Journal of Experimental Botany, Vol. 70, No. 6 pp. 1697–1701, 2019
doi:10.1093/jxb/erz078

eXtra Botany

Insight

Variations in the Calvin–Benson cycle: selection pressures and optimization?

Tina B. Schreier[1*], and Julian M. Hibberd[1*]

[1] Department of Plant Sciences, University of Cambridge, Downing Street, CB2 3EA Cambridge, UK
* Correspondence: tbs32@cam.ac.uk, jmh65@cam.ac.uk

The Calvin–Benson cycle is the basis of carbon fixation in all photosynthetic organisms. However, relatively little is known about the extent to which its operation varies between species. Using a metabolite profiling approach, Arrivault *et al.* (2019) discovered differences in the levels of key Calvin–Benson cycle intermediates amongst C_3 and C_4 species. These differences in metabolite pools were observed between C_3 species as well as between C_3 and C_4 plants. This work raises the interesting possibility that varying selection pressures on components of the Calvin–Benson cycle have led to its independent optimization between species.

In 1954, Melvin Calvin, Andrew Benson and James Bassham published the metabolic pathway used to fix atmospheric CO_2 – the Calvin–Benson cycle (Bassham *et al.*, 1954). Their fundamental discoveries were based on feeding the alga *Chlorella* with ^{14}C-labelled CO_2 and tracing the labelling of metabolites over time (Bassham *et al.*, 1954; Sharkey 2018). They discovered that the cycle is composed of three phases: first, the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) fixes CO_2 using ribulose-1,5-bisphosphate (RuBP) as the acceptor, producing two 3-carbon molecules, 3-phosphoglycerate (3-PGA). Second, ATP and NADPH generated during the photosynthetic electron transport chain (the light-dependent reactions of photosynthesis) are used to phosphorylate and subsequently reduce 3-PGA to triose phosphate (triose-P). Third, the CO_2 acceptor RuBP is regenerated through a series of reactions (Box 1). The majority of enzymes involved in this cycle were discovered earlier or soon afterwards (Horecker *et al.* 1951; Racker *et al.*, 1953; Mayoudan *et al.*, 1957). Since then, it has generally been assumed that operation of the Calvin–Benson cycle is highly conserved among different plant species.

In the vast majority of land plants, along with the light-dependent reactions of photosynthesis, the Calvin–Benson cycle is primarily conducted in the mesophyll cells of leaves. However, Rubisco discriminates poorly between CO_2 and O_2 (Bowes *et al.*, 1971) and fixing an O_2 molecule instead of CO_2 results in photorespiration – an energetically expensive salvage pathway to recover RuBP. Subsequent to the atmospheric CO_2 concentration dropping dramatically 2.3 billion years ago (Bekker *et al.*, 2004), two carbon-concentrating mechanisms evolved, limiting the amount of photorespiration. These modifications to the basic photosynthetic process, C_4 photosynthesis and Crassulacean Acid Metabolism (CAM), each arose multiple times (Sage *et al.*, 2011). C_4 photosynthesis involves the spatial separation of photosynthesis such that components of both the light-dependent reactions and the Calvin–Benson cycle occur in mesophyll and bundle sheath cells (Box 2). Despite the differences in anatomical location of carbon fixation, until now little was known about how the operation of the Calvin–Benson cycle may be different in C_4 versus C_3 plants and also between C_3 plants.

Variation in Calvin–Benson cycle metabolites between species

The Calvin–Benson cycle is without doubt one of the most critical biochemical pathways on earth, as the pathway of carbon assimilation in plants – the heart of photosynthesis. But do all plant species run this pathway in the same way? Arrivault *et al.* (2019) profiled the abundance of Calvin–Benson cycle metabolites from five C_3 plants (including Arabidopsis and several important crops such as rice, wheat and cassava) and four C_4 plants (including maize). Total metabolites were extracted from mature leaves and measured using reverse-phase liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). For reliable quantification, samples were spiked with isotope-labelled internal metabolite standards; 3-PGA was quantified enzymatically. Metabolite profiles of the different plant species were compared using principal component analysis.

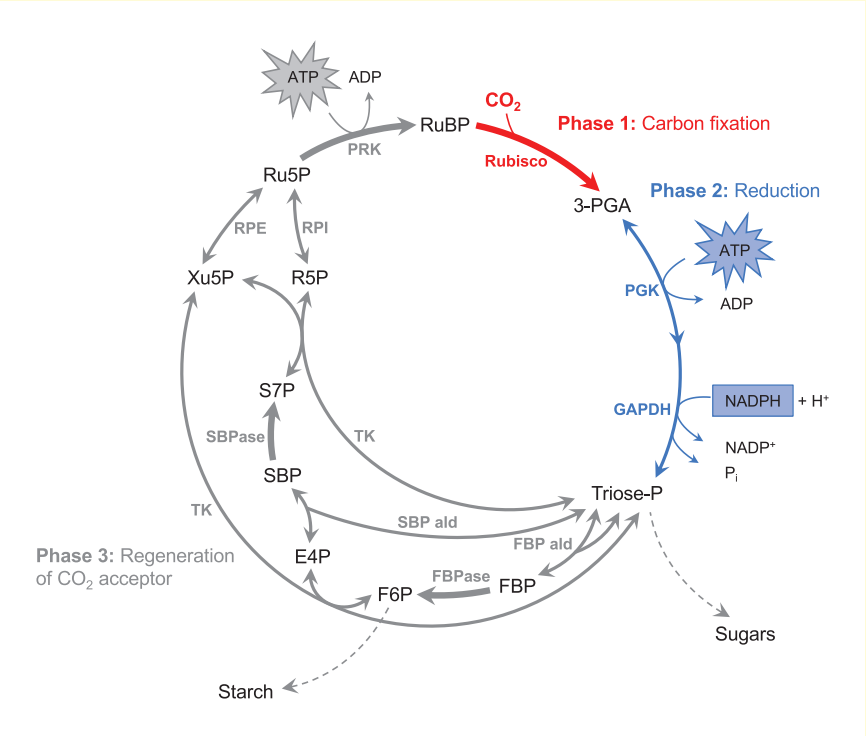
Strikingly, Arrivault *et al.* (2019) discovered substantial differences in the metabolite profiles of Calvin–Benson cycle

Box 1. The Calvin–Benson cycle

The Calvin–Benson cycle is composed of three phases: (1) carbon fixation, (2) reduction and (3) regeneration of the CO_2 acceptor.

Carboxylation is achieved via ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which fixes CO_2 using ribulose-1,5-bisphosphate (RuBP) as the acceptor and in so doing produces two 3-carbon molecules of 3-phosphoglycerate (3-PGA). 3-PGA is subsequently phosphorylated by phosphoglycerate kinase (PGK) and reduced to triose phosphate (triose-P) by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the reduction phase. The cycle uses 3 ATP and 2 NADPH per molecule of fixed CO_2. Triose-P can be transported out of the chloroplast to produce sucrose in the cytosol. Fructose 1,6-bisphosphate aldolase (FBP ald) can convert triose-P into fructose-6-phosphate (F6P), the intermediate used to produce starch. Also, triose-P can be converted to RuBP in a series of regeneration reactions for fixing more CO_2 molecules.

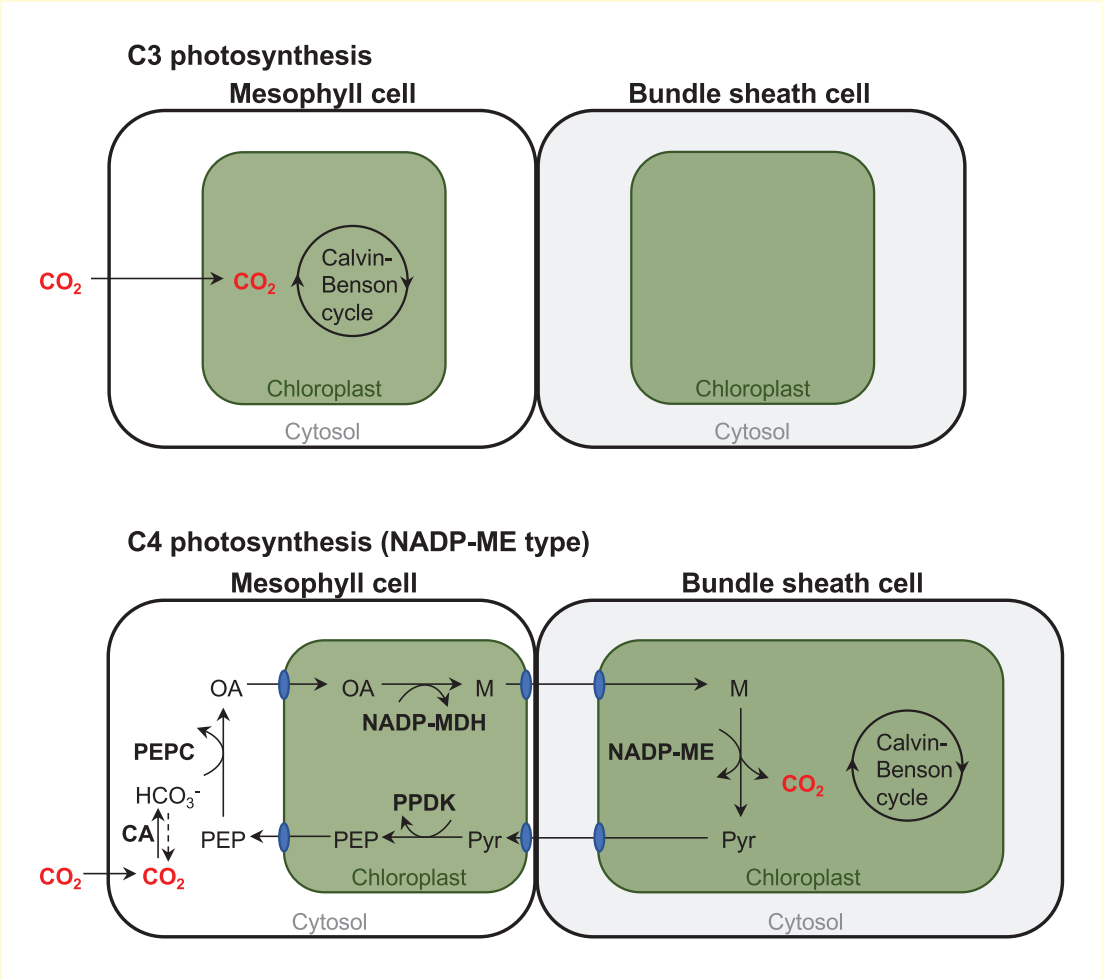
Abbreviations: fructose-1,6-bisphosphate (FBP), fructose-1,6-bisphosphatase (FBPase), erythrose-4-phosphate (E4P), sedoheptulose 1,7-bisphosphate aldolase (SBP ald), sedoheptulose-1,7-bisphosphate (SBP), sedoheptulose-1,7-bisphosphatase (SBPase), sedoheptulose-7-phosphate (S7P), transketolase (TK), ribose-5-phosphate (R5P), xylulose-5-phosphate (Xu5P), ribose-5-phosphate isomerase (RPI), ribulose-5-phosphate epimerase (RPE), ribulose-5-phosphate (Ru5P), phosphoribulokinase (PRK). Enzymes which catalyse irreversible reactions are highlighted by a heavy bold arrow (i.e. Rubisco, FBPase, SBPase and PRK).



intermediates among the five C_3 species that they studied. Intermediates that varied most included the absolute levels of 3-PGA, triose-P, ribulose-5-phosphate (Ru5P) and xylulose-5-phosphate (Xu5P). The relative levels of RuBP compared with levels of intermediates involved in RuBP regeneration were variable among species. Moreover, the authors demonstrated variability in the relative levels of metabolite pairs such as fructose-1,6-bisphosphate (FBP) and fructose-6-phosphate (F6P), which are linked via the irreversible reaction of FBPase; and in the metabolite pair sedoheptulose-1,7-bisphosphate (SBP) and sedoheptulose-7-phosphate (S7P), which are irreversibly interconverted by SBPase. In most cases, the five C_3 species clearly separated from each other in the principal component analysis. The extent to which they separated depended on whether data were normalized to fresh weight, chlorophyll content or protein content. The variation in these intermediates within the five C_3 species point to differences in how plants run the same carbon fixation pathway. This information has consequences for strategies that aim to improve photosynthesis. For example, SBPase can limit the rate of photosynthesis (Zhu *et al.*, 2007) and overexpression can increase photosynthetic efficiency (Lefebvre *et al.*, 2005; Feng *et al.* 2007; Ding *et al.*, 2016; Driever *et al.*, 2017). Thus, altering the activity of

Box 2. The carbon-concentrating mechanism in C_4 plants

In 1966, Hal Hatch and Roger Slack discovered C_4 photosynthesis (Hatch and Slack, 1966), which involves a carbon-concentrating mechanism added onto the regular carbon C_3 fixation pathway. They used ^{14}C-labelling to demonstrate that CO_2 in sugarcane leaves was first fixed into a 4-carbon acid, rather than 3-phosphoglycerate (3-PGA) as in C_3 plants. C_4 plants use phospho*enol*pyruvate carboxylase (PEPC) as their initial carbon-fixing enzyme in the mesophyll cells. The 4-carbon acid (malate or aspartate depending on the type of C_4 photosynthesis) produced in the mesophyll cells then enters bundle sheath cells, where it is decarboxylated and CO_2 is liberated. This carbon-concentrating mechanism allows Rubisco to act almost exclusively as a carboxylase during the Calvin–Benson cycle within the bundle sheath cells. In addition to a reconfiguration of existing metabolic enzymes, the C_4 pathway requires the development of specialized leaf anatomy (Kranz anatomy) which includes an increase in vein spacing and bundle sheath cell size. Classically, three different types of C_4 photosynthesis have been recognized, named after the primary enzyme responsible for the decarboxylation reaction in the bundle sheath cells: NAD-ME, NADP-ME or PEPCK type. Abbreviations: carbonic anhydrase (CA), oxaloacetate (OA), NADP-dependent malate dehydrogenase (NADP-MDH), malate (M), NADP-dependent malic enzyme (NADP-ME), pyruvate (Pyr), pyruvate,orthophosphate dikinase (PPDK), phospho*enol*pyruvate (PEP).



a single enzyme in the Calvin–Benson cycle can impact the rate of photosynthesis, and subsequently biomass and yield. However, the effectiveness of this approach is known to vary between species. The pre-existing variation in levels of the SBP and S7P metabolites between C_3 species reported by Arrivault *et al.* (2019) is therefore important and may provide insight into the variable response of photosynthesis to increasing the amounts of SBPase.

Since the carbon-concentration mechanism of C_4 plants limits photorespiration, it was perhaps less surprising that the first product of photorespiration, 2-phosphoglycolate (2-PG), was less abundant in C_4 species than in C_3 species. Furthermore, C_4 plants had lower levels of RuBP than C_3 plants, consistent with their lower investment in Rubisco. However, even when 2-PG and RuBP levels were omitted from the dataset, C_3 and C_4 metabolite levels almost always separated in the principal

component analysis. These differences were consistent, irrespective of whether data were normalized to fresh weight, chlorophyll content or protein content, and so the changes indicate that the enzymes responsible for generating the metabolites are undertaking catalysis at different rates in different species. The authors coin the term 'operation mode' of the Calvin–Benson cycle to describe these differences – such that the cycle is operating differently between species even though the same enzymes are involved, leading to the observed alterations in the relative levels of intermediates. They therefore propose that differences in the Calvin–Benson cycle between C_3 and C_4 plants are broader than a simple spatial relocation to bundle sheath cells in the latter, and involve adaptation in the cycle's operation mode.

Future perspectives

Establishing that the operation mode of the Calvin–Benson cycle can vary is interesting, especially considering that the structure of the pathway (in terms of enzymes involved and their reaction sequence within the cycle) has been highly conserved. However, over the millions of years since the cycle's first appearance, the ratio of O_2 to CO_2 in the atmosphere has dramatically changed. These changes are thought to have contributed to some plant species evolving carbon-concentration mechanisms. The authors now propose that low CO_2 levels in combination with specific environmental conditions may have led to the development of different Calvin–Benson cycle operation modes. Thus, variation in metabolite profiles observed might reflect distinct selection pressures on how the Calvin–Benson cycle is regulated in different plant lineages. The approach used by the authors to analyse Calvin–Benson cycle intermediates could now be applied to more species, and this would be particularly interesting if these covered a broader range of plant families across diverse environments. This could reveal whether the variation strictly follows phylogenetic taxa or specific environments to which the plants have adapted.

Also, the C_4 plants analysed in Arrivault *et al.* all conduct the NADP-ME type of C_4 photosynthesis. Thus, a promising line of further study would be to explore whether similar changes in Calvin–Benson cycle intermediates are observed in all three types of C_4 metabolism, or whether they are specific to the NADP-ME type.

This work is an excellent starting point for discovering how these different Calvin–Benson cycle modes are controlled at the molecular level. While metabolite profiling enables an unbiased approach to assess variation in levels of intermediates between different species, the underlying causes for these differences remain to be determined. The variation between species could result from differences in gene expression and subsequent protein activities, variation in amino acid sequence impacting on kinetics, or post-translational regulation of the enzymes. Notably, almost all Calvin–Benson cycle enzymes are subject to at least some form of redox regulation, mostly via the thioredoxin (TRX)/ferredoxin (Fd) system (Buchanan and Palmer, 2005; Michelet *et al.* 2013). The integration of these transcript, protein abundance and enzyme activity data to the metabolite levels may reveal the molecular basis of the variation. Moreover, the observed variations in metabolite pools may also be related to demands for certain intermediates, particularly those that are withdrawn from the Calvin–Benson cycle. For example, flux through the cycle can be influenced by exit pathways to allow the synthesis of starch (via F6P), sucrose and isoprenoids (via triose-P), amino acids via the shikimate pathway (via E4P), as well as thiamine and nucleotides (via R5P) (Raines, 2011).

Arrivault *et al.* (2019) report interesting variation in how components of the Calvin–Benson cycle operate in different plant species. This will surely catalyse further studies on how plants have adapted this fundamental and ancient pathway of carbon fixation to different environments.

Keywords: photosynthesis, Calvin–Benson cycle, carbon assimilation, carbon-concentration mechanism (CCM), metabolite profiling, C_4 photosynthesis.

References

Arrivault S, Moraes TA, Obata T, Medeiros DB, Fernie AR, Boulouis A, Ludwig M, Lunn JE, Borghi GL, Schlereth A, Guenther M, Stitt M. 2019. Metabolite profiles reveal inter-specific variation in operation of the Calvin-Benson cycle in both C4 and C3 plants. Journal of Experimental Botany **70,** 1843–1858.

Bassham JA, Benson AA, Kay LD, Harris AZ, Wilson AT, Calvin M. 1954. The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor. Journal of the American Chemical Society **76,** 1760–1770.

Bekker A, Holland HD, Wang PL, Rumble D 3rd, Stein HJ, Hannah JL, Coetzee LL, Beukes NJ. 2004. Dating the rise of atmospheric oxygen. Nature **427,** 117–120.

Bowes G, Ogren WL, Hageman RH. 1971. Phosphoglycolate production catalyzed by ribulose diphosphate carboxylase. Biochemical and Biophysical Research Communications **45,** 716–722.

Buchanan BB, Balmer Y. 2005. Redox regulation: a broadening horizon. Annual Review of Plant Biology **56,** 187–220.

Ding F, Wang M, Zhang S, Ai X. 2016. Changes in SBPase activity influence photosynthetic capacity, growth, and tolerance to chilling stress in transgenic tomato plants. Scientific Reports **6,** 32741.

Driever SM, Simkin AJ, Alotaibi S, Fisk SJ, Madgwick PJ, Sparks CA, Jones HD, Lawson T, Parry MAJ, Raines CA. 2017. Increased SBPase activity improves photosynthesis and grain yield in wheat grown in greenhouse conditions. Philosophical Transactions of the Royal Society B **372,** 20160384.

Feng L, Wang K, Li Y, Tan Y, Kong J, Li H, Li Y, Zhu Y. 2007. Overexpression of SBPase enhances photosynthesis against high temperature stress in transgenic rice plants. Plant Cell Reports **26,** 1635–1646.

Hatch MD. 1987. C4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. Biochimica et Biophysica Acta **895,** 81–106.

Hatch MD, Slack CR. 1966. Photosynthesis by sugar-cane leaves. A new carboxylation reaction and the pathway of sugar formation. The Biochemical journal **101,** 103–111.

Horecker BL, Smyrniotis PZ, Seegmiller JE. 1951. The enzymatic conversion of 6-phosphogluconate to ribulose-5-phosphate and ribose-5-phosphate. The Journal of Biological Chemistry **193,** 383–396.

Lefebvre S, Lawson T, Zakhleniuk OV, Lloyd JC, Raines CA, Fryer M. 2005. Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiology **138,** 451–460.

Mayaudon J. 1957. Study of association between the main nucleoprotein of green leaves and carboxydismutase. Enzymologia **18,** 343–354.

Michelet L, Zaffagnini M, Morisse S, *et al*. 2013. Redox regulation of the Calvin-Benson cycle: something old, something new. Frontiers in Plant Science **4,** 470.

Racker E, Haba GDL, Leder IG. 1953. Thiamine pyrophosphate, a coenzyme of transketolase. Journal of the American Chemical Society **75,** 1010–1011.

Raines CA. 2011. Increasing photosynthetic carbon assimilation in C3 plants to improve crop yield: current and future strategies. Plant Physiology **155,** 36–42.

Sage RF, Christin PA, Edwards EJ. 2011. The C(4) plant lineages of planet Earth. Journal of Experimental Botany **62,** 3155–3169.

Sharkey T. 2018. Discovery of the canonical Calvin–Benson cycle. Photosynthesis Research. doi: 10.1007/s11120-018-0600-2.

Zhu XG, de Sturler E, Long SP. 2007. Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. Plant Physiology **145,** 513–526.