*Photosynthesis Research* **75:** 1–10, 2003.



*Minireview*

# The Calvin cycle revisited

Christine A. Raines
*Department of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK (e-mail: rainc@essex.ac.uk; fax: +44-1206-872592)*

Received 18 April 2002; accepted in revised form 11 July 2002

*Key words:* carbon allocation, overexpression, photosynthesis, primary carbon fixation, transgenic plants

**Abstract**

The sequence of reactions in the Calvin cycle, and the biochemical characteristics of the enzymes involved, have been known for some time. However, the extent to which any individual enzyme controls the rate of carbon fixation has been a long standing question. Over the last 10 years, antisense transgenic plants have been used as tools to address this and have revealed some unexpected findings about the Calvin cycle. It was shown that under a range of environmental conditions, the level of Rubisco protein had little impact on the control of carbon fixation. In addition, three of the four thioredoxin regulated enzymes, FBPase, PRKase and GAPDH, had negligible control of the cycle. Unexpectedly, non-regulated enzymes catalysing reversible reactions, aldolase and transketolase, both exerted significant control over carbon flux. Furthermore, under a range of growth conditions SBPase was shown to have a significant level of control over the Calvin cycle. These data led to the hypothesis that increasing the amounts of these enzymes may lead to an increase in photosynthetic carbon assimilation. Remarkably, photosynthetic capacity and growth were increased in tobacco plants expressing a bifunctional SBPase/FBPase enzyme. Future work is discussed which will further our understanding of this complex and important pathway, particularly in relation to the mechanisms that regulate and co-ordinate enzyme activity.

*Abbreviations:* ATP – adenosine triphosphate; FBPase – fructose-1,6-bisphosphatase; GAPDH – glyceraldehydes-3- phosphate dehydrogenase; LC-MS – liquid crystal-mass spectrometry; NADP – nicotinamide adenine phosphate; NMR – nuclear magnetic resonance; PGKinase – glycerate-3-phosphate kinase or phosphoglycerate kinase; PRKase – ribulose-5-phosphate kinase or phosphoribulokinase; RPI – ribose phosphate isomerase; RPE – ribulose phosphate epimerase; RuBP – ribulose-1,5-bisphosphate; Rubsico – ribulose-1,5-bisphosphate carboxylase/oxygenase; ssu – small subunit unit of ribulose-1,5-bisphosphate carboxylase/oxygenase; TPI – triose-3-phosphate isomerase

## Introduction

The photosynthetic carbon reduction (Calvin) cycle is the primary pathway of carbon fixation and in higher plants is located in the chloroplast stroma. In the 1950s, Calvin and colleagues elucidated the sequence of reactions in this cycle. Following this, the enzymes catalysing the reactions in the pathway were identified and their kinetic properties studied *in vitro*. A major goal in photosynthetic research has been to identify limiting points in the process, in order to produce plants with increased yield. Efforts to improve photosynthetic carbon fixation have been focussed on altering the catalytic properties of Rubisco, as this enzyme was believed to be the major limiting factor (Spreitzer 1993; Hartman and Harpel 1994). However, the emphasis of this research has now shifted, due in part to the lack of success in producing a Rubisco enzyme with reduced oxygenase activity. In addition, during the last decade DNA sequences for all the Calvin cycle enzymes have been isolated and sequenced and antisense transgenic plants with reduced levels of enzymes

have been produced and analysed. Data from these studies is challenging many of the previously held opinions on the importance of individual enzymes in determining the rate of carbon fixation. Previous reviews have considered the application of transgenic plants to the study of chloroplast metabolism (Furbank and Taylor 1995; Stitt and Sonnewald 1995). Two further articles discuss the earlier work on the antisense analysis of the Calvin cycle (Stitt and Schulze 1994; Quick and Neuhaus 1997) and the C4 photosynthetic pathway (Furbank et al. 1997). This review will focus on the interesting data that has emerged from the transgenic analysis of the Calvin cycle that has identified potential targets for manipulation to increase carbon fixation *in vivo*.

## Calvin cycle reactions

The Calvin cycle utilises the products of the light reactions of photosynthesis, ATP and NADPH, to fix atmospheric $CO_2$ into carbon skeletons that are used directly for starch and sucrose biosynthesis (Figure 1) (Woodrow and Berry 1988; Geiger and Servaites 1995; Quick and Neuhaus 1997). This cycle comprises 11 different enzymes, catalysing 13 reactions, and is initiated by the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) which catalyses the carboxylation of the $CO_2$ acceptor molecule, ribulose-1,5-bisphosphate (RuBP). The 3-phosphoglycerate (3-PGA) formed by this reaction is then utilised to form the triose phosphates, glyceraldehyde phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP), via two reactions that consume ATP and NADPH. The regenerative phase of the cycle involves a series of reactions that convert triose phosphates into the $CO_2$ acceptor molecule, RuBP. The majority of the triose phosphate produced in the Calvin cycle remains within the cycle to regenerate RuBP. However, carbon compounds produced in this cycle are essential for growth and development of the plant and therefore triose phosphates exit from the cycle and are used to synthesise sucrose and starch. The Calvin cycle also supplies intermediates to an array of other pathways in the chloroplast, including the shikimate pathway for the biosynthesis of amino acids and lignin, isoprenoid biosynthesis and precursors for nucleotide metabolism and cell wall synthesis (Lichtenthaler 1999) (Figure 1). Clearly, the Calvin cycle occupies a central position in carbon metabolism and a full understanding of the mechanisms that control flux through this pathway, together with those that control the allocation of carbon to intermediate and secondary metabolic pathways, is essential if genetic manipulation of plant metabolism is to be a realised.

## Identifying enzymes that limit photosynthetic carbon flux

Traditionally, analysis of metabolic pathways focussed on the study of the kinetic properties of individual enzymes. This approach led to the identification of a number of 'key' enzymes in the Calvin cycle such as Rubisco, SBPase, FBPase and PRKase. This classification was based on the fact that the activity of these enzymes was regulated by a number of factors, including light, stromal pH [$Mg^{2+}$], and that they catalysed reactions which were more or less irreversible (Portis et al. 1977; Woodrow and Berry 1988). The conclusion from this biochemical analysis was that these 'key' enzymes were likely to have the greatest importance in controlling the rate of $CO_2$ fixation. However, these *in vitro* studies provided no information on the extent to which any single Calvin cycle enzyme controlled the rate of carbon dioxide fixation *in vivo*.

Metabolic control analysis is an alternative approach that can be used to determine the relative importance of an individual enzyme in controlling the flux through a pathway (Fell 1997). To undertake metabolic control analysis of a pathway it is necessary to be able to reduce specifically the amount of an individual enzyme in that pathway; the effect of this reduction on flux can then be compared to the control with the normal level of enzyme acitivity (for fuller description of this approach, see Fell 1997). This analysis can provide a quantitative measure of the control exerted by a single enzyme over the flux through a pathway and can be defined mathematically:

$$C = \frac{\frac{\delta J}{J}}{\frac{\delta E}{E}}$$

where $C$ is the flux control co-efficient; $J$ the original flux through the pathway; $\delta J$ change in flux; $E$ original enzyme activity; $\delta E$ change in enzyme activity. The flux control coefficient can vary from 0, for an enzyme that makes no contribution to control, to 1, for an enzyme that exerts total control. The flux control value for any single enzyme is not a constant and can change depending on the conditions under which the

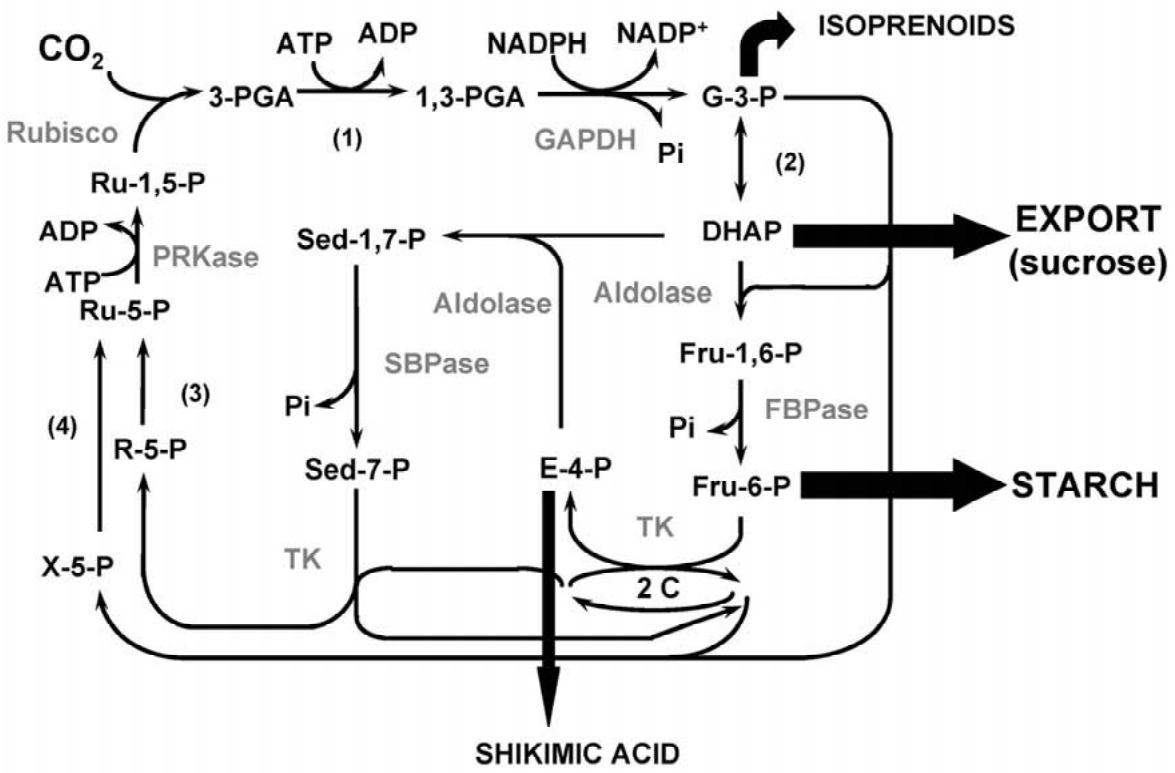


*Figure 1.* The Calvin cycle showing the intermediates from the first stable carbon compound, 3-PGA, to the cabon dioxide acceptor molecule, ribulose-1,5-bisphosphate and the exit points form the cycle into the pathways of sucrose, starch, isoprenoids and shikimic acid . The reactions catalysed by the enzymes whose levels have been manipulated in transgenic plants, are shown in grey. The site of function of the enzymes (1) 3-phosphoglycerate kinase (2) triose phosphate isomerase (3) ribose-5-phosphate isomerase and (4) ribulose-5-phosphate epimerase are also indicated.

analysis is carried out. However, the sum of the flux control co-efficients for the for all the enzymes in a single pathway should equal 1. Therefore, in order that this be maintained, when the control value for one enzyme in the system increases, then the control values for one or more of the other enzymes in the system must decrease. One fundamental difference between this approach and that of earlier studies based on the kinetics of individual enzymes, is that metabolic control analysis allows for all enzymes in a pathway to share control of flux in that pathway.

The contribution that individual enzymes make to the control of carbon flux through the Calvin cycle has been investigated using antisense plants with reduced levels of individual enzymes. The application of metabolic control analysis to address this problem was initiated by Stitt and co-workers using transgenic antisense Rubisco plants (Rodermel et al. 1988). Presently, antisense plants for seven of the eleven enzymes in the Calvin cycle have been analysed using this approach (Table 1). Although outwith the scope of this review it should be noted that antisense plants with reduced Calvin cycle enzyme levels have also been used extensively to study the interactions between electron transport processes and carbon assimilation (Ruuska et al. 1998, 2000a–c; Badger et al. 2000).

## The enzymes Rubisco, sedoheptulose-1,7-bisphosphatase, aldolase and transketolase dominate control of photosynthetic carbon fixation and reduce carbohydrate accumulation and growth

### *Rubisco*

An extensive analysis has been carried out using antisense plants with reduced levels of the enzyme Rubisco and much of this data has been reviewed previously (see the review by Stitt and Schulze 1994). For this

*Table 1.* Calvin cycle antisense plants; a summary of the plant species and promoters used, together with the photosynthetic flux control values

| Enzyme | Amtisense plants | Promoter used | Photosynthesis flux control values | Primary references |
|---|---|---|---|---|
| Rubisco | Tobacco | CaMV | 0–1.0 | Rodermal and Bogorad 1988<br>Stitt and Schulzel 1994<br>Hudson et al (1992) |
| PGKinase | | | | No published data |
| GAPDH | Tobacco | CaMV | <0.2 | Price et al. 1995 |
| TPI | | | | No published data |
| Aldolase | Potato | CaMV | 0.07–0.55 | Haake et al. 1998 |
| Transketolase | Tobacco | CaMV | 0.07–1.0 | Henkes et al. 2001 |
| FBPase | Potato | CaMV | <0.2 | Kossman et al. 1994 |
| SBPase | Tobacco | CaMV | 0.3–0.75 | Harrison et al. 1998 |
| RPE | | | | No published data |
| RPI | | | | No published data |
| PRKase | Tobacco | Tobacco ssu | <0.28 | Paul et al. 1995 |

reason only a brief summary of the most important findings will be included here. The most surprising observation was that halving the activity of Rubisco caused no significant decrease in photosynthesis. This finding applied regardless if the plants were grown in high or low light conditions; when photosynthesis was measured under the conditions in which the plants were grown and flux control co-efficient values obtained were below 0.2 (Stitt et al. 1991; Quick et al. 1991b; Hudson et al. 1992). However, if the tobacco Rubisco antisense plants were grown in ambient $CO_2$ and moderate light (350 $\mu$mol $m^{-2}$ $s^{-1}$) and photosynthesis measured under saturating light and/or saturating $CO_2$, then the control exerted by Rubisco increased significantly, and control coefficient values could reach 0.8. Interestingly, when the antisense Rubisco plants were grown in a greenhouse with high irradiance, high temperature and low humidity, Rubisco had almost complete control of carbon fixation when photosynthesis was measured under the growth conditions (Krapp et al. 1994). The impact of reductions in Rubisco ssu protein on photosynthesis was also greater when the Rubisco antisense plants were grown in limiting nitrogen (Quick et al. 1991a; Fichtener et al. 1993; Masle et al. 1993). These results demonstrated that the flux control co-efficient varied dependent on both the conditions of growth and analysis and that the level of Rubisco protein does not limit photosynthetic capacity under a wide range of conditions. However, it is clear that the biochemical characteristics of this enzyme severely limit the uptake of carbon from the atmosphere. This is evidenced by the high flux control co-efficient obtained in plants with reduced Rubisco content when the analysis is carried out under conditions where photorespiration is favoured, thereby reducing carboxylation efficiency. These results are in agreement with the model of von Caemmerer and Farquhar which predicts that, under high light and limiting $CO_2$ conditions, Rubisco is the single limitation on photosyntheis and would have a control co-efficient of 1 (von Caemmerer et al. 1981; von Caemmerer 2000).

In keeping with the insensitivity of photosynthesis to reductions in Rubisco activity, when the Rubico antisense plants were grown in low light (300 $\mu$mol $m^{-2}$ $s^{-1}$) and nitrogen-sufficient conditions, little or no effect, on growth, or carbohydrate accumulation were observed. However, plants with less than 50% of wild-type Rubisco activity had decreased starch and sucrose levels, increased shoot to root ratio and when only 20% Rubsico acitivity remained, an increase in specific leaf area was observed (Quick et al. 1992; Fichtener et al. 1993). These results suggested that carbon allocation was being shifted in an attempt to maximise photosynthetic capacity in response to reduced Rubisco activity. Further analysis of growth and development in these antisense plants with only 20% of normal Rubisco activity revealed that the timing

of shoot development was altered, possibly due to reduced source strength or to changes in the sink-source balance (Jiang and Rodermel 1995; Tsai et al. 1997).

*Sedoheptulose-1,7-bisphosphatase*

Photosynthetic carbon fixation was reduced in antisense tobacco plants with small decreases in SBPase activity. This was reflected in the control co-efficient values for SBPase of between 0.3 and 0.5 when photosynthesis was measured in greenhouse grown plants (irrandiance of between 600–1500 $\mu$mol $m^{-2}$ $s^{-1}$) under saturating light (Harrison et al. 1998; Raines et al. 2000; Olcer et al. 2001). Even higher flux control values, in the range 0.6–0.75, were obtained when the SBPase antisense plants were grown in controlled environment conditions with a constant high irradiance (800 $\mu$mol $m^{-2}$ $s^{-1}$). These results provide convincing evidence that the activity of SBPase *in vivo* is a major determinant of photosynthetic capacity in tobacco.

Carbohydrate levels in the source leaves of the SBPase antisense plants were also sensitive to small decreases in SBPase acitivity. Starch levels declined linearly as SBPase activity was reduced and flux control values for starch accumulation were as high as 0.6 in plants grown in high light (800 $\mu$mol $m^{-2}$ $s^{-1}$). In plants with less than 20% of wild-type SBPase activity, starch was barely detectable (Raines et al. 2000). Growth analysis of SBPase antisense plants revealed that shoot, leaf and floral biomass declined linearly in response to reductions in SBPase activity. An interesting feature observed in these plants was a biphasic response of leaf and stem morphology to reductions in SBPase activity (Bryant 2000). Plants with large reductions in SBPase activity were the same height or taller than wild-type plants, with thin stems and increased specific leaf area. In contrast, small reductions in SBPase activity resulted in plants that were shorter, had thicker stems and reduced specific leaf area when compared to wild type plants. Similar changes in leaf and stem morphology occur in some species in response to the light environment (Björkman 1981; Evans 1996). It is possible that the reductions in source capacity in the antisense plants mimic the metabolic signals that invoke the acclimatory responses to different light environments.

*Plastid aldolase*

In the first report on the analysis of antisense potato with reduced aldolase levels, photosynthesis did not decrease until aldolase activity was reduced to less than 60% of normal wild-type levels, in plants grown in low light (250 $\mu$mol $m^{-2}$ $s^{-1}$) and ambient $CO_2$. In this analysis flux control co-efficient values for aldolase of between 0.18 (measured in ambient conditions) and 0.32 (measured in saturating light and saturating $CO_2$ conditions) were obtained (Haake et al. 1998).

In a subsequent more extensive analysis antisense aldolase plants were grown in three different environmental conditions and photosynthetic carbon fixation measured (Haake et al. 1999). Under very low light and ambient $CO_2$ (70 $\mu$mol $m^{-2}$ $s^{-1}$ light and 350 ppm $CO_2$) a flux control co-efficient of 0.15 was obtained; increasing both the growth and measuring light level (390 $\mu$mol $m^{-2}$ $s^{-1}$ light and 400 ppm $CO_2$) resulted in a flux control value of aldolase of 0.21 which increased even further to 0.55 at saturating $CO_2$ (390 $\mu$mol $m^{-2}$ $s^{-1}$ light and 800 ppm $CO_2$). These results were surprising as they showed for the first time that a non-regulated enzyme, catalysing a freely reversible reaction, had a significant share of control over photosynthetic carbon flux.

Carbohydrate accumulation was reduced in the aldolase antisense plants and, as with photosynthetic capacity, the extent of this was dependent on growth conditions. In general, reductions in aldolase activity had a greater effect on starch than sucrose and, in light conditions of 400 $\mu$mol $m^{-2}$ $s^{-1}$, the flux control coefficient for aldolase over starch accumulation could be as high as 0.45. In contrast, little effect on sucrose levels was seen until only 30% of normal wild type aldolase activity remained. The changes in sugar levels correlated with the finding that no major decrease in the fresh weight of leaves, stem, root or tubers were evident, regardless of the growth conditions until aldolase activity was less than 50% of wild type (Haake et al. 1999).

*Transketolase*

The unexpected result obtained from analysis of antisense aldolase plants, showing that non-regulated enzymes could exert control over carbon fixation, was reinforced by results obtained from tobacco plants with reduced levels of plastid transketolase (Henkes et al. 2001). When these antisense tobacco plants

were grown in greenhouse conditions (daily irradiance levels were between 170 $\mu$mol $m^{-2}$ $s^{-1}$ to 600 $\mu$mol $m^{-2}$ $s^{-1}$). The flux control value for transketolase over carbon fixation, in ambient $CO_2$and low light (170 $\mu$mol $m^{-2}$ $s^{-1}$), was 0.07. This value increased to 0.32 when photosynthesis was measured at high irradiance (700 $\mu$mol $m^{-2}$ $s^{-1}$) and ambient $CO_2$.

Reductions in transketolase activity had dramatic effects on carbon partitioning between the sucrose and starch biosynthetic pathways. Sucrose levels declined linearly with reductions in transketolase activity and, even in plants with 75% of wild-type tranketolase activity a 25% reduction in the levels of sucrose was evident. In contrast, starch accumulation was unaffected until transketolase activity was reduced to below 60% of wild-type activity. These results were particularly interesting as they are unique when compared to all the other Calvin cycle antisense plants where sucrose levels were maintained at the expense of starch. Another interesting consequence of reduction in transketolase activity was a decrease in aromatic amino acid biosynthesis and intermediates of the shikimic acid pathway, indicating that the availability of carbon skeletons from the Calvin cycle could control the flux of carbon into phenylpropanoid metabolism. Although photosynthetic capacity and sucrose accumulation were sensitive to small reductions in transketolase, the total biomass (fresh weight) of these antisense plants did not change until transketolase activity was below 60% of wild-type. However, further reductions in the levels of this enzyme results in a linear decline in total fresh weight of these plants.

### Glyderaldehyde-3-phosphate dehydrogenase, fructose-1,6-bisphosphatase or phosphoribulokinase have little control over photosynthetic carbon fixation

The antisense analysis described above has provided a picture of the distribution of control that has overturned the preconception that highly regulated enzymes (‘key’) had most control. Further support for this came from the finding that some highly regulated enzymes had almost no control over cabon fixation. In antisense tobacco plants with reduced levels of GAPDH were grown in high light greenhouse conditions (maximum irradiance of 900 $\mu$mol $m^{-2}$ $s^{-1}$) no effects on photosynthesis were observed until GAPDH activity was reduced to below 35% of wild type, even when photosynthetic carbon assimilation was measured under saturating light (irradiance of 1500 $\mu$mol $m^{-2}$ $s^{-1}$) (Price et al. 1995). Similar results were obtained with FBPase antisense potato plants where no effect on photosynthesis occurred until FBPase activity was less than 34% of wild type (Kossman et al. 1994). These plants were grown in low light (250 $\mu$mol $m^{-2}$ $s^{-1}$) and photosynthesis measured as oxygen evolution with saturating light (1000 $\mu$mol $m^{-2}$ $s^{-1}$) and $CO_2$. The flux control coefficients for GAPDH and FBPase were less than 0.2 in most conditions.

Photosynthetic carbon fixation was also insensitive to reductions in the levels of PRKase activity. Analysis of antisense PRKase tobacco plants that had been grown in low light conditions (330 $\mu$mol $m^{-2}$ $s^{-1}$), or in nitrogen deficient conditions, showed that PRKase activity could be reduced to less than 20% of wild-type levels before any decrease in photosynthesis was evident (Paul et al. 1995; Banks et al. 1998). The maximum flux control coefficient for carbon flux through the Calvin cycle obtained for PRKase was 0.25, in plants grown at 330 $\mu$mol $m^{-2}$ $s^{-1}$ and photosynthesis measured under saturating light of between 800-1400 $\mu$mol $m^{-2}$ $s^{-1}$. (Paul et al. 2000). Measurements of *in vivo* PRKase activity revealed that reductions in levels of this enzyme in transgenic plants were compensated for by activating a higher percentage of the remaining PRKase (Banks et al. 1999).

### Can photosynthetic carbon fixation and plant growth rates be increased?

Analyses of antisense plants have provided direct experimental evidence that control over the rate of carbon fixation in the Calvin cycle is shared between a restricted number of enzymes. Two enzymes, Rubisco and SBPase, appear to dominate, however, two non-regulated enzymes, transketolase and aldolase, were also shown to have the potential to contribute significantly to carbon flux through the Calvin cycle (Hudson et al. 1992; Stitt and Schulze 1994; Harrison et al. 1998; Haake et al. 1998, 1999, Henkes et al. 2001; Raines et al. 2000). In contrast, there was no evidence that GAPDH, FBPase or PRKase had any significant control over carbon fixation. Although the level of control exerted by any single enzyme in the cycle varied dependent on environmental conditions and developmental status, the data from the transgenic plants strongly indicated that carbon flux

was co-limited by the enzymes at the branch point in the cycle, between regeneration and export, and the carboxylation reaction of Rubisco.

A model of $CO_2$ uptake developed by Farquar and colleagues partitioned the limitation of photosynthesis between Rubisco activity and the capacity for RuBP regeneration (von Caemmerer et al. 1981, 2000). This model predicts that, under light saturating, $CO_2$ limiting conditions, Rubisco activity is the major determinant of carbon fixation. Application of this model demonstrated that the balance of co-limitation of photosynthetic carbon fixation is poised between carboxylation and regeneration, under a wide range of environmental conditions (von Caemmerer and Farquhar 1984) in a large number of plant species (Evans 1986; Evans and Farquhar 1991; Wullschleger 1993; Leuning 1997). The balance between regeneration and carboxylation capacities is normally maintained such that they co-limit photosynthesis and neither of these capacities dominate control of carbon dioxide flux in the Calvin cycle. This implied that in order to increase photosynthetic capacity both of these parameters would have to be increased simultaneously. A kinetic model of the Calvin cycle, developed by Fell and Poolman, has also predicted that the control of photosynthetic carbon flux is likely to be co-limited by Rubisco and regeneration, with the enzyme SBPase being the major controlling enzyme in this phase of the cycle. Interestingly, this model also predicted that share of control between Rubisco and SBPase was balanced, however, these authors suggested that this co-limitation could be shifted dependent on conditions within the cell (Poolman et al. 2000, 2001). Analysis of the Calvin cycle antisense plants has shown clearly that it is possible to disrupt this balance, such that either regenerative capacity or Rubisco activity dominates control. In addition, this transgenic approach has identified individual enzymes that have significant control of flux through the cycle. These results are highly significant as they provide evidence to suggest that by using genetic manipulation that it might be possible to increase carbon assimilation without changing Rubisco acitivity (Stitt and Schulze 1994; Ruuska et al. 1998; Harrison et al. 2001).

The information obtained from the analysis of the antisense Calvin cycle plants suggested that increasing the levels of SBPase and, to a lesser extent, transketolase or aldolase, may lead to an increase in photosynthetic carbon fixation and growth rates. In support of this hypothesis, expression of a bi-functional cyanobacterial FBPase/SBPase in transgenic tobacco plants has resulted in a stimulation in photosynthetic capacity and in an increase in height and dry weight (Miyagawa et al. 2001). This dramatic effect maybe due to differences in regulatory properties between the bifunctional cyanobacterial FBPase/SBPase, and the plant FBPase and SBPase enzymes. Transgenic tobacco plants have been produced using a full-length *Arabidopsis* SBPase cDNA clone expressed under the control of the CaMV35S promoter. Interestingly, photosynthetic capacity and plant biomass were also increased in these tobacco plants with increased SBPase protein levels (Lefebvre, Lloyd and Raines, unpublished data). This work suggested that an increase in SBPase activity alone may be sufficient to stimulate carbon fixation capacity and plant growth in tobacco (Figure 2). These findings are remarkable and show for the first time that photosynthetic carbon fixation can be improved by increasing the activity of one enzyme in the Calvin cycle. Of particular significance is the clear demonstration that carbon fixation can be improved without any direct manipulation of Rubisco activity or content. It has also overturned earlier assumptions that changing the activity of only one enzyme in the cycle would be likely to have no effect on photosynthetic capacity and be even less likely to impact on yield (see reviews in Paul and Lawlor 2000).

## Future directions

### *Regulation of the Calvin cycle*

Transgenic plants will continue to be excellent tools to use to further our understanding of the regulatory mechanisms which, not only control the flux of carbon within the Calvin cycle, but may also determine the rate of carbon flow into intermediate and secondary metabolism. Although it has been known for over 20 years that thioredoxin regulates the activity of four enzymes within the Calvin cycle, the importance of thioredoxin in controlling flux of carbon *in vivo* is still unknown (Buchanan et al. 1980). The availability of fully active deregulated mutants of FBPase and SBPase enzymes in the cycle together with plants with altered thioredoxin levels will allow these questions to be addressed *in vivo* (Jacquot et al. 1995; Dunford et al. 1998; Raines et al. 2000). Using mutant *Arabidopsis* plants lacking Rubisco activase, together with mutated activase protein sequences, a role for Rubisco activase in the redox modulation of Rubisco activity in light limiting conditions has been demonstrated

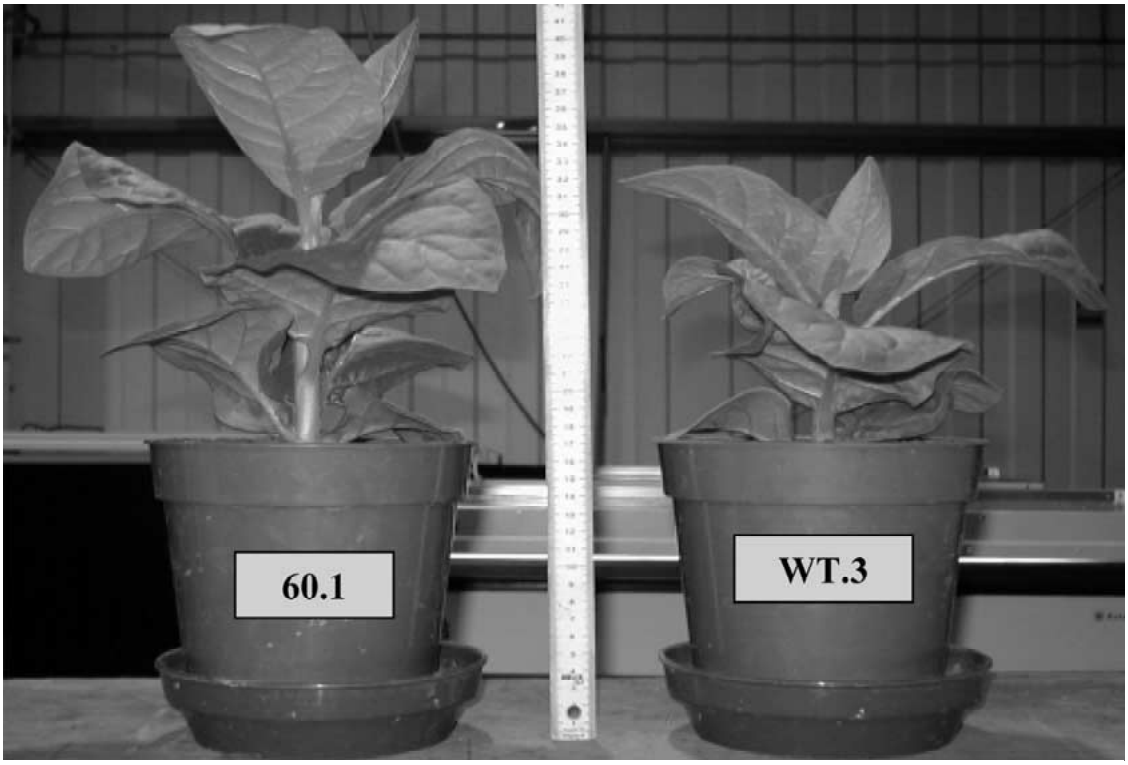


*Figure 2.* Growth of wild type and transgenic *Nicotiana tabacum* plants over-expressing *Arabidopsis* SBPase. Wild type and T1 progeny were analysed after 6 weeks of growth in soil in greenhouse conditions, at an irradiance of between 600–1500 $\mu$mol $m^{-2}$ $s^{-1}$ and 340 ppm $CO_2$.

(Zhang et al. 2002). Recently, a novel chloroplast protein, CP12, was identified that binds to PRKase and GAPDH in a NADPH-dependent manner, possibly regulating the activity of these enzymes (Wedel et al. 1997; Wedel and Soll 1998). Clearly we still have much to learn about the regulatory processes that operate *in vivo* to modulate Calvin cycle enzyme activity, and of the role that they play in controlling the rate of carbon fixation.

*Novel modelling approaches*

Although transgenic analysis has proved to be a powerful approach to determine the control that specific enzymes exert over carbon fixation, it will not be possible to analyse every step in the photosynthetic process, under every environmental variable, in every species. For these reasons modelling of photosynthesis will continue to be a desirable goal. The application of analytical techniques such as LC-MS and NMR will make it possible to obtain accurate measurements of fluxes through the Calvin cycle in both wild type and transgenic plants. Using these data, together with state-of-the-art computing technology it may be possible to produce a dynamic model of these processes (Giersch 2001). This type of model is predictive and would have the potential to identify likely targets for further manipulation, in a wide range of species and environmental conditions.

*Increasing carbon assimilation*

The transgenic over-expression studies carried out using SBPase/FBPase represent a significant step forward in the attempt to identify targets that may be manipulated to increase photosynthesis. However, it remains to be seen if this technology can be applied to crop species or be maintained in field conditions. This not withstanding, these preliminary studies pave the way for biotechnological applications using genetic manipulation of photosynthetic carbon fixation to increase plant growth and yield. At present there is no data available from over-expression studies involving any other Calvin cycle enzymes but, given the impact of reductions in transketolase on photosynthesis and growth, it will be of great interest to see if over-expression of this enzyme will also increase photosynthetic capacity and yield.

## Acknowledgements

The author would like to thank The University of Essex Research Promotion Fund and the BBSRC for supporting our work on the Calvin cycle. Thanks to Dr Julie Lloyd for ideas, discussions and critically reviewing the manuscript. In addition, thanks are also extended to Dr S. von Caemmerer, Professor S.P. Long, Professor J. Whitmarsh, Professor D. Fell and Dr M. Poolman for stimulating discussions and Professor A.R. Portis for providing data in press.

## References

Badger MR, Caemmerer S von, Ruuska S and Nakomo H (2000) Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and Rubsico oxygenase. Phil Trans R Soc London B 335: 1433–1446

Banks FM, Driscoll SP, Parry MAJ, Lawlor DW, Knight JS, Gray JC and Paul MJ (1999) Decrease in phosphoribulokinase activity by antisense RNA in transgenic tobacco. Relationship between photosynthesis, growth and allocation at different nitrogen levels. Plant Physiol 119: 1125–1136

Björkman O (1981) Response to different quantum flux intensities. In: Lange OL, Nobel PS, Osmond CB and Zeigler H (eds) Encyclopedia of Plant Physiology, Vol 12, pp 57–107. Springer-Verlag, Heidelberg

Bryant B (2000) The production and analysis of tobacco plants with reduced levels of the Calvin cycle enzyme sedoheptulose-1,7-bisphosphatase. University of Essex, PhD thesis

Buchanan BB (1980) Role of light in the regulation of chloroplast enzymes. Annu Rev Plant Physiol 31: 341–374

Caemmerer S von (2000) Biochemical Models of Leaf Photosynthesis. CSIRO Publishing, Collingwood, Australia

Caemmerer S von and Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376–387

Caemmerer S von and Farquhar GD (1984) Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced p($CO_2$) on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. Planta 160: 320–329

Dunford RP, Durrant MC, Catley MA and Dyer TA (1998) Location of the redox-active cysteines in chloroplast sedoheptulose-1,7-bisphosphatase indicates that it is allosteric regulation is similar but not identical to that of fructose-1,6-bisphosphatase. Photosynth Res 58: 221–230

Evans JR (1986) The relationship between carbon-dioxide-limited photosynthetic rate and ribulose-1,5-bisphosphate-carboxylase content in 2 nuclear-cytoplasm substitution lines of wheat, and the coordination of ribulose-bisphosphate-carboxylation and electron-transport capacities. Planta 167: 351–358

Evans JR (1996) Effects of light and nutrition on photosynthesis. In: Baker NR (ed) Photosynthesis and the Environment, Vol 5, pp 281–304. Kluwer Academic Publishers, Dordecht, The Netherlands

Evans JR and Farquhar GD (1991) Modeling canopy photosynthesis from the biochemistry of the $C_3$ chloroplast. In: Boote KJ and Loomis RS (eds) Modeling Crop Photosynthesis – from Biochemistry to Canopy, pp 1–16. Madison, Wisconsin, Crop Science Society of America

Fell D (1997) Understanding the Control of Metabolism. Portland Press, London

Fichtner K, Quick WP, Schulze E-D, Mooney HA, Rodermel SR, Bogorad L and Stitt M (1993) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' *rbc*S. V. Relationship between photosynthetic rate, storage strategy, biomass allocation and vegetative plant growth at three different nitrogen supplies. Planta 190: 1–9

Furbank RT and Taylor WC (1995) Regulation of photosynthesis in C3 and C4 plants: a molecular approach. Plant Cell 7: 797–807

Furbank RT, Chitty JA, Jenkins CLD, Taylor WC, Trevanion SJ, Caemmerer S von and Ashton AR (1997) Genetic manipulation of key photosynthetic enzymes in the C-4 plant *Flaveria bidentis*. Aust J Plant Physiol 24: 477–485

Geiger DR and Servaites JC (1994) Diurnal regulation of photosynthetic carbon metabolism in C3 plants. Annu Rev Plant Phys Plant Mol Biol 45: 235–256

Giersch C (2001) Mathematical modelling of metabolism. Curr Opin Plant Biol 3: 249–253

Haake V, Zrenner R, Sonnewald U and Stitt M (1998) A moderate decrease of plastid aldolase activity inhibits photosynthesis, alters the levels of sugars and starch and inhibits growth of potato plants. Plant J 14: 147–157

Haake V, Geiger M, Walch-Liu P, Engels C, Zrenner R and Stitt M (1999) Changes in aldolase activity in wild-type potato plants are important for acclimation to growth irradiance and carbon dioxide concentration, because plastid aldolase exerts control over the ambient rate of photosynthesis across a range of growth conditions. Plant J 17: 479–489

Harrison EP, Willingham NM, Lloyd JC and Raines CA (1998) Reduced sedoheptulose-1,7-bisphosphatase levels in transgenic tobacco lead to decreased photosynthetic capacity and altered carbohydrate partitioning. Planta 204: 27–36

Harrison EP, Olcer H, Lloyd JC, Long SP and Raines CA (2001) Small decreases in SBPase cause a linear decline in the apparent RuBP regeneration rate, but do not affect Rubsico carboxylation capacity. J Exp Bot 52: 1779–1784

Hartman FC and Harpel MR (1994) Structure, function, regulation and assembly of D-ribulose-1,5-bisphosphate carboxylase oxygenase. Annu Rev Biochem 63: 197–234

Henkes S, Sonnewald U, Badur R, Flachmann R and Stitt M (2001) A small decrease of plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism. Plant Cell 13: 535–551

Hudson GS, Evans JR, Caemmerer S, von Arvidsson YBC and Andrews TJ. (1992) Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants. Plant Physiol 98: 294–302

Jacquot J-P, Lopez-Jaramillo J, Chueca A, Cherfils J, Lemaire S, Chedozeau B, Migniac-Maslow m, Decottignies P, Wolosiuk R and Lopez-Gorge J (1995) High-level expression of recombinant pea chloroplast fructose-1,6-bisphosphatase and mutagenesis of its regulatory site. Eur J Biochem 229: 675–681

Jiang C-Z and Rodermel SR (1995) Regulation of photosynthesis during leaf development in rbcS antisense DNA mutants of tobacco. Plant Physiol 107: 215–22

Kossmann J, Sonnewald U and Willmitzer L (1994) Reduction of the chloroplastic fructose-16-bisphosphatase in transgenic potato plants impairs photosynthesis and plant growth. Plant J 6: 637–650

Krapp A, Chaves MM, David MM, Rodriguez ML, Pereira JS and Stitt M (1994) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with

'antisense' *rbc*S. VII. Impact on photosynthesis and growth in tobacco growing under extreme high irradiance and high temperature. Plant Cell Environ 17: 945–953

Leunig R (1997) Scaling to a common temperature improves the correlation between the photosynthesis parameters J(max) and V-cmax. J Exp Bot 48: 345–347

Lichtentahler HK (1999) The 1-deoxy-d-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Phys Plant Mol Biol 50: 47–65

Masle J, Hudson GS and Badger MR (1993) Effects of ambient $CO_2$ concentration on growth and nitrogen use in tobacco (*Nicotiana tabacum*) plants transformed with an antisense gene to the small subunit of ribulose-1,5-bisphosphate carboxylase oxygenase. Plant Physiol 103: 1075–1088

Miyawaga, Y, Tamoi M and Shigeoka S (2001) Overexpression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphat ase in tobacco enhances photosynthesis and growth. Nature Biotech 19: 965–969

Olcer H, Lloyd JC and Raines CA (2001) Photosynthetic capacity is differentially affected by reductions in sedoheptulose-1,7-bisphosphatase activity during leaf development in transgenic tobacco plants. Plant Physiol 125: 982–989

Paul MJ, Knight JS, Habash D, Parry MAJ, Lawlor DW, Barnes SA, Loynes A, and Gray JC (1995) Reduction in phosphoribulokinase activity by antisense RNA in transgenic tobacco: effect on $CO_2$assimilation and growth at low irradiance. Plant J 7: 535–542

Paul MJ and Lawlor D (1999) Genetic manipulation of photosynthesis. J Exp Bot 51 (special issue)

Paul MJ, Driscoll SP, Andralojc PJ, Knight JS, Gray JC and Lawlor DW (2000) Decrease of phosphoribulokinase activity by antisense RNA in transgenic tobacco: definition of the light environment under which phosphoribulokinase is not in large excess. Planta 211: 112–119

Poolman MG, Fell DA and Thomas S (2000) Modelling photosynthesis and its control. J Exp Bot 51: 319–328

Poolman MG, Olcer H, Lloyd JC, Raines CA and Fell DA (2001) Computer modelling and experimental evidence for two steady states in the photosynthetic in Calvin cycle. Eur J Biochem 268: 2810–2816

Portis AR, Chon CJA, Mosbac A and Heldt HW (1977) Fructose- and sedoheptulose-bisphosphatase. The sites of a possible control of $CO_2$ fixation by light dependent changes of the stromal $Mg^{2+}$ concentration. Biochim Biophys Acta 461: 313–325.

Price GD, Evans JR, Caemmerer S von, Yu J-W and Badger MR (1995) Specific reduction of chloroplast glyceraldehyde-3-phosphate dehydrogenase activity by antisense RNA reduces $CO_2$ assimilation via a reduction in ribulose bisphosphate regeneration in transgenic plants. Planta 195: 369–378

Quick WP and Neuhaus HE (1997) The regulation and control of photosynthetic carbon assimilation. In: Foyer CH and Quick WP (eds) A Molecular Approach to Primary Metabolism in Higher Plants, pp 41–62. Taylor and Francis, London

Quick WP, Schurr U, Fichtner K, Schulze E-D, Rodermel SR, Bogorad L and Stitt M (1991a) The impact of decreased Rubisco on photosynthesis, growth, allocation and storage in tobacco plants which have been transformed with antisense *rbcS*. Plant J 1: 51–58

Quick WP, Schurr U, Scheibe R, Schulze E-D, Rodermel SR, Bogorad L and Stitt M (1991b) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' *rbc*S. I Impact on photosynthesis in ambient growth conditions. Planta 183: 542–554

Quick WP, Schurr U, Fichtner K, Schultze E-D, Wendler R, Leegood RC, Mooney H, Rodermel SR, Bogorad L and Stitt M (1992) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' rbcS. IV. Impact on photosynthesis in conditions of altered nitrogen supply. Planta 188: 522–531

Raines CA, Harrison EP, Olcer H and Lloyd JC (2000) Investigating the role of the thiol-regulated enzyme sedoheptulose-1,7-bisphosphatase in the control of photosynthesis. Physiol Plant 110: 303–308

Rodermel SR, Abbott MS and Bogorad L (1988) Nuclear-organelle interactions: nuclear antisense gene inhibits ribulose-1,5-bisphophate carboxylase enzymes levels in transformed tobacco plants. Cell 55: 673–681

Ruuska S, Andrews TJ, Badger MR, Hudson GS, Laisk A, Price GD and Caemmerer S von (1998) The interplay between limiting processes in C-3 photosynthesis studied by rapid-response gas exchange using transgenic tobacco impaired in photosynthesis. Aust J Plant Physiol 25: 859–870

Ruuska S, Andrews TJ, Badger MR, Price GD and Caemmerer S von (2000a) The role of chloroplast electron transport and metabolites in modulating Rubisoc activity in tobacco. Insights from transgenic plants with reduced amounts of cytochrome *b/f* complex or glyceraldehydes 3-phosphate dehydrogenase. Plant Physiol 122: 491–504

Ruuska S, Badger MR, Andrews TJ and Caemmerer von S (2000b) Photosynthetic electron sinks in transgenic tobacco with reduced amonts of Rubisco: little evidence for significant Mehler reaction. J Exp Bot 51: 357–368

Ruuska S, Caemmerer S von, Badger MR, Andrews TJ and Robinson SA (2000c) Xanthophyll cycle, light energy dissipation and electron transport in transgenic tobacco with reduced carbon assimilation capacity. Aust J Plant Physiol 27: 289–300

Spreitzer R (1993) Genetic dissection of Rubisco structure and function. Annu Rev Plant Phys Plant Mol Biol 44: 411–434

Stitt M and Schulze E-D (1994) Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. Plant Cell Environ 17: 465–487

Stitt M and Sonnewald U (1995) Regulation of metabolism in transgenic plants. Annu Rev Plant Physiol Plant Mol Biol 46: 341–368

Stitt M, Quick WP, Schurr U, Schulze ED, Rodermel SR and Bogorad L (1991) Decreased ribulose-1,5-bisphosphate carboxylase/oxygenase in transgenic tobacco transformed with antisense rbcS II. Flux control coefficients for photosynthesis in varying light, $CO_2$ and air humidity. Planta 183: 555–566

Tsai C-H, Miller A, Spalding M and Rodermel S (1997) Source strength regulates an early phase transition of tobacco shoot morphogenesis. Plant Physiol 115: 907–914

Wedel N and Soll J (1998) Evolutionary conserved light regulation of Calvin cycle activity by NADPH-mediated reversible phosphoribulokinase/CP12/glyceraldehyde-3-p hosphate dehydrogenase complex dissociation. Proc Natl Acad Sci USA 95, 9699–9704

Wedel N, Soll J and Paap BK (1997) CP12 provides a new mode of light regulation of Calvin cycle activity in higher plants. Proc Natl Acad Sci USA 94: 10479–10484

Woodrow IE and Berry JA. (1988) Enzymic regulation of photosynthetic $CO_2$ fixation in $C_3$ plants. Annu Rev Plant Physiol Plant Mol Biol 39: 533–594

Wullschleger SD (1993) Biochemical limitations to carbon assimilation in C3 plants - A retrospective analysis of the $A/c_i$ curves of 109 species. J Exp Bot 44: 907–920

Zhang N, Kallis R, Ewy RG and Portis AR (2002) Light modulation of Rubisco in *Arabidopsis* requires a capacity for redox regulation of the larger Rubisco activase isoform. Proc Natl Acad Sci USA 99: 3330–3334