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Topical Review

The end game(s) of photosynthetic carbon metabolism

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Abstract

The year 2024 marks 70 years since the general outline of the carbon pathway in photosynthesis was published. Although several alternative pathways are now known, it is remarkable how many organisms use the reaction sequence described 70 yrs ago, which is now known as the Calvin–Benson cycle or variants such as the Calvin–Benson–Bassham cycle or Benson–Calvin cycle. However, once the carbon has entered the Calvin–Benson cycle and is converted to a 3-carbon sugar, it has many potential fates. This review will examine the last stages of photosynthetic metabolism in leaves. In land plants, this process mostly involves the production of sucrose provided by an endosymbiont (the chloroplast) to its host for use and transport to the rest of the plant. Photosynthetic metabolism also usually involves the synthesis of starch, which helps maintain respiration in the dark and enables the symbiont to supply sugars during both the day and night. Other end products made in the chloroplast are closely tied to photosynthetic CO_2 assimilation. These include serine from photorespiration and various amino acids, fatty acids, isoprenoids, and shikimate pathway products. I also describe 2 pathways that can short circuit parts of the Calvin–Benson cycle. These final processes of photosynthetic metabolism play many important roles in plants.

Introduction

The phrase "path of carbon in photosynthesis" was used in the titles of 22 papers published in the 1940s and 1950s, plus 2 books, and Melvin Calvin's Nobel Lecture (Calvin 1964). The most consequential is the 21st such paper (Bassham et al. 1954), which will be 70 years old in 2024 and is referred to here as "paper 21." This paper has been cited over 320 times (as of August 9, 2023) but laid the groundwork for many thousands of papers. The achievement of Bassham, Benson, and Calvin has been extensively documented (e.g. Nickelsen 2012; Sharkey 2019). Like the Krebs/tricarboxylic acid (TCA)/citric acid cycle, the scheme in paper 21 goes by various names, but here, I will refer to it as the Calvin–Benson cycle. Much current interest in this cycle focuses on sedoheptulose bisphosphatase (SBPase) (Lefebvre et al. 2005; Zhu et al. 2007; Rosenthal et al. 2011; Driever et al. 2017; Simkin et al. 2017). This enzyme is used in preference to transaldolase normally found in the nonoxidative pentose phosphate pathway, which has the effect of directing carbon from trioses to pentoses rather than from pentoses to hexoses and trioses, as is normally depicted for the transaldolase-dependent pentose phosphate pathway (Sharkey 2021)

In 1954, when paper 21 was published, almost nothing was known about how carbon enters the cycle, except that there are 3 sources of pentose and that a pentose (now called RuBP) is carboxylated to generate 3-phosphoglycerate (3-PGA). The carboxylase eventually became known as rubisco (Portis and Parry 2007; Sharkey 2022). A big question about photosynthetic carbon metabolism remained: what are the last steps as carbon leaves the cycle? While paper 21 did not track the phosphorylation status of the intermediates, it became clear that ATP drives the cycle forward. Therefore, the phosphate added to make RuBP must be

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- The last steps of photosynthetic carbon metabolism in the endosymbiont chloroplast provide important precursors for plants.
- The last steps of photosynthetic carbon metabolism are essential in order to release phosphate from the organic phosphate pool for reuse in ATP.
- Photorespiration can make serine as an end product of photosynthetic carbon metabolism although the estimates of 40% serine export seem high.
- A stromal G6P shunt involving the oxidative pentose phosphate pathway consumes carbon from the Calvin–Benson cycle and consumes ATP. It is normally not functioning during the day.
- A cytosolic G6P shunt reinjects free glucose, fructose, and sucrose into the Calvin–Benson cycle helping it refill very quickly after periods of low light.

released so that ATP can be regenerated. All the phosphate added by phosphoglycerate kinase is immediately released by glyceraldehyde 3-phosphate dehydrogenase. One third of the phosphate added by phosphoribulokinase is released by fructose bisphosphatase (FBPase) and one third by SBPase, but the last third must be released during end-product synthesis. This sets a reasonable boundary for photosynthetic carbon metabolism: it begins with CO_2 and ends only when an equivalent amount of carbon in intermediates is dephosphorylated.

Glucose is often regarded as the ultimate product of photosynthetic carbon metabolism, but unphosphorylated glucose only occurs in part of the starch utilization pathway. The bulk of carbon fixed in photosynthesis ends up as either starch or sucrose (Fig. 1). If starch is made, all photosynthetic carbon metabolism is said to occur inside chloroplasts. However, as an endosymbiont, the chloroplast supplies the host cell with reduced carbon, and starch synthesis capacity is typically not sufficient to account for the high rates of CO_2 fixation that could be observed using isolated intact chloroplasts (Walker and Herold 1977).

Sucrose metabolism

Carbon export from chloroplasts during CO_2 fixation depends on the obligatory export of triose phosphate and the import of inorganic phosphate (Heldt et al. 1977; Fliege et al. 1978). Phosphate and triose phosphate exchange occurs at a triose phosphate/phosphate antiporter (TPT), an abundant protein on the inner envelope of the chloroplast and a member of a family of related phosphate antiporters (Bockwoldt et al. 2019). The evolution of these antiporters is

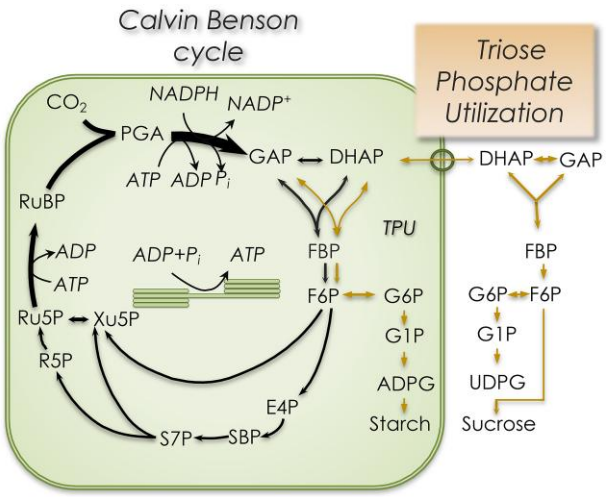

Figure 1. The carbon dioxide that enters the Calvin–Benson cycle is metabolized primarily to starch and sucrose (lines on right side of the figure). Since both sucrose and starch synthesis can be readily traced back to the triose phosphates, this is often called triose phosphate utilization. PGA, 3-phosphoglycerate; TPI, triose phosphate isomerase; GAP, glyceraldehyde 3-phosphate (distinguished from glycerol 3-phosphate denoted G3P); DHAP, dihydroxyacetone phosphate; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; E4P, erythrose 4-phosphate; glyco-TDP, glycoaldehyde thiamine diphosphate; SBP, sedoheptulose 1,7-bisphosphate; S7P, sedoheptulose 7-phosphate; R5P, ribose 5-phosphate; Xu5P; xylulose 5-phosphate; Ru5P, ribulose 5-phosphate.

thought to have been an essential step to establishing the endosymbiotic nature of chloroplasts (Weber et al. 2006; Linka and Weber 2010). The export of triose phosphate supports the synthesis of sucrose in the cytosol of plant cells (Bird et al. 1974). As sucrose is a phosphate-free molecule, its production marks the end of photosynthetic carbon metabolism.

Sucrose synthesis involves the formation of sucrose 6-phosphate from fructose 6-phosphate (F6P) and uridine diphosphate (UDP)-glucose by the enzyme sucrose-phosphate synthase (SPS). The sucrose phosphate is readily broken down to sucrose by SPS. Sucrose-phosphate synthase is highly regulated, especially by phosphorylation (Huber and Huber 1996; Hardin et al. 2003). Several sites can be phosphorylated with different effects, and it is dephosphorylated by SPS protein phosphatase, a type 2A protein phosphatase (Huber and Huber 1996). This regulation also involves a 14-3-3 protein; such proteins are often involved in regulating phosphorylation (Bachmann et al. 1996). While SPS activity is lower and more sensitive to metabolite concentrations at night, it is not turned off completely (Jones and Ort 1997), allowing sucrose synthesis to occur during both day and night.

Sucrose is a disaccharide that plays several central roles in plants (Salerno and Curatti 2003). A similar disaccharide, trehalose, is more widespread phylogenetically. The answer to the question "why sucrose?" is unclear (Salerno and Curatti 2003). In plants, trehalose metabolism appears to be specialized for carbohydrate status signaling, especially trehalose

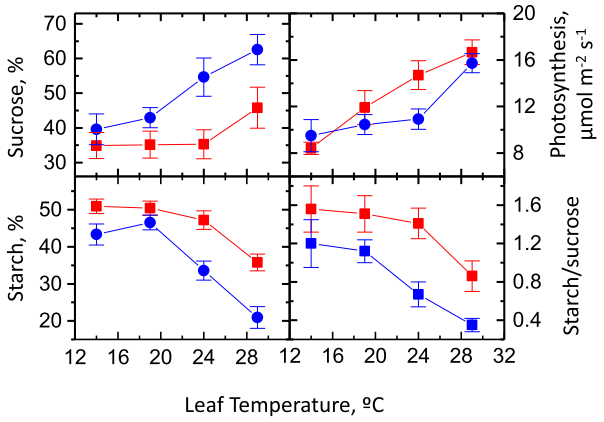

Figure 2. Sucrose and starch synthesis as a function of temperature in tomato with and without a SPS gene from maize. Squares are data from transformed plants, and circles are from untransformed plants. The proportion of sucrose synthesis from CO_2 fixation increases with temperature, while starch synthesis declines. The SPS gene had little effect on the rate of photosynthesis, which increased over a temperature range of 14 °C to 29 °C. Data are from Laporte (1997).

6-phosphate (Lastdrager et al. 2014; Fichtner and Lunn 2021; Peixoto et al. 2021) and its interactions with sucrose nonfermenting-related kinase (SnRK) and target of rapamycin (TOR) regulatory mechanisms (Jamsheer et al. 2019; Ryabova et al. 2019; Sharma et al. 2022).

Starch metabolism

The identification of the TPT explained carbon export from the chloroplast during the day, but what about carbon export at night? Starch accumulates throughout the day in most plants. At low rates of photosynthesis, most carbon is partitioned to sucrose, but as the photosynthetic rate increases, due to either increased light or increased CO_2 availability, more and more carbon is partitioned to starch (Sharkey et al. 1985; Mullen and Koller 1988). The ratio of starch to sucrose is higher in plants growing in short days (Sulpice et al. 2014; Xu et al. 2023) and can be low when leaves are incubated at low temperature (Pollock and Lloyd 1987). The ratio of partitioning to starch versus sucrose can be regulated by activity of SPS (Galtier et al. 1993). Increasing SPS activity by transforming tomato (*Solanum lycopersicum*) with an SPS gene from maize (*Zea mays*) increased the proportion of carbon going to sucrose and decreased the proportion going to starch (Fig. 2, redrawn from Laporte (1997)). The starch/sucrose ratio declined with increasing temperature in both the transformed line and the controls.

Another critical control point for sucrose synthesis is the ratio of FBPase activity in the stroma to that in the cytosol. In the stroma, this regulation involves light activation, among other mechanisms. In the cytosol, FBPase activity is regulated by fructose 2,6-bisphosphate (Stitt 1990). F6P is phosphorylated and dephosphorylated at the 2 position, a process that integrates many different signals. The regulation of FBPase, and its mirror image, i.e. the regulation of phosphofructokinase, prevents a futile cycle in which ATP is used to convert F6P to F1,6BP, and FBPase removes that phosphate, consuming ATP. Regulation involving fructose 2,6-bisphosphate ensures that these reactions are occurring in only 1 direction, toward sucrose during photosynthesis and consuming glucose only when photosynthesis is not occurring. The regulation at FBPase is more complete than the regulation of SPS so that at night, sucrose cannot be produced from triose phosphates but it can be produced from hexose phosphates.

Starch breakdown then supplies the plant with sugars at night. Starch is broken down at a constant rate at night; more than 90% of the carbon present in starch at the end of the day is released from starch at night (Lu et al. 2005; Pokhilko et al. 2014; Flis et al. 2019). As a result, the chloroplast supplies reduced carbon to the host at an almost constant rate day and night (Sulpice et al. 2014).

It was assumed that at night, the sugars in starch broke down to triose phosphates, which were then exported on the TPT. However, deuterium labeling showed that when starch breaks down and is converted to sucrose, the carbon skeletons are never broken down to triose phosphates (Schleucher et al. 1998). Instead, maltose, the product of β-amylase, is the primary export sugar resulting from starch breakdown (Weise et al. 2004). Initial attempts to show a gradient for maltose export were unsuccessful, leading to the finding that β-maltose is the primary exported sugar. β-maltose, but not total maltose, shows a significant gradient in favor of export from chloroplasts (Weise et al. 2005).

β-amylase cleaves 2 glucose residues from α-1,4 maltodextrins (glucose chains) to generate β-maltose. However, this enzyme cannot work on maltotriose, only longer maltodextrins. To finish breaking down starch remnants, an enzyme called disproportionating enzyme 1 (DPE1) (1 for the plastidial form) uses maltotriose in this reaction:

$$\text{Glu3} + \text{Glu3} \rightleftharpoons \text{Glu5} + \text{Glu}$$

where Glu3 is maltotriose and Glu5 is maltopentaose. Other reengagements are possible, but the net effect is that maltodextrins can be broken down primarily to produce maltose but some glucose as well. Glucose in the chloroplast can exit through a glucose transporter, which was first reported in 2000 (Weber et al. 2000). A maltose transporter was discovered soon after (Niittylä et al., 2004). Once in the cytosol, the maltose is acted on by DPE2 (Chia et al. 2004; Lu and Sharkey 2004) (Fig. 3). DPE2 is similar to DPE1 but contains a 150 amino acid insert (Steichen et al. 2008). However, unlike DPE1, DPE2 transfers the nonreducing glucose to a soluble heteroglycan (SHG) (Fettke et al. 2006). This large-molecular-weight polysaccharide primarily comprises arabinose, galactose, ribose, and some glucose (Yang and Steup 1990). The glucose transferred to the heteroglycan can be liberated by phosphorolysis. In this way, the energy in the glucose–glucose bond of maltose can be conserved in glucose 1-phosphate (G1P), which can be isomerized to glucose 6-phosphate (G6P) or used to make UDP-glucose.

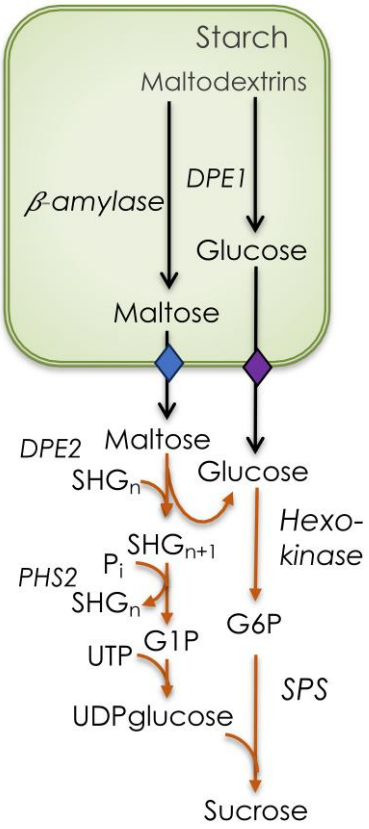

Figure 3. Conversion of starch to sucrose at night. Various starch-degrading enzymes break the starch down to linear maltodextrins, which are hydrolyzed by β-amylase to generate β-maltose. Once the maltodextrin reaches a degree of polymerization of 3, it is acted on by DPE1. Mostly maltose, but some glucose too, leaves the chloroplast through specific transporters. Maltose is acted on by disproportionating enzyme 2 (DPE2) to add 1 glucose to a SHG and glucose. Starch phosphorylase 2 (PHS2) removes a glucose from the SHG to produce G1P. The glucose is acted on by hexokinase to make G6P. SPS generates sucrose phosphate, which is then dephosphorylated to sucrose.

Currently, there is much interest in how starch breakdown is regulated. The remarkably constant rate of starch breakdown at night has attracted much attention (Lu et al. 2005; Graf et al. 2010; Scialdone et al. 2013; Fernandez et al. 2017; Mengin et al. 2017). Transitory starch is phosphorylated by a glucan water dikinase (Ritte et al. 2002) and phosphoglucan water dikinase (Kötting et al. 2005). Without this phosphorylation, starch becomes more difficult to break down. Some plants accumulate nontransitory starch as they age (Chu et al. 2022), possibly due to the accumulation of unphosphorylated starch, which is no longer accessible to the plant, over wks or mos.

Photorespiration

It was recently hypothesized that photorespiration can provide some capacity for end-product synthesis in the form of glycine and serine (Harley and Sharkey 1991; Busch et al. 2018; Fu et al. 2023). It has long been recognized that O_2 inhibits photosynthesis. This is sometimes called the (green) Warburg effect (Kutschera et al. 2020); the red effect is related to cancer metabolism (Liberti and Locasale 2016). Rabinowitch (1945) used the term "photorespiration" to describe metabolism that begins upon illumination and ends upon darkness. Decker (1955) described a postillumination burst of CO_2 release from leaves believed to result from the light-dependent process that releases CO_2. Wilson and Calvin (1955) found that glycolate was produced in their reactions and that this could be suppressed by using 1% CO_2. Bassham and Kirk (1962) established that the production of glycolate (and glycine) could be affected by O_2. However, the major advance in the field of photorespiration was the discovery that the CO_2-fixing enzyme rubisco also fixes O_2 (Bowes et al. 1971; Ogren and Bowes 1971). The 2-phosphoglycolate formed in this reaction is a potent inhibitor of triose phosphate isomerase (Anderson 1971; Flügel et al. 2017; Li et al. 2019). Phosphoglycolate also inhibits phosphofructokinase (Kelly and Latzko 1976), but at higher concentrations. Phosphofructokinase inside chloroplasts is not operational during photosynthesis when FBPase is functioning. NADPH provides 1 mechanism for ensuring that phosphofructokinase is not active in the light (Cséke et al. 1982). The pathway for metabolizing 2-phosphoglycolate formed by the oxygenation of RuBP was proposed by Tolbert (1971).

In the view of photorespiration presented above, this process is not a method for end-product synthesis, since all the carbon that enters the pathway is either released as CO_2 or rephosphorylated to reenter the Calvin–Benson cycle. However, for many years, it appeared as though photorespiration could add capacity for end-product synthesis. An early example was reported by Jolliffe and Tregunna (1973), who showed that under high CO_2 and low temperature conditions, the photosynthetic rate under low O_2 conditions (low photorespiration) was lower than the rate in 21% O_2. Sharkey (1985) proposed that when end-product synthesis limits photosynthesis, CO_2 assimilation becomes insensitive to CO_2 and O_2 (in other words, photorespiration does not decrease the rate of CO_2 assimilation). We now know that photorespiration in fact increases the capacity for photosynthesis when starch and sucrose synthesis rates are maximal. This may be explained by the hypothesis that glycine, and more likely serine, leaves photorespiratory metabolism, providing both reduced nitrogen and carbon for use in leaf metabolism (Busch et al. 2018). If this process occurs at a rate proportional to the rate of photorespiration, and if end-product synthesis sets the upper bound of CO_2 assimilation, then photorespiration increases the upper bound. As a result, as CO_2 levels increase and the rate of photorespiration decreases, the overall rate of CO_2 assimilation will also decrease.

Under low light conditions, increasing CO_2 or decreasing O_2 levels stimulate photosynthesis, as would be expected if the rate of photorespiration is reduced (Fig. 4). However, as light levels increase, the upper bound of the rate of CO_2 assimilation falls under the control of end-product synthesis, and increased CO_2 or decreased O_2 levels reduce

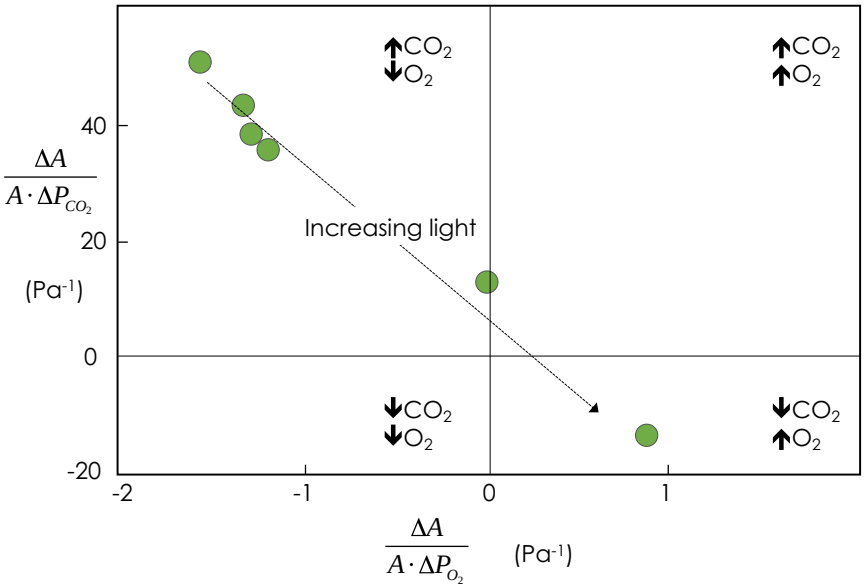

Figure 4. Assimilation-weighted sensitivity to CO_2 (vertical axis) and O_2 (horizontal axis) as affected by increasing light levels. At limiting light levels, the energy cost of photorespiration results in a reduction in CO_2 assimilation capacity. As light levels increase, the upper bound becomes sensitive to end-product synthesis. Photorespiration stimulates CO_2 assimilation by adding end-product synthesis capacity in the form of serine export. Redrawn from Sharkey (1990). Arrows indicate the response of photosynthesis to the indicated gases.

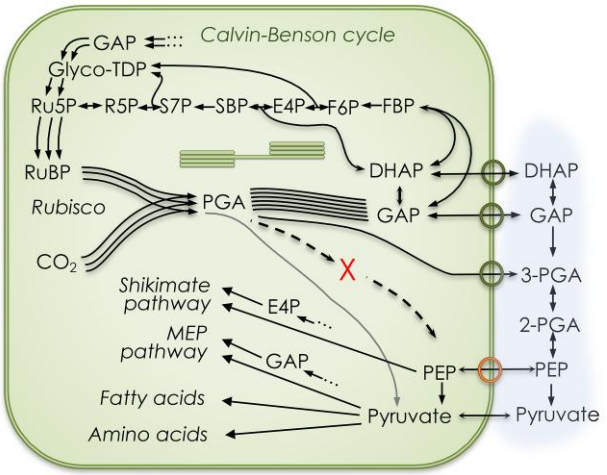

Figure 5. Chloroplast metabolism emphasizing the lower glycolytic pathway. The TPT (circles on the chloroplast membrane) exchanges DHAP, GAP, and PGA. The PEP/phosphate antiporter exchanges PEP for phosphate. The production of PEP inside the chloroplast is restricted by very low phosphoglyceromutase and enolase activities (denoted with X). Pyruvate is generated at a rate of ~0.5% of total carboxylations, directly providing pyruvate for chloroplast reactions (line connecting rubisco to pyruvate). Additional products of the Calvin–Benson cycle include shikimate pathway products (e.g. aromatic amino acids), isoprenoids, and pentoses. See Raines (2011, 2022) (Fig. 1) for a more complete depiction of export sites from the Calvin–Benson cycle.

photorespiration, thereby reducing the overall rate of CO_2 assimilation. The value of 30% to 40% of carbon diverted from 2-phosphoglycolate to serine found from curve fitting (Busch et al. 2018), and isotopically non–steady-state mass flux analysis (Fu et al. 2023) seems high. It is possible that other metabolisms occur that have the same effect as serine export for nitrogen metabolism.

While serine export can explain many observations of reversed sensitivity, some observations are too extreme to be accounted for by serine export from photorespiration. Sharkey and Vassey (1989) determined that the rate of CO_2 assimilation decreased by 20% upon switching from 200 to 20 kPa O_2. This decline was almost entirely the result of a reduced rate of starch synthesis. The amount of stromal G6P fell by 73%, likely due to the inhibition of phosphoglucoisomerase (PGI). This enzyme is known to be out of equilibrium in the stroma (Gerhardt et al. 1987; Schleucher et al. 1999) and can be inhibited by some Calvin–Benson cycle intermediates, especially erythrose 4-phosphate (Preiser et al. 2020). In this case, the reversed sensitivity to O_2 is not a function of increased end-product synthesis capacity associated with photorespiration. Instead, it is a function of a metabolic "traffic jam" in which increases in the levels of some Calvin–Benson cycle intermediates reduce the capacity for end-product (in this case starch) synthesis.

There is currently substantial interest in devising alternative pathways for metabolizing 2-phosphoglycerate, the metabolite that initiates photorespiration (Peterhänsel and Maurino 2011; Peterhänsel et al. 2013; Dalal et al. 2015; Xin et al. 2015; Engqvist and Maurino 2017; South et al. 2019). On the other hand, some researchers believe that photorespiration plays an important role in nitrogen metabolism (Rachmilevitch et al. 2004; Bloom 2015; Busch et al. 2018) and that plants experience evolutionary pressure to maintain photorespiration.

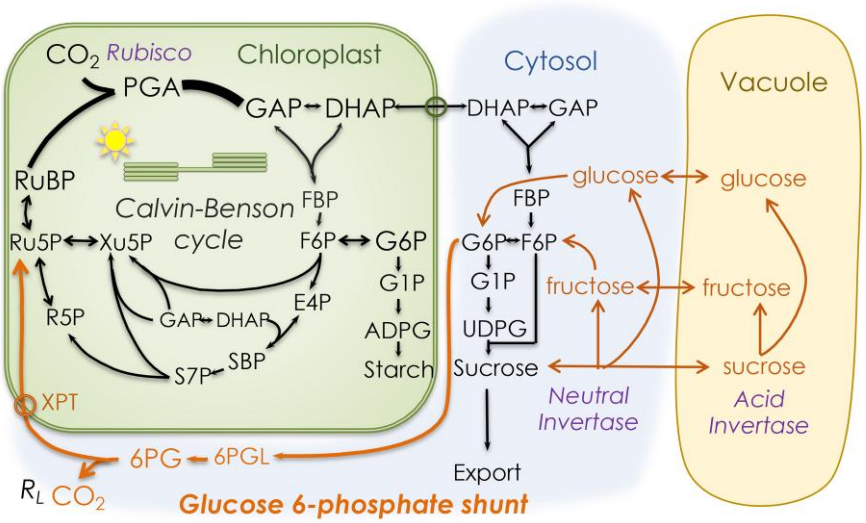

Figure 6. Model of photosynthetic carbon metabolism including sources of unlabeled carbon that is imported into the Calvin–Benson cycle. The import of unlabeled carbon that enters as hexose slows the rate of labeling of Calvin–Benson cycle intermediates. The G6P shunt also releases moderately labeled CO_2 that can be detected as $^{12}CO_2$ emitted into a 99+% $^{13}CO_2$ atmosphere. Abbreviations as in Fig. 1 plus 6PGL, 6-phosphogluconolactone; 6PG, 6-phosphogluconate; XPT, xylulose 5-phosphate/phosphate antiporter (also transports Ru5P). A detailed flux map based on this model is available in Xu et al. (2022).

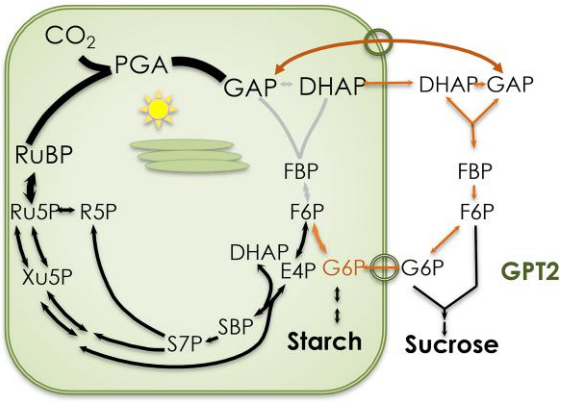

Figure 7. Mechanism for the cytosolic bypass of critical enzymes of the Calvin–Benson cycle. Triose phosphate isomerase, aldolase, and FBPase can be bypassed when the G6P transporter GPT2 is induced. However, the low capacity of PGI causes G6P to build up in the stroma, ultimately activating stromal G6PD, causing a futile cycle that consumes ATP. The ATP is replaced by cyclic electron flow, which has been observed in plants carrying out cytosolic bypass.

The lower glycolysis metabolism of chloroplasts

The reduction step and several steps in the regeneration portion of the Calvin–Benson cycle are gluconeogenic, that is, the reverse of glycolysis, from 3-PGA to F6P and G6P. The lower branch of glycolysis from PGA to phospho*enol*pyruvate (PEP) appears to be absent from photosynthesizing chloroplasts. Phosphoglyceromutase and enolase activities were found to be <1% of the activities in the cytosol (Stitt and ap Rees 1979; Schulze-Siebert et al. 1987; Hoppe et al. 1993) (X in Fig. 5). The TPT (circles on the chloroplast membrane in Fig. 5) exchanges phosphate, dihydroxyacetone phosphate (DHAP), glyceraldehyde 3-phosphate (GAP), and PGA. The metabolism in the stroma and cytosol determines the concentration gradients of these 4 molecules, and they are exchanged based on their concentration gradients. A triose phosphate isomerase can isomerize DHAP and GAP, but whole-leaf triose phosphate concentrations are not in isomerase equilibrium (Li et al. 2019). A nonphosphorylating glyceraldehyde 3-phosphate dehydrogenase (GAPN) that converts GAP to PGA is present and can provide NADPH in the cytosol. This reaction is irreversible. There are also phosphorylating GAPD enzymes, i.e. GAPC1 and GAPC2 (Guo et al. 2014). The amount of PGA that can be converted to GAP depends on the energetic status of the cytosol.

Several biosynthetic pathways require PEP (e.g. the shikimate pathway and its many products). PEP is imported from the cytosol by a PEP/P_i antiporter (bottom ring on the chloroplast membrane in Fig. 5). When the antiporter is absent, as in the Arabidopsis (*Arabidopsis thaliana*) *cue1* (chlorophyll a/b binding protein [CAB] underexpressors) mutants, the plants do not grow well (Voll et al. 2003). Voll et al. (2003) found that the *cue1* mutant could be rescued by either expressing a gene encoding a PEP/P_i antiporter (PPT) or by expressing a pyruvate phosphate dikinase (PPDK) gene in the stroma. The finding that expressing a PPT gene in the mutant background rescued this mutant indicates that the chloroplast does not have sufficient phosphoglycerate mutase to support the requirement for PEP in the chloroplast.

The reaction from PEP to pyruvate catalyzed by pyruvate kinase is not easily reversed, but the PPDK reaction is

energetically favorable because it, in essence, uses the energy of 2 ATPs. The rescue of the *cue1* mutant by PPDK means that there is sufficient pyruvate to satisfy the need for PEP inside the chloroplast. Thus, either pyruvate is transported into the chloroplast or the small amount of pyruvate made by rubisco (Andrews and Kane 1991) is sufficient for the plant's needs.

Other chloroplast-localized metabolic pathways require pyruvate, e.g. the methylerythritol 4-phosphate (MEP) pathway (the source of carotenoids among other classes of molecules), fatty acid synthesis, and the synthesis of branched chain amino acids. There are 4 possible sources of pyruvate in the chloroplast stroma: PEP that enters through the PPT could be converted to pyruvate by pyruvate kinase inside the chloroplast, pyruvate could be imported from the cytosol by passive permeation, pyruvate could be transported by the BASS2 importer (Furumoto et al. 2011), or pyruvate could be supplied as a side reaction of rubisco (Andrews and Kane 1991). One source of cytosolic pyruvate is malic enzyme acting on malate from the vacuole.

The pyruvate paradox

When $^{13}CO_2$ is fed to leaves, intermediates of the Calvin–Benson cycle are labeled very quickly at first, but the rate of labeling slows considerably when 80% to 90% of the intermediates are labeled. All the intermediates of the Calvin–Benson cycle are labeled with very similar kinetics (Szecowka et al. 2013; Ma et al. 2014; Xu et al. 2021; Xu et al. 2022). This includes 2-PGA (Szecowka et al. 2013), which should only be present in the cytosol, not the chloroplast, and PEP (Hasunuma et al. 2010; Ma et al. 2014; Xu et al. 2022), which should mix with any PEP in the cytosol. However, pyruvate, which should be produced from PEP, is labeled much more slowly (Szecowka et al. 2013; Xu et al. 2022) than all the other molecules shown in Fig. 5. Although whole-leaf pyruvate is relatively unlabeled after 20 min, isoprene, 40% of which is derived from pyruvate, is labeled to the same degree as Calvin–Benson cycle intermediates (Delwiche and Sharkey 1993; Sharkey et al. 2020), indicating that the metabolically active pyruvate pool is labeled to the same degree as Calvin–Benson cycle intermediates. Perhaps a very large amount of pyruvate in the vacuole is labeled very slowly, masking the very rapid labeling of a small, metabolically active pyruvate pool in the chloroplast (and presumably also in the cytosol). It is possible that pyruvate is generated in the chloroplast by rubisco (Andrews and Kane 1991) and that this rubisco-derived pyruvate is heavily labeled and is used preferentially in the MEP pathway and presumably for fatty acid synthesis and other stromal reactions requiring pyruvate.

In summary, the intermediates of the Calvin–Benson cycle, starch synthesis precursors (e.g. ADP-glucose) and both PEP and pyruvate, constitute a large metabolically active pool of carbon that is labeled with similar kinetics. Of all these molecules, only pyruvate is also present in a metabolically inactive pool.

Shunts that bypass Calvin–Benson cycle reactions

The radioactive isotope ^{14}C (Ruben and Kamen 1940) played a critical role in elucidating the carbon pathway in photosynthesis. However, when 99+% labeled CO_2 is fed to photosynthesizing leaves, Calvin–Benson cycle intermediates do not become fully labeled (Mahon et al. 1974; Hasunuma et al. 2010; Szecowka et al. 2013; Ma et al. 2014), indicating that either nonmetabolic pools of most Calvin–Benson cycle intermediates are present or a source of unlabeled carbon enters the cycle.

A model of photosynthetic carbon metabolism has been used in metabolic flux analysis that includes oxidative pentose phosphate pathway reactions forming a shunt that bypasses a substantial portion of the Calvin–Benson cycle (Xu et al. 2022). This model (Fig. 6) can account for the lack of complete labeling and a nonphotorespiratory CO_2 release known as day respiration (R_d) or respiration in the light (R_L).

The G6P shunt occurring in the cytosol appears to operate continuously and at a reasonably constant rate, as assessed by measuring R_L (Tcherkez et al. 2017; Schmiege et al. 2023). A similar shunt can operate in the stroma, but this appears to occur only in response to stress (Sharkey et al. 2020), although a recent report describes a very large stromal shunt that operates under either low or high CO_2 levels (Wieloch et al. 2022). A different shunt can be induced to bypass missing enzymes in the Calvin–Benson cycle. This shunt involves GAP leaving the chloroplast via the TPT, its conversion to G6P, and its reimport through an inducible G6P transporter called GPT2 (Kammerer et al. 1998).

Ordinarily, there is no exchange of G6P across the chloroplast envelope, and large gradients of G6P between the stroma and cytosol have been reported (Gerhardt et al. 1987; Sharkey and Vassey 1989; Schleucher et al. 1998; Schleucher et al. 1999). Keeping the G6P level low in the chloroplast has the advantage of preventing stimulation of the stromal G6P dehydrogenase (G6PD), which would lead to a futile cycle. The stromal G6PD is normally inactive during the day due to the thioredoxin-dependent reduction of a disulfide bridge (Wenderoth et al. 1997; Née et al. 2009; Née et al. 2014; Cardi et al. 2016; Yoshida et al. 2019). However, the deactivation is mostly the result of an increase in K_m rather than a reduction in overall capacity (Scheibe et al. 1989; Née et al. 2014). In addition, G6P can overcome the redox regulation of G6PD (Cossar et al. 1984; Preiser et al. 2019). Therefore, G6PD activity in the stroma can be very sensitive to G6P concentration. This G6P sensitivity likely explains why regulatory mechanisms are in place to limit the G6P concentration in the stroma, including the lack of G6P exchange with the cytosol and the limited capacity at the stromal PGI. However, plants lacking otherwise essential enzymes such as stromal triose phosphate isomerase, aldolase, and FBPase can survive by making use of the cytosolic versions of these enzymes and letting G6P back into the stroma through the inducible GPT2 (Fig. 7). The low capacity of

PGI will cause G6P to build up, stimulating the futile G6P shunt in the stroma. This leads to cyclic electron flow to make up for the ATP lost in the futile cycle. Therefore, although there is evidence for the cytosolic bypass mechanism described here (Gotoh et al. 2010; Livingston et al. 2010; Li et al. 2019), plants that depend on this bypass mechanism are generally compromised.

Conclusion

The core of photosynthetic carbon metabolism is the Calvin–Benson cycle. To keep this cycle going, end products must be generated that result in the release of phosphate for reuse in ATP. Chloroplasts produce many end products. The rates of sucrose, starch, and serine synthesis (from photorespiration) can affect the gas exchange behavior of leaves. These processes, plus some bypass mechanisms, are the end game of photosynthetic CO_2 fixation, but they mark the beginning of the next phase, such as using the resources for maintenance, to build the plant, to flower, and to reproduce. The production of end products of photosynthesis also represents a starting point for designing ways to modify photosynthesis to enhance food, fuel, and fiber production for human use. There has been substantial progress in understanding the end-product metabolism of photosynthesis, but regulation of G6P in the stroma and the pyruvate paradox remain important areas for research (Outstanding questions box).

OUTSTANDING QUESTIONS BOX

- The apparent large flux of serine out of photorespiration requires additional study. What is the fate of all of that serine?
- G6P in the chloroplast appears to be strongly controlled by regulation of G6P isomerase. How this regulation occurs and whether it can be modified to improve how photosynthesis meets human interests is an important area for research.
- Solving the pyruvate paradox also is an outstanding concern that should be investigated. The ability of rubisco to make pyruvate has been reported once but needs to be independently verified, and then, the consequences of this pyruvate production need additional study.

Author contributions

T.D.S. conceived the idea and wrote the paper.

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Data availability

No new data were created for this work.

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