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Control of Photosynthetic Carbon Fixation and Partitioning: How can Use of Genetically Manipulated Plants Improve the Nature and Quality of Information about Regulation?

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Control of photosynthetic carbon fixation and partitioning: how can use of genetically manipulated plants improve the nature and quality of information about regulation?

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SUMMARY

The study of regulation has previously involved indirect, and largely correlative, approaches. In the following contribution I illustrate the uses and limitations of these traditional approaches, and then discuss how molecular genetics provide a new tool to test directly ideas about regulation *in vivo* in the intact organism.

1. THE PATHWAYS, AND WHY THEY ARE REGULATED

The pathways of sucrose and starch synthesis (Stitt *et al.* 1987) are summarized in figure 1. Inorganic phosphate (P_i) and CO_2 are converted to triose phosphate in the chloroplast. Most of the triose phosphate is exported to the cytosol and converted to sucrose, releasing P_i which returns to the chloroplast via the phosphate translocator (TPT) in counter-exchange with triose phosphate. Some triose phosphate may be retained in the chloroplast and converted to starch.

Unless the rate of sucrose and starch synthesis are coordinated with the rate of CO_2 fixation, the Calvin cycle will be inhibited, due to (i) depletion of metabolites and lack of ribulose 1,5-bisphosphate (if triose phosphates are removed too quickly), or (ii) accumulation of phosphorylated metabolites and depletion of P_i and inhibition of ATP synthesis (if triose phosphates are removed too slowly). Within this window, the relative rates of sucrose and starch synthesis need to be additionally regulated in response to the rate of sucrose export, to allow starch storage, and to allow some photosynthate to be diverted into anapleurotic pathways for amino acid synthesis during NO_3^- or NH_4^+ assimilation.

2. IDENTIFICATION AND CHARACTERIZATION OF REGULATORY STEPS: THE BIOCHEMICAL APPROACH

When confronted with a metabolic system containing a large number of enzymes, an understandable first aim is to reduce the complexity by identifying one or a small number of 'parameters' or 'key regulatory' enzymes, which control flux through the system. Several criteria have been proposed to pin-point such enzymes (Rolleston 1972; Newsholm & Start 1973), for example: (i) the enzyme should be present at activities which are not greatly above the required flux through the pathway; (ii) the enzyme should possess 'regulatory properties', e.g. product inhibition, allosteric regulation, phosphorylation sites; (iii) the enzyme should catalyse an 'irreversible' (thermodynamically 'non-equilibrium') reaction; and (iv) there should be characteristic reciprocal changes of flux and substrate concentration *in vivo*. Application of these criteria to the pathways of sucrose and starch synthesis illustrates the strengths, but also several short-comings, of this purely biochemical approach.

Firstly, metabolic pathways often contain more than one 'regulatory enzyme'. For starch, the reactions catalysed by ADPglucosepyrophosphorylase (AGPase), starch synthase and branching enzyme are all irreversible. Although AGPase is widely accepted to be a key regulating step (Preiss 1982) it would be premature to exclude a role for other enzymes in some conditions (see Smith & Martin 1993). The pathway of sucrose synthesis also contains several non-equilibrium reactions, catalysed by the cytosolic fructose-1,6-bisphosphatase (cFBPase), sucrose-phosphate synthase (SPS), sucrose-6-phosphatase, as well as the hydrolysis of pyrophosphate (Stitt *et al.* 1987). In addition, the transport step catalysed by the TPT may sometimes be displaced from equilibrium (Gerhardt *et al.* 1987). At least two of the enzymes in the pathway of sucrose synthesis (cFBPase, SPS) exhibit reciprocal changes of substrate concentration and flux *in vivo*.

Secondly, enzymes are often susceptible to regulation by multiple effectors, and these are commonly highly interactive. This means it is difficult to extrapolate from *in vitro* to *in vivo*. For example, cFBPase is

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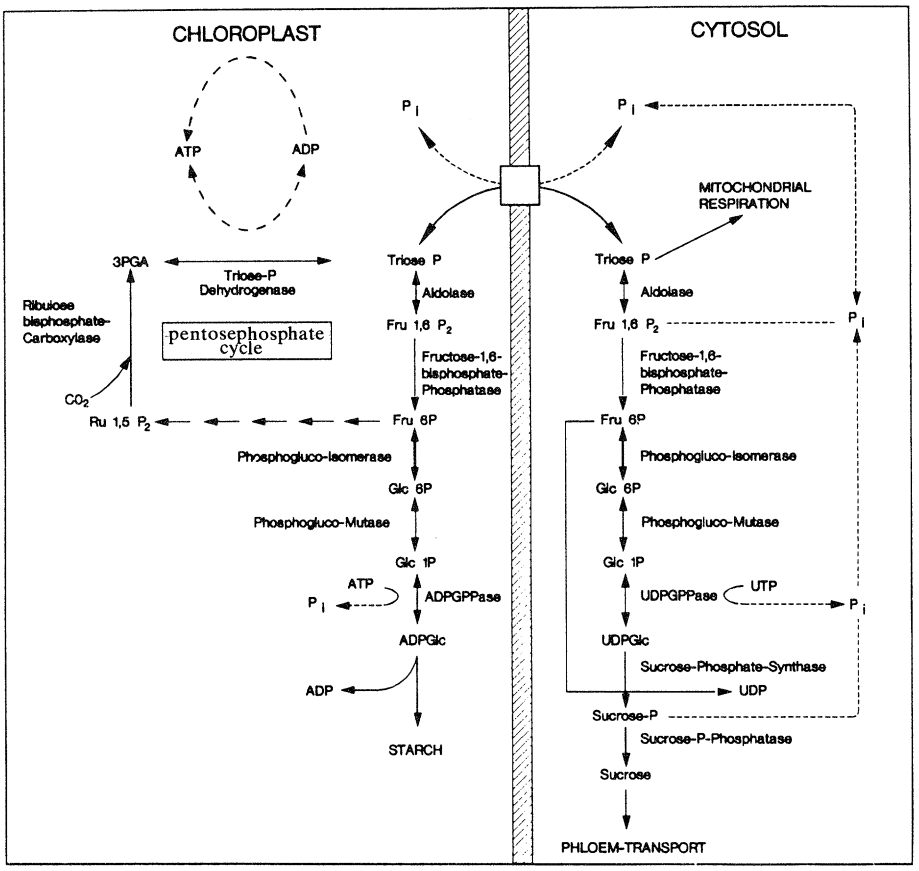

Figure 1. Pathway of sucrose and starch metabolism. Reproduced with permission from Stitt (1993).

inhibited by the signal metabolite fructose 2,6-bisphosphate (Fru2,6bisP), AMP, P_i (Stitt *et al.* 1987; Stitt 1990), Ca^{2+} (Brauer *et al.* 1990), and these interact with the pH, the Mg^{2+} concentration, and the substrate concentration (see Stitt *et al.* 1987). SPS is activated by glucose 6-phosphate (Glc6P) and inhibited by inorganic phosphate (P_i) at an allosteric site (Doehlert & Huber 1984), is inhibited by sucrose in some species (Stitt *et al.* 1987) and is also inhibited by protein phosphorylation (Huber & Huber 1992; Siegl *et al.* 1990).

Thirdly, activity of the various enzymes in a pathway is highly coordinated. This can be illustrated for the cFBPase and SPS, by briefly considering how changes in Fru2,6bisP and protein phosphorylation act in parallel to alter their activity in response to an increased rate of photosynthesis, or to an accumulation of sucrose in the leaf (see Stitt *et al.* 1987; Stitt 1991). When the rate of photosynthesis increases (figure 2*a*), a rising concentration of glycerate 3-phosphate (3PGA) and probably, a decrease in P_i inhibit the enzyme responsible for synthesis of Fru2,6bisP (Fru6P,2-kinase), leading to a decrease of Fru2,6bisP and activation of cFBPase. This will be potentiated by rising concentrations of substrate as triose phosphate rise in the cytosol. The increased production of hexose phosphate then leads to allosteric activation of SPS. Rising Glc6P and falling P_i are further implicated as mechanisms to inhibit SPS-kinase (Huber & Huber 1992) and activate protein phosphatase 2A (Weiner *et al.* 1994), allowing dephosphorylation (activation) of SPS. When sucrose accumulates in the leaf SPS is deactivated (see Stitt *et al.* 1988), presumably due to modulation of SPS-kinase or PP2A via mechanisms which we are currently investigating. As a result the fructose 6-phosphate (Fru6P) concentration rises, leading to activation of Fru6P,2-kinase and inhibition of Fru-2,6-bisphosphatase, a rise of Fru2,6bisP, inhibition of the cFBPase, and decreased export of triose phosphate out of the chloroplast. The resulting shortfall of P_i is accompanied by a restriction on 3PGA reduction, and starch synthesis is activated because AGPase is stimulated by a rising 3PGA/P_i quotient.

3. REGULATION AND CONTROL

These studies provide many insights into details but they do not yet reveal the key site(s) at which partitioning is controlled. An enzyme may be regulated to maintain a functional balance within a pathway, or to initiate and maintain a change in flux through the pathway. Much regulation will be involved with the former; coordinating the activities of enzymes and maintaining concentrations of cofactors and substrates in a range allowing operation of the other enzymes in the pathway. The more complex and integrated system, the more frequently parameters will correlate with flux as a consequence, rather than cause, of regulation.

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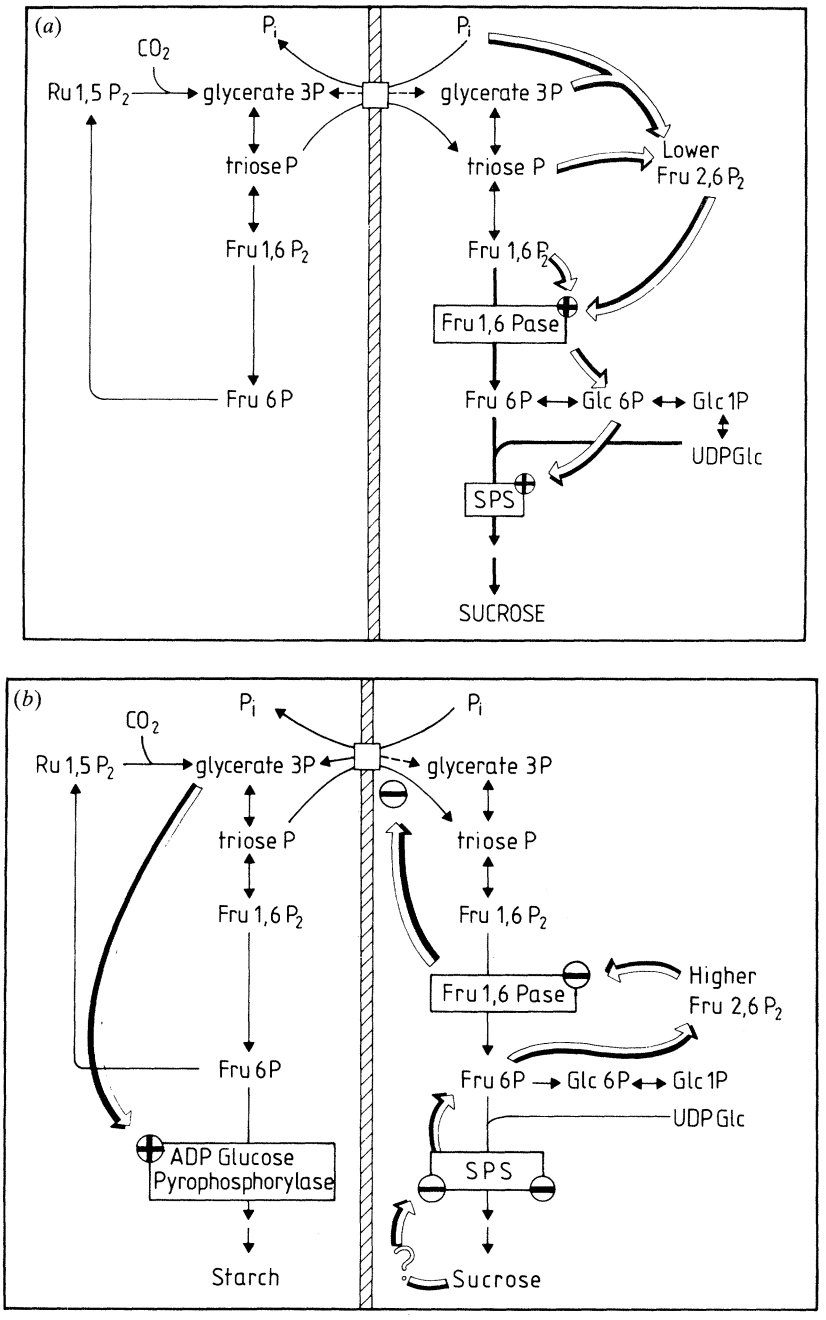

Figure 2. Regulation of sucrose synthesis. (*a*) Feedforward; (*b*) Feedback. Reproduced with modification from Stitt (1990*b*).

4. USE OF MOLECULAR GENETICS

Genetically manipulated plants allow us to: (i) dramatically decrease or remove an enzyme, to determine whether it is essential; (ii) generate changes in protein amount to assess its contribution to control of flux; (iii) introduce a protein with changed properties to assess how this effects its operation *in vivo*; and (iv) introduce a heterologous protein to disrupt a process; or (v) allow a process which would not usually occur. I will illustrate these approaches with examples, starting with a study of a single enzyme to introduce the topic, and then the more complicated case of an interaction between the pathways for starch and sucrose synthesis.

5. CONTRIBUTION OF RUBISCO TO THE CONTROL OF PHOTOSYNTHETIC FLUX

A direct way to decide whether a particular enzyme contributes to the control of flux through a pathway would be to decrease the amount of protein slightly (say 30%) without changing the amount of any other enzyme, and to monitor the resulting change in flux. If the flux changes proportionally (say 30%) then flux is controlled (limited in the strict sense of the word) by the enzyme. If pathway flux is unaffected, the enzyme is not controlling flux. If flux changes by a smaller value (say 10%) than the enzyme contributes to control, but is not the only site of control ('co-limitation').

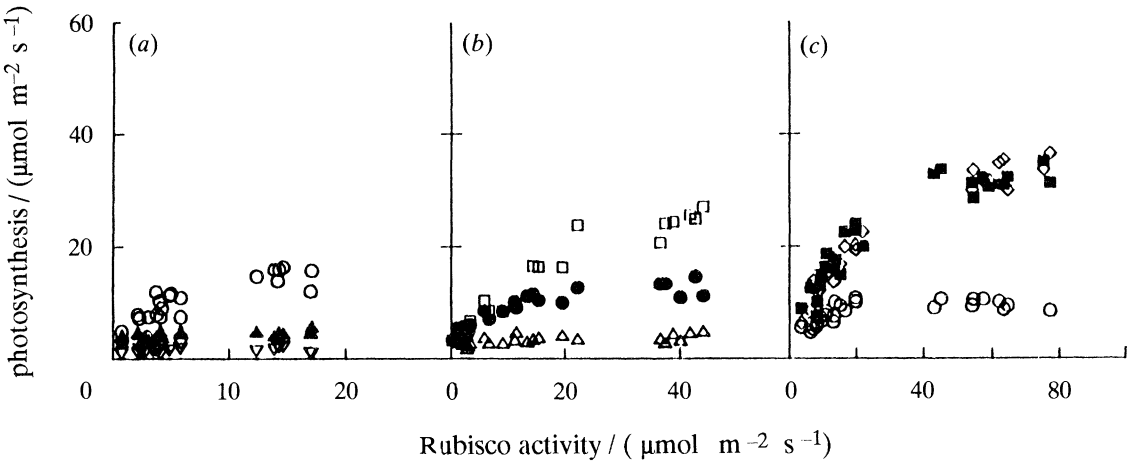

Figure 3. Relation between Rubisco content and photosynthesis in 'antisense' *rbc*S tobacco plants grown at three different irradiances; solid symbols show measurements carried out at the growth irradiance corresponding to (*a*) 100 (triangles), (*b*) 300 (circles) and (*c*) 1000 (squares) μmol m^{-2} s^{-1} irradiance. Photosynthesis was also measured at 50 (open, inverted triangles), 100 (open, upright triangles), 300 (open circles), 1000 (open squares) and 2000 (open diamonds) μmol m^{-2} s^{-1}. Other conditions were 28°C and 350 p.p.m. CO_2. Reproduced from Quick *et al.* (1992) with permission.

This thought-experiment has been formalised (Kascer & Porteous 1987) as the flux control coefficient,

$$C = \frac{\mathrm{d}J/J}{\mathrm{d}E/E},$$

where E and J are the enzyme amount and flux in the wild-type, dE is the experimentally induced change in the enzyme amount, and dJ is the resulting change in flux. The flux control coefficient can be conveniently derived from the slope of a normalized plot of flux versus enzyme amount (both being normalized such that the average wild-type values for flux and enzyme amount are geometrically equivalent).

This approach is quantitative, can be applied in systems where control is shared, and does not require prior knowledge of all the details of regulation mechanisms. There are some experimental constraints. Although strictly speaking, small perturbations should be used, experimental errors in flux and enzyme measurements means that changes of 25–30% or more are needed for practical reasons. It is important to check that other enzyme amounts are not changed, due to side effects of the genetic manipulation. For this reason only well back-crossed mutant lines, and at least two independent transformant lines to avoid artefacts due to accidental gene disruption, should be used. Material with a large decrease in the enzyme amount is basically unsuitable for evaluation of control, for two reasons. Firstly, indirect effects on the expression of other enzymes may develop. Secondly, the flux control coefficient usually increases as the enzyme amount is decreased, relative to the wild-type, and large changes will therefore tend to provide inaccurate and overestimated values. Material with large changes can give interesting and important insights into mechanisms to compensate for a lesion in metabolism, but this aspect is not considered further in the present contribution.

Rodermel *et al.* (1988) produced a series of tobacco plants containing approximately 60%, 35% and under 20% of the wild-type Rubicso content. We grew this material in controlled climate chambers at three different irradiances (figure 3) or three different nitrogen supplies (figure 4). After 5–6 weeks, the amount of Rubisco was quantitated by immunoassay and activity assay in each plant, and the rate of photosynthesis was measured (Quick *et al.* 1991, 1993; Lauerer *et al.* 1993).

The contribution of Rubisco to control of photosynthesis in growth conditions is revealed by measurements carried out in the ambient growth conditions (solid symbols in figures 3*a–c* and 4*a–c*). In well-fertilized plants, 30% or more of the Rubisco could be removed without a large inhibition of ambient photosynthesis, and a relatively low flux control coefficient ($C = 0.1$–0.3) can be estimated. The flux control coefficient was higher (0.4–0.5) when the plants were grown on limiting nitrogen (figure 3*a*).

Photosynthesis was also measured at irradiances and CO_2 concentrations which differed from the growth irradiance (figure 3*a–c*, open symbols; figure 4*a–c*, open symbols; figure 4*d–f*, all symbols). Short-term changes led to large changes in the flux control coefficient; in general it rose as the measuring CO_2 concentration was decreased, or as irradiance was increased. Values approaching unity (i.e. total limitation by Rubisco) were obtained when shade-grown plants were suddenly exposed to high irradiance (figure 4*f*).

Thus even though Rubisco has often been considered to be a major limiting factor in photosynthesis (Farquhar & Von Caemmerer 1982; Woodrow & Berry 1988), these results show that Rubisco does not exert major control over photosynthesis over a wide range of growth conditions. Its contribution varies dramatically depending on the short-term conditions, underlining the danger of extrapolating from one condition to another, or from short- to long-term conditions. Indeed, the leaves adjust to escape from

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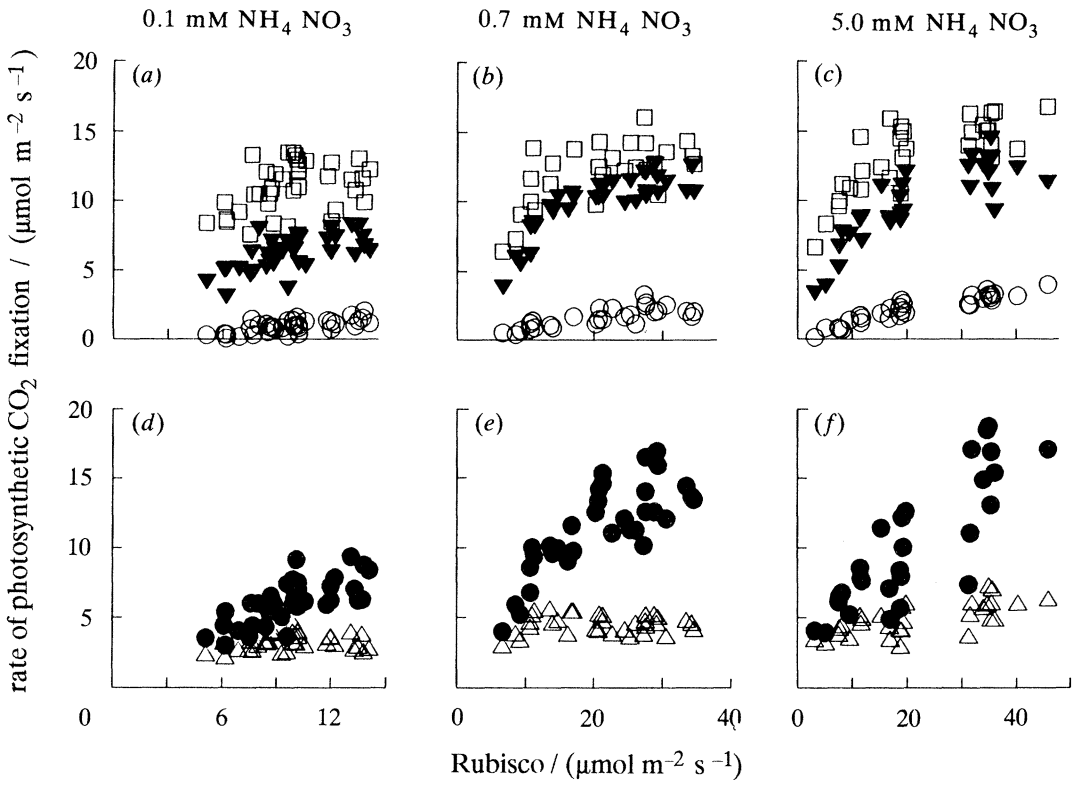

Figure 4. Relation between Rubisco content and photosynthesis in 'antisense' *rbc*S tobacco plants grown at 0.1 (*a*,*d*), 0.7 (*b*,*e*) and 5 (*c*,*f*) mM NH_4NO_3, 20°C, 350 p.p.m. CO_2 and 330 μmol m^{-2} s^{-1} irradiance. Photosynthesis was measured in ambient growth conditions (*a*–*c*, solid triangles), and also at ambient irradiance with 100 (open circles) and 1000 (open squares) p.p.m. CO_2 (*a*–*c*), and at 100 (open triangles) and 1000 (solid circles) μmol m^{-2} s^{-1} irradiance (*d*–*f*). Reproduced from Lauerer *et al.* (1993) with permission.

a one-sided limitation: when low irradiance-grown plants are suddenly exposed to 1000 μmol m^{-2} s^{-1}, photosynthesis is totally limited by Rubisco (figure 4*f*) whereas when the plants have grown at high irradiance, Rubisco only exerts a small limitation (figure 4*a*, solid symbols). This involves an increase in the percentage of protein allocated to Rubisco (see Lauerer *et al.* 1993).

It is interesting that plants tend to over-invest in Rubisco when they have a good supply of nitrogen. Rubisco represents up to 40% of leaf nitrogen, and additional organic nitrogen invested in Rubisco would act as a store, which simultaneously brings side-advantages for the plant by allowing better use of short-term increases in irradiance (figure 4) and (see Quick *et al.* 1991) improved water use efficiency.

6. FLUX CONTROL COEFFICIENTS FOR STARCH SYNTHESIS

A preliminary analysis of starch synthesis has been carried out using classical mutants with decreased plastic phosphoglucose isomerase (pPGI) (*Clarkia xantiana*; Jones *et al.* 1986), plastid phosphoglucomutase (pPGM) (*Arabidopsis thaliana*; Caspar *et al.* 1986), AGPase (*Arabidopsis thaliana*; Lin *et al.* 1988) and branching enzyme (*Pisum sativum*; Battacharyya *et al.* 1990). These represent four of the five enzymic steps required to synthesize starch. The results are provisional, in so far that they represent a comparison across species.

Fluxes to sucrose and starch were measured by providing $^{14}CO_2$ to leaf discs and measuring label incorporation into sucrose and starch, over a relatively short time period (to avoid additional effects due to accumulation of carbohydrates in the leaf). This approach has the advantage that fluxes are measured. The sucrose and starch content of the leaf (which are frequently measured as a guide to partitioning) are less reliable, because they depend on the rate of export and degradation, and past history, as well as the momentary rate of synthesis. There is therefore a risk that the content may be affected by indirect factors, other than the rate of synthesis.

The rate of starch synthesis (solid symbols) and sucrose synthesis (open symbols, see later for discussion) are summarized in a normalized control plot in figure 5. Fluxes were measured under short-term conditions of low (figure 5*a*–*d*) and high irradiance (figure 5*e*–*h*). In low irradiance (i.e. at low pathway flux) only AGPase exerted significant control over the flux to starch, as expected on the basis of biochemical studies (see above). Even so, AGPase only exerted partial control ($C \simeq 0.3$); the remaining control is probably located in the reactions associated with the efficiency of light-harvesting and photosynthetic rate in non-saturating irradiance. AGPase continued to exert control (figure 4*g*) in high irradiance but, surprisingly, some control was now also exerted by pPGI and pPGM (figure 4*e*,*f*). This was rather unexpected because these are typical examples of enzymes which catalyse readily reversible reactions

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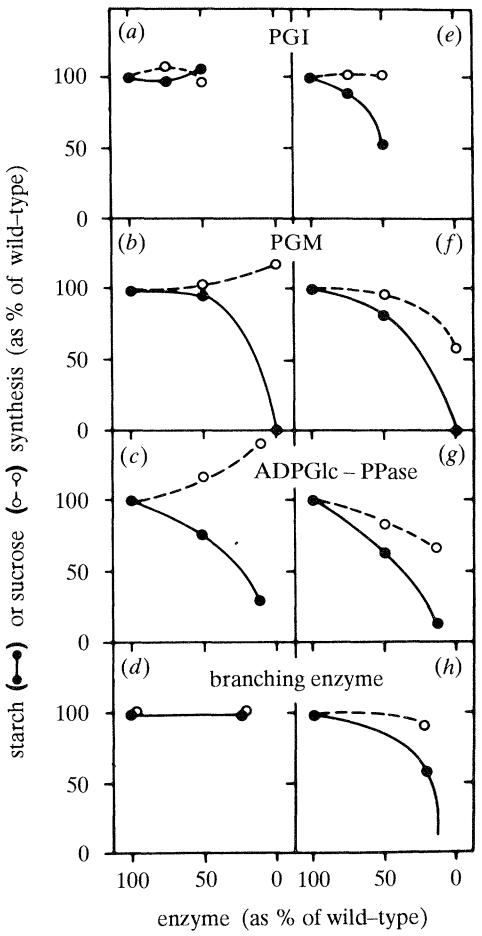

Figure 5. Control plot of the rate of starch synthesis in mutants with decreased activity of enzymes in the pathway of starch synthesis. Flux was measured as $^{14}CO_2$ incorporation into starch (solid circles) and sucrose (open circles) under (*a–d*) low irradiance (150 μmol m^{-2} s^{-1}), and (*e–h*) high irradiance (1000 μmol m^{-2} s^{-1}).

(their reactants lie close to equilibrium (Stitt *et al.* 1987), and they often operate in the opposite direction, for example during phosphorolytic starch degradation) and were therefore considered to be present in 'excess'. A reduction in the rate of starch synthesis did not result in a consistent increase in the rate of sucrose synthesis (open symbols). Instead (data not shown), the rate of photosynthesis was inhibited.

On the basis of these results it can be predicted that it will not be possible consistently to generate a large increase in the rate of starch synthesis by increasing AGPase alone because (i) it often only exerts partial control, and (ii) other enzymes will soon become limiting if AGPase is increased. Our results also indicate it will be difficult to manipulate the rate of sucrose synthesis and export by altering starch synthesis alone. In agreement, Stark *et al.* (1992) recently reported that overexpression of the plant AGPase gene did not increase starch synthesis in potatoes. A mutated non-regulatable *E. coli* AGPase did increase tuber starch synthesis by 30% when overexpressed in the tubers, but was lethal when it was expressed under the control of a constitutive promotor, and was therefore also present in the leaf. Their results illustrate that the homologous enzyme is regulated to

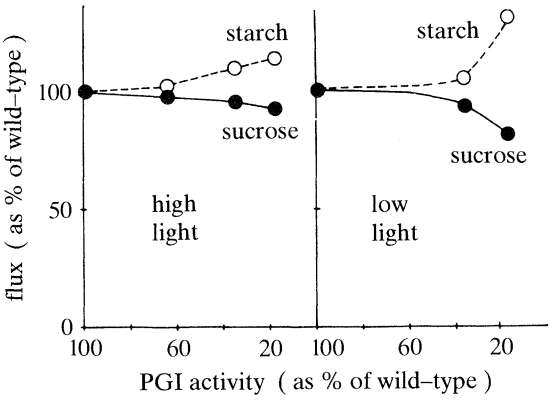

Figure 6. Control plot of the relation between sucrose synthesis and cytosolic phosphoglucose isomerase activity. Flux was measured as $^{14}CO_2$ incorporation into sucrose (solid circles) and starch (open circles) under (*a*) high irradiance (1000 μmol m^{-2} s^{-1}), and (*b*) low irradiance (150 μmol m^{-2} s^{-1}).

avoid dramatic effects on flux, and that removal of regulatory properties leads to serious disruption of leaf metabolism emphasizing, as discussed above, that much regulation occurs to maintain a functional balance, rather than to alter flux *per se*. The lethal effects could, speculatively, be due to sequestration of adenine nucleotides as ADPGlc, draining of intermediates from the Calvin cycle and/or inhibition of sucrose synthesis.

7. FLUX COEFFICIENTS FOR SUCROSE SYNTHESIS

I will present results for a classical mutant with decreased cytosolic phosphoglucose isomerase (cPGI), and transgenic plants with increased ability to hydrolyse PP_i, and will briefly discuss recent results from other laboratories for SPS, TPT and phloem loading.

Studies with a *Clarkia xantiana* mutant reveal that cPGI does not exert any significant control over the rate of sucrose synthesis (figure 6). Nevertheless, in low irradiance a fivefold decrease did result in a significant reduction in sucrose synthesis, providing another example of an enzyme which catalyses a readily reversible reaction, but is not present in large excess.

The studies with the cPGI mutant provide indirect information about the regulation of the cFBPase *in vivo*. Sucrose synthesis is inhibited in these mutants because decreased cPGI leads to higher Fru6P, which in turn activates Fru6P,2-kinase and inhibits Fru2, 6bisphosphatase, resulting in higher Fru2,6bisP and inhibition of cFBPase. These plants can therefore be used to quantify the effectiveness of the feedback regulation of the cFBPase, as summarized in table 1 (see Neuhaus & Stitt (1989) for more details). In the metabolic conditions prevailing under limiting irradiance, feedback operates but there is certainly not a stoichiometric relation between inhibitor concentration and flux inhibition; a 30% increase of Fru6P resulted in a twofold larger relative increase (60%) of Fru2,6bisP, which in turn resulted in a 20% inhibition of sucrose synthesis. A different picture emerged in

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Table 1. *Effectiveness of Fru2,6bisP as a regulator of the cytosolic fructose-1,6-bisphosphatase* in vivo, *investigated in mutants with fivefold decreased activity of cytosolic PGI*

(The results are calculated from Neuhaus *et al.* (1989).)

	value in mutant (as % of wild-type)			
	concentration			
measuring conditions	Fru6P	Fru2,6bisP	triose phosphate	rate of sucrose synthesis
low irradiance	131	161	99	123
high irradiance	152	210	122	108

high irradiance; a 55% rise in Fru6P resulted in a doubling of Fru2,6bisP, but no significant inhibition of sucrose synthesis resulted. This was explained because triose phosphates rise in the latter conditions. As cFBPase has a sigmoidal substrate saturation response, a relatively small increase of the triose-phosphate concentration will overcome the feedback inhibition by Fru2,6bisP. The example illustrates that the effectiveness of regulation mechanisms defined by biochemical studies, depends on a variety of interactions *in vivo*. These cannot be predicted with certainty, but can be uncovered by using genetically altered plants.

To investigate whether the rate of PP_i hydrolysis limits sucrose synthesis, tobacco plants were transformed with alkaline pyrophosphatase from *E. coli* (Sonnewald 1991) (table 2). The transformants contained two- to threefold less PP_i, higher UDPGlc and decreased pools of the other phosphorylated intermediates (Jelitto *et al.* 1992), as would be expected if the reversible reaction catalysed by UDPglucose pyrophosphorylase is shifted towards UDPGlc formation by improved removal of PP_i. There was a small but non-significant increase in the rate of sucrose synthesis and decrease in the rate of starch synthesis, indicating that PP_i hydrolysis is only a minor factor regulating sucrose synthesis. Sucrose did accumulate in the leaves, but this was due to secondary effects including decreased growth (Stitt *et al.* 1992) and phloem transport (U. Sonnewald, personnal communication).

SPS has frequently been assigned a cardinal role in the regulation of sucrose synthesis (Huber & Huber 1992). The extracted activity correlates strongly with sucrose synthesis and export (for references, see Stitt *et al.* 1987), and the enzyme is strongly regulated via metabolites and protein phosphorylation (see above). Galthier *et al.* (1993) recently reported that tomato plants overexpressing the maize SPS gene contained increased sucrose and decreased starch, and concluded that SPS is a major determinant of partitioning. However, a rather large (three- to sevenfold) increase in SPS activity was needed to produce a quite moderate change in sucrose and starch content, and it cannot be excluded that these changes are indirect. Also the maize gene product is apparently not susceptible to regulation by protein phosphorylation in tomato leaves, leaving it an open question whether the endogenous protein could control sucrose synthesis. In collaborative experiments with U. Sonnewald, we recently observed that overexpression of spinach leaf SPS in tobacco, or a 50% reduction of potato leaf SPS expression using antisense, had no significant impact on partitioning; instead the activation state of the SPS appeared to be adjusted to compensate for the changed protein (U. Sonnewald, K.-P. Krause, M. Stitt, unpublished data). More studies are needed to clarify the significance of SPS for the control of partitioning, and to evaluate whether protein amount, or post-translational modification are decisive.

Finally, a recently published study indicates that membrane transport proteins may be more important than previously thought. Riesmeier *et al.* (1993) transformed plants with antisense to the envelope carrier TPT, and found marked changes in sucrose and starch contents in response to relatively small (30–40%) changes in TPT. It will be important in the future to learn to what extent the recently cloned phloem H^+/sucrose co-transporter (Riesmeier *et al.* 1992) controls the rate of sucrose export. A decreased rate of export could lead, via an increase of sucrose and inactivation of SPS (see above, figure 2*b*), to a lower rate of sucrose synthesis.

8. LONGER-TERM EFFECTS OF CARBOHYDRATE ON EXPRESSION OF GENES

So far, I have considered the regulation of metabolism in response to photosynthetic rate and the accumulation of sucrose in the leaf. However, what happens in the long-term, when carbohydrate accumulates in the leaf? Do additional changes complicate the picture?

Von Schaewen *et al.* (1990) transformed tobacco

Table 2. *Expression of* E. coli *pyrophosphatase in the cytosol of potato leaves does not significantly increase the rate of sucrose synthesis*

	wild-type	transformant
metabolites/(nmol g FW^{-1})		
PP_i	9.7 ± 2.5	5.0 ± 0.6
UDPGlc	72 ± 3.3	80 ± 3.1
Glc6P	260 ± 44	105 ± 5.0
rate of synthesis/(μmol C m^{-2} s^{-1})		
sucrose	4.6 ± 0.8	5.3 ± 0.6
starch	3.5 ± 0.4	3.3 ± 0.5
carbohydrate content/(μmol g FW)		
sucrose	8.8 ± 1.0	24.7 ± 3.5
starch	200 ± 23	118 ± 28

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plants with invertase from yeast, directing the enzyme to the cell wall to prevent apoplastic phloem loading. The result was an accumulation of carbohydrates in the leaf, visual bleaching of the leaves, but the changes in metabolism did not resemble those expected as a result of inhibition of sucrose synthesis and depletion of phosphate (see Stitt *et al.* (1992) for details). The visual bleaching in plants expressing yeast invertase in their apoplast was accompanied by a general decrease in the extracted activity of Calvin cycle enzymes (Stitt *et al.* 1991). Identical changes in photosynthesis, chlorophyll and enzyme activities can be produced by feeding glucose via the transpiration stream to detached spinach leaves (Krapp *et al.* 1991). In experiments with autotrophic *Chenopodium rubrum* cells and cold-girdled leaves, Krapp *et al.* (1993) showed that accumulating carbohydrates repress several genes for photosynthetic enzymes. A similar conclusion drawn by Sheen (1989) from her studies of reporter gene expression in a maize protoplast transient expression system.

Thus, accumulation of carbohydrate in the leaf not only leads to modification of carbon *via* regulation of metabolic pathways; it also can result in regulation of gene expression with the result that the rate of photosynthesis is decreased in the mid- to long-term. Simultaneously it can be speculated that the resources released from the leaf can be invested elsewhere to correct the sink-source balance. The precise signal, and the transduction pathway, are at present unknown, and it is likely that molecular genetics will be needed in the future to elucidate them.

9. CONCLUSIONS

Summarizing, although AGPase is the major regulatory enzyme in the pathway of starch synthesis, partitioning is more easily and reproducibly controlled from the cytosol in leaves. The relative importance of the individual cytosolic enzymes is not yet clear, and it would be premature to conclude that SPS is the only or major factor. It is also evident that the effectiveness of the feedback regulation depends on the conditions, being readily overriden in conditions allowing rapid photosynthesis.

On a more general note, whereas study of regulation has been dominated by the idea that flux is controlled by one or a small number of 'regulatory' enzymes, results obtained using genetically altered plants reveal that enzymes which have been widely viewed as limiting or regulatory may not have such a large impact as previously thought, probably because in the past it has been difficult to distinguish between regulation aimed at maintaining a balance within the path, and control in the sense of changing flux through the pathway. It is also emerging that enzymes which catalyse reversible reactions are not necessarily present in a large excess. We need to learn more about the contribution of transport proteins and the relative importance of changes in protein properties as compared to protein amount. It is also now clear that the importance of a particular enzyme or mechanism can vary, depending on the conditions in which the plant has developed, and on the short-term conditions in which the measurements are carried out. Finally, metabolic regulation of gene expression may sometimes operate to allow a plant to escape from one-sided limitations, and it is dangerous to analyse regulation solely in the context of metabolism (just as it is dangerous to assume that increased expression necessarily means a change in metabolism).

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