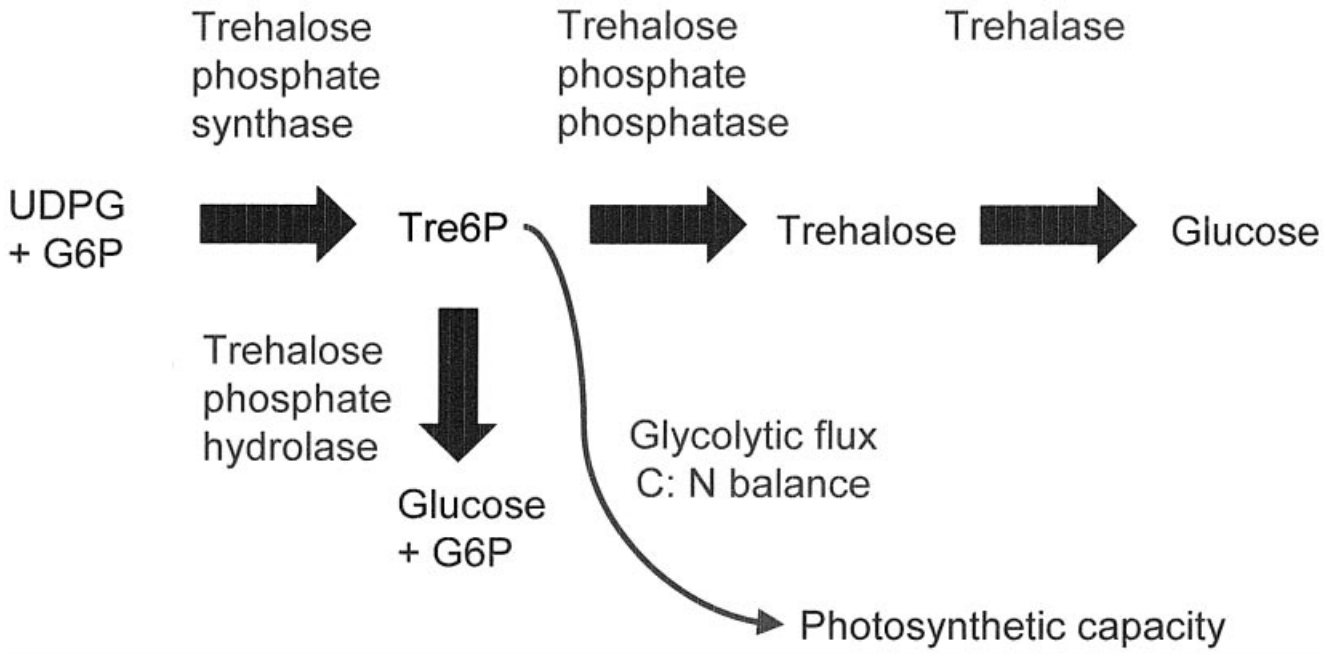

Fig. 2. Trehalose synthesis pathway and model for its relationship with regulating photosynthetic capacity.

Their elucidation remains an important challenge and one likely to benefit from omic technologies, particularly metabolite and transcript profiling that enable integrated analysis of metabolic networks.

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