Autotrophy: Calvin Cycle

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Anabolism

- In anabolism or biosynthesis, cells use free energy to construct more complex molecules and structures from smaller, simpler precursors.
- Catabolic and anabolic pathways may differ in enzymes, regulation, intracellular location, and use of cofactors and nucleoside diphosphate carriers.
- Although many enzymes of amphibolic pathways participate in both catabolism and anabolism, some enzymes are involved only in one of the two processes.

Autotrophs

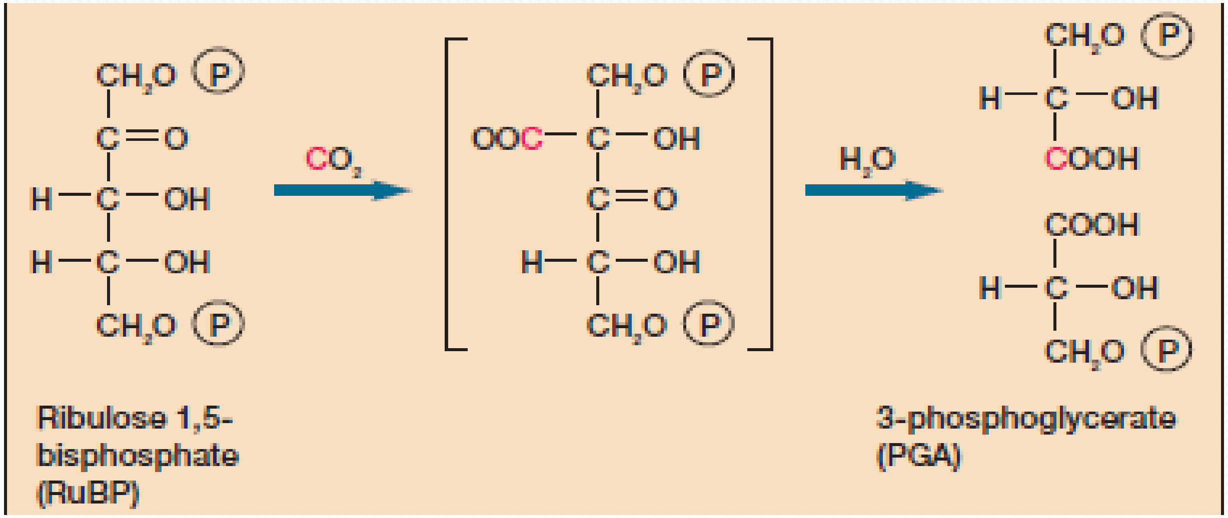
- **Autotrophs** use ATP and NADPH from photosynthesis or from oxidation of inorganic molecules to reduce CO2 and incorporate it into organic material.
- Microorganisms can fix CO_2 or convert this inorganic molecule to organic carbon and assimilate it in three major ways:
  - Calvin cycle/Calvin-Benson cycle/ Reductive pentose phosphate pathway/ C3 pathway
  - Reductive TCA pathway : is used by some archaea (*Thermoproteus, Sulfolobus)* and by bacteria such as *Chlorobium* and *Desulfobacter*.
  - Acetyl CoA pathway: is found in methanogens, sulfate reducers, and bacteria that can form acetate from CO_2 during fermentation (acetogens).

Calvin Cycle

- Although the Calvin cycle is found in photosynthetic eucaryotes and most photosynthetic procaryotes, it is absent in the Archaea, some obligately anaerobic bacteria, and some microaerophilic bacteria.
- The Calvin cycle is found in the chloroplast stroma of eucaryotic microbial autotrophs.
- Cyanobacteria, some nitrifying bacteria, and thiobacilli possess **carboxysomes (polyhedral inclusion** bodies that contain the enzyme ribulose-1,5-bisphosphate carboxylase), may be the site of CO_2 fixation or may store the carboxylase and other proteins.
- Calvin cycle divided into three phases:
  - **The Carboxylation Phase** (***Fixation of CO_2 into 3-Phosphoglycerate***)
  - **Reduction phase** (***Conversion of 3-Phosphoglycerate to Glyceraldehyde 3-Phosphate***)
  - **Regeneration phase** (***Regeneration of Ribulose 1,5-Bisphosphate from Triose Phosphates***)

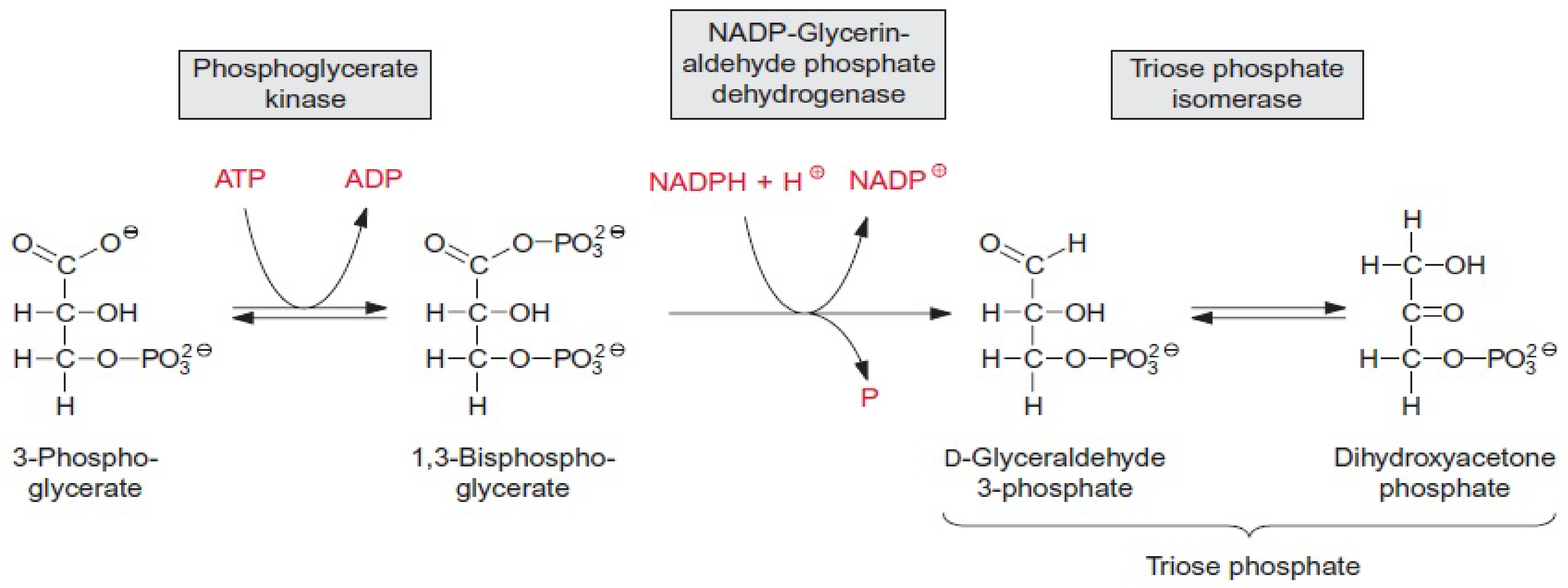
The Carboxylation Phase

- Calvin and his associates illuminated a suspension of green algae in the presence of radioactive carbon dioxide (14CO2) for just a few seconds, then quickly killed the cells, extracted their contents, and observed that the first compound that became labeled was **3-phosphoglycerate**, with the 14C predominantly located in the carboxyl carbon atom.
- The enzyme **ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco)** catalyzes the covalent attachment of CO2 to the five-carbon sugar ribulose 1,5-bisphosphate and cleavage of the unstable six-carbon intermediate to form two molecules of 3-phosphoglycerate.



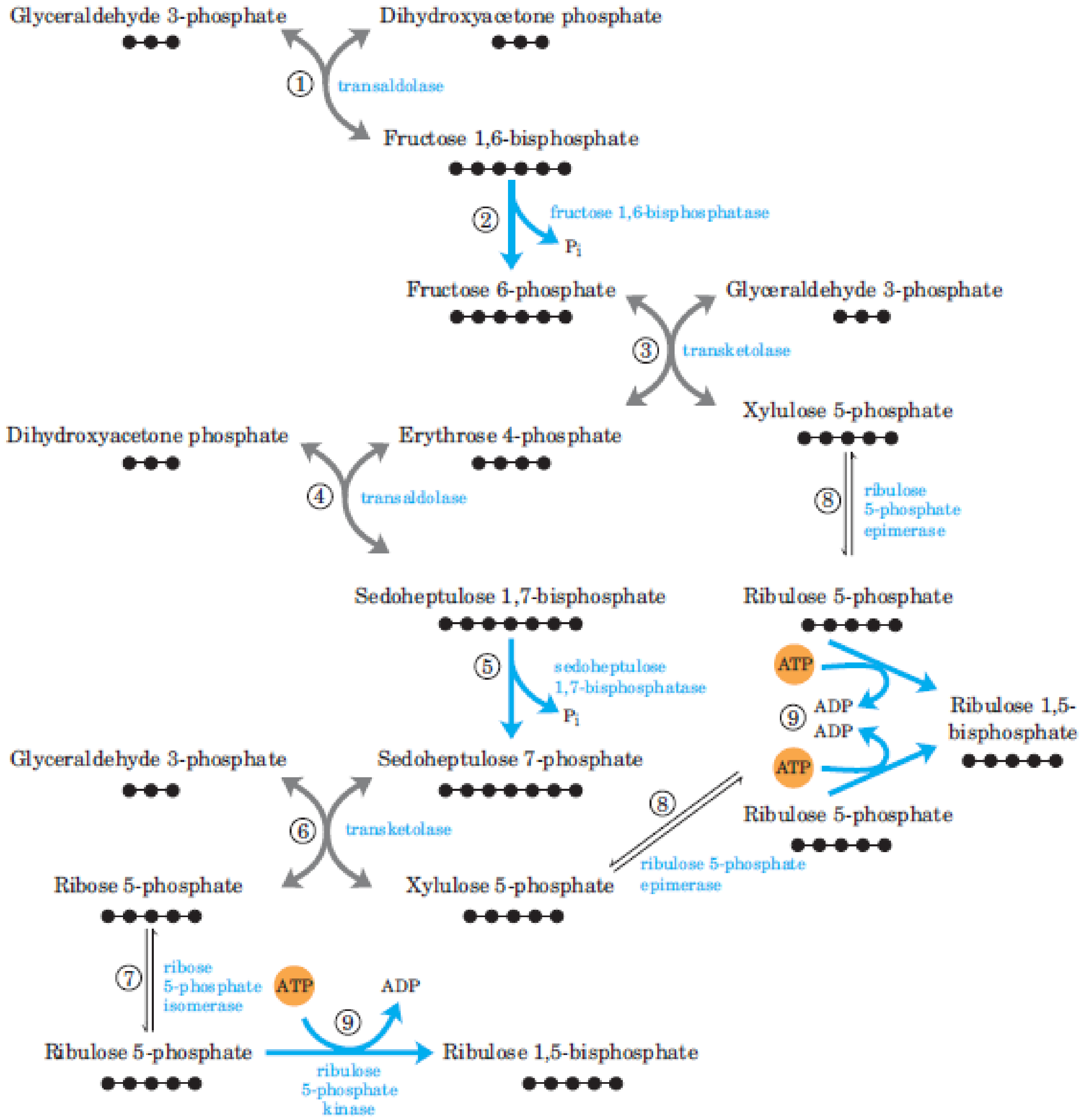
Reduction phase

- After PGA is formed by carboxylation, it is reduced to glyceraldehyde 3-phosphate.
- The reduction, carried out by two enzymes, is essentially a reversal of a portion of the glycolytic pathway, although the glyceraldehyde 3-phosphate dehydrogenase differs from the glycolytic enzyme in using NADP rather than NAD^+
- Triose phosphate isomerase then interconverts glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.



Regeneration Phase

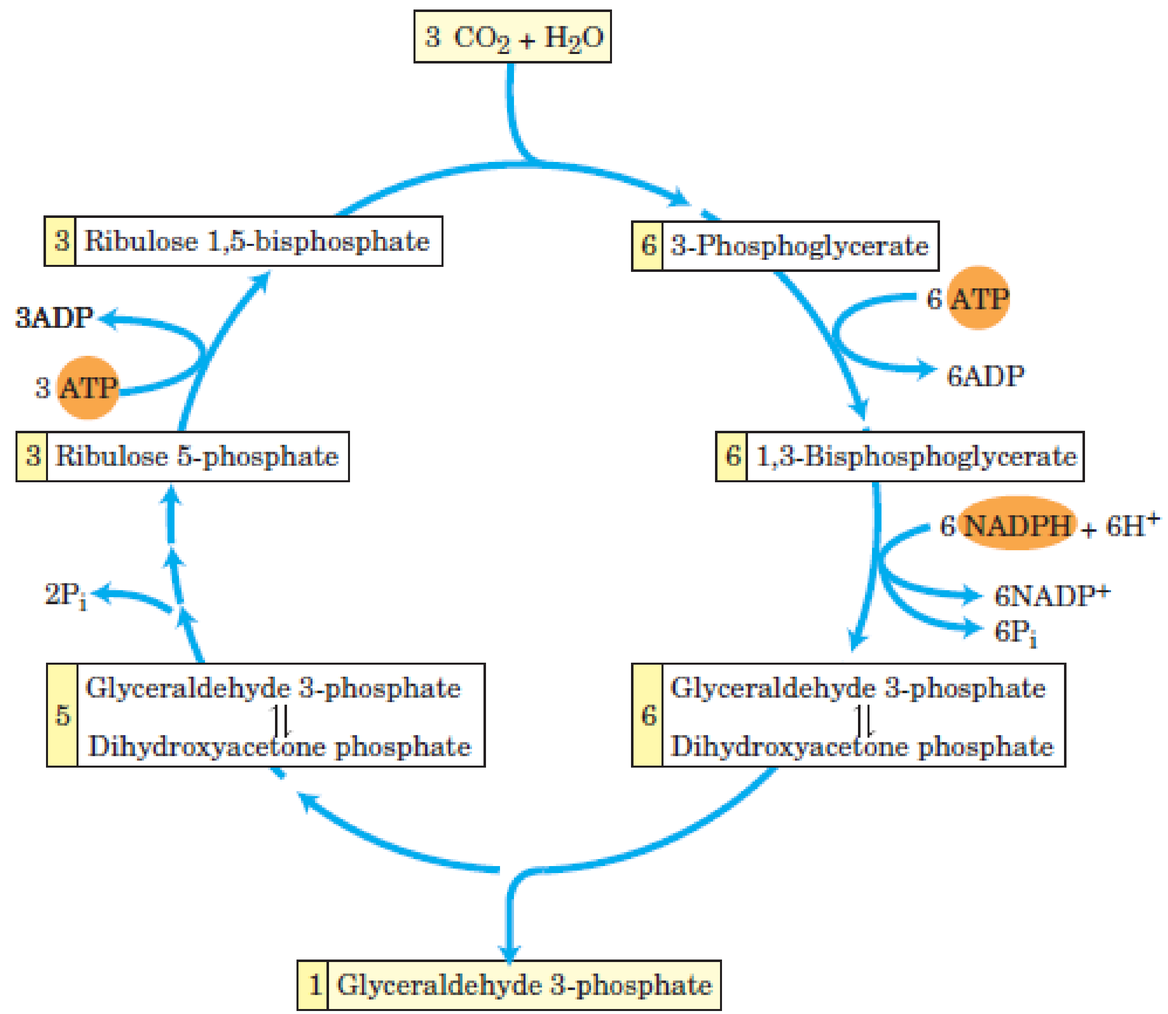
- The first reaction in the assimilation of CO_2 into triose phosphates consumes ribulose 1,5-bisphosphate and, for continuous flow of CO_2 into carbohydrate, ribulose 1,5-bisphosphate must be constantly regenerated.
- This is accomplished in a series of reactions that, together with stages 1 and 2, constitute the cyclic pathway.



Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate
① transaldolase
Fructose 1,6-bisphosphate
② fructose 1,6-bisphosphatase
P_i
Fructose 6-phosphate
Glyceraldehyde 3-phosphate
③ transketolase
Xylulose 5-phosphate
Dihydroxyacetone phosphate
Erythrose 4-phosphate
④ transaldolase
⑧ ribulose 5-phosphate epimerase
Sedoheptulose 1,7-bisphosphate
Ribulose 5-phosphate
⑤ sedoheptulose 1,7-bisphosphatase
P_i
ATP
ADP
⑨
ADP
ATP
Ribulose 1,5-bisphosphate
Glyceraldehyde 3-phosphate
Sedoheptulose 7-phosphate
⑧
Ribulose 5-phosphate
⑥ transketolase
ribulose 5-phosphate epimerase
Ribose 5-phosphate
Xylulose 5-phosphate
⑦ ribose 5-phosphate isomerase
ATP
ADP
⑨
Ribulose 5-phosphate
Ribulose 1,5-bisphosphate
ribulose 5-phosphate kinase

Summary of Calvin Cycle

$3CO_2$ + 3RuBP + $3H_2O$ + 9ATP + 6NADP + $6H^+$ ⟶ Glyceraldehyde 3-phosphate + 3RuBP + 9ADP + 8Pi + 6 $NADP^+$



Summary of Calvin Cycle

- The incorporation of one CO_2 into organic material requires three ATPs and two NADPHs.
- The formation of glucose from CO_2 may be summarized by the following equation.

$$6CO_2 + 18ATP + 12NADPH + 12H^+ + 12H_2O \longrightarrow Glucose + 18ADP + 18Pi + 12NADP^+$$

Regulation of Calvin Cycle

- There is a light-dependent regulation of the cycle enzymes, as 3rd step requires reduced NADP.
- There are two **regulation** systems at work when the **cycle** must be turned on or off:
  - the thioredoxin/ferredoxin activation system, which activates some of the **cycle** enzymes;
  - and the RuBisCo enzyme activation, active in the **Calvin cycle**, which involves its own activase.

THIOREDOXIN/FERREDOXIN ACTIVATION SYSTEM

- The thioredoxin/ferredoxin system activates the enzymes
  - glyceraldehyde-3-P dehydrogenase,
  - glyceraldehyde-3-Phosphatase,
  - fructose-1,6-bisphosphatase,
  - sedoheptulose-1,7-bisphosphatase,
  - ribulose-5-phosphatase kinase.
- This happens when light is available, as the ferredoxin protein is reduced in the photosystem electron chain when electrons are circulating through it.
- Ferredoxin then binds to and reduces the thioredoxin protein, which activates the cycle enzymes by severing a cystine bond found in all these enzymes.
- This is a dynamic process as the same bond is formed again by other proteins that deactivate the enzymes.
- The implications of this process are that the enzymes remain mostly activated by day and are deactivated in the dark when there is no more reduced ferredoxin available.

RUBISCO ENZYME ACTIVATION

- The enzyme Rubisco has its own activation process, which involves a more complex process.
- It is necessary that a specific lysine amino acid be carbamylated in order to activate the enzyme.
- This lysine binds to RuBP and leads to a non-functional state if left uncarbamylated.
- A specific activase enzyme, called Rubisco activase, helps this carbamylation process by removing one proton from the lysine and making the binding of the carbon dioxide molecule possible.
- Even then the Rubisco enzyme is not yet functional, as it needs a magnesium ion to be bound to the lysine in order to function.
- This magnesium ion is released when the inner pH drops due to the active pumping of protons from the electron flow.
- Rubisco activase itself is activated by increased concentrations of ATP caused by its phosphorylation.
- RuBisCO is only active during the day as its substrate, ribulose 1,5-bisphosphate, is not generated in the dark.

Questions

- What is autotrophy? Write down the various pathways of autotrophic nutrition found in prokaryotes.
- Write an essay on calvin cycle (C_3-pathway).
- Discuss regulation of calvin cycle
- Diagrammatically represent the calvin cycle.