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 C3 and C4 species. These differences in metabolite pools were observed between C3 species as well as between C3 and C4 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 CO2 – the Calvin–Benson cycle (Basham et al., 1954). Their fundamental discoveries were based on feeding the alga Chlorella with 14C-labelled CO2 and tracing the labelling of metabolites over time (Basham 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 CO2 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 CO2 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 CO2 and O2 (Bowes et al., 1971) and fixing an O2 molecule instead of CO2 results in photorespiration – an energetically expensive salvage pathway to recover RuBP. Subsequent to the atmospheric CO2 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, C4 photosynthesis and Crassulacean Acid Metabolism (CAM), each arose multiple times (Sage et al., 2011). C4 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 C4 versus C3 plants and also between C3 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 C3 plants (including Arabidopsis and several important crops such as rice, wheat and cassava) and four C4 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
intermediates among the five C₃ 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-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).
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 C3 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 C4 plants limits photorespiration, it was perhaps less surprising that the first product of photorespiration, 2-phosphoglycolate (2-PG), was less abundant in C4 species than in C3 species. Furthermore, C4 plants had lower levels of RuBP than C3 plants, consistent with their lower investment in Rubisco. However, even when 2-PG and RuBP levels were omitted from the dataset, C3 and C4 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 C₃ and C₄ 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 O₂ to CO₂ 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 CO₂ 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 C₄ plants analysed in Arrivault et al. all conduct the NADP-ME type of C₄ 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 C₄ 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, C₄ photosynthesis.

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