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THE PATHS OF CARBON AND THEIR REGULATION

Conference of the Origins of Chloroplasts
Session 3
Origin and Evolution of the Chloroplast Metabolism

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Two questions arise under consideration of the paths of carbon and their regulation with respect to the objectives of this conference and this session. One might hope to gain some clues regarding the possible evolutionary relationship between photosynthetic, oxygenic bacteria, and chloroplasts from eukaryotic cells from a comparison of carbon pathways and their regulation. Secondly, one might attempt to write a scenario for the evolution of the carbon pathways from the beginning of the most primitive development of these organisms onward.

The Carbon Pathways of Chloroplasts

The reductive pentose phosphate (RPP) cycle, the Calvin cycle, is ubiquitous to all eukaryotic photosynthetic plants, to the cyanobacteria, and to many photosynthetic bacteria (Norris et al., 1955; Fuller, R. C., 1978). This pathway (Fig. 1) is required for the synthesis of sugar phosphates from CO₂, utilizing reduced pyridine nucleotides and ATP derived from the light reactions in photosynthetic organisms.

Additional pathways of CO₂ fixation supplementary to the RPP cycle exist in certain higher plants. These are the pathways of the "C-4" plants which include certain tropical grasses and other species from diverse families, and the plants that exhibit crassulacean acid metabolism (CAM) such as the succulents, including the cactuses. Neither of these photosynthetic carbon fixing pathways is relevant to the purposes of our present discussion since the evolution of such pathways has occurred in much later times than the evolution of the chloroplasts.

The RPP, or Calvin cycle (Bassham et al., 1954) consists of three stages. The first stage is made of the phosphorylation with ATP of ribulose 5-phosphate (Ru5P) to give ribulose 1,5 bisphosphate (RuBP), followed by the
carboxylation and hydrolytic split of the resulting 6-carbon enzyme-bound compound to give 2 molecules of 3-phosphoglyceric acid (3-PGA). This stage is unique to the RPP cycle.

The second stage of the cycle begins with the phosphorylation of PGA to give phosphoryl PGA and ADP. The reaction is mediated by the enzyme phosphoglyceric acid kinase. The resulting acyl phosphate is then reduced with NADPH in the presence of triose phosphate dehydrogenase yielding oxidized NADP⁺ and 3-phosphoglyceraldehyde (Gal3P) plus inorganic phosphate (Pᵢ). These reactions are essentially the reverse of steps of the glycolytic pathway.

The third stage of the cycle consists of all of the remaining reactions which work together to convert 5 molecules of the triose phosphate (Gal3P) to 3 molecules of Ru5P. The reactions are mediated by aldolase, transketolase, and isomerases, epimerases and bisphosphatases. Sedoheptulose-1,7-bisphosphatase (SBPase) is unique to the RPP cycle. The transketolase, aldolase, and isomerization reactions are common to the oxidative pentose phosphate cycle.

The oxidative pentose phosphate cycle (OPP cycle) includes the hexose monophosphate shunt (Fig. 2). This cycle beginning with glucose-6-phosphate (G6P) includes the oxidation of G6P to 6-phosphogluconic acid (6PGluA), mediated by glucose-6-phosphate dehydrogenase and transferring electrons to NADP⁺ producing NADPH. The second step is a further oxidation of 6PGluA to CO₂ and Ru5P, with two more electrons being transferred to NADP⁺. The resulting NADPH is widely used in plant cells for the biosynthesis of fatty acids from acetyl CoA. Subsequent steps of the OPP cycle result in the conversion of 3 molecules of Ru5P to 1 molecule of fructose-6-phosphate and 1 molecule of Gal3P which in turn can be converted back to F6P and G6P thus completing the cycle (when and if it functions as a complete closed cycle) (Kaiser & Bassham, 1979c).
Regulation of RPP and OPP Cycles in Chloroplasts.

The regulation of the RPP cycle in chloroplasts has been worked out over the past twelve years or so (for reviews see Bassham, 1971, 1979). During photosynthesis the rate-limiting steps (Fig. 1) are those mediated by phosphoribulokinase, ribulose-1,5-bisphosphate carboxylase, and the two phosphatases involved in the conversion of SBP and fructose-1,6-bisphosphate (FBP) to their respective monophosphates (Bassham & Krause, 1969).

The principal sites at which carbon is removed from the cycle during photosynthesis are as follows: 1. GA3P is exported from the chloroplast to the cytoplasm where it can be used for subsequent biosynthetic reactions. 2. F6P is converted to glucose-6-phosphate (G6P) which is the starting point for the synthesis of starch; and 3. The oxygenase reaction mediated by RuBP carboxylase/oxygenase (in which oxygen binds competitively at the CO₂ binding site) oxidizes RuBP to PGA and phosphoglycolate. Free glycolate is exported from the chloroplast after which its metabolic fate is different in algae and higher plants. In higher plants the glycolate is converted in peroxisomes and mitochondria via the glycolate pathway, producing 1 molecule of CO₂ and 1 molecule of glycerate from each 2 molecules of glycolate. The glycerate is reincorporated into the chloroplast (Tolbert, 1971). The CO₂ evolved is considered to be the product of photorespiration. This production of glycolate and the whole pathway of photorespiration can be considered as a late era phenomenon, occurring only after the level of CO₂ has declined to its present low level, and is not relevant to the question of the evolution of the chloroplast or its metabolic cycles.

Regulation of the rate-limiting steps of the RPP cycle in the light is required to balance the levels of cycle intermediates when there is a change.
in the relative proportions of cycle metabolites exported or converted to starch. The export of Gai3P, controlled in part by cytoplasmic Pi (Heldt, 1976; Walker, 1976), should vary inversely with the conversion of F6P to starch when there is a constant rate of carboxylation. Therefore, in order for physiological concentrations of each sugar phosphate to be maintained within the chloroplast, the activities of the FBPase and SBPase must be finely adjusted in comparison with the activity of phosphoribulokinase and RuBP carboxylase.

It appears that the internal Pi level in the chloroplasts serves to provide the inverse relation between the rate of export of Ga13P to the cytoplasm and conversion of F6P to starch. Since Ga13P export is balanced against Pi import, chloroplast internal Pi concentration increases with increased rate of triose phosphate export. This increased Pi concentration in the chloroplast in turn slows the rate of conversion of glucose-1-phosphate (G1P) with ATP to adenosine diphosphoglucose (ADPG) and inorganic pyrophosphate (PPi).

If PGA should accumulate in the chloroplast, as would be expected when the rate of triose phosphate export declines, it would be advantageous for the rate of starch formation to accelerate. The stimulation of the ADPG pyrophosphorylase reaction by PGA and its inhibition by Pi were reported for the isolated enzyme (Priess, 1967; Sanwal et al., 1968). When concentrations of controlling factors (pH, Pi, PGA and ATP) observed in whole chloroplast in the light and dark (Kaiser & Bassham, 1979a) were administered to reconstituted chloroplasts, strong regulation of ADPGlucose formation was observed (Kaiser and Bassham, 1979b).

For regulation of the light-dark transition from the RPP cycle to glycolysis and the OPP cycle, those enzymes of the RPP cycle which were
rate limiting in the light become inactivated in the dark (Bassham & Kirk, 1960; Pedersen et al, 1966). This inactivation is the result of changes in Mg$^{2+}$ ion concentration, pH, and the level of reduced to oxidized cofactors (Breazeale et al., 1978; Schürmann et al., 1976; Schürmann and Buchanan, 1975; Buchanan et al., 1979; Anderson, 1973). Besides the dark induced decline in the activities of the four rate-limiting steps from the light, there appears to be some evidence for a decrease in the activity of triose phosphate dehydrogenase in the dark (Buchanan et al, 1979).

In the OPP cycle, the key regulated step is the oxidation of glucose 6-phosphate to 6-phosphogluconate mediated by glucose 6-phosphate dehydrogenase. The principal mechanism of regulation appears to be by the ratio of NADPH/NADP$^+$ (Lendzian & Bassham, 1975, 1976). High ratios of NADPH/NADP$^+$ decrease the activity of the enzyme and this effect is more pronounced at pH 8.2 than at pH 7.6 (the light and dark pH's of chloroplast stroma respectively). Considerable additional inactivation of glucose 6-phosphate dehydrogenase at the physiological levels of NADPH/NADP$^+$ in the light occurs in the presence of physiological levels of ribulose-1,5-bisphosphate. With such high ratios of NADPH/NADP$^+$ and in the presence of 0.5 mM RuBP, the activity of the enzyme is reduced to less than 1% of its level under conditions of full activity.

**Regulation of RPP and OPP Cycles in Cyanobacteria.**

Regulation of carbon metabolism in the cyanobacteria (Review, see Stanier and Cohen-Bazire, 1975) exhibits many similarities to the regulation of the RPP and OPP cycles in chloroplasts of green algae and higher plants. Except for glucose-6-phosphate dehydrogenase, which increases in specific activity somewhat in the dark (Pelroy et al, 1972), all enzymes of the OPP and RPP cycles are synthesized under both light and dark conditions. In
Aphanocapsa 6714 the rate limiting steps of the RPP cycle in the light appear to be those mediated by FBPase and SBPase and by phosphofructokinase and perhaps the carboxylase (Pelroy et al, 1976). Even the time required to reactivate FBPase and SBPase on going from dark to light (20 - 30 sec) is reminiscent of that observed with Chlorella pyrenoidosa (Bassham & Kirk, 1968). Aphanocapsa, when metabolizing glucose in the dark, however, maintains a high level of NADPH, a condition not likely to be found in chloroplasts of eukaryotic cells. Phosphoribulokinase activity was dependent on the presence of sulfhydryl reducing agents such as dithiothreitol, a response similar to that reported for the enzyme from eukaryotic cells (Anderson, 1973). Perhaps cyanobacteria, like chloroplasts of eukaryotic cells, regulate SBPase, FBPase, phosphoribulokinase, and even triose phosphate dehydrogenase through the levels of reduced to oxidized ferridoxin, acting through the intermediacy of thioredoxin as has been shown in the case of eukaryotic cells (Breazeale et al., 1978). The presence of the thioredoxin system in bacterial systems including cyanobacteria has been reported (Buchanan and Wolosiuk, 1976).

It appears that the OPP cycle can operate to some extent in Aphanocapsa even in the presence of high ratios of NADPH to NADP⁺. For cyanobacteria such as Anacistis nidulens which are only poorly permiable to glucose (Pelroy et al, 1972) such high ratios of NADPH/NADP⁺ would not be expected. In view of the specific inhibition of the eukaryotic chloroplast glucose 6-phosphate dehydrogenase by RuBP mentioned earlier, it is interesting that one study of glucose 6-phosphate dehydrogenase activity in crude, cell free extracts of a variety of cyanobacteria showed this enzyme to be inhibited by RuBP (Pelroy et al., 1972). Other studies of the same enzyme partly purified from Anabaena found NADPH but not RuBP to be an inhibitor (Grossman
& McGowan, 1975). Considering the reported complex nature of the Anabaena enzyme (Stanier & Cohen-Bazire, 1977), it seems possible that just as in the case of chloroplasts, the bacterial enzyme may be regulated both by NADPH/NADP⁺ ratio and by RuBP concentration, perhaps with requirements for regulatory behavior that are lost during the purification of the enzyme.

Although the cyanobacteria synthesize glycogen as a carbohydrate storage product rather than starch, there appear to be similarities in the regulation of the synthetic pathway. The rate limiting enzyme, ADPG pyrophosphorylase, is activated 8 to 25 fold by 3-phosphoglycerate and is inhibited by inorganic phosphate (Levi & Preiss, 1976). The enzyme from other prokaryotic cells is not activated by phosphoglycerate. It is significant that the cyanobacterial enzyme exhibits this regulatory property characteristic of eukaryotic photosynthetic organisms.

Endosymbiotic Origin of Chloroplasts from Oxygenic Photosynthetic Bacteria.

What are the implications of the similarities between carbon pathways and their regulation in chloroplasts of higher plants and in the oxygenic photosynthetic bacteria? As pointed out by Stanier and Cohen Bazire (1977), the similarities in carbon metabolism between the cyanobacteria and chloroplasts of higher plants are striking, but the pigment systems are quite different. All photosynthetic oxygenic organisms contain chlorophyll a, but whereas higher plants have chlorophyll b as the principal second pigment, the cyanobacteria contain phycobilins. The red algae contain a light harvesting pigment system very similar to that of the cyanobacteria making the origin of the rhodophyta chromoplast from cyanobacteria an attractive evolutionary hypothesis. For the origin of chloroplasts of higher plants and green algae, one must look for other precursors, possibly the oxygenic photosynthetic
bacteria isolated by Lewin and his colleagues from the marine organism, Didemnum (Lewin, 1976; Lewin & Withers, 1975; Withers et al., 1978). These organisms, for which the name Prochloron sp. has been proposed, have Chl a/Chl b ratios in the range of 4.4 to 6.9, contain carotene and zeaxanthine, and generally resemble green algae in the arrangement of paired or stacked thylakoids. Moreover, a chlorophyll protein isolated from these prokaryotes is indistinguishable from that obtained from the chloroplast of higher plants.

From the foregoing it seems clear that although similarities in carbon metabolism and its regulation point to a common evolutionary origin for all photosynthetic oxygenic organisms, they do not provide a basis for establishing a sequential relationship between the cyanobacteria and green chloroplasts of eukaryotic cells.

Origin of Carbon Metabolism in Chloroplasts.

What can we deduce about the origin of chloroplast carbon metabolism in the first primitive organisms and its subsequent evolution? Much has been written on chemical evolution and subsequent biochemical evolution. Broda (1975) has provided an excellent comprehensive description of the evolution of bioenergetic processes which brings together a great many of the excellent reviews and original papers which have appeared on this subject over the years. The following speculations are based in part on information reviewed by Broda. No claim is made either for originality or for agreement with all the existing literature which in at least some cases is still controversial.

It is widely believed that after the earth had reached its approximate present size and structure, most of the primary atmosphere had been lost, and the new atmosphere was formed by outgassing of the earth's rocks. There
is considerable controversy over how reducing this primitive earth's atmosphere was, but it is generally conceded that it was basically free of oxygen. Various simple organic molecules are thought to have been formed from hydrides (methane, water, ammonia, hydrogen sulfide) and perhaps from CO, CO₂, N₂, and H₂ under the influence of electrical discharges from storms, ultra violet radiation, heat and volcanism, and perhaps catalysis by certain mineral clays. Under a variety of assumed conditions, amino acids, sugars, and more complex biological building blocks have been formed in laboratory experiments employing these various agents. From concentrations of such organic chemicals, primitive life could have evolved in the form of very simple cells capable of utilizing the wealth of organic substrate in their environment and of reproducing themselves.

All life requires energy and the earliest organisms must have derived their vital supply of energy by carrying out various oxidation-reduction reactions on the abiotically formed materials available to them. Thus, we can suppose that the fermentative pathway of glycolysis from hexoses to pyruvate followed by either lactogenesis of formation of alcohol and CO₂ may have evolved in very primitive organisms as a means for generating the energy currency of living cells, ATP.

Although a variety of sugars, including pentoses and hexoses, would be expected to be formed from the condensation or formaldehyde in the ponds of the primitive earth, it is apparent that pentoses became the sugar of choice for nucleotides and hence for genetic material. Since thermodynamics somewhat favors the formation of hexoses over pentoses, it seems possible that the supply of pentoses may have become exhausted, necessitating the evolution of biochemical pathways to convert hexoses to pentoses. The most
primitive of such pathways may well have been the conversion of hexose phosphates to pentose phosphates via the reactions mediated by transketolase, transaldolase, and isomerases.

Further depletion of the reduced compounds in the primitive environment particularly as the atmosphere became less reducing (due to \(H_2\) escape from earth) could have led to a shortage of the supply of endogenous long chain hydrocarbons required for the synthesis of fatty acids needed for cellular membranes. Assuming that at this point carbohydrates were still available, a way had to be found for the conversion of carbohydrate to fatty acids. If acetyl CoA became the building block for the synthesis of fatty acids, these two-carbon fragments could have been generated by pyruvate formation via glycolysis and oxidation of pyruvate with the electrons being used for fatty acid synthesis. Since this supply of electrons would be stoichiometrically inadequate, the oxidative stages of the OPP cycle may have evolved in order to provide a supply of reduced NADPH. The oxidative pentose phosphate cycle is used primarily for that purpose in many types of cells to this day.

The evolution of the reductive pentose phosphate cycle would have occurred very much later, at a time when the endogenous supply of carbon in forms other than \(CO_2\) had become depleted. Long before this happened, we may suppose that photosynthetic organisms analogous to modern day photosynthetic bacteria had developed which were capable of utilizing light energy to drive the process of photophosphorylation. We can surmise that the first photophosphorylation was of the cyclic type in which electrons are cycled from the product of the photochemical reaction (electron acceptor) to the resulting oxidized pigment via an electron donor, with the energy of electron flow being used to form a proton gradient across membranes in order to drive the
conversion of ADP and inorganic phosphate to ATP. A later stage of evolution would lead to non-cyclic photophosphorylation, that is a net flow of electrons from donor to acceptor. With the establishment of photophosphorylation and photochemical electron flow, the stage was set for the development of a great diversity of types of photochemical metabolism, many of which are seen in present day photosynthetic bacteria, both aerobic and anaerobic.

Depending upon the oxidation level of available substrate and the oxidation level of cellular material, photosynthetic bacteria may absorb CO₂ and reduce it to various organic molecules required for cell synthesis. Long before supplies of endogenous reduced carbon were exhausted, it seems likely that the reductive pentose phosphate cycle may have evolved in photosynthetic bacteria. The evolution of the RPP cycle from the oxidative pentose phosphate pathway requires the evolution of the reaction converting R5P to RuBP, utilizing 1 molecule of ATP and the subsequent carboxylation reaction which produces 2 molecules of PGA. A third unique reaction is involved in the RPP cycle, namely the conversion of SBP to sedoheptulose-7-phosphate. The enzyme for this reaction, SBPase is closely related to FBPase and could easily have evolved from it. Even though phosphoribulokinase (PRK) is similar in function to phosphofructokinase, it should be noted that PRK activity is turned on in the light and off in the dark whereas PFK, an enzyme of glycolysis, has to be regulated in exactly the opposite way. Since RuBP has no other known function than to serve as substrate for the photosynthetic carboxylation reaction of the RPP cycle, it would appear that both the PRK and RuBP carboxylase enzymes had to evolve simultaneously. The evolution of these two enzymes has to be considered as one of the most significant events in evolutionary history, given the present day importance of the carbon-fixing reactions in the biosphere. The evolution of the genetic information, both chloroplastic
and nuclear, for the various subunits of RuBPCase is an extremely interesting problem which has been discussed elsewhere at this meeting.

Although it has just been suggested that the formation and carboxylation of RuBP were evolved by certain photosynthetic bacteria, another possibility might be that these steps could have evolved in anaerobic chemolithotrope living in proximity to anaerobic fermenters producing hydrogen and CO₂.

Presumably, oxygenic prokaryotic photosynthetic bacteria, the cyanobacteria and others, evolved in time from some primitive photosynthetic bacteria and retained the capability of carrying out the reductive pentose phosphate cycle. With the evolution of O₂ formation by oxidation of water in oxygenic photosynthetic bacteria, the resulting electrons and ATP could be used to drive the RPP cycle. In addition, a smaller portion of the electron flow could be used for the reduction of nitrite and the reduction of sulfate.

Finally, with the incorporation of these oxygenic prokaryotes into eukaryotic cells, the last stages of the evolution of chloroplastic carbon metabolism could proceed with the development of the special regulatory mechanism required in these organelles.
REFERENCES


Kaiser, W. M. and Bassham, J. A. 1979b. Light-dark regulation of starch
metabolism in chloroplasts. II. Effect of chloroplastic
metabolite levels on the formation of ADP-glucose by

Kaiser, W. M. and Bassham, J. A. 1979c. Carbon metabolism of chloroplasts
in the dark: Oxidation pentose phosphate cycle versus

Lendzian, K. and Bassham, J. A. 1975. Regulation of glucose 6-phosphate
dehydrogenase in spinach chloroplasts by ribulose 1,5

Lendzian, K. and Bassham, J. A. 1976. NADPH/NAD⁺ ratios in photosynthesizing

pyrophosphorylase of the blue-green bacterium Synechococcus


Lewin, R. A. and Withers, N. W. 1975. Extraordinary pigment composition of

Norris, L., Norris, R. E., and Calvin M. 1955. A survey of the rates and
products of short-term photosynthesis in plants of nine phyla.

levels of intermediate compounds during photosynthesis in

CO₂ fixation and glucose assimilation by Aphanocapsa 6714.
Preiss, J., Ghosh, H. P., and Wittkop, J. 1967. Regulation of the 
Biosynthesis of Starch in Spinach Leaf Chloroplasts. In 

Sanwal, G. G., Greenberg, E., Hardie, J., Cameron, E. C., and Preiss, J. 
1968. Regulation of starch biosynthesis in plant leaves: 
Activation and Inhibition of ADP-glucose pyrophosphorylase. 
Plant. Physiol. 43: 417-427.

Two proteins function in the regulation of photosynthetic CO₂ 

Schürmann, P. and Buchanan, B. B. 1975. Role of ferredoxin in the activation 
of sedoheptulose diphosphatase in isolated chloroplasts. 


Stocking, C. R. and Heber, U. eds. Berlin and New York: 
Springer-Verlag.

Withers, N. W., Alberti, R. S., Lewin, R. A., Thornber, J. D., Britton, G., 
and Goodwin, R. W. 1978. Photosynthetic unit size carotenoids, 
and chlorophyll-protein composition of Prochloron sp., a 
procaryotic green algae. Proc. Natl. Acad. Sci. USA 75: 
2301-2305.
FIGURE CAPTIONS

Figure 1: The Reductive Pentose Phosphate (RPP) Cycle. Regulated steps are indicated by \( \bigcirc \), unique steps by asterisk. Abbreviations: G1P, glucose 1-phosphate; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; E4P, erythrose 4-phosphate; Ru5P, ribulose 5-phosphate; Xu5P, xylulose 5-phosphate; DHAP, dihydroxyacetone phosphate; Gal3P, glyceraldehyde 3-phosphate; FBP, fructose 1,6 bisphosphate; SBP, sedoheptulose 1,7 bisphosphate; RuBP ribulose 1,5-bisphosphate; ADPG, adenosine diphosphoglucone; PGA, 3-phosphoglycerate.

Figure 2: The Oxidative Pentose Phosphate (OPP) Cycle. Regulated steps are indicated by \( \bigcirc \), unique steps by asterisk. Glycolytic steps are indicated by dashed line. Abbreviations (see Figure 1), and 6PGLuA, 6-phosphogluconate.
Bassham

Fig. 1
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