

Discovery of the Canonical Calvin-Benson Cycle

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Abstract

It has been 65 years since the Calvin-Benson cycle was first formulated. In this paper the development of the concepts that are critical to the cycle are traced and the contributions of Calvin, Benson, and Bassham are discussed. Some simplified views often found in text books such as ascending paper chromatography and the use of the “lollipop” for short labeling are discussed and further details given. Key discoveries that underpinned elucidation of the cycle such as the importance of sedoheptulose phosphate and ribulose 1,5-bisphosphate are described. The interchange of ideas between other researchers working on what is now called the pentose phosphate pathway and the development of the ideas of Calvin and Benson are explored while the gluconeogenic aspects of the cycle are emphasized. Concerns raised about anomalies of label distribution in glucose are considered. Other carbon metabolism pathways associated with the Calvin-Benson cycle are also described. Finally, there is a section describing the rift between Calvin and Benson.

Keywords

Bassham; Benson; Calvin; Calvin-Benson cycle; $^{14}\text{CO}_2$; gluconeogenesis; pentose phosphate pathway; rubisco

Introduction

In 1947, Andrew Benson and Melvin Calvin published a paper titled “The Dark Reductions of Photosynthesis”. Over the next seven years they published “The Path of Carbon in Photosynthesis” papers I to XXIII proposing many different cycles. As late as 1952, in “The Path of Carbon in Photosynthesis XX” Calvin and Massini (1952) proposed two carboxylations, a role for malate, and multiple, confusing suggestions for how carbon reduction occurred (no less than five potential reduction steps). But then in 1954 (submitted in 1953), with Al Bassham as first author, “The Path of Carbon in Photosynthesis XXI” was published laying out the pathway as we understand it today, including the correct stoichiometry of reducing requirements and ATP (Bassham et al. 1954). This series of reactions is the primary mechanism by which the energy of sunlight is stored as reduced carbon in plants, algae and many bacteria. Here I describe the development of core concepts of the pathway, the relationship to the discovery of the non-oxidative pentose phosphate pathway and the roles of Calvin, Benson, and Bassham. Also included is some information on metabolic pathways intimately associate with the cycle including photorespiration, non-photorespiratory respiration in the light, and a cytosolic bypass of the gluconeogenic reactions of the cycle.

Setting the stage for the discovery of the Calvin-Benson cycle

During the early twentieth century, the basic mechanism of photosynthesis was under intense study. It became clear that conversion of carbon dioxide to sugars using the energy in light could be separated into two distinct phases, light absorption and carbon metabolism. This led to the unfortunate practice of calling photosynthetic carbon metabolism “the dark reactions” a practice some trace back conceptually to Blackman (1905). The terms “dark reactions” or “light-independent” reactions are (too) slowly leaving common usage (Buchanan 2016b). Two alternative names in use, “photosynthetic carbon metabolism” and “photosynthetic carbon reduction cycle” are descriptive, but since there are a number of carbon metabolism pathways that allow for photoautotrophic growth (Buchanan and Arnon 1990; Bar-Even et al. 2012) a more specific name is needed for the carbon metabolism found in plants, algae, and most bacteria. This is sometimes called the reductive pentose phosphate pathway but this obscures the fact that most reactions are gluconeogenic and the pentose phosphate pathway components of the Calvin-Benson cycle are not redox reactions. Melvin Calvin was awarded the Nobel prize for elucidation of this pathway (Calvin 1964) and so when textbooks avoid saying the dark reactions they usually call it the Calvin cycle. For reasons given below I will call it the Calvin-Benson cycle. This cycle is sometimes seen a synonymous with “C₃” photosynthesis and indeed is at the heart of C₃ photosynthesis. However, it is also at the heart of C₄ photosynthesis and even C₂ (also called C₃-C₄ intermediate photosynthesis). These refer to mechanisms of CO₂ supply but all rely on the Calvin-Benson cycle.

Early thinking about carbon fixation in photosynthesis was described by Willstätter and Stoll (1918) [cited in Benson (2002)]. They hypothesized that photo-excited chlorophyll would interact with carbon dioxide, likely making formaldehyde that would undergo further reactions to become sugar. In 1938, Sam Ruben and Martin Kamen, working at Berkeley, used ¹⁴C to study carbon fixation in photosynthesis. They found no support for formaldehyde as a first product and put forward that the first product should be a carboxylic acid (Ruben et al. 1939). That work was very important in moving the thinking away from formaldehyde formation to carboxylation as the first step in carbon fixation.

The short half-life of ¹⁴C made it unsuitable since methods for separating the products of photosynthesis required more time than the short half-life would allow. Nuclear physicists, including Ernest Lawrence, inventor of the cyclotron and Director of the Radiation Laboratory (which became the Lawrence Berkeley National Laboratory) predicted that the isotope ¹⁴C should exist. With some effort, the method for making it was discovered (Benson 2002; Ruben and Kamen 1940). Following the premature death of Ruben and the end of World War 2, Lawrence recruited Melvin Calvin to make use of the discovery of ¹⁴C. Calvin was more interested in oxidation-reduction reactions and so recruited Benson to return to Berkeley to be

Director of the Photosynthesis Laboratory within Calvin's Bio-Organic Laboratory which was part of the Rutherford Laboratory at the University of California. Andrew Benson, who had gotten his Bachelor's degree at Berkeley, returned from Cal Tech in 1942, where he had gotten his PhD in carbohydrate chemistry and confirmed some of the findings of Ruben and Kamen using ^{11}C . Benson had a teaching appointment at Berkeley that ended in 1943, whereupon he left Berkeley and entered the Civilian Public Service. Benson was a conscientious objector to war, and had met frequently with the photosynthesis expert Robert Emerson while at Cal Tech for advice concerning this status. Of the work with ^{11}C , Benson concluded that "the many photosynthesis experiments performed by Ruben and Kamen (Ruben et al. 1939) during four years of hectic effort yielded no real information on the path of carbon in photosynthesis" despite also noting the importance of the work in moving the field away from the concept of direct interaction of CO_2 with chlorophyll. Thus, Benson's involvement with photosynthesis, interacting with Robert Emerson at Cal Tech prior to 1942 and working with ^{11}C and Ruben and Kamen at Berkeley 1942-1943, significantly predated Calvin's involvement.

In the first report of results with ^{14}C , Benson and Calvin (1947) reported that they had held algae in the light while bubbling with N_2 to keep out CO_2 . They then turned off the light and quickly added $^{14}\text{CO}_2$ and incubated for 5 min. They found a small amount of label in sugars providing strong evidence that it was possible for sugars to be made after the light was turned off. This paper was important because it demonstrated "that the reduction of CO_2 to sugars and the intermediates in that reduction does not involve the primary photochemical step itself" and that photosynthetic carbon metabolism "cannot be a simple reversal of the respiratory system of reactions." The CO_2 fixation that occurs after turning the light off was ascribed to the presence of reducing power but is now understood to result from an accumulation of RuBP when photosynthesizing material is deprived of light (Eichelmann and Laisk 1999). In fact the accumulation of RuBP in low CO_2 was an important clue about its role in carboxylation (Buchanan and Wong 2013; Bassham et al. 1954 (Fig. 6)).

Further experiments exploring the relationship between photosynthesis and respiration were described in Benson and Calvin (1950). In that paper they reported that no isocitrate or glutarate became labeled in the light although label quickly appeared in these compounds in the dark. The occurrence of respiration in the light is still debated (Heskel and Tang 2018; Tcherkez et al. 2017) but the lack of label in some of the citric acid cycle metabolites has been confirmed frequently (Gauthier et al. 2010; Szecowka et al. 2013). Benson and Calvin (1950) also reported the oxygen-dependent labeling of phosphoglycolate and glycine, which, 20 years later, would be found to result from the oxygenation of RuBP (Bowes et al. 1971) and to be the first step in photorespiration.

The 1947 paper (Benson and Calvin 1947) proposed three components of photosynthetic carbon metabolism; 1. that the central cycle had four reduction steps, 2. there were two carboxylations, and 3. it included several reactions from the tricarboxylic acid cycle. Many cycles were proposed before the correct one and most involved reactions of the tricarboxylic acid cycle, sometimes explicitly invoking citrate (Benson et al. 1952b) even as other publications the same year were showing that no label appears in citrate, ruling out a role for the tricarboxylic acid cycle (Calvin and Massini 1952). Many years later it would be shown that some bacteria use a reverse tricarboxylic acid cycle (Buchanan and Arnon 1990) but this is not the path of carbon in photosynthesis in plants and algae. With benefit of hindsight it is clear that the concepts embodied in the various early cycles were not progressing toward discovery of the canonical cycle.

It was a fundamental change to drawing on glycolysis (more specifically gluconeogenesis) and carbohydrate chemistry, away from any tricarboxylic acid cycle reactions that distinguishes the canonical cycle published in paper XXI (Bassham et al. 1954) from the early cycles. The Calvin-Benson cycle is normally thought of as mostly the reversal of the non-oxidative pentose phosphate pathway but I believe it was acceptance of the gluconeogenic nature of the energy reactions that allowed the intellectual leap that occurred between paper XX and XXI. Of the 11 enzymes in the Calvin-Benson cycle, five are

gluconeogenic (3-phosphoglycerate 3-PGA) kinase, glyceraldehyde 3-phosphate (GAP) dehydrogenase, triose phosphate isomerase, fructose biphosphate (FBP) aldolase, and fructose-1,6-bisphosphatase), three are from the non-oxidative pentose phosphate pathway, (transketolase, pentose phosphate epimerase, and pentose phosphate isomerase), and three are unique (or nearly so) to photosynthesis (sedoheptulose-1,7-bisphosphatase, phosphoribulokinase, and rubisco). I will focus on the gluconeogenic components of the Calvin-Benson cycle, as shown in Fig. 1 [redrawn from an unnumbered scheme in Bassham et al. (1954)], to better understand the essence of how CO₂ is converted to carbohydrate. This scheme shows that the reducing power is supplied by oxidation of water and I have added shading to show the significance of gluconeogenic reactions. The importance of ribulose 1,5-bisphosphate (RuBP) (called RDP in the original scheme) was also a critical step in elucidation of the cycle and notably this was published by Benson (1951), independent of Calvin. Calvin had been away from the lab for an extended period while Benson worked out the identity of RuBP (Buchanan and Wong 2013) but even though Calvin did not put his name on the paper he did change the title to remove “ribulose diphosphate”, eliminating the most important thing about the report from the title.

3-Phosphoglyceric acid

Initially it was thought that 2-PGA was the first product (Calvin 1949) and it is not clear when this idea was abandoned. However, 3-phosphoglyceric acid was identified as the compound mostly heavily labeled when algae were provided with ¹⁴CO₂ for very short periods (Benson et al. 1950). The primacy of PGA was not universally accepted, but in time even strong skeptics conceded that PGA is the first product (Fager and Rosenberg 1950) [see discussion on page 37 of Benson (2002)]. Malate is also strongly labeled but Bassham et al. (1954) ruled out a role for malate based on the intermolecular labeling of sedoheptulose bisphosphate. At nearly the same time a role for malate in photosynthesis of sugar cane was being discovered, especially in the work from Constance Hartt in 1943 and 44 [cited in Benson (2002)]. She had done experiments with inhibitors of glycolysis and found a role for GAP dehydrogenase, ten years before this reaction would come to be seen as the central mechanism of reduction in photosynthetic carbon metabolism. Malate (and aspartate) plays an essential role in C₄ photosynthesis but in the algae and C₃ plants used by Benson and Calvin, malate is not part of the path of carbon towards sugars.

It is now well accepted that two molecules of 3-PGA are produced by the redox-neutral carboxylation of RuBP. The very strong anionic character of PGA (negative charges on both the phosphate and carboxylic acid) made it hard to elute from the chromatography columns that were being used. This, and the need to separate many compounds that get labeled during photosynthetic carbon metabolism led to the use of paper chromatography to separate the labeled compounds (Benson et al. 1950). Benson devised a new elution mixture consisting of butanol/propionic acid/water that proved an effective second dimension to the phenol/water solution used in the first direction. The paper used was very large (43 by 61 cm, 17 by 24 inches) and hung from trays containing the eluent (Benson et al. 1950). Textbooks usually show ascending chromatography but this is incorrect, it was, necessarily, descending.

Sedoheptulose and ribulose

Armed with paper chromatography and ¹⁴CO₂, Benson first worked to determine the identity of the major products labeled during photosynthesis. A major discovery was that sedoheptulose monophosphate was labeled before significant label was found in hexoses (Benson et al. 1952a). Free sedoheptose (the aldose, sedoheptulose is the corresponding ketose) was known to accumulate in some succulents including sedum, from which it gets its name (La Forge and Hudson 1917). However, free sedoheptose or sedoheptulose was not found in the material being used to study the path of carbon. Sedoheptulose phosphate was found in all plant and algal material that was studied (Benson et al. 1951). Horecker and Smyrniotis (1952) followed up by checking for sedoheptulose monophosphate in yeast and incorporated this molecule into the models they were developing of the conversion of pentoses to hexoses in what

would come to be called the pentose phosphate pathway. Benson's discovery of the role of sedoheptulose phosphate in photosynthesis played an important role in leading to the discovery of the non-oxidative pentose phosphate pathway (Horecker and Smyrniotis 1952).

Also critical to working out the photosynthetic carbon metabolism pathway was the discovery of ribulose biphosphate (at the time called diphosphate, but bis indicates the phosphates are on different carbon atoms, not linked together as in ADP) (Benson 1951). Benson used ^{32}P and ^{14}C to show that there were two phosphates on the ribulose backbone. Given that 3-PGA was the first product there was a search for the "two carbon acceptor". Instead, it turned out to be a five-carbon acceptor, RuBP, that Calvin hypothesized first results in a six-carbon intermediate (2-carboxy-3-ketopentitol 1,5-bisphosphate) that is quickly converted to two 3-PGA molecules. The existence of this intermediate was demonstrated 30 years later (Schloss and Lorimer 1982). Since two molecules of PGA are made [most of the time (Andrews and Kane 1990)] the enzyme is a dismutase so Calvin named it carboxydismutase. Benson also noted that ribulose biphosphate would spontaneously oxidize to glycolate but it would be nearly 20 years before this reaction could be catalyzed and is the initial reaction of photorespiration (Bowes et al. 1971).

The 1954 paper

A comparison of paper XX (in 1952) and XXI (submitted in 1953) shows how quickly the canonical pathway was conceived. While the Calvin-Benson cycle is typically considered to be inspired by the pentose phosphate pathway, it was the role of gluconeogenic reactions that provided the framework for understanding the path of carbon in photosynthesis. Reactions from PGA to fructose 6-phosphate (F6P), gluconeogenic reactions, include the reactions in which carbon reduction occurs (and 2/3 of the ATP is used). The simplified scheme in Fig. 1, redrawn from the 1954 paper, shows the essential concepts: 1. RuBP is the "C2 donor" that is carboxylated, 2. reduction occurs by a reversal of the oxidative step in glycolysis, a carboxylic acid is reduced to an aldehyde resulting in a sugar (glyceraldehyde 3-phosphate), 3. 5/6 of the resulting sugar must be recycled to maintain the reaction, the first step of which is catalyzed by the glycolytic enzyme aldolase. Once the "two-carbon" acceptor (the top two carbons of RuBP) was identified and the gluconeogenic nature of the primary processes were identified, it was only left to work out the sugar rearrangements, the compounds included in "B" in Fig. 1 ("A" is fructose 6-phosphate). The rearrangements are similar to the pentose phosphate pathway that was being worked out simultaneously (e.g. (Horecker and Smyrniotis 1953b, a, 1952) but in my opinion insights from Benson were more helpful in elucidating the pentose phosphate pathway than vice versa. In any case the 1954 paper was submitted a few days before the paper describing the non-oxidative branch of the pentose phosphate pathway (Horecker et al. 1954). The 1954 paper, "The Path of Carbon in Photosynthesis. XXI", provides the single biggest step forward in elucidating what we now call the Calvin-Benson cycle.

The cycle

The cycle proposed in the 1954 paper (Fig. 2) is still widely accepted as correct. In a break with previous depictions of the path of carbon, this depiction employs multiple curved lines that necessarily cross one another. This can make it harder to follow. In Fig. 3 (supplemental Fig. S1 is a slightly simplified version in Powerpoint that can be modified for teaching) the cycle is shown as two linear branches. In the top branch three pentose phosphates are converted to six triose phosphates with the consumption of three CO_2 , nine ATP and six NADPH. These reactions account for the carboxylations and energy usage of the Calvin-Benson cycle. The bottom branch of the cycle converts five out of six triose phosphates (GAP + dihydroxyacetone phosphate (DHAP)) to three pentose phosphates getting back to the beginning of the top branch. The sixth triose phosphate is the net production of sugar by the Calvin-Benson cycle. The reactions in the top of Fig. 3 occur three times to produce one triose phosphate that can leave the cycle while the bottom reaction scheme happens just once. This makes stoichiometry difficult for students learning the Calvin-Benson cycle for the first time. What is meant by one turn of the Calvin-Benson cycle? One CO_2 fixed or one occurrence of FBPase action? All reactions of the tricarboxylic acid cycle

proceed with the same stoichiometry so it is easy to follow and to describe stoichiometries, not so in the Calvin-Benson cycle.

Some of the enzymes involved in the reactions in the top half of Fig. 3 were known, for example the gluconeogenic reaction enzymes GAP dehydrogenase and PGA kinase. Rubisco was unknown as was phosphoribulokinase. Pentose phosphate isomerase had been described by Horecker et al. (1951).

The bottom branch comprises two sequences of aldolase → bisphosphatase → transketolase. The aldolase reaction is common in organic chemistry and used to make carbon-carbon bonds. Two carbonyls react to make a molecule with a carbonyl and a hydroxyl at the β carbon, an aldol (Fig. 4). In photosynthesis the two aldolase reactions add a keto-sugar to an aldo-sugar (See ketose and aldose structures in Supplemental Fig. S2).

The fructose bisphosphatase reaction was well-known from gluconeogenesis, it was only later that it became clear that the sedoheptulose bisphosphatase activity is a separate enzyme in plants, though not in some bacteria (Martin et al. 2000). Calvin, Benson, and Bassham had decided to propose the reaction catalyzed by transketolase and were reassured when Racker et al. (1953) published evidence for an enzyme that carried out this reaction (and gave it the name transketolase).

Position-specific labeling

The 1954 paper has data on the labeling of specific carbons. Fig. 5 shows the scheme by which carbon 3 of ribulose-5 phosphate is heavily labeled, carbons 1 and 2 have some label while carbons 4 and 5 have very little label. It appears that it was Calvin that had the insight that RuBP had three different sources (two molecules of Xu5P and one of R5P) leading to a very specific labeling pattern.

Observing the pattern in Fig. 5 and Table 1 requires labeling for very short periods. The label in carbons 1-2 and 4-5 get mixed when RuBP is converted to two molecules of PGA so label will equilibrate between these positions very quickly. Labeling for periods as short as 0.4 s were reported by Bassham et al. (1954). Textbooks and reminiscences of both Benson (Buchanan and Wong 2013) and Calvin (1964) describe a “lollipop” apparatus for fast labeling. This was a flattened circular glass container that with a stopcock at the bottom that was opened to allow the contents to drain into hot methanol to stop the reaction. I have experimented with a replica built by N. Edward Tolbert, who had been a post-doc with Calvin. The lollipop holds about 150 ml of algal culture. It takes between 5 and 10 seconds to drain under optimal conditions. It would take some time to get the CO₂ uniformly distributed, potentially giving rise to artifacts resulting from large changes in CO₂ availability. To overcome these problems Bassham et al. (1954) did not use the lollipop but instead used a quenched flow system (Fig. 6) developed by Bassham (Bassham 2003) that allowed them to introduce ¹⁴CO₂ while changing the overall CO₂ concentration by less than 15% and allowed for exposure times as short as 0.4 s. Over the years many different labeling devices were reported, some involving gas pressure to speed movement of liquids and some involving injecting the quenching solution directly into the solution of photosynthesizing algae (Calvin 1949). In the 1954 paper a system for exposing plant leaves for as short as 0.4 s is also described. The most amusing depiction of one of the labeling systems is in Wilson and Calvin (1955). There, very clearly, is a someone fishing in the algal pond (Fig. 7, see also prospect.rsc.org/blogs/cw/2011/07/15/8170/).

The carboxylation step

The 1954 paper assumed that RuBP was carboxylated by an enzyme Calvin called carboxydismutase. Calvin postulated that CO₂ would be added to the 2,3-enediol of RuBP, which turned out to be correct. However, no experimental evidence was known to support the proposal that photosynthetic organisms could carboxylate RuBP. Soon after the 1954 paper there were several reports of activity of leaf and algal extracts that could carboxylate RuBP (Quayle et al. 1954; Jakoby et al. 1956; Weissbach et al. 1956). However, in 1952 (Calvin and Massini 1952), and as late as 1964 (Calvin 1964), Calvin thought it

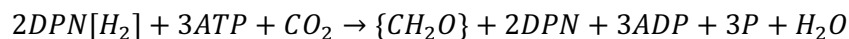
possible that there could be a reductive carboxylation in the light resulting in one PGA and one triose phosphate molecule. He hypothesized that this would require a cyclic disulfide compound similar to lipoic acid (also called thioctic acid) important in the oxidative decarboxylation of pyruvic acid. Calvin discussed his thioctic acid theory in the 1954 paper.

The enzyme that catalyzes the carboxylation of RuBP, known variously as carboxydismutase, ribulose-1,5-bisphosphate carboxylase/oxygenase, or rubisco¹), turns out to be the most abundant enzyme in the leaf, and some say most abundant enzyme on Earth. Benson frequently visited Sam Wildman at Cal Tech. Wildman had isolated a major protein from leaves he called Fraction 1 Protein. Benson and Jaques Mayaudon, using RuBP synthesized by Benson, discovered that Fraction 1 Protein was rubisco. Benson's work showing that Fraction 1 protein was rubisco was eventually published as Mayaudon (1957).

Stoichiometry and energetics

The reducing power needed for the reduction reaction of the cycle was presumed to come from NAD (called DPN, diphosphopyridine nucleotide, at that time) by analogy with gluconeogenesis. It is not clear, and the papers were not cited, that Bassham et al. were aware of earlier work showing that chloroplasts could reduce NADP⁺ (TPN at that time) (Vishniac and Ochoa 1951; Tolmach 1951; Arnon 1951). It is now well established that NADPH is the major source of reductant although GAP dehydrogenase in the Calvin-Benson cycle can use either NADH or NADPH (McGowan and Gibbs 1974).

The overall stoichiometry was given in equation A' of the 1954 paper (page 1769)



or in today's terminology



Given this stoichiometry, Bassham et al. (1954) calculated that the overall energy efficiency of the Calvin-Benson cycle would be 0.88. Calvin was excellent in working out thermodynamics and it is likely that the energetics reported in the 1954 paper were worked out by Calvin. Other papers by Calvin (Bartrop et al. 1954) indicate that he was working to explain the primary photochemistry while others in his group (primarily Benson) were given the less exciting task of discovering the path of carbon. Calvin was consumed with a much bigger theory.

The thioctic acid theory

The thioctic acid theory was an attempt to explain how reducing power generated by chlorophyll absorption of light was transferred to the carbon compounds of the Calvin-Benson cycle and resulted in

¹ The name rubisco was coined by David Eisenberg in 1979 as a joke at Sam Wildman's retirement party (Wildman 2002). The name emphasized the recent change in nomenclature from diphosphate to bisphosphate and the recent discovery that the enzyme is also an oxygenase (Bowes et al. 1971). In Sam's next paper he used the acronym rubisco, but soon after many variations in capitalization appeared. Style manuals provide clear guidelines for capitalization in this case. If the abbreviation is pronounced as a word (making it an acronym), and if it is so well known that people recognize the acronym more easily than the words it stands for, it is not capitalized (examples - laser and scuba). Wildman did not capitalize rubisco in his first publication using it (Wildman 1981). If an acronym is not quite so universally known but is more than four letters, the first letter (only) is capitalized (Hepes and Unesco). I have found no style manuals that would accept RuBisCO, RubisCO or any of the other capitalization schemes that are used. Because rubisco is so central to photosynthesis and better recognized than the words it stands for I believe it should be all lower case like "laser", which is more easily understood than light amplification by stimulated emission of radiation. The use of Rubisco is tolerable, but other capitalization schemes should be abandoned. I will use rubisco here.

oxygen evolution (Barltrop et al. 1954; Calvin and Barltrop 1952). The theory turned out to be wrong and should simply fade away like so many good ideas that do not hold up. However, a discussion of the thioctic acid theory gives insight into the working relationship between Calvin and Benson. While Benson, Bassham, and others in the laboratory were carrying out the work that would lead to elucidation of the cycle, and later Benson and Mayaudon were discovering that Fraction 1 protein was rubisco, Calvin was hard at work developing the thioctic acid theory that would connect light absorption with oxygen evolution and carbon metabolism. A photochemical reaction would reduce one of the sulfhydryls of 6-thioctic acid while the other would become oxidized. The reduced sulfur would reduce NADP⁺ to NADPH while the oxidized sulfur would lead to oxygen. This work fit Calvin's interests in redox reactions (Calvin 1989), while Benson's background left him ideally suited to the carbohydrate work that led to understanding the cycle. Had the thioctic acid theory been true it would have been an even bigger discovery than the Calvin-Benson cycle. The 1954 paper that was subtitled "The cyclic regeneration of carbon dioxide acceptor" was used by Calvin to put forward his thioctic acid theory. It didn't really fit in the paper and none of the data in the paper addressed the issue, but it was an exciting theory from the head of the lab and so in it went. Alas, the very promising idea did not pan out so that the very long 1954 paper had both the highs (Calvin-Benson cycle) and lows (Calvin's thioctic acid theory) from the lab. In the meantime, Benson was pursuing the next big question – how the carboxylation step occurs. Benson synthesized large quantities of RuBP for assays and consulted with Sam Wildman, who had been working on Fraction 1 protein, the most abundant protein in leaves.

Benson admits that he had stopped reporting his activities to Calvin, the head of the lab. Calvin apparently decided that the breakdown in communication could not be tolerated and fired Benson in December, 1954 (Benson 2010; Buchanan and Wong 2013). Some of the work that Benson was involved in at the time on the carboxylase and its relationship to Fraction 1 protein that had recently been described by Sam Wildman (Wildman 2002), was eventually published (Mayaudon 1957) but without Benson's name and possibly without the only listed author (Jacques Mayaudon) knowing that the manuscript had been submitted for publication (Benson 2010). In Calvin's Nobel acceptance speech (Calvin 1964) Benson was mentioned just once; he was identified as just one of many students and collaborators that assisted Calvin. Only two papers with Benson are cited. The record clearly shows a number of critical discoveries that are primarily the work of Benson. These include adaptation of paper chromatography for discovery of compounds in photosynthetic carbon metabolism (Benson et al. 1950), discovery of sedoheptulose (Benson et al. 1951), discovery of RuBP (Benson 1951), and discovery that Fraction 1 Protein is rubisco (Mayaudon 1957). None of the papers describing these contributions were cited in Calvin's Nobel Prize address.

Given the relative inputs into elucidating the pathway, many people expand the common Calvin cycle name to Calvin-Benson cycle or Calvin-Benson-Bassham cycle. Bassham worked well with both Calvin and Benson. Bassham was responsible for the quenched flow system of the 1954 paper and he was credited with switching the quenching solution to boiling methanol, which improved results and he carried out many of the chemical reactions that Benson oversaw that proved the existence of sedoheptulose 7-phosphate. He also continued studies on the energetics of photosynthesis reactions (Bassham and Krause 1969). Bassham himself preferred that the cycle be given a title that was descriptive instead of based on who should get credit, he used photosynthetic carbon reduction cycle, which I also favor but that argument seems to have been lost. I use Calvin-Benson cycle here and apologize to one author who, in my role as editor, I once made change a manuscript from using Calvin-Benson cycle to the simpler Calvin cycle.

Historical concerns

As with most great discoveries, many observations were explained but some observations did not fit perfectly.

Equilibrium of triose phosphate isomerase

The cycle as proposed (Fig. 2) should result in equal label in carbons 3, 4 and 5 of sedoheptulose but in very short labeling times (0.4 s) carbon 4 had significantly less label than 3 or 5 (Bassham et al. 1954). Carbon 4 comes from DHAP. Triose phosphate isomerase converts labeled GAP to DHAP. The equilibrium is such that there should be ~20 times more DHAP than GAP (Sharkey and Weise 2012; Meyerhof and Junowicz-Kocholaty 1943). Bassham et al. (1954) hypothesized that triose phosphate isomerase was, in fact, not in equilibrium, leading to a slower incorporation of label into DHAP than into GAP and accounting for the reduced labeling in carbon 4 of sedoheptulose. Because GAP is normally in much lower concentration than DHAP, it is often not measured and equilibrium is assumed (Bassham and Krause 1969). However, my lab recently measured DHAP and GAP enzymatically and found just 11 times more DHAP than GAP in whole leaf extracts (Li et al. 2018). This would have a significant advantage since the free energy change of the aldolase reaction will depend on the product of the concentration of GAP and DHAP.

$$\Delta G = \Delta G'^o + R \cdot T \cdot \ln \frac{[FBP]}{[GAP] \cdot [DHAP]}$$

Values can be taken from Bassham and Krause (1969): $\Delta G'^o = -21.9$ kJ/mol, $[FBP] = 0.097$ mM, total triose phosphate concentration = 0.672 mM. The effect of the ratio of GAP to DHAP is shown in Fig. 8A. It shows that when the triose phosphates are near equilibrium ($[DHAP]/[GAP]=21$) the ΔG for the formation of FBP is -0.89 kJ/mol but when the ratio is 10, the ΔG is -2.49 kJ/mol. Thus, there is much more energy available to drive the aldolase reaction if TPI is not in equilibrium (Fig. 8). The higher concentration of GAP in non-equilibrium conditions will also help in formation of SBP for the same reason.

A simple time-step model (Fig. 8B) shows the rate of change of the specific activity of GAP and DHAP when the TPI activity is sufficient to cause the ratio to be 20 (close to equilibrium, solid lines) or 10 (close to the measured value, dashed lines). The difference results not only from the faster conversion of GAP to DHAP, increasing the rate of label appearance in DHAP (solid green line above the dashed green line) but also dilution of label in GAP by unlabeled DHAP being converted to GAP (solid blue line less than dashed blue line).

Transaldolase versus aldolase/SBPase

The original cycle did not include transaldolase even though the pentose phosphate pathway published at almost the same time did (Horecker et al. 1954). Transaldolase, working in the direction needed for photosynthesis, would transfer the top three carbons from F6P to E4P to make S7P and GAP. This reaction involves aldol (carbonyl with a β -hydroxyl) chemistry, in effect transferring an aldol from one molecule (F6P) to a second molecule (E4P to become S7P). This is why Horecker and Smyrniotis (1953b) named it transaldolase. Transketolase could then transfer two carbons from S7P to the GAP that resulted from transaldolase action on F6P to make Xu5P and R5P. The cycle proposed in the 1954 paper did not follow phosphate status of the intermediates but the scheme never followed that path that would occur if transaldolase were involved. Gibbs (1966) pointed out that no evidence had been presented to prove that transaldolase does not have a role in photosynthesis. With transaldolase it would be possible to do without SBPase. However, SBPase has been shown to be a critical enzyme for photosynthesis (Raines et al. 1999; Harrison et al. 1998) and in fact is the target of efforts to improve photosynthetic carbon metabolism (Driever et al. 2017; Rosenthal et al. 2011; Lefebvre et al. 2005). Nevertheless, transaldolase is found in photosynthetic tissue (Caillau and Quick 2005) and could play a role, perhaps in tandem with

aldolase/SBPase, in converting triose phosphates to pentose phosphates. Perhaps the effects of manipulating the expression of SBPase (Driever et al. 2017; Rosenthal et al. 2011; Lawson et al. 2006; Harrison et al. 2001; Ölçer et al. 2001) result from changing a balance between SBPase and transaldolase fluxes.

Gibbs effects and flexibility of aldolase and transketolase

Kandler and Gibbs (1956) developed methods for looking at the label in each carbon of glucose. Previously, fragments of glucose could be analyzed and specific labeling patterns had to be inferred. If everything worked as described in the 1954 paper, Gibbs said that glucose should be labeled most heavily and the same in carbons 3 and 4. Carbons 1, 2, 5, and 6 should have less than 3 and 4 but the same as each other. However, they found that carbon 4 had significantly more label than 3 while carbons 5 and 6 had significantly less than 1 and 2. Carbon 4 comes from GAP while carbon 3 comes from DHAP. The lack of TPI equilibrium (Fig. 8B) can easily explain the greater labeling in carbon 4 than carbon 3.

Ebenhöh and Spelberg (2018) have provided an explanation for the greater labeling in carbons 1 and 2 than 5 and 6. They point out that transketolase can catalyze reactions that are not part of the canonical Calvin-Benson cycle. Many keto sugars can provide the two-carbon fragment that gets transferred and many aldo sugars can receive the two-carbon fragment. The two-carbon fragment can be donated to G6P to yield octulose 1,8-bisphosphate. Aldolase may also be flexible. Aldolase could cleave octulose 1,8-bisphosphate into DHAP and arabinose 5-phosphate. Based on similar flexibility of these enzymes in animals (Horecker et al. 1982), Flanigan et al. (2006) proposed that the canonical Calvin-Benson cycle is not complete. Evidence for the occurrence of octulose bisphosphate in photosynthesizing leaves has been published (Flanigan et al. 2006) but it appears that only small amounts of carbon follow this path and it does not appear to change photosynthetic behavior or responses. It is likely there are other minor carbon reaction pathways that occur during photosynthesis but there is no evidence that any of these change our understanding of the primary path of carbon in the Calvin-Benson cycle.

While Ebenhöh and Spelberg (2018) do an in depth analysis modeling the various reactions that can occur, a simplified explanation is shown in Fig. 9 that can account for the unequal labeling (Gibbs and Kandler 1957; Kandler and Gibbs 1956). Starting with the labeled Ru5P from Fig. 4, this can be converted to Xu5P which can then be acted on by transketolase to transfer the top two carbons to E4P making F6P, which can isomerize to G6P. Any carbon that follows this path would have label in carbons 1 and 2 but not 5 or 6. This glucose would also add to the excess of label in carbon 4 compared to 3. The more complete analysis of Ebenhöh and Spelberg (2018) gives similar results.

Incomplete labeling of cycle intermediates and a glucose-6-phosphate shunt

Although the label distribution within molecules is consistent with the canonical Calvin-Benson cycle, there is a problem with the absolute amount of label. Mahon et al. (1974) showed that the specific activity of the intermediates (especially PGA) labeled quickly up to about 80% but that the last 10 to 20% labeled much more slowly. They concluded that “The low specific activity of 3-PGA at 1% O₂ is not completely understood.” Work from my laboratory found that isoprene, which is made directly from Calvin-Benson cycle products, showed the same rapid labeling up to 80 to 90% but then much slower labeling of the last 10 to 20% (Delwiche and Sharkey 1993) and this slow labeling pool in isoprenoid synthesis has been seen by many other labs (Schnitzler et al. 2004; Karl et al. 2002; Loreto et al. 1996). Szecowka et al. (2013) also saw that Calvin-Benson cycle intermediates show a fast and slow labeling pool, with the slow labeling pool in the range of 15% of the total.

One candidate for the slow labeling pool is maltodextrins made during starch synthesis. Starch is very regularly branched but this regular branching occurs because of trimming of maltodextrins. When starch is being synthesized a “preamylopectin” is made first that has a higher degree of branching than amylopectin (Mouille et al. 1996; Ball et al. 1996). Isoamylase 1 is required to cleave the extra branches

resulting in regularly branched, crystalline starch (Streb et al. 2008). The branches that are removed may be maltodextrins with a degree of polymerization around 4 to 8 (my guess). These branches can be broken down by starch phosphorylase inside the chloroplast to recover the carbon as glucose 1-phosphate which then can feed the G6P pool. In other words, there is an overcycling of carbon during starch synthesis. In an analysis presented later I assume about one third of the carbon initially made into starch is recycled back to G1P and G6P.

If these maltodextrins are the slow labeling pool, then it becomes important how they get back into the Calvin-Benson cycle. In the stroma, G6P and F6P are not in equilibrium (Gerhardt et al. 1987; Sharkey and Vassey 1989; Szecowka et al. 2013) indicating a kinetic limitation. What is more, the enzyme has a much higher K_m for G6P than F6P. This would preclude a significant flux of carbon from G6P back into the Calvin-Benson cycle. I suggest that the slow labeling pool reenters the Calvin-Benson cycle by an additional path of carbon in photosynthesis, the G6P shunt (Sharkey and Weise 2016) (and see below).

Additional paths of carbon in photosynthesis

The canonical Calvin-Benson cycle accounts for the bulk of the carbon flow associated with photosynthetic conversion of CO₂ to carbohydrate. There is other metabolism that is associated with this process, notably carbon concentrating mechanisms such as C₄ and CAM. In addition, there are additional paths of carbon closely connected to the reactions of the Calvin-Benson cycle. These are difficult to measure given the large fluxes through the canonical pathway but several pathways can be described.

A glucose-6-phosphate shunt

Glucose-6-phosphate dehydrogenase (G6PDH) could supply carbon from the G6P pool through the oxidative branch of the pentose phosphate pathway. It has long been known that G6PDH activity is reduced during the day (Anderson et al. 1974; Lenzian 1980; Johnson 1972) but generally the inhibition is about 50% and significant activity of G6PDH can be found in crude extracts of leaves in the light (A.L. Preiser, unpublished). If G6PDH is active in photosynthesizing tissue it catalyzes the first step in a G6P shunt (Sharkey and Weise 2016) (Fig. 10). The G6P shunt releases one CO₂ for every CO₂ fixed, it is balanced for NADPH (two consumed, two produced) but results in a net consumption of 3 ATP. Injection of carbon into the Calvin-Benson cycle as hexose phosphate can be problematic if there are no triose phosphates available for the SBP aldolase reaction and the transketolase reactions. However, injection of carbon as Ru5P would get around this problem and quickly fill the Calvin-Benson cycle with intermediates.

The G6P shunt would feed carbon from the G6P pool back into the Calvin-Benson cycle as Ru5P. This carbon would have the specific activity of G6P, which could have a lower specific activity than Calvin-Benson cycle intermediates if it were in equilibrium with a large pool of carbon in maltodextrins being released by isoamylase during preamylopectin maturation. This also requires that phosphoglucoisomerase be limiting. It has been found to be out of equilibrium (Dietz et al. 1985; Schleucher et al. 1999; Gerhardt et al. 1987; Schnarrenberger and Oeser 1974; Sharkey and Vassey 1989).

A time-step model assuming that maltodextrins are the slow-labeling pool can fit data from Canvin (1979) (Fig. 11). At this time starch metabolism is my best guess for the slow-labeling pool of carbon that causes the Calvin-Benson cycle to label quickly to 80% but then label much more slowly for the last 20%.

Respiration during photosynthesis

It was clear from the early work that the photosynthetic organisms Calvin and Benson were studying did not use a reversal of the citric acid cycle; “while the light is on there is practically no glutamic, citric, and/or isocitric acids formed” (Calvin 1949) [see also Tcherkez et al. (2005)]. There are organisms that use a reverse citric acid cycle for photoautotrophic carbon fixation (Buchanan and Arnon 1990; Evans et al.

1966) but the algae and plants used by Benson and Calvin do not have a functional citric acid cycle, in either direction, in the light. But when carbon dioxide exchange is modeled, a “day” respiration term is always needed (Farquhar et al. 1980). This parameter, called R_d but also R_L for respiration in the light, has been difficult to measure, at least in part because measuring CO_2 release while much larger amounts of CO_2 are being fixed by rubisco (Gong et al. 2018; Tcherkez et al. 2017; Tcherkez et al. 2005). Although variable, typical rates are about 5% of the rate of net CO_2 uptake. Gong et al. (2018) report that an isotope dilution method for measuring R_d under physiological conditions gives values for R_d between 0.7 and 2.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The citric acid cycle could account for just 0.005 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of R_d and pyruvate dehydrogenase (for lipid synthesis) 0.058 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Tcherkez et al. 2005). However, the G6P shunt, operating at 10% of the rate of carboxylation, would release 1 to 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (depending on how much was refixed before getting to the atmosphere). This carbon would be relatively unlabeled, as is found for R_d . Therefore, I put forward the hypothesis that R_d is primarily CO_2 released in the G6P shunt.

A cytosolic bypass of the gluconeogenic reactions

The reactions from PGA to triosephosphate to fructose bisphosphate are considered to be in equilibrium. Once FBPase is dephosphorylated the carbon is committed. Inside the chloroplast the commitment is to RuBP regeneration and starch synthesis while in the cytosol the commitment is to (primarily) sucrose synthesis (Stitt et al. 1987) as long as hexose phosphates are not exchanged across the chloroplast membranes. Plants have two glucose-6-phosphate/phosphate antiporters (GPT) but these are generally not expressed in photosynthesizing leaves (Kammerer et al. 1998). Confirming the expression data, it has been shown that there can be a very large gradient of G6P between the chloroplast (low) and cytosol (high) (Sharkey and Vassey 1989; Gerhardt et al. 1987; Szecowka et al. 2013). Kunz et al. (2010) showed little or no transport activity in liposomes made from lipids from wild type *Arabidopsis* leaves. However, CAM plants can export G6P when breaking down starch (Neuhaus and Schulte 1996) and plants growing in high CO_2 (Leakey et al. 2009) or transferred to high light (Li et al. 2018) express GPT2. Plants lacking starch synthesizing enzymes phosphoglucosomerase, phosphoglucomutase, or ADPglucose pyrophosphorylase gain the ability to import G6P into chloroplasts (Kunz et al. 2010). If G6P from the cytosol can reenter the Calvin-Benson cycle then the gluconeogenic reactions from GAP to F6P could be bypassed by using cytosolic enzymes. This would be especially beneficial for starch synthesis. Plants lacking phosphoglucosomerase but constitutively expressing a glucose-6-phosphate transporter accumulate high levels of starch (Niewiadomski et al. 2005) proving that the chloroplast gluconeogenic reactions can be bypassed by carbon that is exported as triose phosphate, converted to G6P, and then reimported into the chloroplast.

Normally, starch synthesis is thought to be regulated the activity of ADPglucose pyrophosphorylase (Preiss et al. 1967) but, at least in some circumstances, phosphoglucosomerase can limit starch synthesis (Dietz et al. 1985; Sharkey and Vassey 1989). Overexpression of PGM can increase starch synthesis (Uematsu et al. 2012) supporting the idea that supply of G6P from the Calvin-Benson cycle by PGI can limit the rate of starch synthesis. If GPT2 is present, gluconeogenic reactions in the cytosol could contribute G6P for starch synthesis (Fig. 12). This G6P does not easily enter the Calvin-Benson cycle because the K_m of phosphoglucosomerase for G6P is very high relative to the K_m for F6P (Schnarrenberger and Oeser 1974)(AL Preiser, unpublished), making this phosphoglucosomerase similar to a diode in electronics that allows current to flow in one direction but not the other.

Calvin-Benson cycle enzymes and cyclic electron flow.

The Calvin-Benson cycle is connected to photosynthetic electron transport in a number of ways including the supply of NADPH and ATP, Changes in the pH of the stroma, changes in the concentration of Mg^{2+} and reduction of dithiols on some enzymes causing them to be activated (Buchanan 2016a). Recently, another connection between electron transport and the Calvin-Benson cycle has been identified. It has been reported that loss of several enzymes leads to increased cyclic electron flow (Strand et al. 2015;

Livingston et al. 2010; Gotoh et al. 2010; Li et al. 2018). To explain this behavior, Li et al. (2018) hypothesize that the affected gluconeogenic enzymes are bypassed by exporting carbon through the TPT and following the path of the cytosolic bypass shown in Fig. 12. However, PGI is kinetically limited, especially in the direction G6P to F6P, needed to bring carbon back into the cycle. In this case, G6P in the stroma builds up, and this causes an increase in activity of G6PDH, increasing the rate of carbon going through the G6P shunt shown in Fig. 10. The simultaneous operation of the cytosolic glycolytic bypass and the G6P shunt is shown in Fig. 13. The increased rate of the G6P shunt would cause an ATP deficit that could be sensed and translated into higher rates of cyclic electron flow. One candidate for the sensing mechanism is the concentration of H₂O₂ since it stimulates cyclic electron flow (Strand et al. 2016; Strand et al. 2015).

Epilog

The discovery of the Calvin-Benson cycle has a human interest component. Calvin fired Benson in 1955, just after the cycle was deciphered and before the carboxydismutase activity was demonstrated in plant extracts. Calvin alone was awarded the Nobel Prize and made only the briefest mention of Benson's role in his Nobel address (Calvin 1964). Benson was the carbohydrate expert and his contributions were seminal. The beginning of a rift is evident in the Benson paper reporting the discovery of RuBP. While Calvin declined to put his name on the paper since the work was done in Calvin's absence, as head of the group he exercised editorial prerogative to change the title making it bland and not emphasizing the importance of RuBP. Benson said "Calvin didn't recognize that the ribulose-1,5-diphosphate made the whole cycle." I believe Benson was upset with this failure to recognize the importance of Benson's finding based on the interview in 2012 (Buchanan and Wong 2013) and that this led to a deepening rift between these two scientists.

Benson, on the other hand could be harsh in his judgements as well. For example, of Willstätter and Stoll he said their 1913 work was "classic" and "elegantly informative" but their 1918 work was "a forlorn volume of 448 pages of futile laboratory experiments" (Benson 2002). Of Sam Ruben, Benson opined that "Sam Ruben published about 30 papers...but... made no progress with respect to the absorption and conversion of carbon dioxide to carbohydrates" (Buchanan and Wong 2013) even though his work is credited with changing the thinking from formaldehyde as the first intermediate to thinking the first intermediate must be a carboxylic acid. In the same interview Benson observed that Calvin "didn't know very much about carbohydrate chemistry." Benson said that Al Bassham made the most important contributions of anyone in the lab to the cycle (presumably after Benson and Calvin) but then said of the great many papers Bassham wrote, damning with faint praise, that "they were good papers but not novel."

The rift between Benson and Calvin grew and Benson felt that Calvin was not "noticing my feverish efforts with a Belgian scientist, Jacques Mayaudon. Calvin seemed totally disinterested in my work. Thus, in the fall of 1954, I was not telling him what Jacques and I were doing" (Benson 2010). But Calvin was the head of the lab and really needed to know what Benson was doing. Perhaps Calvin did not need to fire Benson with no warning, but he did. Perhaps Calvin should have been more generous toward Benson in describing the discovery of the cycle in his Nobel acceptance speech (Calvin 1964), but he was not. In studying the papers from that time and reminiscences published by each of the major players a picture emerges of the rift between Calvin and Benson and the role each one played. While it appears that Benson was not blameless in this rift, regarding the (lack of) credit that Calvin gave to Benson in the decades following the discovery in 1954, it is safe to say that Calvin "didn't have to do that. He could have done it right" (Buchanan and Wong 2013).

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Table 1. Position specific labeling of pentose phosphates published by Bassham et al. (1954) is very good evidence for the cycle they proposed.

Carbon number	Relative radioactivity
1	11
2	10
3	69
4	5
5	3

Figure legends

Fig. 1. An unnumbered scheme in Bassham et al. (1954), redrawn from the original. Shading was added to emphasize the importance of gluconeogenic reactions in the Calvin-Benson cycle. The abbreviation RDP in the original was changed to RuBP – ribulose 1,5-bisphosphate. PGA – phosphoglyceric acid. [H] stands for some source of redox energy. With benefit of hindsight we know that A corresponds to F6P while B corresponds to pentose phosphates, erythrose 4-phosphate and sedoheptulose phosphate.

Fig. 2. The original depiction of the Calvin-Benson cycle. I have added text showing the aldolase → transketolase, aldolase → transketolase repeat motif (the bisphosphatase enzymes are not shown). Reproduced with permission from Bassham, J.A., Benson, A.A., Kay, L.D., Harris, A.Z., Wilson, A.T., and 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.

Fig. 3. The Calvin-Benson cycle reactions shown in two steps, conversion of pentose to triose (top) and triose to pentose (bottom). The carboxylation reaction results in three five-carbon molecules becoming six three-carbon molecules. One of these can leave the cycle but the other five must be converted back into five-carbon molecules. The conversion of triose phosphates to pentose phosphates is simply two rounds of aldolase, bisphosphatase, transketolase. Reactions in the purple shaded area are pentose phosphate pathway reactions, and in the blue area are gluconeogenic reactions. DHAP = dihydroxyacetone phosphate, E4P = erythrose 4-phosphate, F6(B)P = fructose 6(bis)-phosphate, G6P = glucose 6-phosphate, GAP = glyceraldehyde 3-phosphate, R5P = ribose 5-phosphate, Ru5P = ribulose 5-phosphate, S7(B)P = sedoheptulose 7(bis)-phosphate, Xu5P = xylulose 5-phosphate

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Fig. 13. The cytosolic bypass can lead to high rates of the G6P shunt. Plants lacking FBPase (Livingston et al. 2010) or FBP aldolase (Gotoh et al. 2010) have high rates of cyclic ATP synthesis, perhaps because the cytosolic bypass occurs and stimulates the G6P shunt, consuming ATP that then must be replaced by cyclic electron flow. DHAP = dihydroxyacetone phosphate, E4P = erythrose 4-phosphate, F6(B)P = fructose 6(bis)-phosphate, G6P = glucose 6-phosphate, GAP = glyceraldehyde 3-phosphate, R5P = ribose 5-phosphate, Ru5P = ribulose 5-phosphate, RuBP = ribulose 1,5-bisphosphate, S7(B)P = sedoheptulose 7(bis)-phosphate, Xu5P = xylulose 5-phosphate

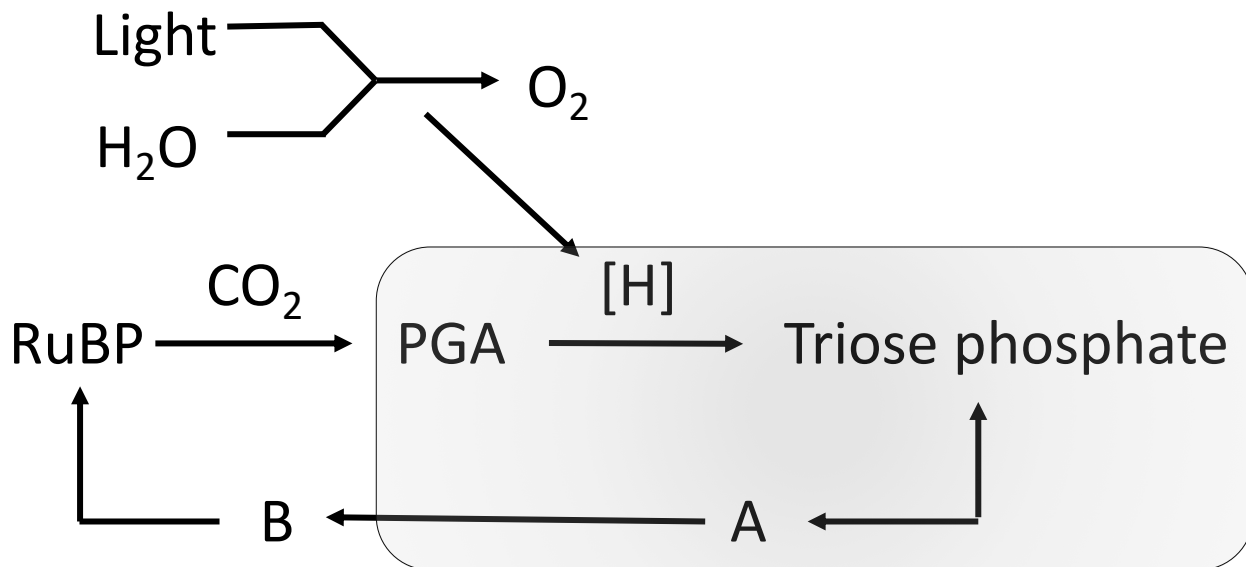


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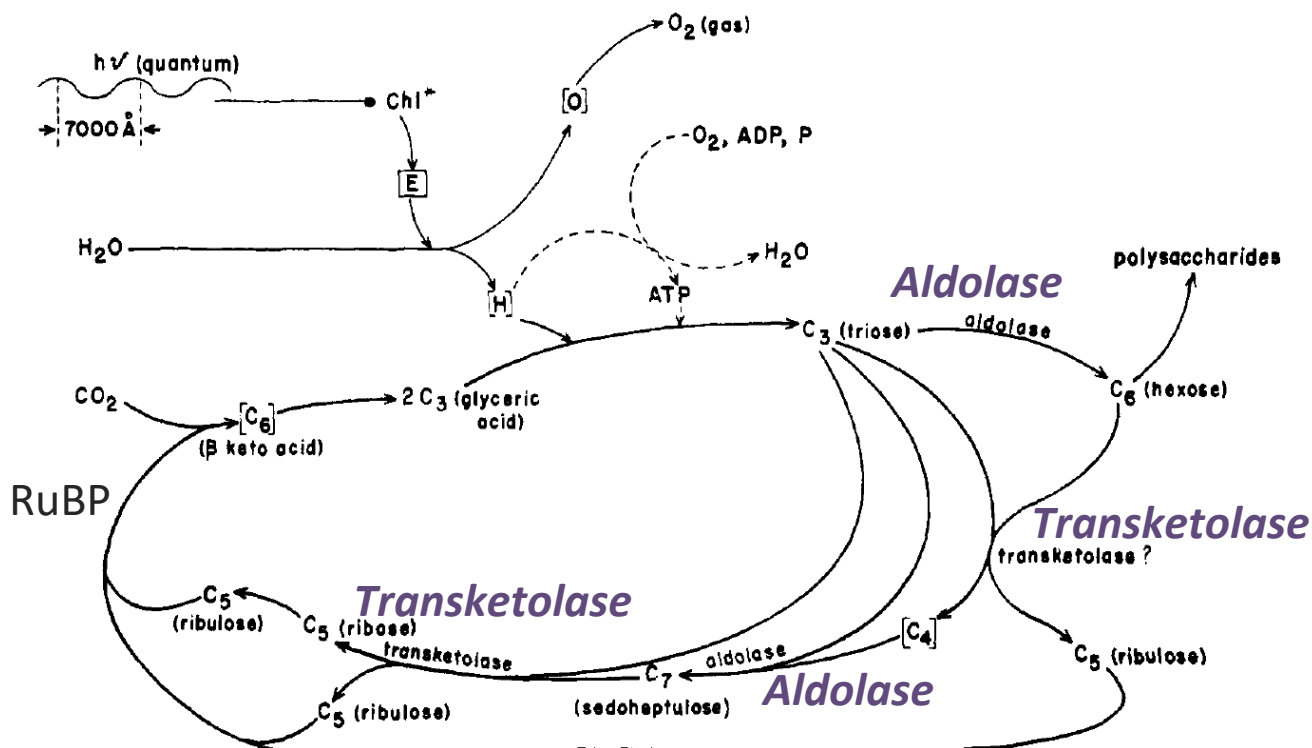


Fig. 7.—Proposed cycle for carbon reduction in photosynthesis. Heavy lines indicate transformations of carbon compounds, light lines the path of conversion of radiant energy to chemical energy and the subsequent use of this energy stored momentarily in some compound (E), to form a reducing agent [H] and oxygen from water.

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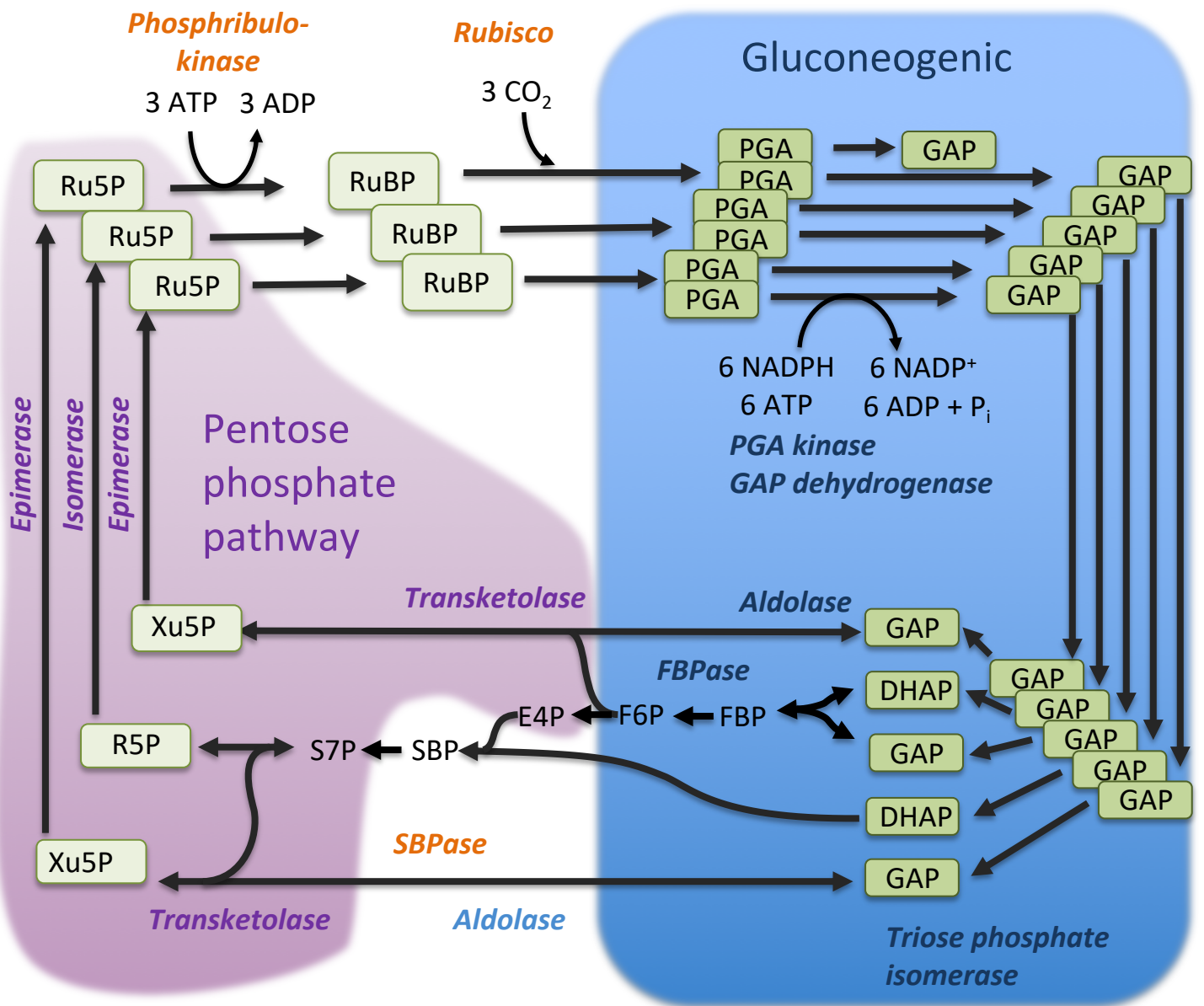


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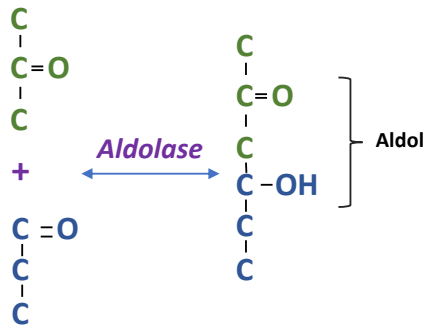


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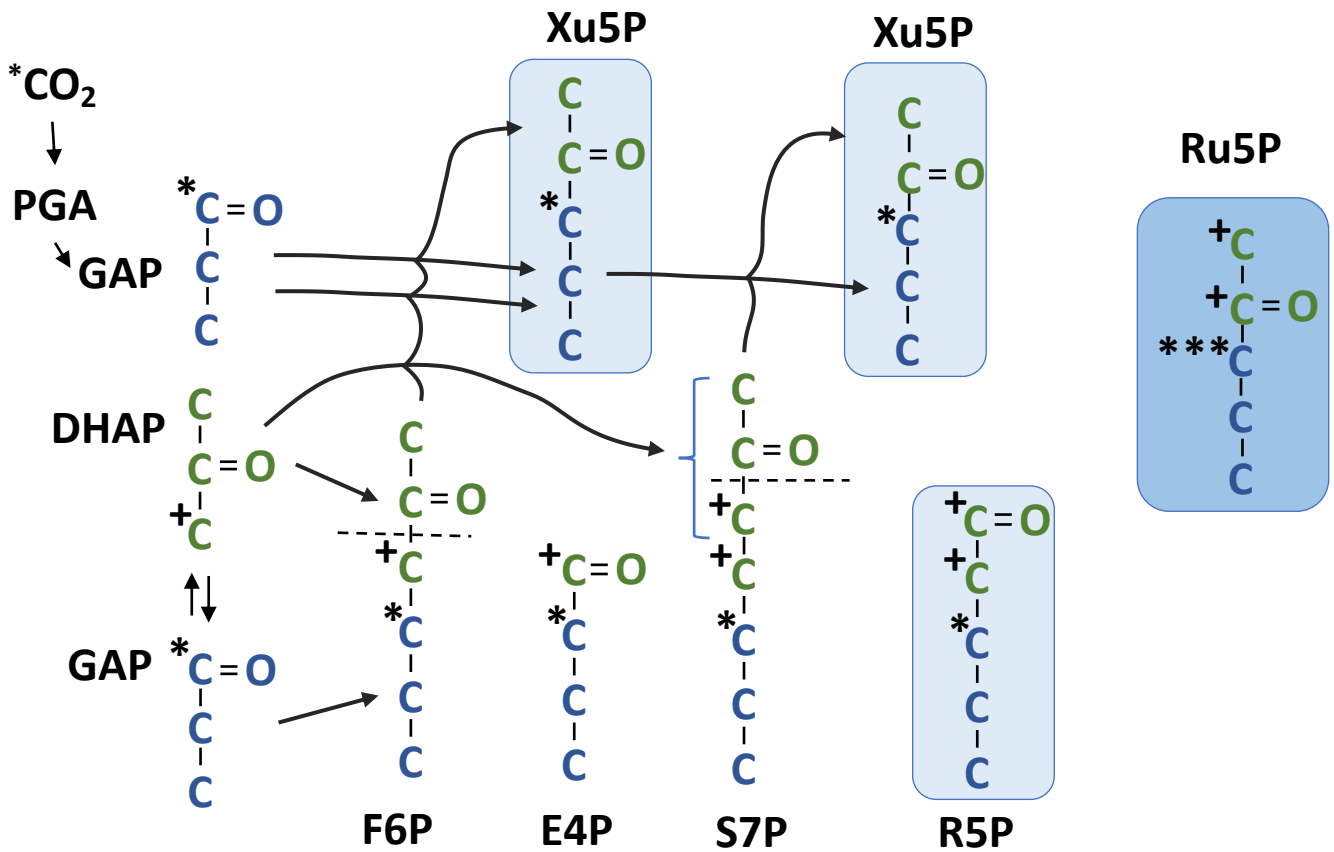


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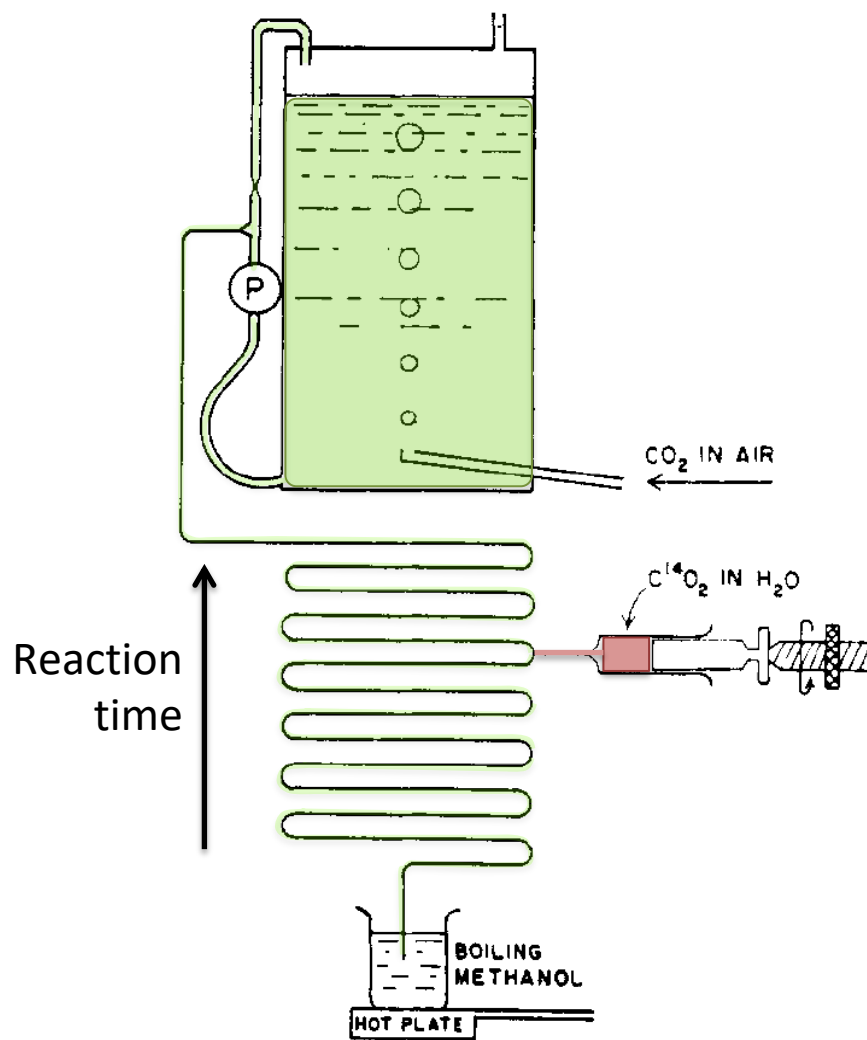


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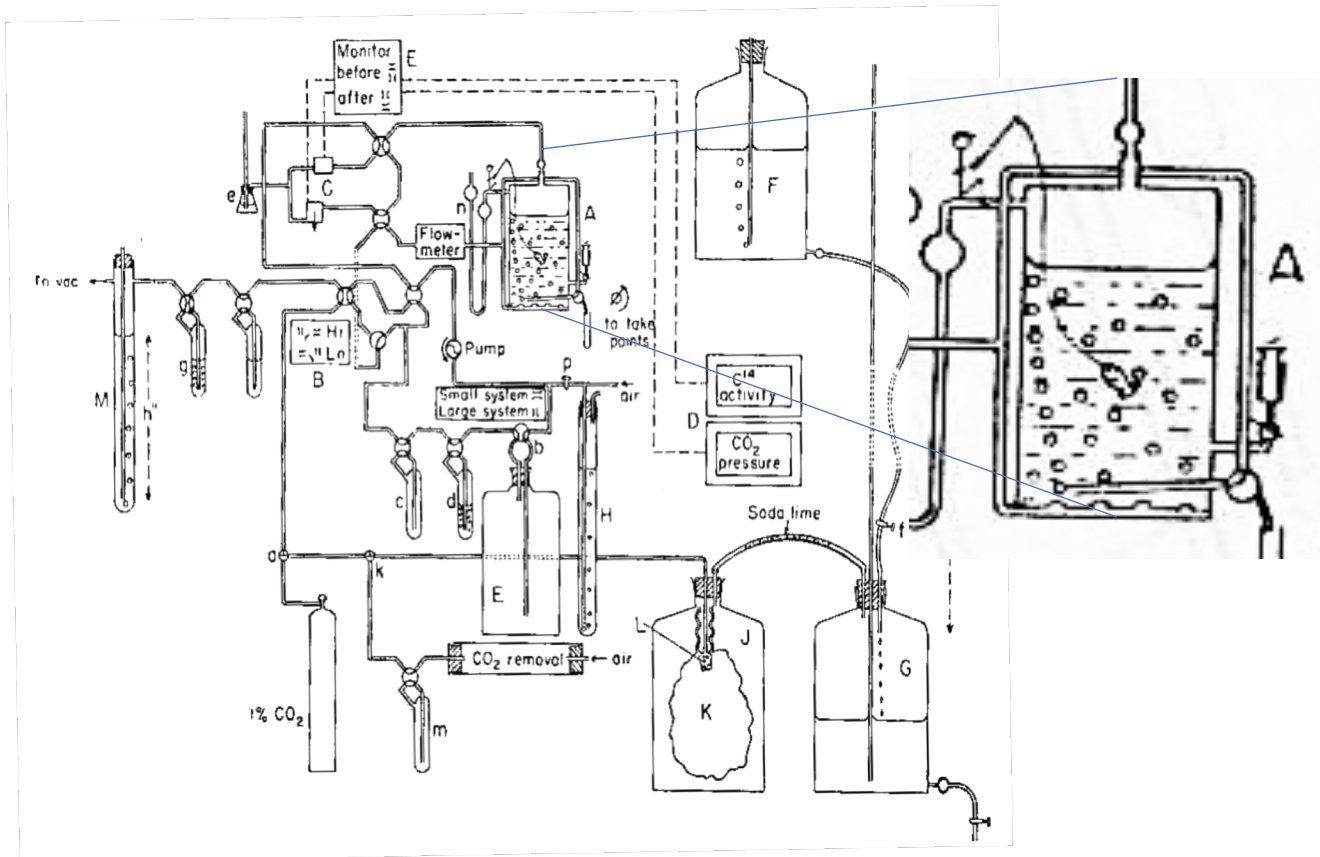


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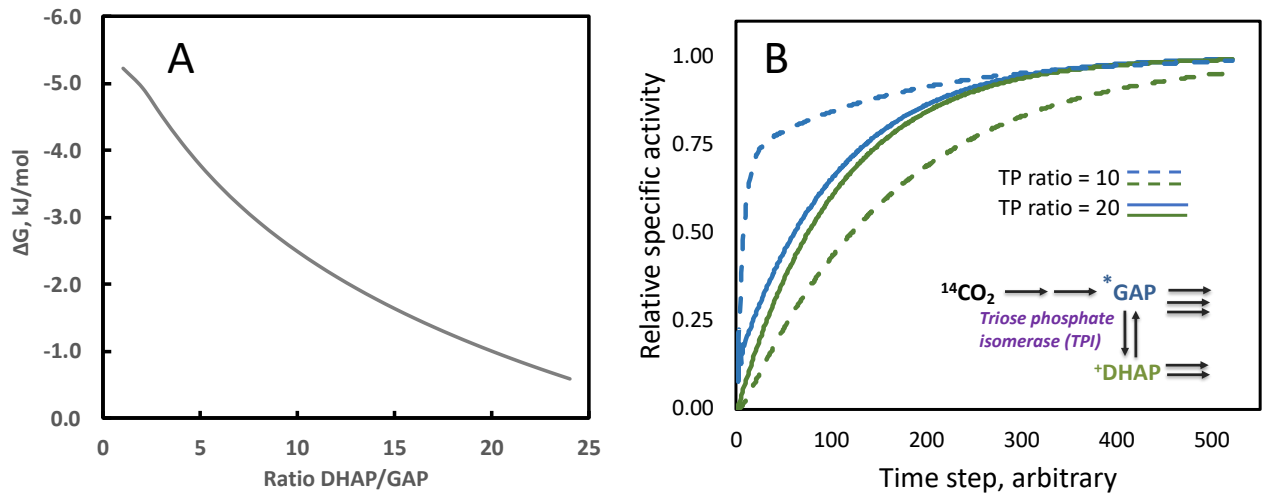


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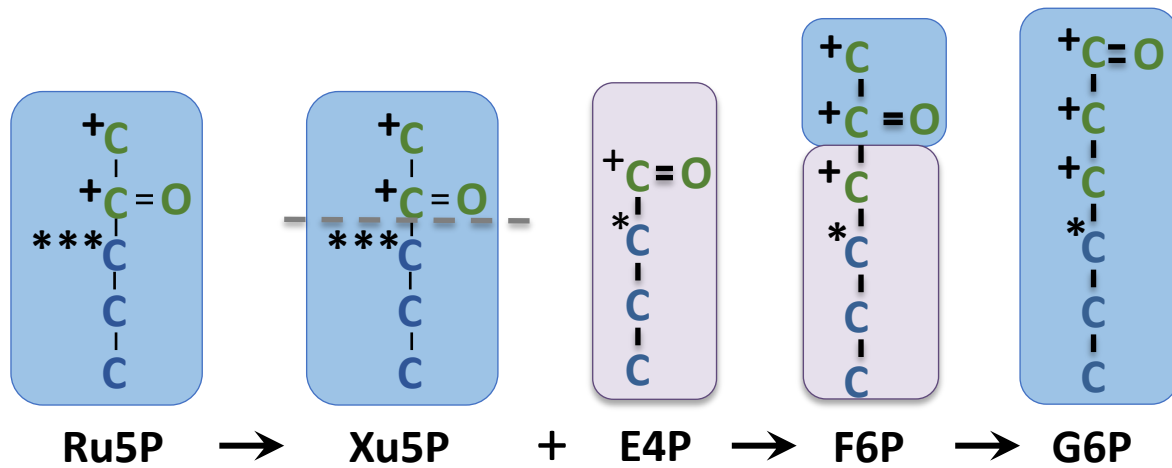


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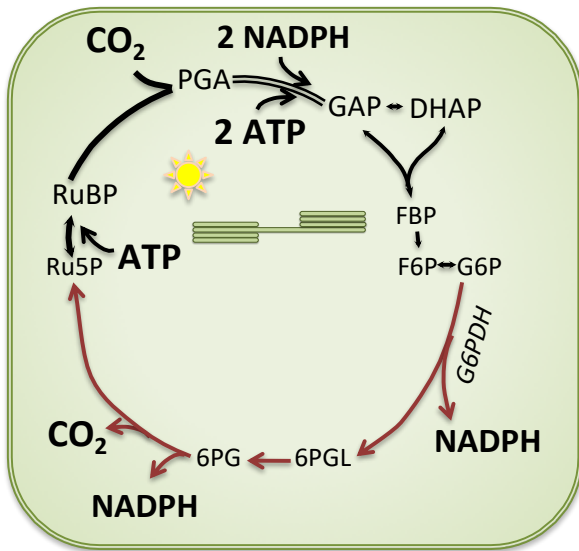


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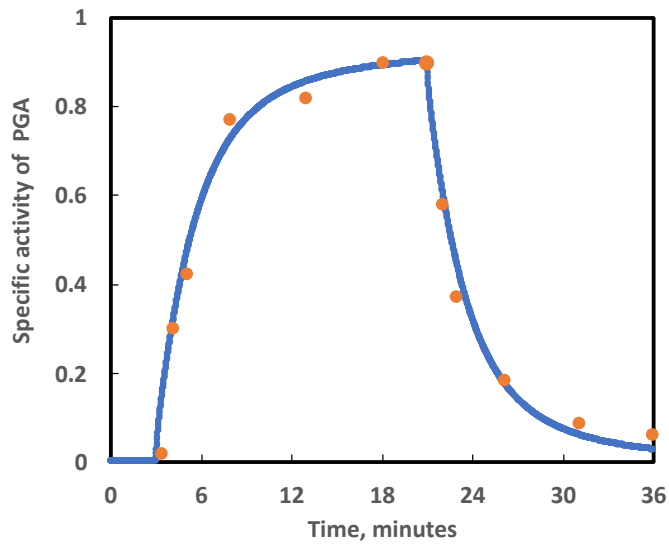
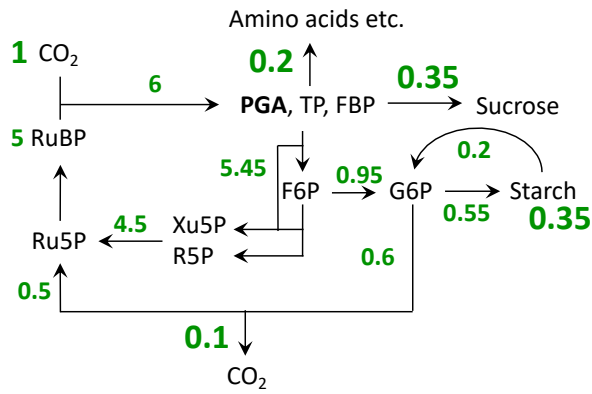


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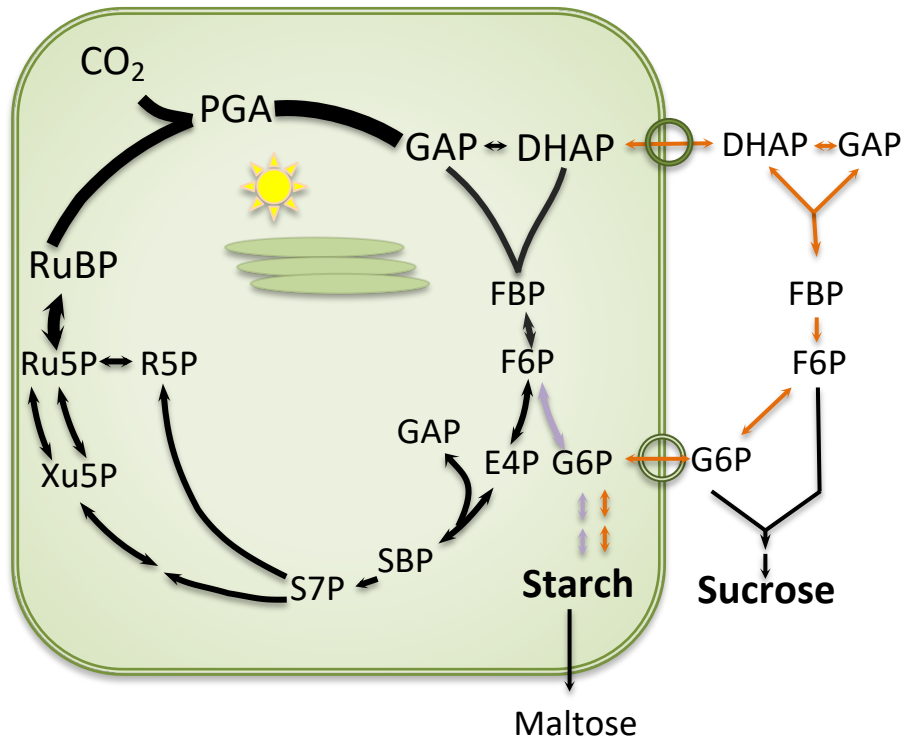


Fig. 12. The glycolytic bypass and G6P import can supplement starch synthesis. This pathway appears to be activated in plants missing starch synthesizing enzymes and in CAM plants, which make much higher amounts of starch than C₃ plants. DHAP = dihydroxyacetone phosphate, E4P = erythrose 4-phosphate, F6(B)P = fructose 6(bis)-phosphate, G6P = glucose 6-phosphate, GAP = glyceraldehyde 3-phosphate, R5P = ribose 5-phosphate, Ru5P = ribulose 5-phosphate, , RuBP = ribulose 1,5-bisphosphate, S7(B)P = sedoheptulose 7(bis)-phosphate, Xu5P = xylulose 5-phosphate

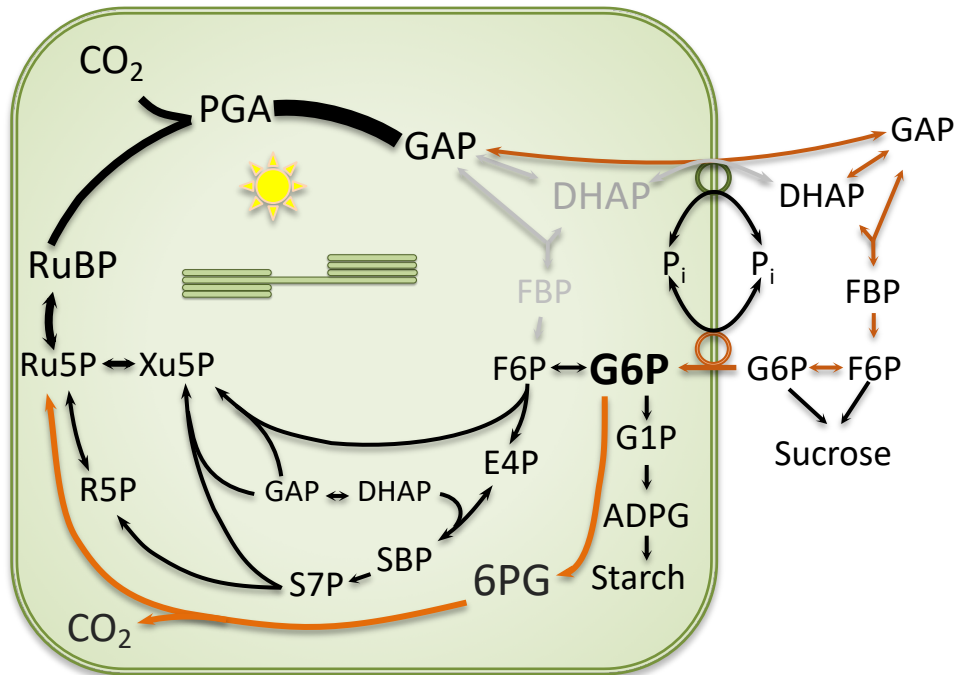


Fig. 13. The cytosolic bypass can lead to high rates of the G6P shunt. Plants lacking FBPase (Livingston et al. 2010) or FBP aldolase (Gotoh et al. 2010) have high rates of cyclic ATP synthesis, perhaps because the cytosolic bypass occurs and stimulates the G6P shunt, consuming ATP that then must be replaced by cyclic electron flow. DHAP = dihydroxyacetone phosphate, E4P = erythrose 4-phosphate, F6(B)P = fructose 6(bis)-phosphate, G6P = glucose 6-phosphate, GAP = glyceraldehyde 3-phosphate, R5P = ribose 5-phosphate, Ru5P = ribulose 5-phosphate, RuBP = ribulose 1,5-bisphosphate, S7(B)P = sedoheptulose 7(bis)-phosphate, Xu5P = xylulose 5-phosphate

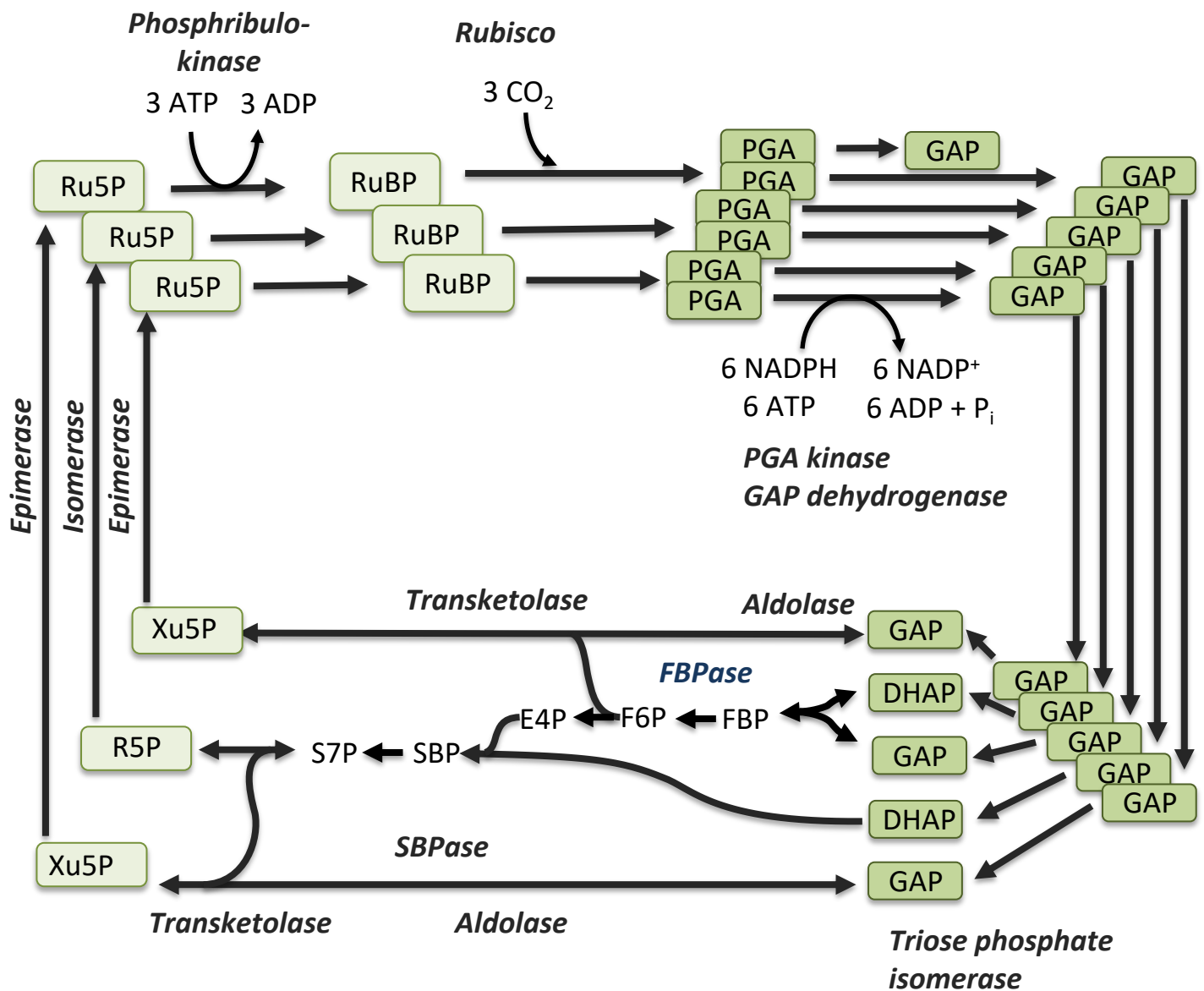


Fig. S1. The Calvin-Benson cycle reactions shown in two steps, conversion of pentose to triose (top) and triose to pentose (bottom). The carboxylation reaction results in three five-carbon molecules becoming six three-carbon molecules. One of these can leave the cycle but the other five must be converted back into five-carbon molecules. The conversion of triose phosphates to pentose phosphates is simply two rounds of aldolase, bisphosphatase, transketolase. DHAP = dihydroxyacetone phosphate, E4P = erythrose 4-phosphate, F6(B)P = fructose 6(bis)-phosphate, G6P = glucose 6-phosphate, GAP = glyceraldehyde 3-phosphate, R5P = ribose 5-phosphate, Ru5P = ribulose 5-phosphate, S7(B)P = sedoheptulose 7(bis)-phosphate, Xu5P = xylulose 5-phosphate

