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REVIEW ARTICLE

Sink regulation of photosynthesis

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Abstract

The concept that photosynthetic flux is influenced by the accumulation of photo-assimilate persisted for 100 years before receiving any strong experimental support. Precise analysis of the mechanisms of photosynthetic responses to sink activity required the development of a battery of appropriate molecular techniques and has benefited from contemporary interest in the effects of elevated CO_2 on photosynthesis. Photosynthesis is one of the most highly integrated and regulated metabolic processes to maximize the use of available light, to minimize the damaging effects of excess light and to optimize the use of limiting carbon and nitrogen resources. Hypotheses of feedback regulation must take account of this integration. In the short term, departure from homeostasis can lead to redox signals, which cause rapid changes in the transcription of genes encoding photosystems I and II. End-product synthesis can exert short-term metabolic feedback control through Pi recycling. Beyond this, carbohydrate accumulation in leaves when there is an imbalance between source and sink at the whole plant level can lead to decreased expression of photosynthetic genes and accelerated leaf senescence. In a high CO_2 world this may become a more prevalent feature of photosynthetic regulation. However, sink regulation of photosynthesis is highly dependent on the physiology of the rest of the plant. This physiological state regulates photosynthesis through signal transduction pathways that co-ordinate the plant carbon : nitrogen balance, which match photosynthetic capacity to growth and storage capacity and underpin and can override the direct short-term controls of photosynthesis by light and CO_2. Photosynthate supply and phytohormones, particularly cytokinins, interact with nitrogen supply to control the expression of photosynthesis genes, the development of leaves and the whole plant nitrogen distribution, which provides the dominant basis for sink regulation of photosynthesis.

Key words: Source, sink, photosynthesis, carbon, nitrogen, feedback regulation.

Introduction

Almost a century after the first demonstration of photosynthesis as a light-driven process by Ingenhousz in 1779, an hypothesis was put forward that the accumulation of photoassimilate in leaves also has a role in regulating photosynthetic rate (Boussingault, 1868). As the accumulation of end-products is a function of the balance between photosynthesis and use by the growth processes of the plant, Boussingault's hypothesis essentially pointed out that there is an interrelationship between photosynthesis and growth rather than a one-way relationship. Only recently have data been obtained that allow an appreciation of the mechanisms of 'sink' regulation of photosynthetic rate. The photosynthetic machinery represents a huge investment of resources and it is logical that the extent of this investment responds to the economy of the whole plant. At low light, for example, leaves readily adapt to shade environments and lower rates of assimilate production by altering chloroplast protein and pigment composition to optimize light capture and light use efficiency. Conversely, in response to high assimilate availability sinks are elaborated and new ones developed. Inability to establish new sink capacity results in accumulation of assimilate in leaves. Plants that can readily increase sink size such as potato and citrus suffer less from feedback inhibition.

Improvements in crop yields during the twentieth century have been achieved through improved agricultural practice, irrigation, fertilizer use, and pest and disease control creating the conditions under which photosynthesis can flourish. Improvement in crop harvest index through breeding has meant that abundant photosynthate is invested in harvested sinks. Altered assimilate partitioning as increased harvest index has reached a ceiling for many crops and has been achieved without large increases in overall biomass. As further substantial

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increases in intrinsic yield potential by this means cannot reasonably be expected, further improvements in yield to sustain a world population expected to reach ten billion by 2050 will depend on overall increases in biomass through increased carbon fixation per unit leaf area. Molecular genetic techniques have provided the imminent possibility of direct genetic manipulation of the photosynthetic apparatus to increase net photosynthetic rate. Any technology that increases plant photosynthesis has the potential to cause a net removal of CO_2 from the atmosphere. However, interest in climate change and research on the effects of elevated CO_2 on photosynthesis has emphasized the two-way relationship between photosynthesis and growth and it is possible that regulation of photosynthesis by the sink could frustrate direct genetic manipulation of photosynthesis. Improvement of crop yield through enhancing photosynthesis therefore depends on an understanding of the nature of the control mechanisms, particularly signal transduction pathways that control photosynthetic capacity, and their physiological context, together with a precise knowledge of the impact of metabolic and environmental cues.

This review focuses on the mechanisms through which sink regulates the source: essentially, regulation caused by a departure from homeostasis within the chloroplast, mesophyll cell and whole plant caused by significant changes in the environment and physiology of the plant. The dominant mechanisms through which the sink regulates the source are not simple linear pathways but networks with many points of reciprocal control. They determine the limits within which photosynthesis can be productive and underpin the source/sink interaction. A model is proposed here where co-ordination of whole plant carbon to nitrogen balance provides the dominant and flexible basis for sink regulation of photosynthesis.

Homeostatic regulation of photosynthesis

Source leaves sustain high rates of photosynthesis over a wide range of conditions. This depends not only on precise co-ordination between reactions in the thylakoid membranes and stroma but also on an exchange of metabolites and assimilatory power with the cytosol and with other organelles such as the mitochondria. Since source activity drives sink metabolism, photosynthetic control must also be responsive to the needs of the whole plant and, in particular, optimal use of carbon and nitrogen resources. During evolution metabolic crosstalk between these pathways has conferred a physiological advantage by preventing feast and famine scenarios. Any hypothesis of adaptive mechanisms of sink regulation must appreciate this integration. Models where just one component is decreased, for example the capacity for CO_2 assimilation, would represent an extreme stress situation for the plant as the production of assimilatory power would exceed the rate of its utilization and irradiance, previously optimal for photosynthesis, would become excessive. Evidence suggests that the relative capacities for electron transport and metabolism are evenly matched (Ott *et al*., 1999). In addition, measurements of the steady-state redox poise of the chloroplast stroma suggest that under saturating light the capacity of metabolic reactions is adequate to prevent over-reduction of the electron transport chain. Molecular regulation ensures that the capacities of energy-producing and energy-utilizing reactions are approximately equal. The degree to which metabolites control investment in one component such as the light-harvesting machinery as opposed to other components such as those of electron transport, ATP synthesis and CO_2 assimilation is not known. Much of the research on photosynthetic regulation has focused on responses to environmental fluctuations rather than metabolic triggers. The information concerning the co-ordinate control of genes encoding electron transport components and those associated with assimilatory processes is patchy, to say the least.

ATP and reducing power (NADPH, NADH, and ferredoxin) are generated simultaneously in the chloroplast during light-dependent electron transport and photophosphorylation (Fig. 1). They are consumed in the reductive assimilation of inorganic elements (C, N, S) from which ATP and reductant can be regenerated by oxidative processes such as respiration. Respiration includes oxidative phosphorylation in the mitochondria that enables the reducing power of NADPH to be converted into ATP. There are a large number of homeostatic mechanisms in plant cells that stabilize the ATP/reductant balance during photosynthesis (Noctor and Foyer, 1998*a*, 2000). These mechanisms are necessary because even slight imbalances between the rates of ATP generation and utilization will lead to marked fluctuations in the cell ATP/ADP ratio. Photosynthetic electron transport alone has a high degree of flexibility in regulating ATP/reductant balance during photosynthesis. Pseudocyclic and cyclic photophosphorylation are putative mechanisms by which the ATP/reductant balance can be matched to the varying requirements of metabolism. All of the above mechanisms contribute to the adenylate status of the photosynthetic cell. A multitude of small but crucial contributions to the stability of ATP production are made by ATP-linked pathways in other parts of the cell requiring co-operation in energy metabolism between the chloroplast and the rest of the cell. Extrachloroplastic compartments contribute to chloroplastic ATP requirements by supplying ATP directly or by accepting reducing equivalents and so supporting ATP synthesis within the chloroplast (Noctor and Foyer, 1998*a*, 2000). Respiratory carbon flow, for example, is necessary in the light for the production of oxoacids such

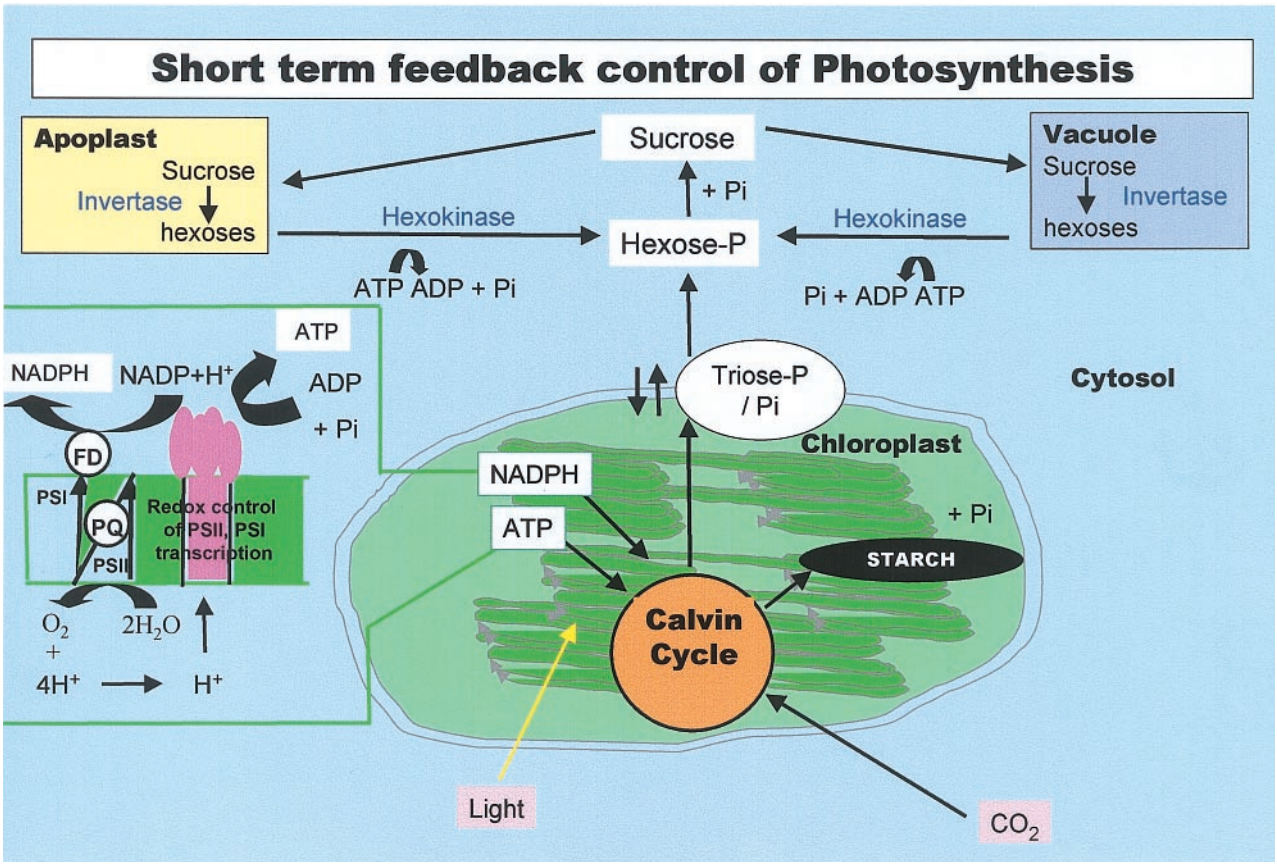

Fig. 1. Short-term feedback regulation of photosynthesis. Redox control of chloroplast gene expression permits transcriptional responses within minutes of perturbation of the redox state of the plastoquinone pool. The plastoquinone pool exerts regulatory control over the synthesis of photosystem I and II to balance its own reduction by photosystem II with its own oxidation by photosystem I (for further details see Pfannschmidt *et al.*, 1999). The rate of triose phosphate utilization in sucrose and starch synthesis can exert short-term control of photosynthesis through recycling of Pi to reactions of photosynthesis and, in particular, photophosphorylation.

as 2-oxoglutarate (Kromer*et al.*, 1988; Kromer, 1995). This is essential for the light-driven incorporation of ammonia into amino acids in the chloroplast. Other oxoacids accept amino groups in transamination reactions occurring in different subcellular compartments. NAD(P)H produced as a result of this oxidative carbon flow can be re-oxidized by the respiratory electron transport system in the mitochondria, thereby generating ATP. This ATP is then transported into the chloroplast by the adenylate translocator on the chloroplast envelope, adding to the ATP produced by photophosphorylation (Kromer and Heldt, 1991; Kromer, 1995; Noctor and Foyer, 2000).

Departure from homeostasis

Despite this enormous flexibility, changes in the environment or in metabolic demands because of changing source–sink relationships, can push the homeostatic mechanisms to their limits. It is therefore crucial that departures from redox and adenylate homeostasis are sensed, and that redox and adenylate signals are used to elicit changes in gene expression to restore the energy status of the cell and to prevent damage. While the AMP : ATP ratio is important in signal transduction in mammalian cells, there is no evidence to date for a comparable system in chloroplasts or photosynthetic cells. In animals the AMP : ATP ratio determines the activity of an AMP-activated protein kinase, which is a key component of a signalling kinase cascade. It is possible that there is a similar system in plants that is activated by environmental stresses that deplete cellular ATP (Hardie *et al.*, 1998) as may occur, for example, under water stress in plants (Tezara *et al.*, 1999). In contrast 'redox' control of the transcription and translation of photosynthetic genes is better characterized. Two key metabolic factors in photosynthetic cells have been shown to regulate photosynthesis. These are the reduction state of key components of the chloroplasts and utilization of triose phosphate in end-product synthesis downstream of the light-dependent photosynthetic reactions (Fig. 1).

Feedback regulation from redox signals

Redox signals arising from the plastoquinone pool and from the accumulation of hydrogen peroxide control photosynthetic gene expression (Allen, 1995; Karpinski *et al*., 1999; Pfannschmidt *et al*., 1999). For example, oxidized plastoquinone signals that PSII is rate-limiting and rapidly initiates the transcription of PSII reaction centre genes and decreases the transcription of genes encoding PSI reaction centre proteins. Reduced plastoquinone signals that PSI is rate-limiting and this initiates the transcription of PSI reaction centre genes while repressing the expression of those encoding PSII reaction centre proteins (Pfannschmidt *et al*., 1999). This redox regulatory system controlling chloroplast transcription appears to have been conserved through evolution from the prokaryotic ancestor. Also important are the redox signals that originate in the chloroplast and are transported to other compartments of the photosynthetic cell. A key mechanism in this regard is photorespiration. During photorespiratory carbon flow huge amounts of H_2O_2 are generated in the peroxisomes as a result of the oxidation of glycollate. H_2O_2 is a well-known redox signal involved in the regulation of gene transcription (Foyer *et al*., 1997). Changes in the rate of photorespiration, and hence the availability of CO_2, may be signalled to the rest of the cell via photorespiratory H_2O_2 production. Most important, perhaps, is the finding that such signals arising in the chloroplast initiate systemic changes in gene expression. Changes in the plastoquinone redox state and in H_2O_2, therefore, serve to initiate signal transduction sequences that act both locally and at long distances from the source of the signal to facilitate changes in gene expression at (Karpinski *et al*., 1999). It is also interesting to consider the role of mitochondria in the generation of redox signals that modulate gene expression. In animals, hyperglycemia leads to enhanced superoxide, and hence H_2O_2, production in the mitochondria leading to pathogenesis, nerve damage and other complications of diabetes (Nishikawa *et al*., 2000). Plant mitochondria are situated in a glucose-rich environment, particularly when carbohydrate accumulates in plant cells. The importance of glucose enrichment in modifying redox signal production by mitochondria and their role in determining the fate of plant cells, for example, by initiating apoptosis, as occurs in animal cells, is not known at present.

Feedback regulation from triose phosphate utilization

Inorganic phosphate (Pi) incorporated in the sugar phosphate end-products of photosynthesis needs to be recycled to the reactions of photosynthesis, in particular, photophosphorylation which is very sensitive to Pi concentration (Quick and Mills, 1988) in order to sustain photosynthetic rate. The rate of end-product (sucrose, starch, amino acids) synthesis determines the rate at which Pi is recycled back to the reactions of photosynthesis and anything that restricts triose phosphate utilization could effectively limit photosynthesis. Manipulation of Pi supply to chloroplasts directly or with Pi-sequestering compounds such as mannose and 2-deoxyglucose has shown that photosynthesis is inhibited if the Pi concentration of the chloroplast incubation medium were either too low or too high (Cockburn *et al*., 1967; Herold, 1980). Pi limitation can be diagnosed by the absence of low O_2-mediated stimulation of photosynthesis (O_2 insensitivity) and pronounced oscillations of photosynthetic CO_2 assimilation during abrupt large transitions in the driving forces for photosynthesis such as changes in light intensity, and CO_2, particularly at low temperature (Stitt, 1986; Sage and Sharkey, 1987; Stitt and Grosse, 1988). In the short term, restriction of Pi serves to increase the transthylakoid ΔpH gradient preventing over-reduction of PSI and increasing energy dissipation. It also provides a flexible system for regulating assimilate partitioning. High 3-PGA/Pi activates ADPglucose pyrophosphorylase and starch synthesis (Preiss, 1988; Preiss and Romeo, 1994). A similar series of events (low ATP/ADP ratio, 3-PGA and RuBP accumulation and deactivation of Rubisco; Stitt and Quick, 1989) can occur in intact leaves, at least in the short term, when sucrose accumulates in leaves due to low demand from sinks (Huber *et al*., 1992). Under these conditions sucrose phosphate synthase (SPS) activity decreases because of an increase in the phosphorylation state of the enzyme (Huber *et al*., 1989; Foyer, 1990; Siegl and Stitt, 1990). SPS has been shown to be a substrate for SNF-1-related protein kinases (SnRKs; Sugden *et al*., 1999) which may be important in modulating SPS activity when sucrose accumulates. Decreased hexose phosphate utilization by sucrose synthesis stimulates fructose-2,6-bisphosphate (F26BP) synthesis. In some plants this results in decreased cytosolic FBPase activity (Neuhaus *et al*., 1990; Stitt, 1990). As a result of the decreased Pi liberation imposed by low cytosolic FBPase activity more carbon is retained in chloroplasts for starch synthesis. The importance of F26BP in controlling sucrose synthesis has been emphasized in transgenic tobacco where elevated F26BP concentrations, produced by the activity of a modified mammalian gene encoding 6-phosphofructo-2-kinase, led to decreased fluxes of carbon to soluble sugars, organic acids and amino acids while enhancing starch accumulation (Scott *et al*., 1995).

It has been suggested that invertase may control the sucrose content of leaves and be involved in a 'futile cycle' of sucrose synthesis and degradation involving hexokinase, similar to mechanisms observed in non-photosynthetic tissues (Foyer, 1988; Huber, 1989). In this hypothesis accumulation of sucrose in the cytosol is converted to hexoses by vacuolar or apoplastic invertase

and the products are converted to hexose phosphates by hexokinase followed by re-synthesis of sucrose. This futile cycle could maintain a high sugar-phosphate requirement with less Pi recycled back to the chloroplast. An examination of the leaves of a wide range of species has shown that invertase does not have a decisive role in controlling carbohydrate partitioning in leaves (Kingston-Smith *et al*., 1999). In addition, no strong evidence of futile cycling around sucrose and hexoses has been found in leaves (Kingston-Smith *et al*., 1999).

The long-term regulation of photosynthesis by low rates of sucrose synthesis or by sucrose cycling leading to restriction of Pi supply would, however, be of no adaptive advantage. Over-capacity and continued unnecessary production of proteins would be surprising in terms of resource allocation. Excess electron transport capacity would increase vulnerability to damage due to the formation of active oxygen species particularly when exposed to high light (Noctor and Foyer, 1998*b*; Foyer and Harbinson, 2000). However, triose-phosphate utilization by end-product synthesis may exert short-term feedback control of photosynthesis in the field at the extreme of source/sink imbalance, before longer-term adaptive mechanisms re-establish greater equilibrium. Hence plants grown with CO_2 enrichment tend towards Pi limitation (Harley *et al*., 1992; Socias *et al*., 1993; RiviereRolland *et al*., 1996). Low rates of sucrose synthesis during Pi deficiency due to low demand from sinks restrict the recycling of Pi back to the chloroplast and limit the rate of photosynthesis (Pieters *et al*., 2001). In drought conditions SPS activity is decreased and photosynthesis may be limited by low sucrose synthesis in a similar manner (Sharkey and Seemann, 1989). The metabolic basis for regulation of photosynthesis during water stress, however, remains a contentious issue (Tezara *et al*., 1999; Cornic, 2000). The importance of sucrose synthesis in recycling Pi is illustrated in transgenic tomatoes over-expressing SPS and a higher capacity for sucrose synthesis. Photosynthesis is modified in response to the increased triose-P utilization capacity which leads to increased O_2 sensitivity (Micallef *et al*., 1995) and, in some cases, increased rates of photosynthesis (Galtier *et al*., 1995) as well as increased sucrose export and biomass accumulation (Laporte *et al*., 1997).

Recent evidence (Hurry *et al*., 2000) suggests that low Pi as a result of low rates of sucrose synthesis can cause long-term adaptive changes in photosynthetic capacity at the level of gene expression. Exposure of warm-grown plants to chilling temperatures (5–10 °C) inhibits photosynthesis because slow sucrose synthesis in the cold limits Pi recycling (Stitt and Grosse, 1988; Strand *et al*., 1999). Evidence obtained from *Arabidopsis* mutants *pho1-2* and *pho2-1* with decreased and increased shoot phosphate, respectively, provides compelling evidence that Pi sequestration during the early stages of low temperature acclimation can trigger changes associated with cold acclimation of leaves. In particular, increased Rubisco expression, changes in expression of other Calvin cycle enzymes to minimize sequestration of Pi in metabolites, and increased expression of sucrose biosynthesis enzymes (Hurry *et al*., 2000). Pi sequestration, therefore, can exert short-term feedback regulation and also induce long-term adaptive regulation of photosynthesis and increased photosynthetic capacity.

The role of starch in feedback regulation

As seen from the previous section, starch synthesis is promoted when sucrose synthesis is restricted and in many plant species leaf starch serves as a transient sink to accommodate excess photosynthate that cannot be converted to sucrose and exported. The capacity of starch synthesis, particularly under high light and high CO_2, enables many plants to achieve a higher rate of photosynthesis than could be sustained by sucrose synthesis alone because the synthesis of starch contributes to triose-phosphate utilization in the chloroplast. This is illustrated nicely in *Arabidopsis thaliana* mutants lacking ADP-glucose pyrophosphorylase where the inability to utilize triose phosphate when grown with CO_2 enrichment limits the capacity for photosynthesis (Sun *et al*., 1999). Growth with CO_2 enrichment usually leads to starch accumulation in leaves (Figs 2, 3). The accumulation of starch occurs within hours after transfer from air to high CO_2 and elevated starch persists throughout the period of growth with CO_2 enrichment (Fig. 3). An immediate effect is a decrease in the activation state of Rubisco (Fig. 4) without any change in the total and maximal activities and hence amounts of Rubisco. The decrease in Rubisco activation state can persist over a long period (Fig. 5). Changes in soluble sugar contents are small in contrast to changes in the leaf starch content (Figs 2, 3).

There has long been speculation that excessive accumulation of starch may impair chloroplast function. Indeed, the earliest proposal for a mechanism of sink regulation of photosynthesis suggested that accumulation of starch, the first photosynthetic end-product to be identified, limited photosynthesis directly through physical limitations such as restriction of CO_2 diffusion or rupturing chloroplasts (Ewart, 1896; Warren Wilson, 1966; Wildman, 1967; Neales and Incoll, 1968). There are large interspecific variations in the relative amounts of sucrose and starch accumulated (Huber, 1981) and differences in partitioning and most obviously in starch accumulation have been proposed as a potential basis for explaining interspecific differences in feedback regulation of photosynthesis. Some species make sugar alcohols rather than starch and sucrose, others particularly monocotyledons, accumulate fructan instead of starch. A robust

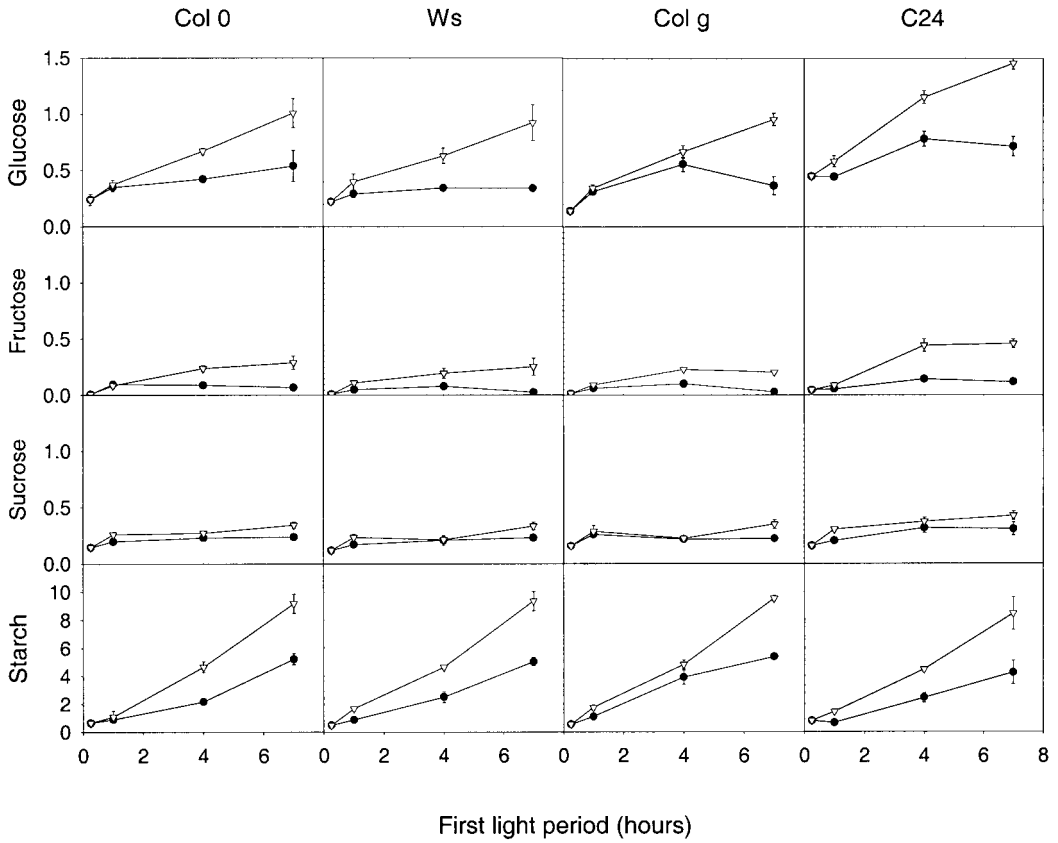

Fig. 2. Response of shoot carbohydrate content of *Arabidopsis thaliana* ecotypes to transfer to elevated CO_2 (1000 ppm, open symbols) during first photoperiod after transfer. Plants were transferred 4 weeks after sowing and the other growing conditions were 20 °C, 10 h photoperiod, 300 μmol quanta m^{-2} s^{-1}. Control plants were maintained at 350 ppm CO_2 (closed symbols). Col O, Columbia; Ws, Wassilewskija; Col g, Columbia glabrous. Plants were grown in soil and given full nutrition and irrigation throughout.

correlation between high starch content in leaves and low assimilation rate can be found (Ewart, 1896; Nafziger and Koller, 1976; Mauney *et al*., 1979; Sasek *et al*., 1985). Further, in species where starch synthesis is low, such as rice and wheat, removal of sinks to promote carbohydrate accumulation in leaves does not inhibit photosynthesis (Austin and Edrich, 1975; Koide and Ishihara, 1992; Nakano *et al*., 1995). Using hot wax collar and leaf excision treatments it has been found that feedback inhibition of photosynthesis was greater in starch-storing species than those that favoured sucrose synthesis (Goldschmidt and Huber, 1992). However, in the same study a starchless mutant of *Nicotiana sylvestris* also showed feedback effects on photosynthesis. Other observations have shown that feedback inhibition does occur in low starch-accumulating species such as wheat when sinks are removed (Birecka and Dakic-Wlodkowska, 1963; King *et al*., 1967; Azcon-Bieto, 1983). These studies cast doubt on a simple relationship between starch accumulation and feedback regulation. Only under extreme conditions where low nitrogen, high CO_2 and water stress are combined is there direct evidence that starch accumulation can restrict CO_2 diffusion (Nakano *et al*., 2000) and disrupt chloroplasts (Pritchard *et al*., 1997).

Recent evidence suggests that night-time hexose content derived from starch may provide signals for longer term feedback regulation through modulation of gene expression (Cheng *et al*., 1998). To date, there has been confusion concerning the nature of the starch degradation product exported from the chloroplast. Bearing this in mind several models could be formulated. A simple model based on the amount of glucose liberated by starch degradation, particularly in situations of high leaf starch accumulation, may be used to describe the role of starch in sugar signalling. There may be speculation that, for example, glucose is the predominant form in which carbon derived from starch degradation is exported from the chloroplast (Trethewey and ap Rees, 1994; Schleucher *et al*., 1998). A change in the amount or timing of glucose production from starch and its export from the chloroplast under situations of high starch accumulation could provide a feedback signal. Indeed, this would have more direct access to sensing components than hexoses generated and sequestered in the vacuole. A hexokinase associated with the outer membrane of the chloroplast may function to phosphorylate glucose during starch mobilization (Stitt *et al*., 1978; Wiese *et al*., 1999) (see later for discussion of role of hexokinases).

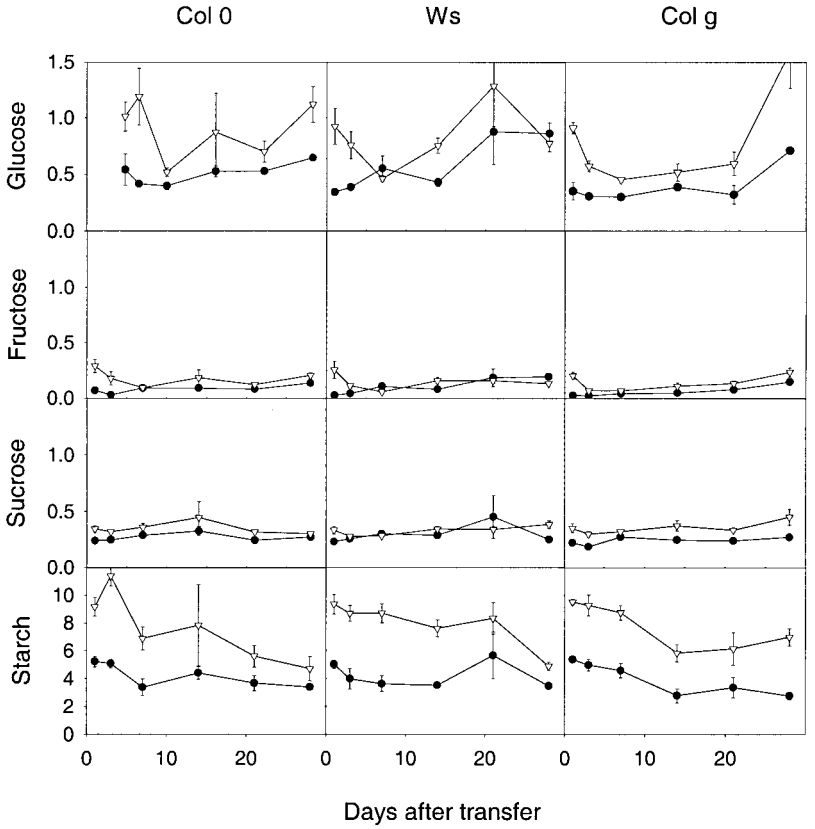

Fig. 3. Response of shoot carbohydrate content of *Arabidopsis thaliana* ecotypes to transfer to elevated CO_2 (1000 ppm, open symbols) over several days. Plants were transferred 4 weeks after sowing and the other growing conditions were 20 °C, 10 h photoperiod, 300 μmol quanta m^{-2} s^{-1}. Control plants were maintained at 350 ppm CO_2 (closed symbols). Col O, Columbia; Ws, Wassilewskija; Col g, Columbia glabrous. Plants were grown in soil and given full nutrition and irrigation throughout.

The timing of starch degradation at night varies between species. In many cases, there is a lag in starch degradation until there is a drop in leaf sucrose suggesting that high sugar contents have a feedback effect on starch degradation (Trethewey and ap Rees, 1994; Cheng *et al*., 1998). This provides an additional mechanism (other than Pi/triose phosphate exchange) by which information on the carbohydrate status of the chloroplast may contribute information on the assimilate status of the leaf. Thus far, relatively few studies have concerned the role of starch turnover in regulating photosynthetic gene expression (Cheng *et al*., 1998) possibly because of the present very poor understanding of the factors that control starch turnover in leaves. However, starch turnover may have a role in signalling assimilate abundance, something that has previously been overlooked.

Interactions with other organisms: the establishment of a new sink

Interactions of leaves with other organisms that can act as an additional sink within the leaves can have a profound effect on the source–sink relationships of the plant. Following pathogen attack or approach by interactive microbes there is often a decrease in the export of sucrose from the infected leaves and an increase of import to infection sites (Farrar, 1992; Ayres *et al*., 1996). The induction of host apoplastic invertase and host hexose transporters are crucial to the establishment of increased sugar transport capacity to the new sink (Tang *et al*., 1996). They may serve to divert carbon en route to the phloem for export. In powdery mildew infections of pea, wheat and *Arabidopsis*, glucose, rather than sucrose, is the major form of carbon transferred to the fungus (Sutton *et al*., 1999). In *Arabidopsis* this marked increase in host transport capacity is linked to the induction of a specific member of a family of hexose transporters, sugar transport protein 4 (STP4; Truernit *et al*., 1996). The SUT4 subfamily of sucrose transporters are expressed predominantly in minor veins in source leaves where high capacity sucrose transport is needed for phloem loading (Weise *et al*., 2000). Some pathogen infections induce programmed cell death with the formation of necrotic tissue within which 'green islands' of healthy tissue persist to feed the pathogen and these areas show enhanced rates of photosynthesis. Little is known about the signals that facilitate this enhancement of photosynthesis, but recently it has been demonstrated that injection of salicylic acid

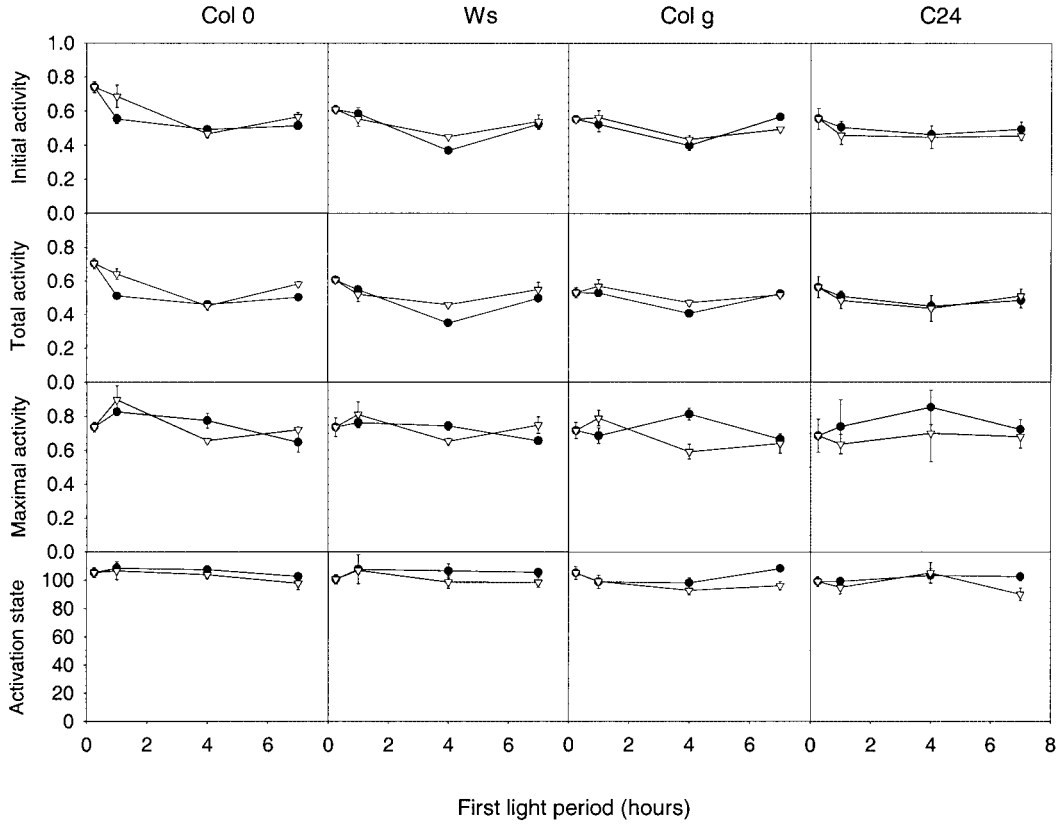

Fig. 4. Response of Rubisco activity of *Arabidopsis thaliana* ecotypes to transfer to elevated CO_2 (1000 ppm, open symbols) during the first photoperiod after transfer. Plants were transferred 4 weeks after sowing and the other growing conditions were 20 °C, 10 h photoperiod, 300 μmol quanta m^{-2} s^{-1}. Control plants were maintained at 350 ppm CO_2 (closed symbols). Col O, Columbia; Ws, Wassilewskija; Col g, Columbia glabrous. Plants were grown in soil and given full nutrition and irrigation throughout.

(a key signal in the plant eliciting defence responses during pathogen attack) into corn increased photosynthetic rates (Zhou *et al*., 1999). There is evidence that the changes in leaf apoplastic invertases and leaf sugar composition, particularly the increase in hexose content, that occur upon infection and in other stress conditions are involved in the induction of host defence responses (Herbers *et al*., 1996, 2000). Infection by pathogens indicates that there are mechanisms that enable co-ordinate regulation of genes of carbon metabolism in plants. It is possible that changes in sugars that accompany pathogen infection are involved in the whole-scale changes in gene expression and resource allocation that accompany plant/pathogen interactions. This has formed part of a strong body of evidence that emerged during the 1990s that sugars in leaves can repress the expression of photosynthesis genes.

Molecular regulation of photosynthesis by sugars

The development of current concepts on the role of sugars in the repression of photosynthetic gene expression coincided with an explosion of research on effects of climate change and, in particular, the effects of elevated CO_2 on plant physiology. This emphasized the two-way interaction between photosynthesis and growth. It had been known for many years that sucrose and glucose included as osmotica in protoplast cultures inhibited photosynthesis, but it was thought that this was due to osmotic stress rather than a direct effect of sugars (Fleck *et al*., 1982; Vernet *et al*., 1982). More recently, it was shown that sugars also repress the expression of photosynthetic genes (Sheen, 1990). In transgenic plants, expression of yeast invertase in the cell wall was found to result in progressive chlorosis in the leaves from the tip downwards as the leaves matured (von Schaewen *et al*., 1990; Stitt *et al*., 1991). The chlorotic areas had a high carbohydrate content, especially soluble sugars, and photosynthesis was gradually inhibited over several days as sugars accumulated in the maturing leaves. In these tissues the ATP/ADP ratio was increased, while the 3-PGA pool was low, distinct from the pattern in the short term due to low Pi when sucrose accumulates. Photosynthesis was clearly limited by the rate of RuBP regeneration and by the rate of carboxylation (von Schaewen *et al*., 1990; Stitt *et al*., 1991). This was

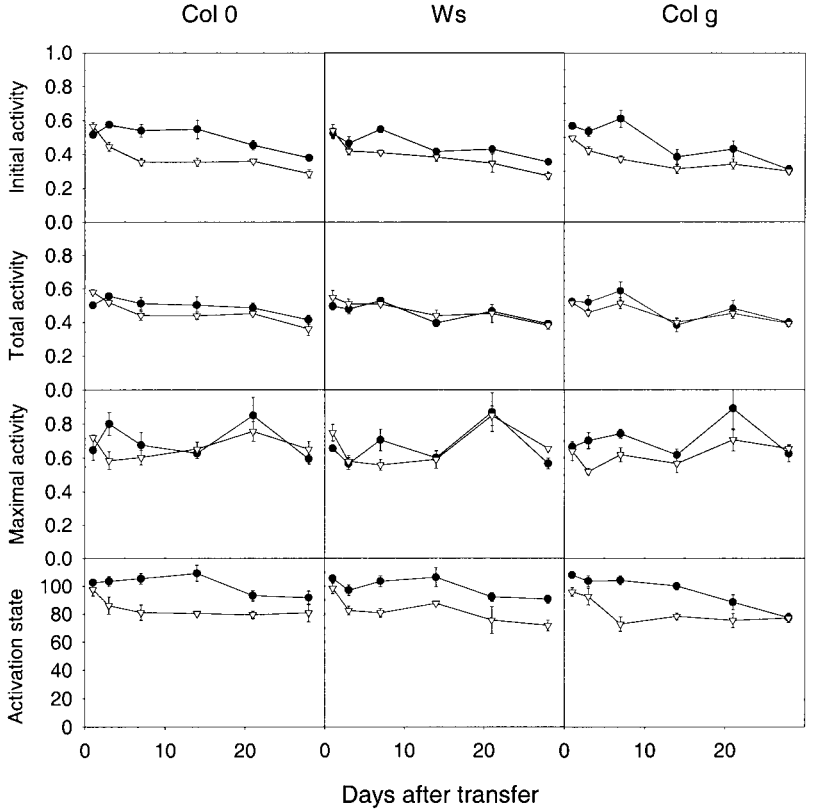

Fig. 5. Response of Rubisco activity of *Arabidopsis thaliana* ecotypes to transfer to elevated CO_2 (1000 ppm, open symbols) over several days. Plants were transferred 4 weeks after sowing and the other growing conditions were 20 °C, 10 h photoperiod, 300 μmol quanta m^{-2} s^{-1}. Control plants were maintained at 350 ppm CO_2 (closed symbols). Col O, Columbia; Ws, Wassilewskija; Col g, Columbia glabrous. Plants were grown in soil and given full nutrition and irrigation throughout.

consistent with a large decrease in the activities of Calvin cycle enzymes, including Rubisco. When glucose was supplied through the transpiration stream of mature spinach leaves, Rubisco was lost from the leaves over a 7 d period (Krapp *et al*., 1991). This finding was also confirmed in cell suspension cultures (Schafer *et al*., 1992; Krapp *et al*., 1993). The interpretation of sugar feeding experiments is complicated to some extent by the simultaneous changes in other metabolites as a result of the metabolism of sucrose or as a result of increased flux through pathways such as nitrogen assimilation stimulated by sugar feeding, which may function as signals too (Ferrario-Mery *et al*., 2000). In addition, experiments on sugar feeding are largely conducted *in vitro* with artificially high levels of sugar. Hence, these studies indicated that sugar-mediated control of gene expression can occur in plants. The extent to which it actually occurs under physiological conditions remains debatable.

Growth of plants at elevated CO_2 to drive the productivity of the source provides a more physiological means of exploring carbohydrate control of photosynthetic gene expression as a mechanism of sink regulation of photosynthesis. Growth with elevated CO_2 depresses photorespiration in C_3 plants. The resulting higher rate of photosynthetic CO_2 assimilation provides more carbohydrate for metabolism and export to sinks. Sink activity is stimulated due to direct effects of enhanced substrate availability and also through the stimulation of the expression of genes encoding proteins involved in the catabolism of sucrose probably via pathways analogous to those involved in the regulation of gene expression in response to sugars in leaves (Farrar and Williams, 1991; Koch, 1996; Pollock and Farrar, 1996). When sinks cannot use all of the assimilate generated by high rates of photosynthesis, sugar accumulation in leaves has direct effects on expression of ADPglucose pyrophosphorylase (Muller-Rober *et al*., 1991) and other carbohydrate-responsive enzymes of sucrose and starch metabolism (Koch, 1996) resulting in adaptive changes in assimilate partitioning. Sugar accumulation also represses the expression of the sucrose transporter (Chiou and Bush, 1998). Direct effects on photosynthesis are seen as a decrease in levels of nuclear-encoded transcripts, such as the small subunit of Rubisco (*rbcS*) (Moore *et al*., 1998), together with chlorophyll-binding protein (*Cab*) and Rubisco activase in tomato, whereas levels of plastid-encoded transcripts including *rbcL*, *PsaA*, *PsaB*, and *PsbA* (encoding the large subunit of Rubisco and core proteins in

photosystem I and II) do not decrease in this species (Van Oosten *et al*., 1994; Van Oosten and Besford, 1995). In wheat and *Arabidopsis* elevated CO_2 decreases both *rbcS* and *rbcL* transcripts as well as transcript abundance of other Calvin cycle enzymes (Nie *et al*., 1995; Cheng *et al*., 1998).

One approach to unravelling the molecular mechanisms and signal transduction pathways through which sugars control photosynthetic gene expression has been to use genetic screens based on either sugar-regulated gene expression or the arrest of development by high sugar concentrations. This has led to the isolation of a large number of sugar-sensing mutants. These include the sucrose-uncoupled (*sun*) mutants (Dijkwel *et al*., 1997), the reduced sucrose response (*rsr*) mutants (Martin *et al*., 1997), low and high β-amylase (*lba* and *hba*) mutants (Mita *et al*., 1997), glucose-insensitive (*gin*), carbohydrate-insensitive (*cai*) and mannose-insensitive germination (*mig*) mutants (Smeekens and Rook, 1997). These screening strategies have been useful tools in the isolation of mutants in the sugar-mediated regulation of gene expression. However, none of these mutants has yet identified a component of a primary sugar-signalling cascade. The recent identification of *gin1* and *sun6* mutated in interacting hormone pathway components, demonstrates the highly integrated nature of sugar sensing and the need for more specific screening strategies capable of discriminating between the primary sugar-signalling cascades and secondary pathways subject to their control.

An alternative approach, and one that has resulted in more definitive information thus far on primary components of sugar signalling in plants, has been the search for plant homologues to proteins known to be involved in sugar signalling in yeast. SNF1 is a global regulator of carbon metabolism in yeast and is activated in response to low cellular glucose levels. SNF1-related protein kinases (SnRKs) have been described in many species. *A. thaliana*, for example, has at least seven different isoforms (Halford and Hardie, 1998). Three important biosynthetic enzymes of plant metabolism, sucrose phosphate synthase (SPS), nitrate reductase (NR) and HMG-CoA reductase (HMGR) are phosphorylated and inactivated by SnRK1. Recent evidence suggests that the phosphorylation control exerted by SnRK1 in inactivating SPS can be overridden by metabolites such as G6P (Toroser *et al*., 2000). Transgenic plants expressing antisense SnRK1 have demonstrated that it is required for sucrose-induced sucrose synthase gene expression in potato (Purcell *et al*., 1998).

Whereas SnRK1 has been linked most closely to sucrose induction and carbon starvation, hexokinase as a sugar sensor has been most linked to carbon surfeit and, in plants, to repression of genes for photosynthesis. As for SnRK1 kinase, the first evidence of the involvement of sugar kinases in the transmission of sugar signals came from yeast (Zimmermann and Scheel, 1998). In higher plants, the weight of evidence and argument has suggested hexokinases have a similar function as key sensors and signal transmitters (Graham *et al*., 1994; Jang and Sheen, 1994; Jang *et al*., 1997; Pego *et al*., 1999). Like SnRKs, they are universal in higher plants and have been localized in the cytosol, plastids and mitochondria (Miernyk and Dennis, 1983; Schnarrenberger, 1990; Renz *et al*., 1993; Galina *et al*., 1999). Up to four hexokinase genes have shown to be present in plants (Renz *et al*., 1993; Giese *et al*., 2000). Transgenic plants expressing antisense hexokinase genes have been shown to be sugar hyposensitive to glucose, whereas overexpressors were sugar hypersensitive (Jang *et al*., 1997). In contrast, overexpression of yeast sugar sensor hexokinase II led to elevated hexokinase activity, but to a decrease in sugar sensitivity. In a similar fashion, *Arabidopsis* hexokinase genes expressed in the yeast *hex1 hxk2* double mutant restored the catalytic, but not the regulatory function (Jang *et al*., 1997). These results suggest that hexokinase is a bifunctional enzyme with catalytic and sugar-sensing activities in plants. However, the precise way in which hexokinase exercises this sensory role is not known and still remains largely mysterious in all organisms (Smeekens, 1998). There is still equivocation regarding the separation and definition of the metabolic and sensory roles of hexokinase (Halford *et al*., 1999; Moore and Sheen, 1999). Detailed characterization of the effects on metabolism in transgenic plants with altered hexokinase activity would counter the contention that changes in metabolites downstream of hexokinase are responsible for perturbed sugar-sensing phenotypes.

An added complexity has come from the finding that trehalose metabolism, the genes for which have recently been found to be universal in plants, may have a role in sensing carbon status perhaps through an interaction with hexokinase (Goddijn and Smeekens, 1998). In yeast trehalose metabolism performs a signalling role (Blasquez *et al*., 1993). Evidence from current research expressing *Escherichia coli otsA* and *otsB* genes in transgenic tobacco that encode trehalose phosphate synthase (TPS) and trehalose phosphate phosphatase, respectively, have produced effects consistent with an impact on sugar signalling (Paul *et al*., 2001). Significantly, photosynthetic capacity per unit leaf area in transgenics expressing the TPS transgene is higher than in wild type. In yeast, trehalose-6-phosphate (T6P) interacts with hexokinase impacting on carbon metabolite sensing. Such an interaction may modify the perception of carbon status. T6P may have a similar role in plants. Plants expressing the TPS transgene and with elevated T6P perceive a carbon deficit and up regulate photosynthesis. Neither breeding nor genetic modification has thus far improved photosynthetic capacity per unit leaf area. This research

shows that it is possible and may represent a significant advance towards increasing the CO_2 fixation of crops (Paul *et al*., 2001).

Modulation of Rubisco protein content is considered to be a major means by which the rate of photosynthesis is adjusted to match sink activity in plants grown at high CO_2. The simplest mechanistic model is that turnover of sucrose and starch are sensed by hexokinase which initiates a signal transduction pathway leading to loss of Rubisco from leaves (van Oosten and Besford, 1996; Moore *et al*., 1998, 1999). The induction of apoplastic invertase activity correlates with the repression of photosynthetic genes in a number of species (Moore *et al*., 1998). However, this is only part of the story as CO_2 enrichment accelerates the normal development of leaves. Plants grown with high CO_2 tend to progress through the life cycle more rapidly and enter senescence much earlier than those grown in air (Catsky *et al*., 1976; Thomas, 1984; Guitman *et al*., 1991) an effect enhanced by nitrogen limitation (Miller *et al*., 1997, 2000). The high CO_2-mediated loss of Rubisco may, therefore, be explained by senescence rather than sugar-mediated repression transcription. The effects of sugars on Rubisco expression depends on leaf age (Van Oosten and Besford, 1995). Enhanced senescence is also observed in transgenic tobacco plants with increased hexokinase activity (Dai *et al*., 1999) and this may also be related to more rapid leaf development. Sugars are only a part of the repertoire of signals that co-ordinate the source–sink interaction and must be considered in the wider context of other important factors. Nitrogen availability is a dominant limiting factor in the natural environment and plants invest excess nitrogen in Rubisco when the nitrogen supply is abundant (Stitt and Schulze, 1994). Carbohydrate accumulation in leaves leads to nitrogen release from Rubisco, which can be used for growth processes. Carbohydrate repression of photosynthesis may, therefore, be viewed as a mechanism that optimizes the whole plant carbon to nitrogen balance. Sugar signalling is dependent on the nitrogen status, as well as the carbon status, of the plant.

Carbon/nitrogen balance regulates photosynthesis

The decline in Rubisco content that accompanies nitrogen deficiency can be prevented in leaves that are shaded to prevent carbohydrate accumulation (Paul and Driscoll, 1997). This result indicates that there is a close interaction between carbon and nitrogen signalling that regulates Rubisco levels. A consistent picture is emerging that the carbon and nitrogen status of plants interacts to regulate photosynthesis and that a surfeit of carbon metabolites and a deficit of nitrogen is necessary for nitrogen remobilization from Rubisco. Support for the crucial involvement of nitrogen deficiency has been provided by a number of studies. Firstly, at high CO_2 when nitrogen supply is high and carefully monitored to keep up with growth rate, there is no loss of Rubisco protein (Habash *et al*., 1995; Atkinson *et al*., 1997; Ludewig *et al*., 1998; Geiger *et al*., 1999). Importantly, the carbohydrate content of the leaves still rises in these conditions (Geiger *et al*., 1999). Secondly, sink growth can be slowed by exposure to low temperatures or to low phosphate nutrition without nitrogen deficit. In such studies carbohydrate accumulation in leaves is not associated with loss of Rubisco protein (Paul and Stitt, 1993; Stitt *et al*., 1995; Nielsen *et al*., 1998, for low Pi; Hurry *et al*., 1995; Strand *et al*., 1997, for low temperatures). Thirdly, where individual leaves are exposed to high CO_2 and the rest of the plant is kept at ambient CO_2, carbohydrate content in the individual leaves at high CO_2 rises without this resulting in loss of Rubisco (Sims *et al*., 1998). These data strongly suggest that a simple hypothesis whereby sugars alone mediate sink regulation of photosynthesis, is too simplistic. Taken together these data suggest that photosynthesis responds to and is controlled by whole plant source–sink balance, controlled by whole plant nutrient balance, principally by the carbon to nitrogen status.

Mechanisms by which carbon/nitrogen balance regulates photosynthesis

Carbon metabolism is inextricably linked to nitrogen metabolism and any effect of a change in carbon abundance impacts on nitrogen metabolism and vice versa (Noctor and Foyer, 2000; Lewis *et al*., 2000). Utilization of sucrose in growing sinks depends on the simultaneous provision of amino acids. The assimilation of nitrate and ammonium in shoots and roots, to produce amino acids requires ATP, reductant and carbon skeletons. These are provided by photosynthesis, glycolysis and respiration. Co-ordination is essential to prevent uncontrolled competition for energy and carbon skeletons between the pathways of nitrogen assimilation, carbohydrate production and CO_2 assimilation. The integration of these pathways requires the interaction of a concerted repertoire of signals that allows graded molecular regulation of the expression of genes for carbon and nitrogen metabolism including those for photosynthesis. Alterations of carbon flux through hexokinase in combination with the prevailing rate of N assimilation may modify the size of the pools of 2-oxoglutarate, acetyl-CoA and possibly the AMP : ATP ratio which could form part of the signal transduction pathway modifying expression of Rubisco and genes involved in photosynthesis. In microorganisms, 2-oxoglutarate and glutamine

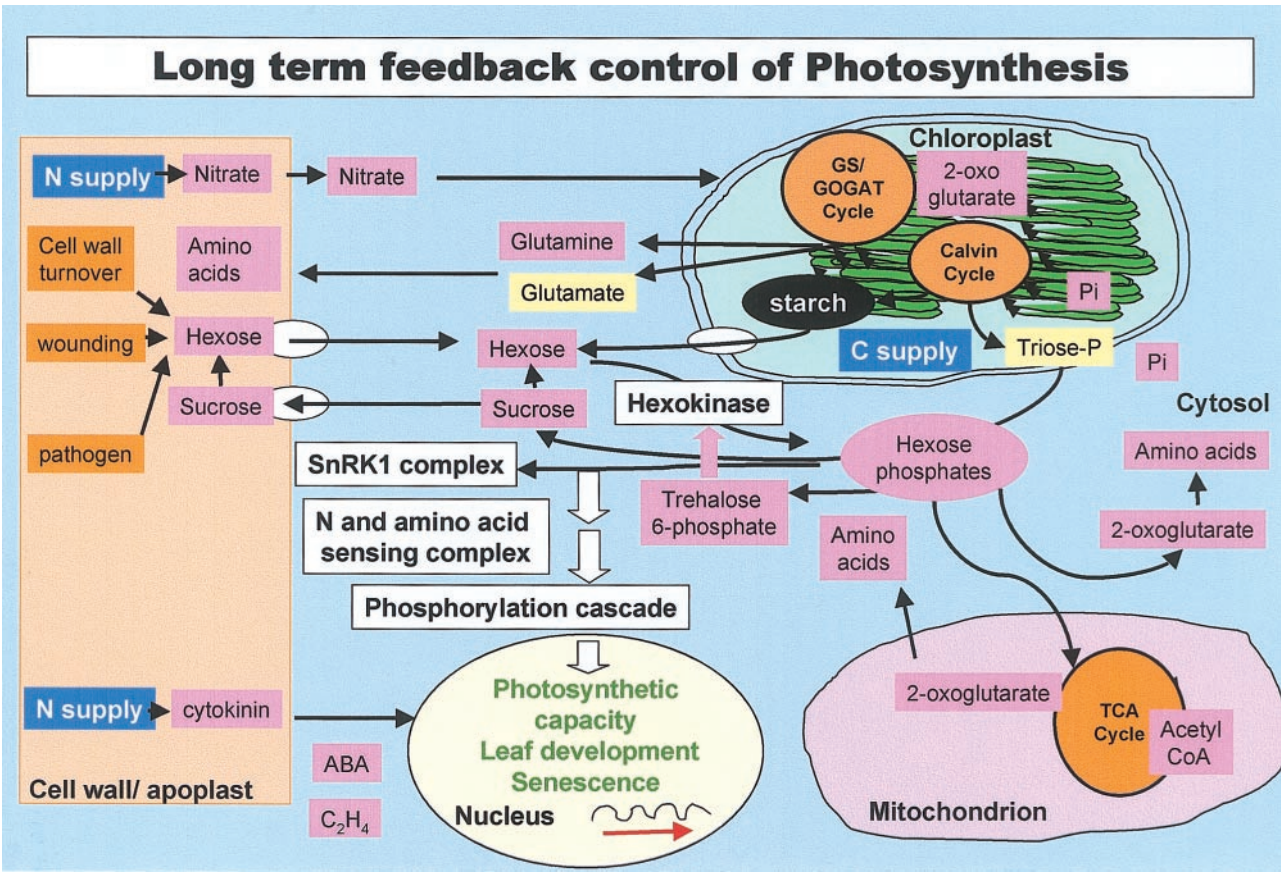

Fig. 6. Long-term feedback regulation of photosynthesis. Simplified model of source/sink control of photosynthetic capacity and leaf development. Photosynthate supply and phytohormones, particularly cytokinins, interact with nitrogen supply through signal transduction pathways to control the expression of photosynthesis genes and development and senescence of leaves. Signalling and putative signalling molecules are in pink boxes. Putative sensing components are highlighted in white.

signal carbon and nitrogen sufficiency, respectively, to co-ordinate carbon : nitrogen balance (Kamberov *et al.*, 1995). They are involved in the regulation of the *Nrt* regulon, low levels of 2-oxoglutarate inhibiting the transcription of *GlnA* (necessary for synthesis of glutamine synthase) by forming a stable PII–NRII complex which is capable of dephosphorylating and inactivating the enhancer-binding transcription factor NRI, which controls *GlnA* expression. This complex is not formed in the presence of high levels of 2-oxoglutarate (Galvez *et al.*, 1999). In *Synechococcus* PII kinase activity is dependent on 2-oxoglutarate and ATP and nitrogen deprivation leads to a 10-fold activation of the PII gene (Bohme, 1998). Glutamine content is sensed by the Utase/UR protein (Kamberov *et al.*, 1995) and is linked to PII levels (Galvez *et al.*, 1999). Recently *GLB1*, encoding a homologue of the bacterial PII protein, has been isolated from *A. thaliana* (Hsieh *et al.*, 1998). Protein kinases, such as GCN2 (Hinnebusch, 1994) that mediate cellular responses to low amino acid levels that arise from low nitrogen are known in yeast and similar sensing mechanisms probably exist in plants too. In plants, glutamine and 2-oxoglutarate are also key metabolite signals in the carbon/nitrogen interaction. Glutamine acts antagonistically with 2-oxoglutarate (and with sucrose) in the regulation of the transcription of nitrate reductase (Ferrario-Mery *et al.*, 2000; Vincentz *et al.*, 1992; Morcuende *et al.*, 1998). Such data provide evidence of crosstalk of signals derived from carbon (2-oxoglutarate and sucrose) and nitrogen metabolism (glutamine). Although glutamine has no direct effect on Rubisco transcript abundance (Stitt *et al.*, 1995), glutamine responds sensitively to nitrate and sucrose supply (Scheible *et al.*, 1997; Morcuende *et al.*, 1998; Lancien *et al.*, 1999). It is greatly increased, for example, in transgenic plants expressing Fd GOGAT in the antisense orientation (Ferrario-Mery *et al.*, 2000). Expression of phosphoenolpyruvate carboxylase gene transcription is tightly controlled by nitrogen supply particularly in the presence of cytokinin. PEP carboxylase is activated by changes in the phosphorylation state of the protein and activity increases as the leaf glutamine pool rises favouring increased carbon flux through the anapleurotic pathway (Duff and Chollet, 1995; Murchie

et al., 2000). Like 2-oxoglutarate, acetyl-CoA has a pivotal role in carbon metabolism in chloroplasts being involved in the pathways of amino acid, fatty acid and nucleotide metabolism. Acetate and therefore acetyl-CoA directly influences carbon metabolism by regulating gene expression (Sheen, 1990) and may exert a broad control over carbon metabolism through signalling (Koch, 1996).

A series of *Nature* papers (Sweet and Wareing, 1966; Treharne and Stoddart, 1968; Wareing *et al*., 1968) confirmed that plant growth regulators, especially cytokinin were involved in the regulation of photosynthetic rate and source/sink balance. Cytokinins in roots respond strongly to nitrogen supply (Samuelson *et al*., 1992; Wagner and Beck, 1993). Movement of cytokinins in the transpiration stream from the roots to the shoots stimulates the expression of photosynthesis genes including Rubisco (Lerbs *et al*., 1984), carbonic anhydrase (Sugiharto *et al*., 1992), light-harvesting chlorophyll *a*/*b* binding protein (Flores and Tobin, 1989) and phosphoenolpyruvate carboxylase (Suzuki *et al*., 1994). Cytokinin stimulates the expression of the *pZmCip1* gene in leaves, which has homology to the response regulator element of bacterial two component signalling systems (Sakakibara *et al*., 1998). Cytokinins delay leaf senescence and offset effects of sugars and light (Wingler *et al*., 1998; Jordi *et al*., 2000). Abscisic acid (ABA) is also involved in the sugar-mediated regulation of gene expression (Arenas-Huertero *et al*., 2000; Finkelstein and Lynch, 2000), the glucose-specific accumulation of ABA being essential for hexokinase-mediated glucose responses. Recent results obtained with sugar-signalling mutants of *A. thaliana* suggest that ABA plays a direct role in mediating the photosynthesis to respiration ratio in leaves and also the inhibition of lateral root development by nitrate. Evidence has also been presented for crosstalk between sugar and ethylene signalling (Zhou *et al*., 1998). These phytohormones are known to be involved in senescence and hence should be viewed as components that control the expression of photosynthetic genes as well as controlling the senescence of leaves co-ordinating photosynthetic activity with the physiological state of the plant (Fig. 6).

Conclusions

Photosynthesis is inextricably linked to whole plant physiology by reciprocal controls. A metabolic signalling network involving information on the carbon and nitrogen status of different tissues interacts with phytohormone signalling pathways and redox signals to control photosynthetic gene expression and leaf development. This highly integrated signal transduction network, which forms the basis of the source–sink interaction, regulates photosynthetic activity by determining the amount of photosynthetic apparatus present during leaf development and senescence, overriding direct control of photosynthesis by light and CO_2. A more comprehensive understanding of the mechanisms and their operation in whole plants will provide a means to enhance photosynthesis and crop productivity.

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