Department of Chemistry and Biochemistry
University of Lethbridge

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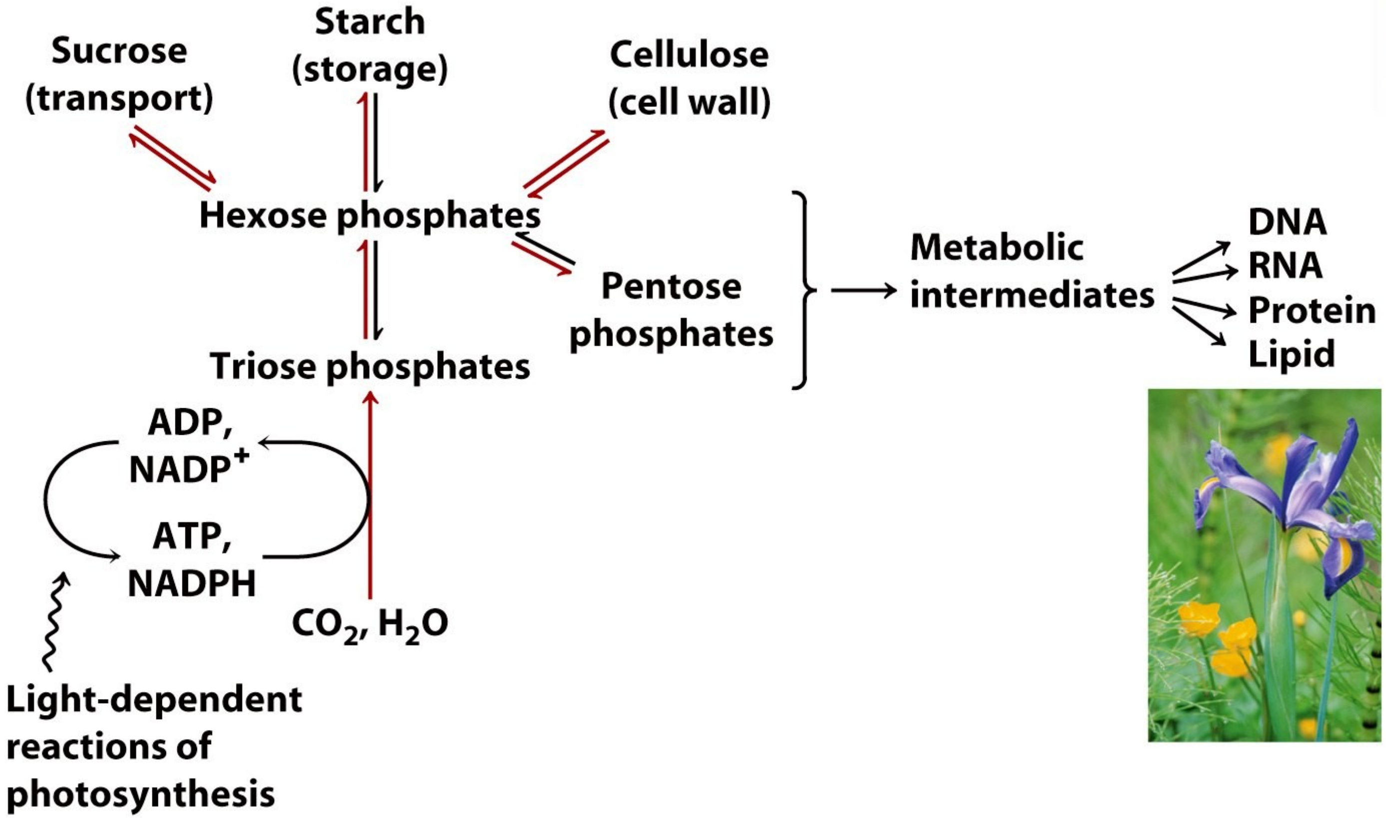
FIAT LUX

III. Metabolism

Carbohydrate Synthesis

CO_2 Assimilation

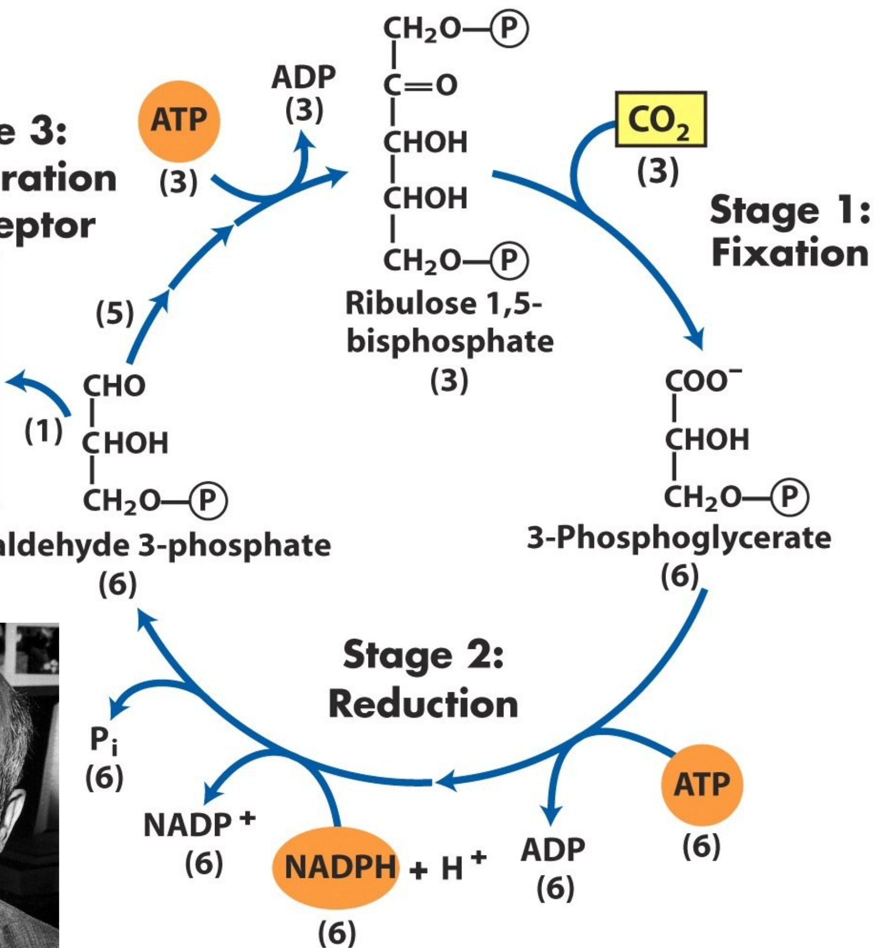


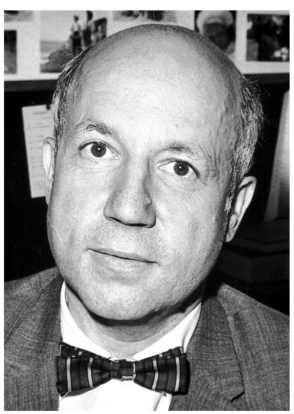
Autotrophic plants convert CO_2 into organic compound (triose phosphates)

Biosynthesis occurs in chloroplasts

CO_2 fixation requires ATP and NADPH

Carbon Assimilation





Melvin Calvin, 1911–1997

The Calvin cycle

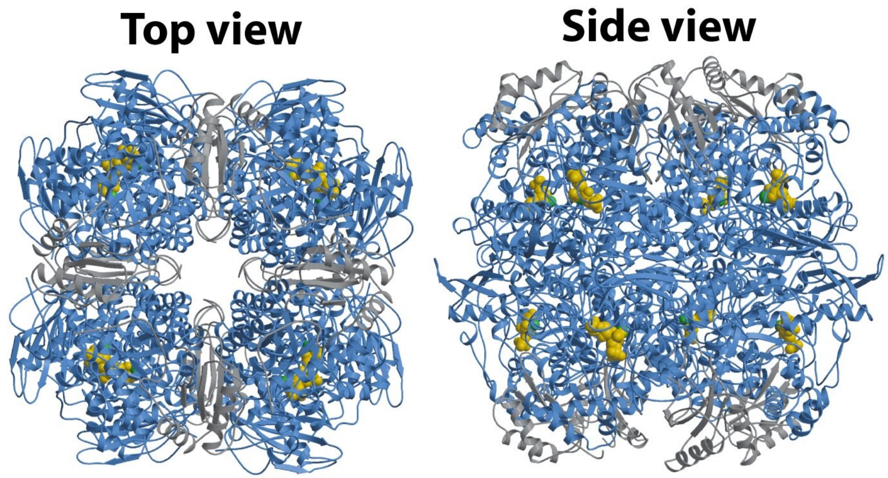
→ cyclic three <u>stage</u> process (many steps)

I) Fixation:
CO_2 is condensed with 5C-sugar (R15BP) to yield two 3C sugars

II) Reduction:
R15BP at the expense of ATP and NADPH

III) Regeneration:
Six 3C sugars to three 5C sugars to keep cycle going
→ regeneration of R15BP

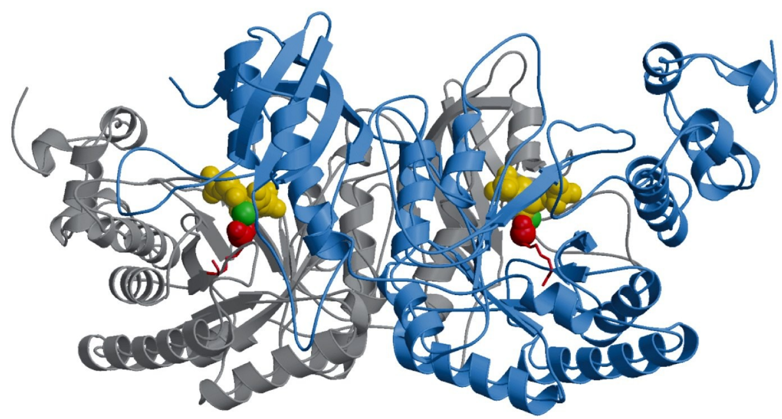
Stage 1) Carbon Fixation - RUBISCO



Spinach RUBISCO

Carbon fixation requires the enzyme Ribulose 1,5-bisphosphate carboxylase/oxygenase → RUBISCO

Most abundant protein in nature.



Bacterial RUBISCO

Two enzymatic activities:

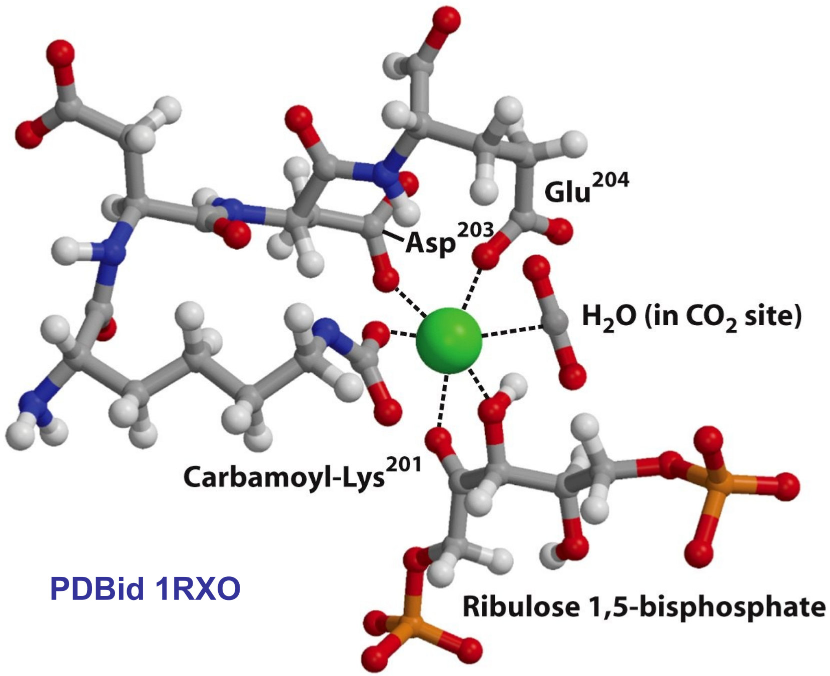
a) Covalently attaches CO_2 to R1,5BP

b) cleaves 6C sugar into 2x 3C sugars (3-phosphoglycerate)

More Later: RUBISCO also has an undesirable oxygenase activity (ie. fixes O_2 not CO_2)

Bacterial RUBISCO is simpler.
→ similar intersubunit contacts

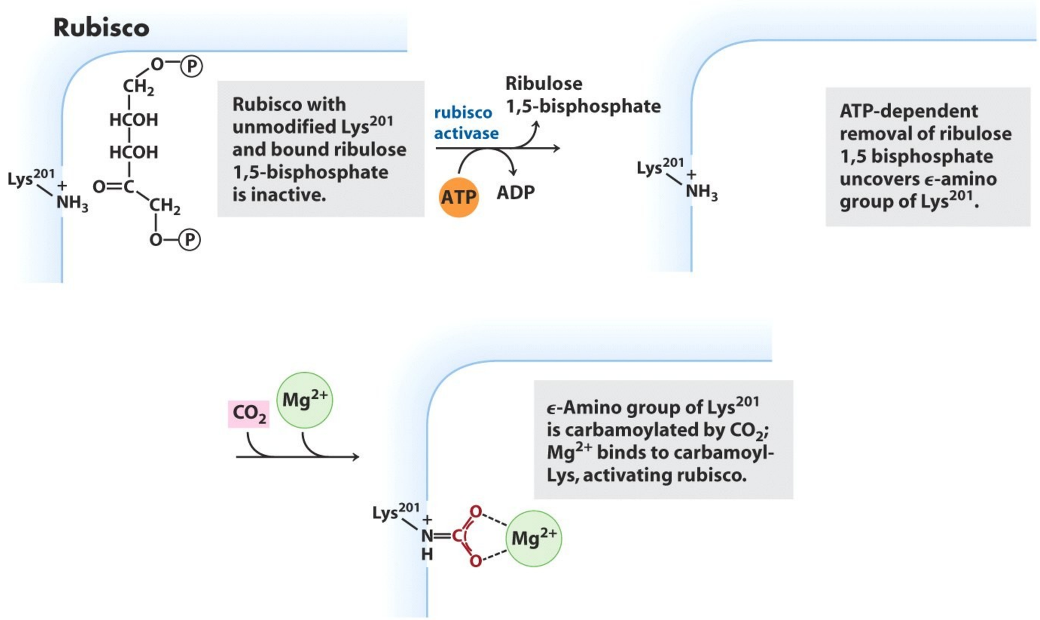
Catalytic Mechanism of RUBISCO



PDBid 1RXO

RUBISCO needs to be activated by the addition of CO_2 to an active-site Lysine.

Activation is catalyzed by **RUBISCO activase**



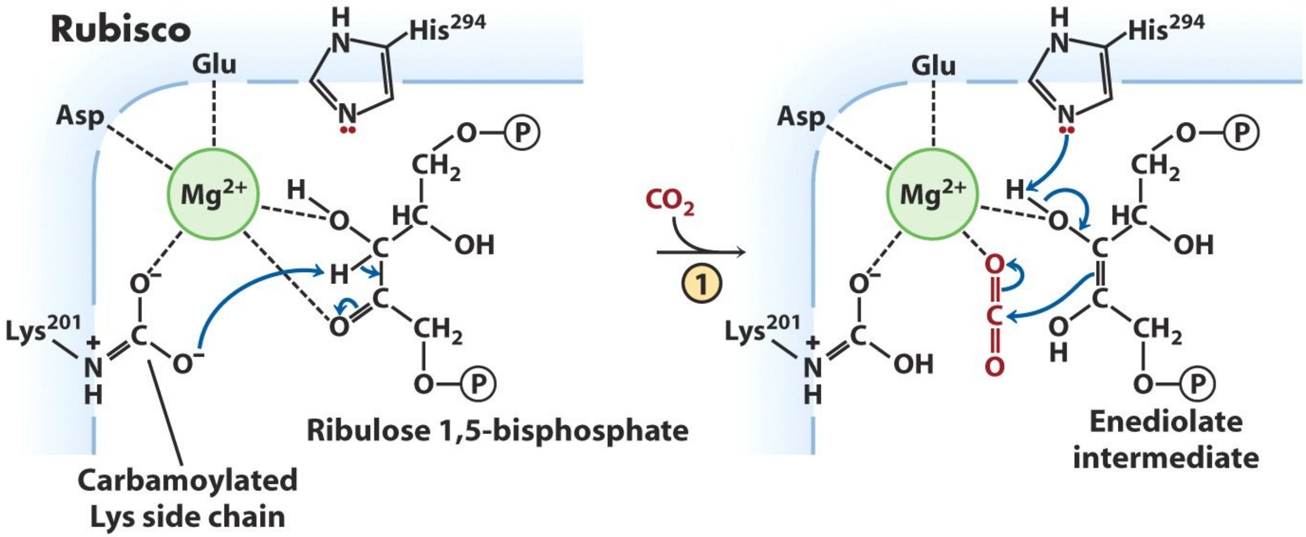
RUBISCO activase

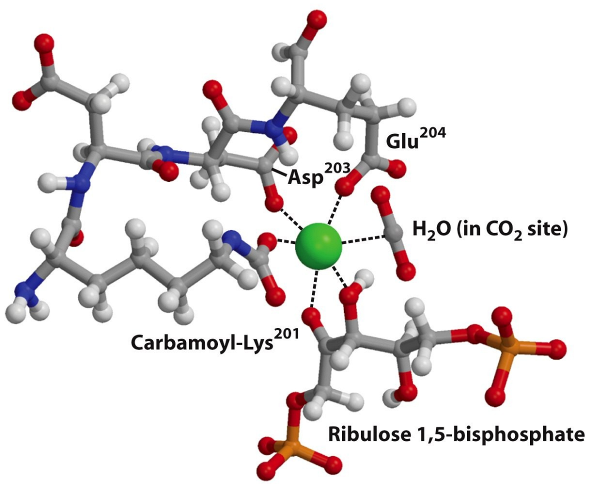
a) displaces R1,5BP of inactive RUBISCO

b) allows CO_2 to bind

CO_2 then reacts with Lys201 of RUBISCO and Mg^{2+} binds to carbamoyl-Lys

Catalytic Mechanism of RUBISCO



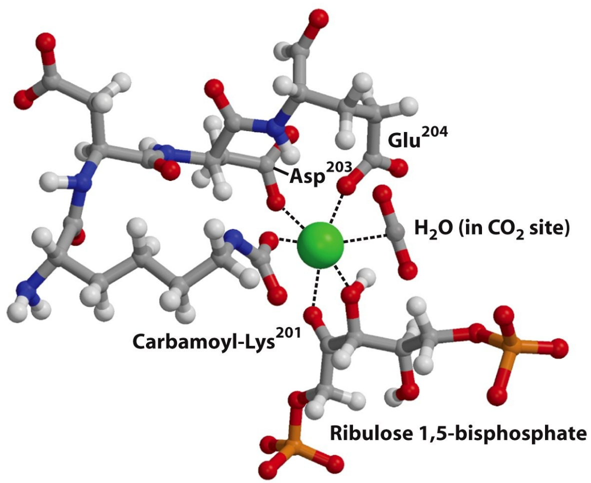


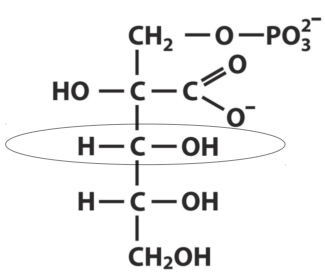
Carbamoylated lysine, stabilized by Mg^{2+}, abstracts a H^+ from the central carbon of R15BP.
→ (forms a carbanion that is) stabilized as an endiol intermediate

Endiol attacks CO_2 forming C-C bond

Catalytic Mechanism of RUBISCO

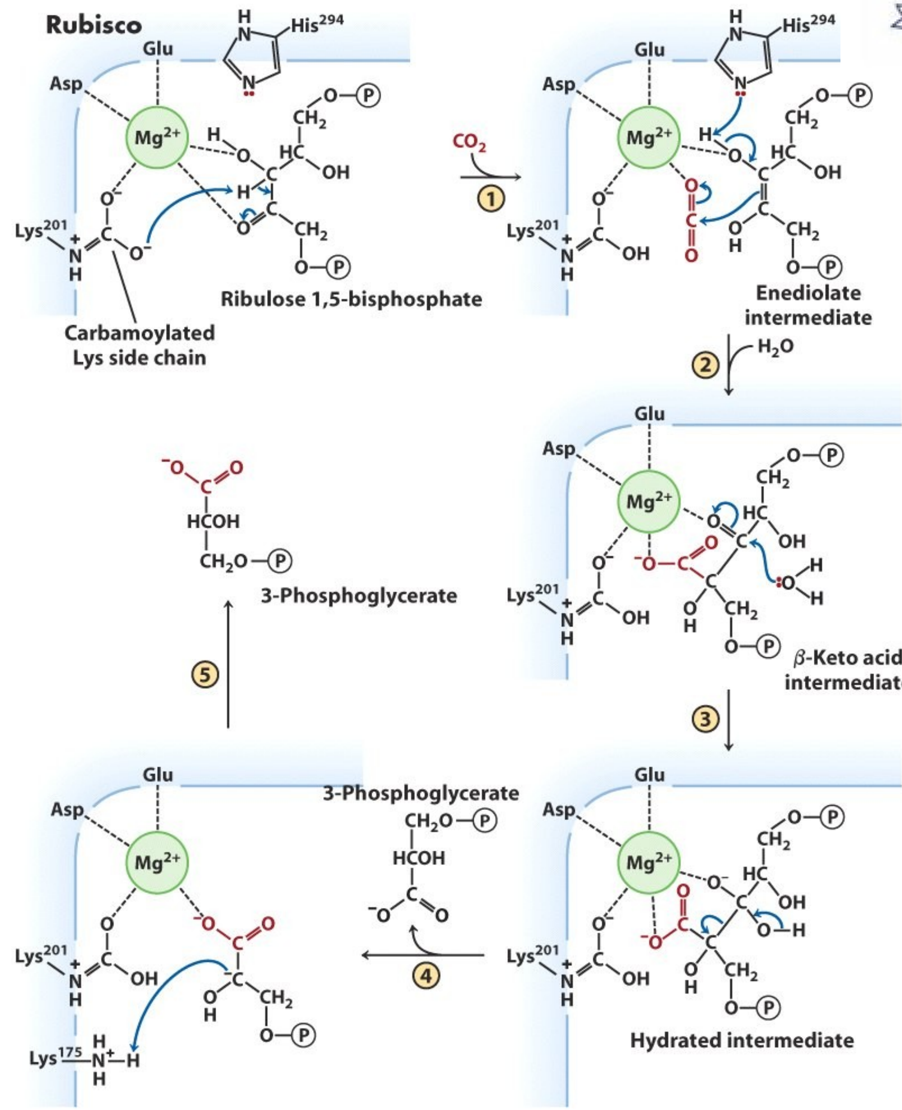
Mg²⁺ plays a central role in the catalytic mechanism at all stages.



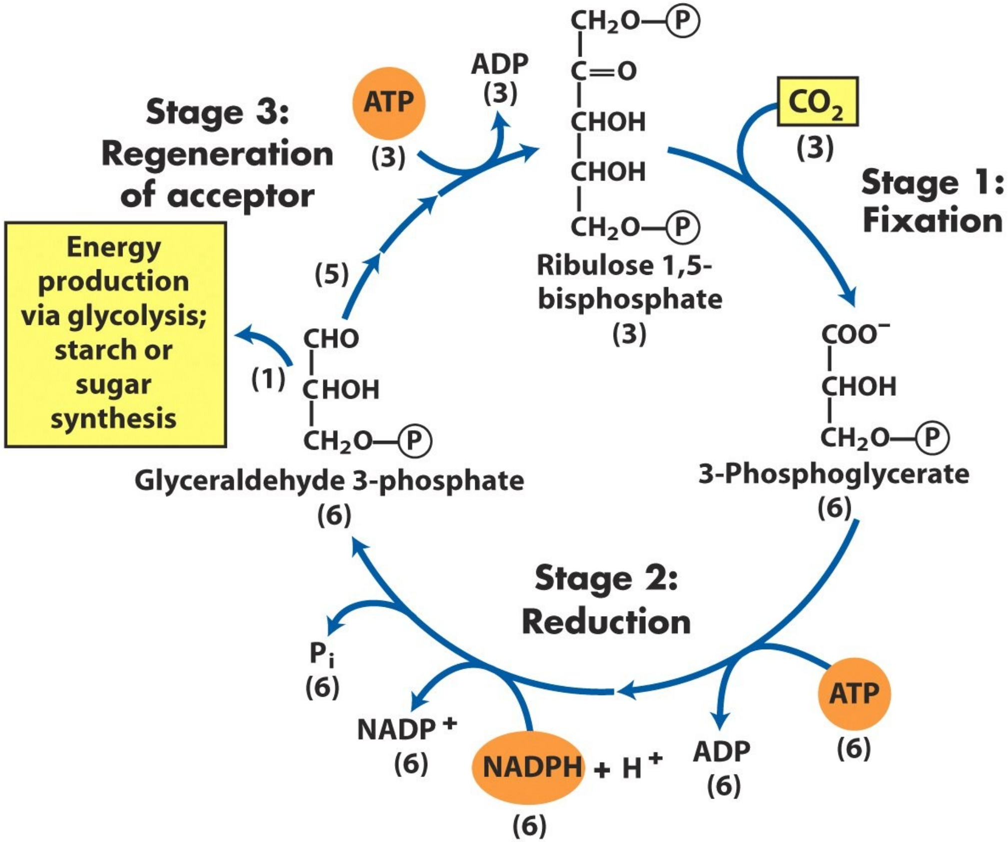


2-Carboxyarabinitol 1-phosphate

RUBISCO inhibitor



Calvin Cycle – Stage 2



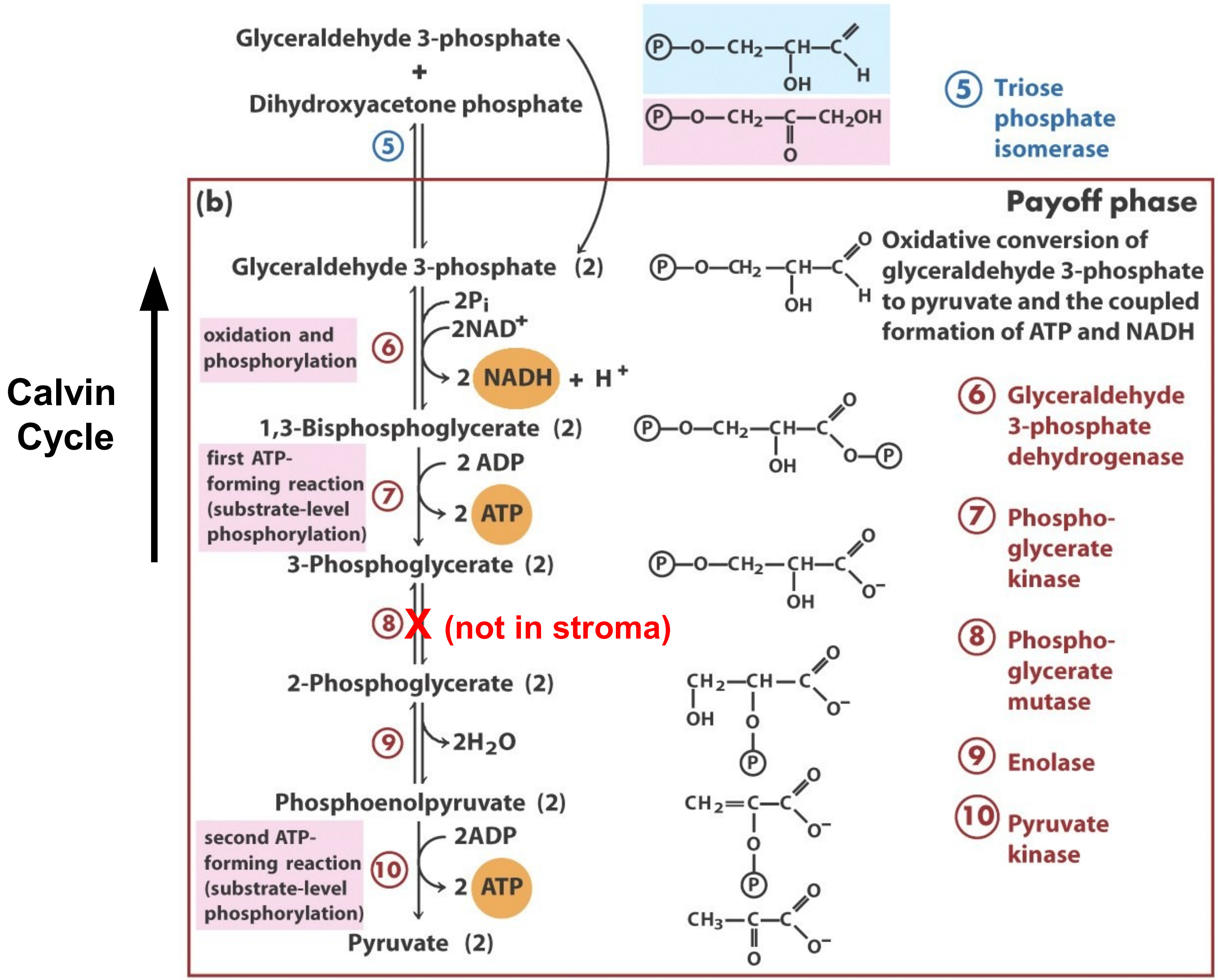
The second stage is a reduction
→ set of two reaction

Reversal of the equivalent steps in glycolysis
→ BUT NADPH is the cofactor

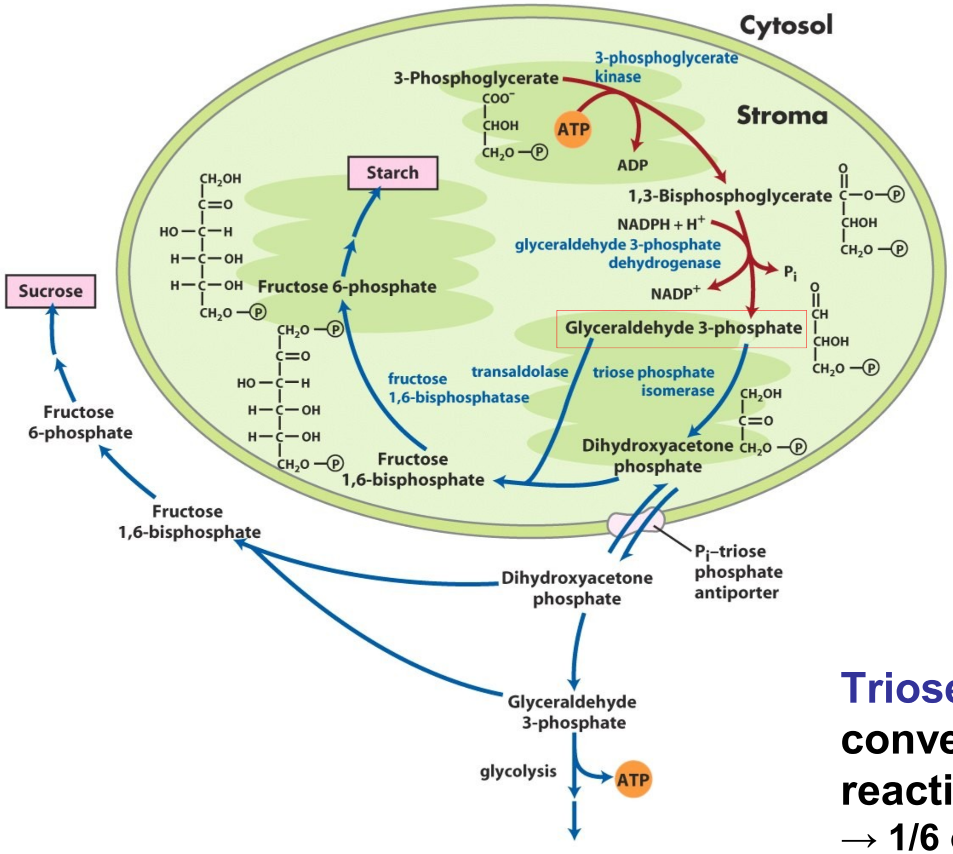
Chloroplast stroma contains all glycolytic enzymes except phosphoglycerate mutase (3PG → 2PG)

Stromal and cytosolic enzymes are isozymes, they catalyze the same reaction but are products of different genes.

Calvin Cycle – Stage 2



Fate of Glyceraldehyde 3-phosphate



Stage 1 – R15BP + CO2 → 2 3PG
Stage 2 – 3PG → G3P
Stage 3 – 6 G3P → 3 R15BP + DHAP

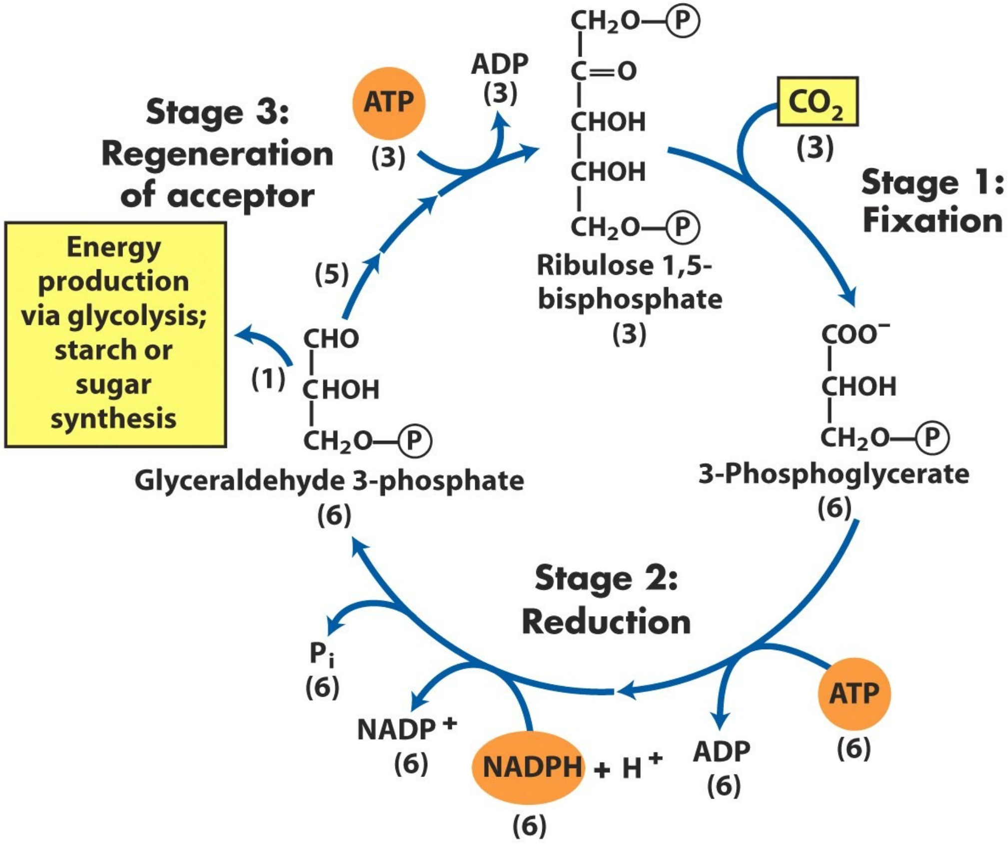
5 of 6 G3P molecules produced are converted back to 3 R15BP

1 in 6 G3P molecules produced is exported to cytosol as DHAP (anabolism/catabolism)

Triose phosphate isomerase (isozyme) converts G3P to DHAP for transaldolase reaction

→ 1/6 of G3P exported (as DHAP) for gluconeogenesis or glycolysis

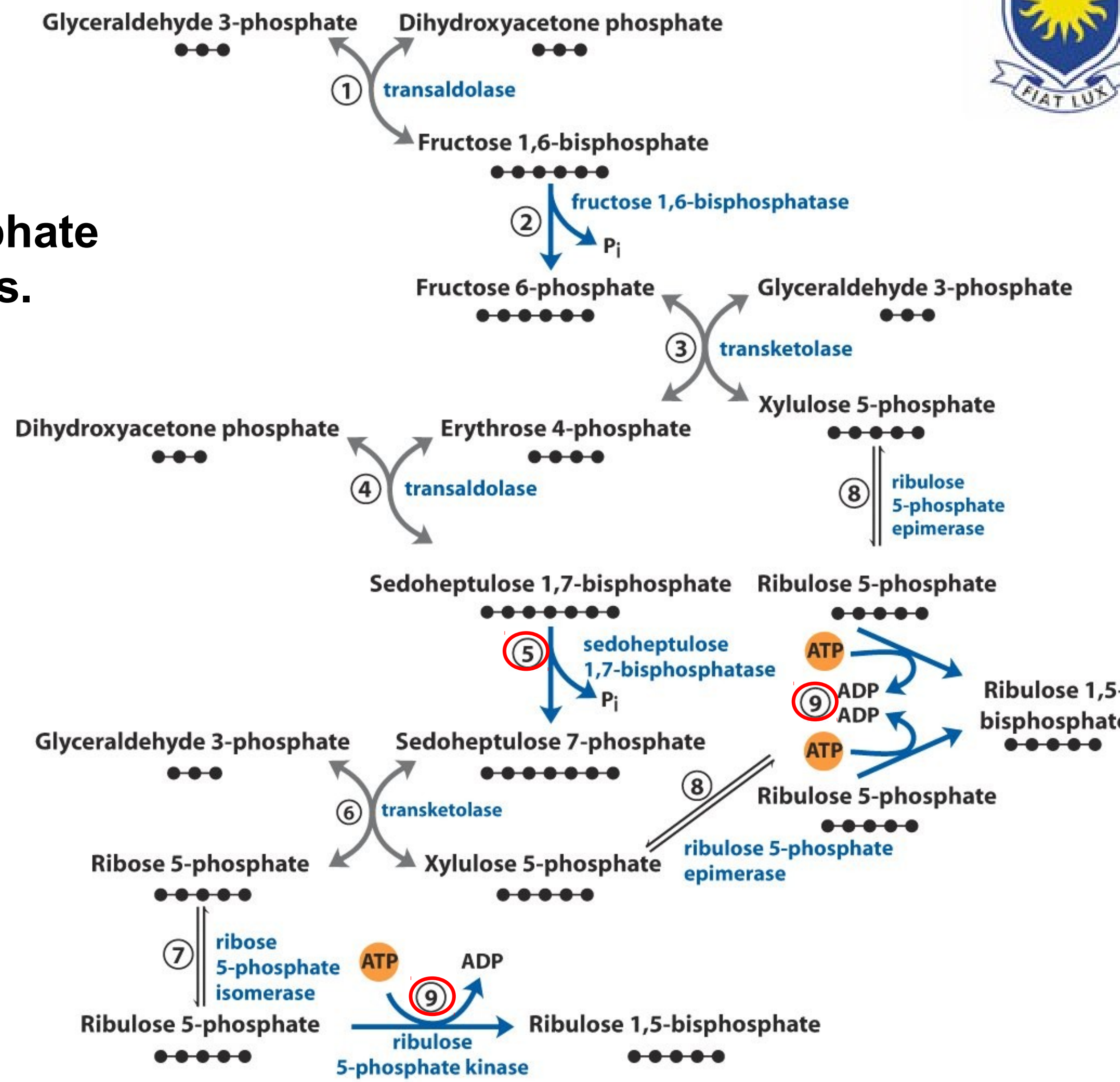
Calvin Cycle – Stage 3



The third stage is the regeneration of the CO_2 acceptor (R15BP)

→ multiple reactions that convert 6 3C-sugars to 3 5C sugar and a 3C-sugar

Calvin Cycle – Stage 3

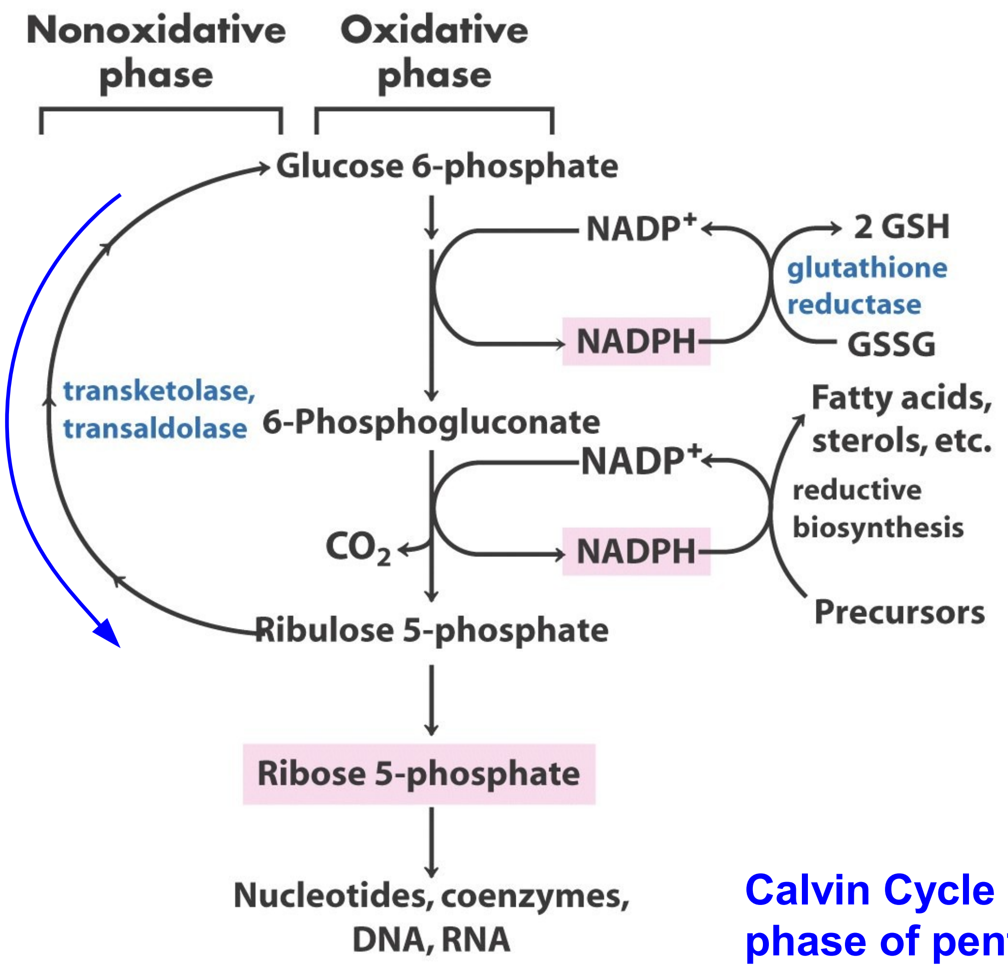


Regeneration of Ribulose 1,5-Bisphosphate from triose phosphates.

Similar to the pentose phosphate pathway in reverse

Enzymes 5 & 9 (right) Are unique

Pentose Phosphate Pathway (review)



Alternative path to oxidize Glucose.

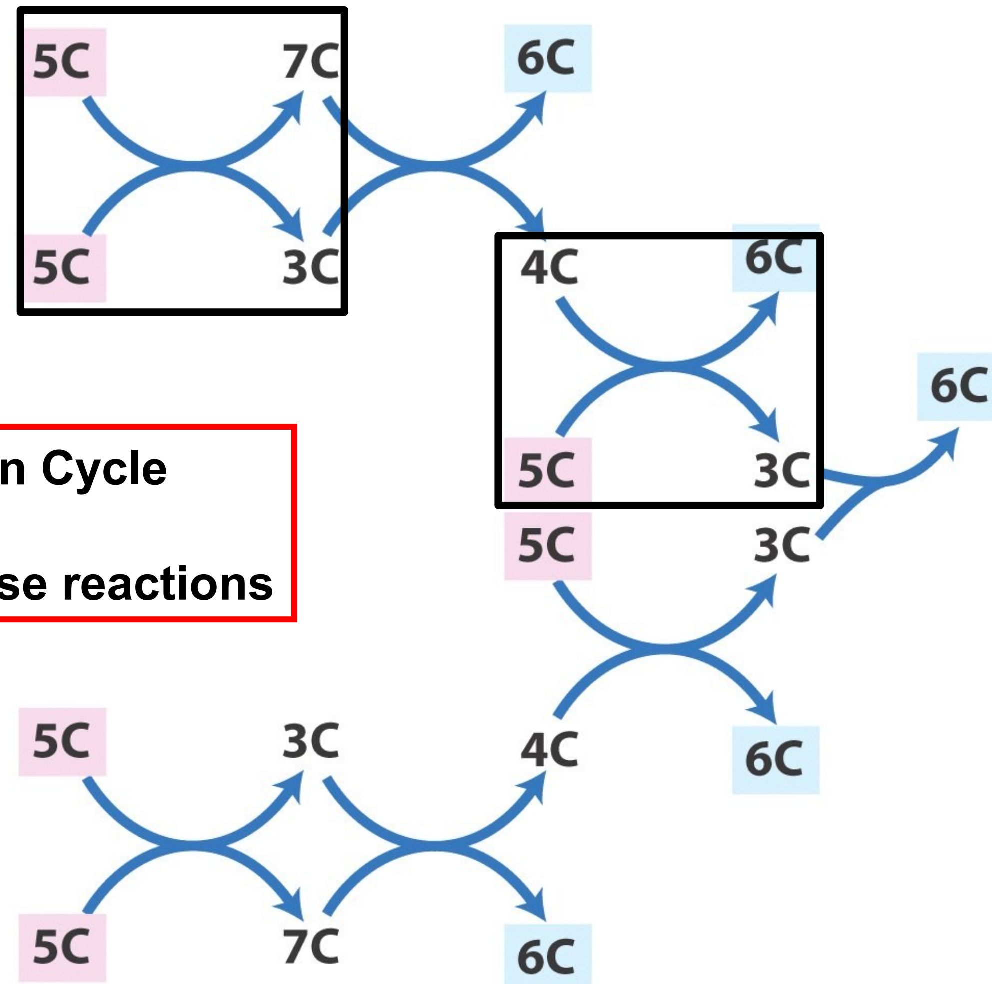
The electron acceptor is $NADP^+$.

NADPH is needed for reductive biosynthesis.

Products are pentose phosphates.

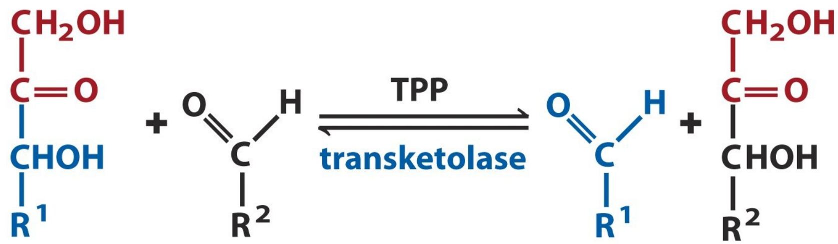
Calvin Cycle (stage 3) is similar to nonoxidative phase of pentose phosphate pathway <u>in reverse</u>

Nonoxidative Reactions of the Pentose Phosphate Pathway



Reaction reversed in Calvin Cycle (stage 3) are boxed
- both transketolase reactions

Transketolase

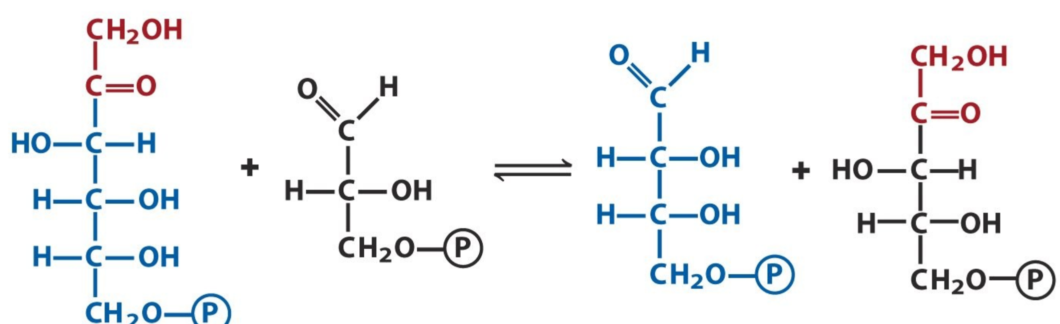


Ketose donor — Aldose acceptor

Transketolase → **transfer of 2-carbon group**
→ **TPP-mediated**

Reaction (below) is reversed in Calvin-Cycle:

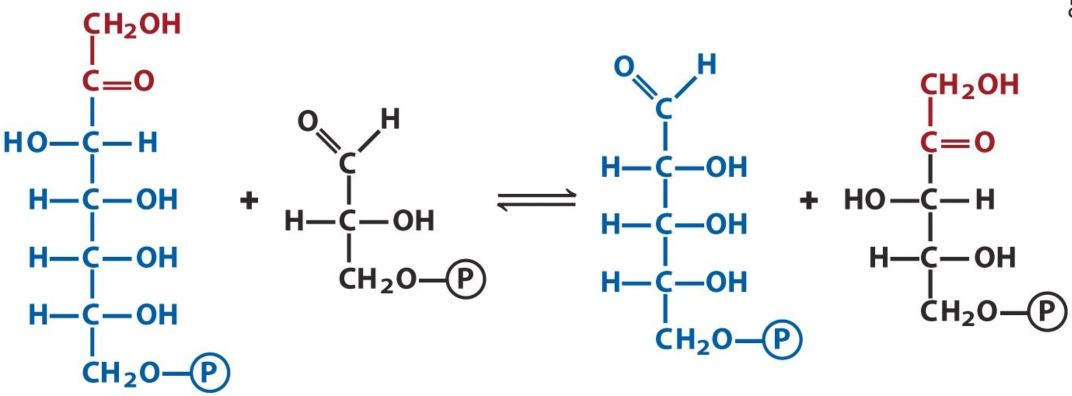
Conversion of 6C and 3C sugars to 4C and 5C sugars



Fructose 6-phosphate — Glyceraldehyde 3-phosphate — Erythrose 4-phosphate — Xylulose 5-phosphate

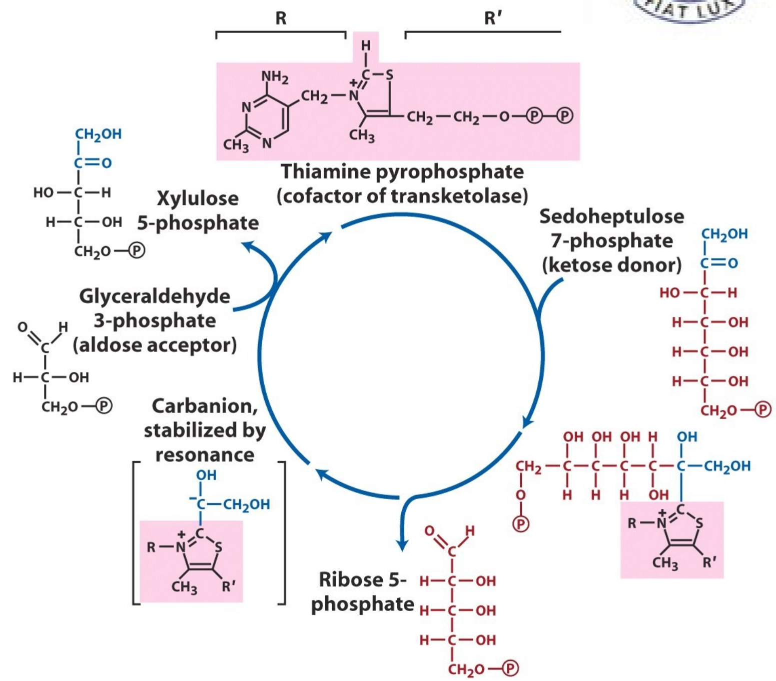
Transketolase

Reaction (below) is reversed in Calvin-Cycle:



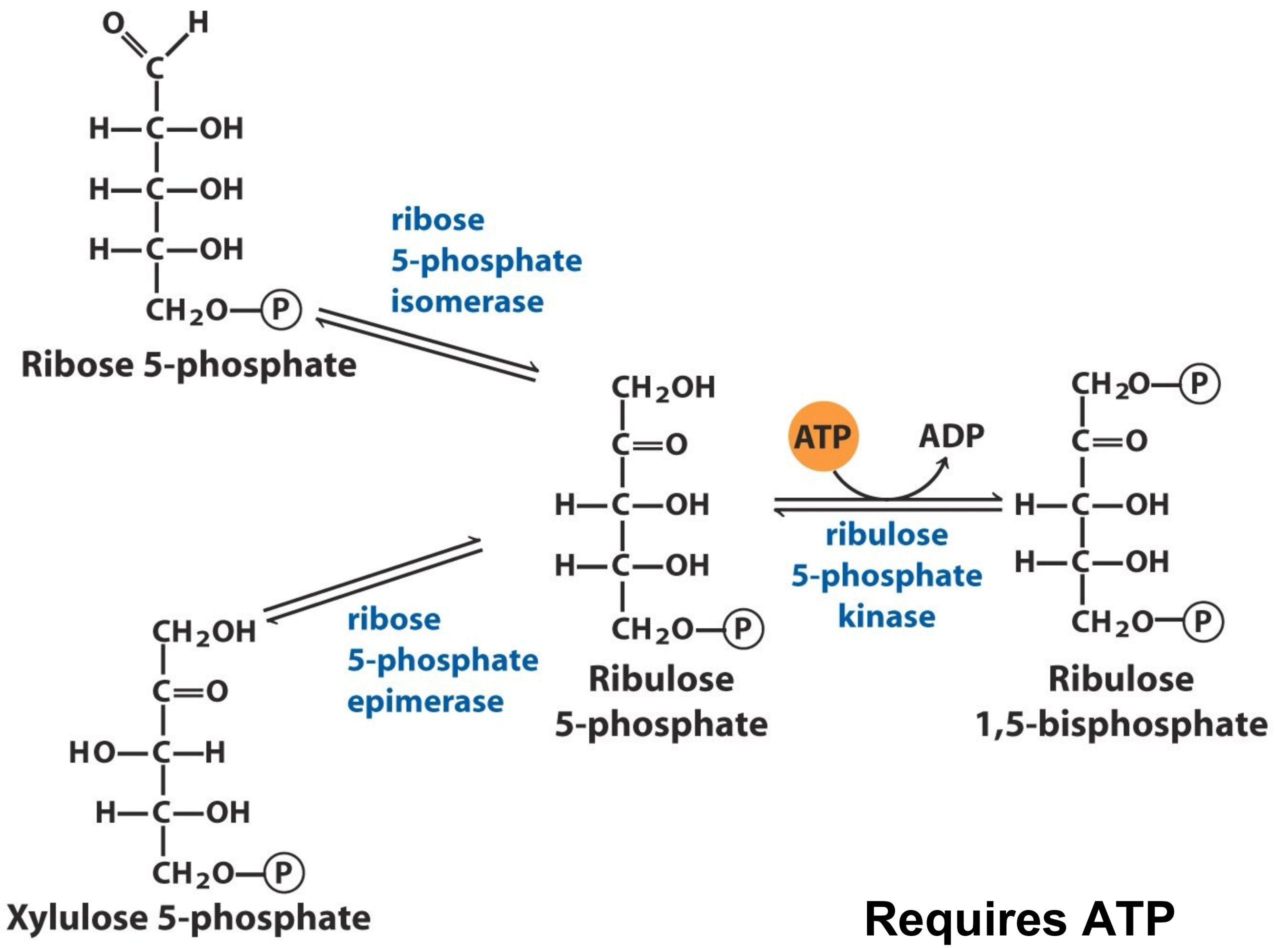
Sedoheptulose 7-phosphate | Glyceraldehyde 3-phosphate | Ribose 5-phosphate | Xylulose 5-phosphate

Conversion of 7C and 3C sugars to two 5C sugars.



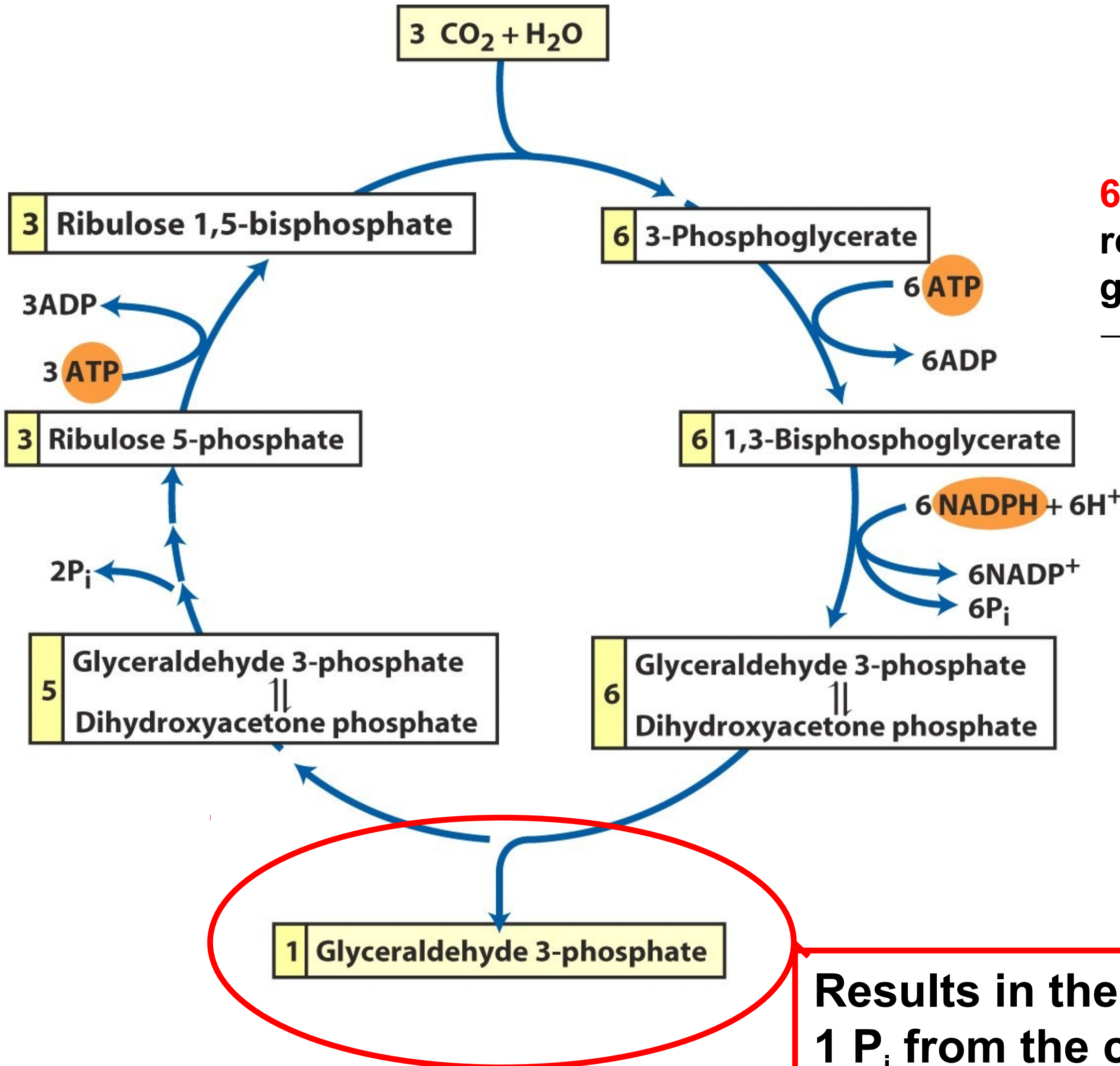
Transketolase reaction mechanism
- **requires TPP cofactor**

Regeneration of ribulose 1,5-bisphosphate



Requires ATP

Calvin Cycle Stoichiometry

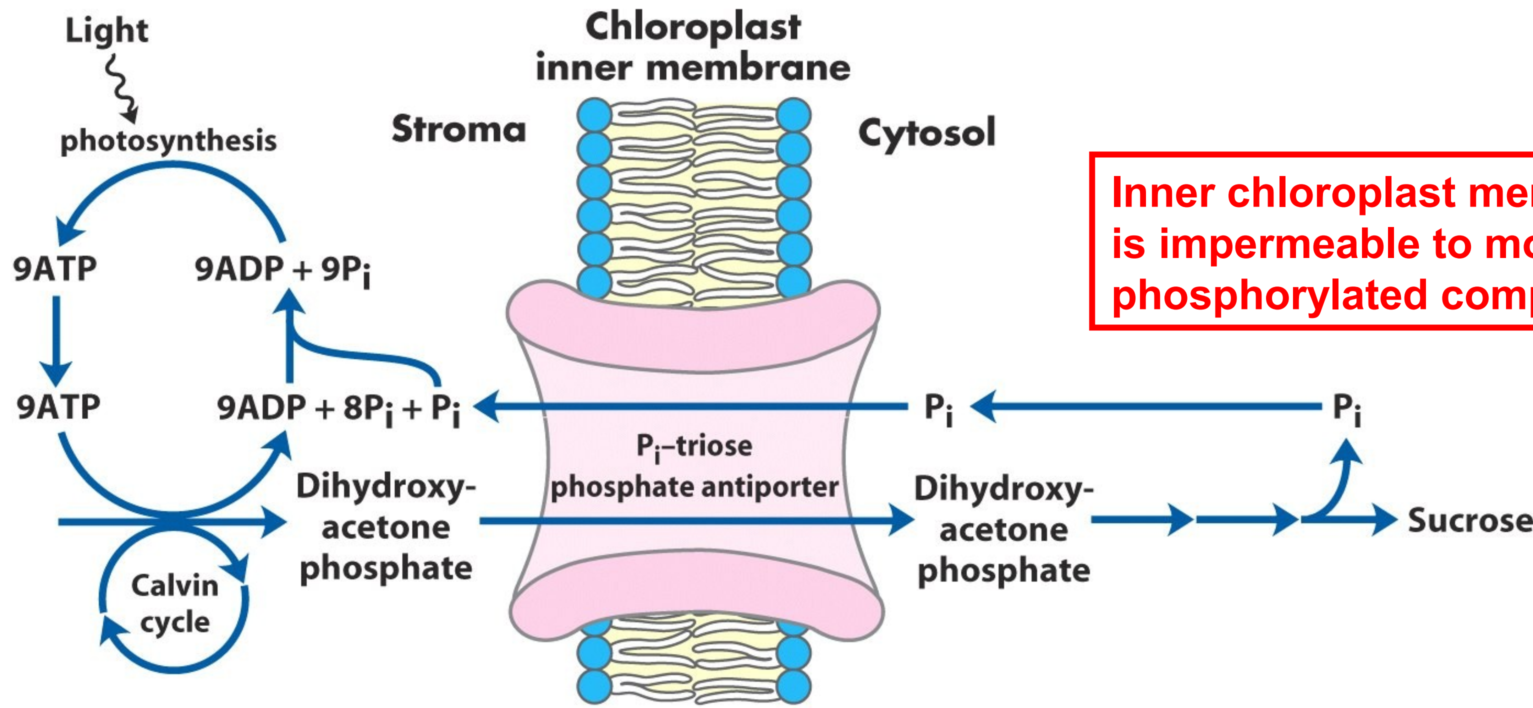


6 NADPH and 9 ATP are required to produce a single glyceraldehyde-3-phosphate → 2:3 ratio

Results in the net loss of 1 P_i from the chloroplast

Phosphate Import & Triose Sugar Export

Inner chloroplast membrane is impermeable to most phosphorylated compounds



P$_i$–triose phosphate antiporter

Glyceraldehyde 3-phosphate produced by Calvin cycle is converted to dihydroxyacetone phosphate by triose phosphate isomerase

→ DHAP$_{(stroma\text{->}cytosol)}$ and P$_{i\ (cytosol\text{->}stroma)}$ are exchanged 1:1

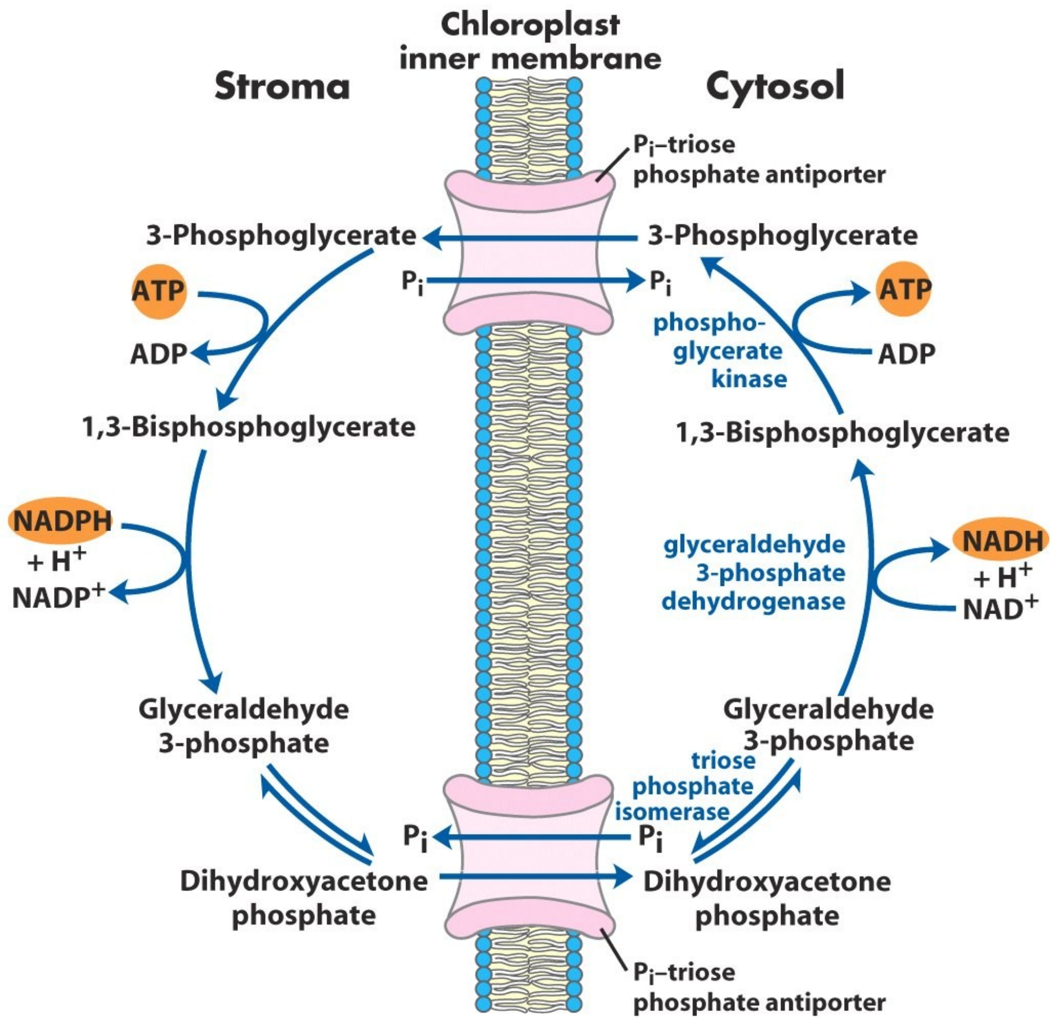
What About ATP and NADPH?

ATP and NADPH cannot cross the chloroplast membrane.

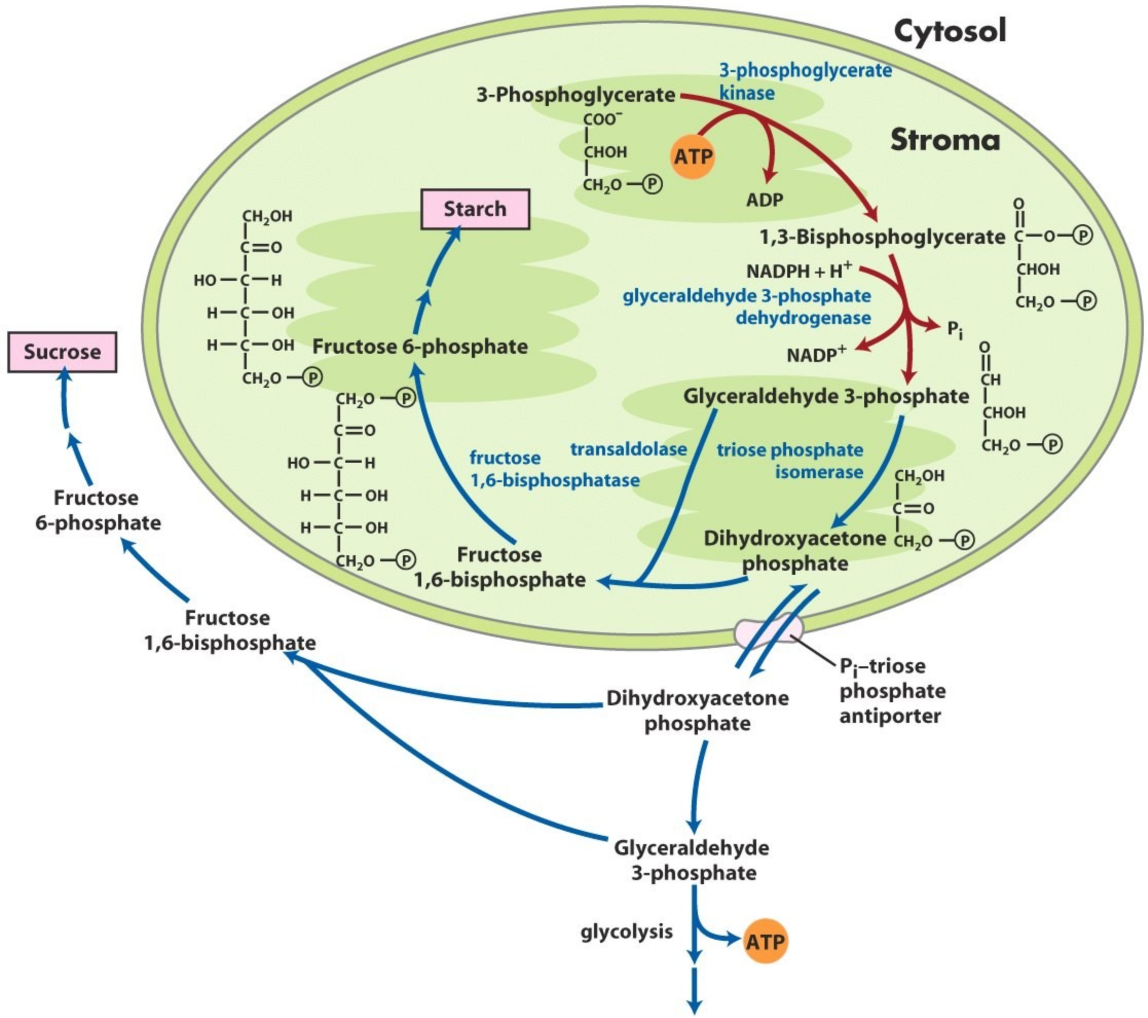
P_i-triose phosphate antiport system 'indirectly' moves ATP and reducing equivalents across the membrane.

DHAP is transported to the cytosol where it is converted to 3-phosphoglycerate (+ ATP + NADH)

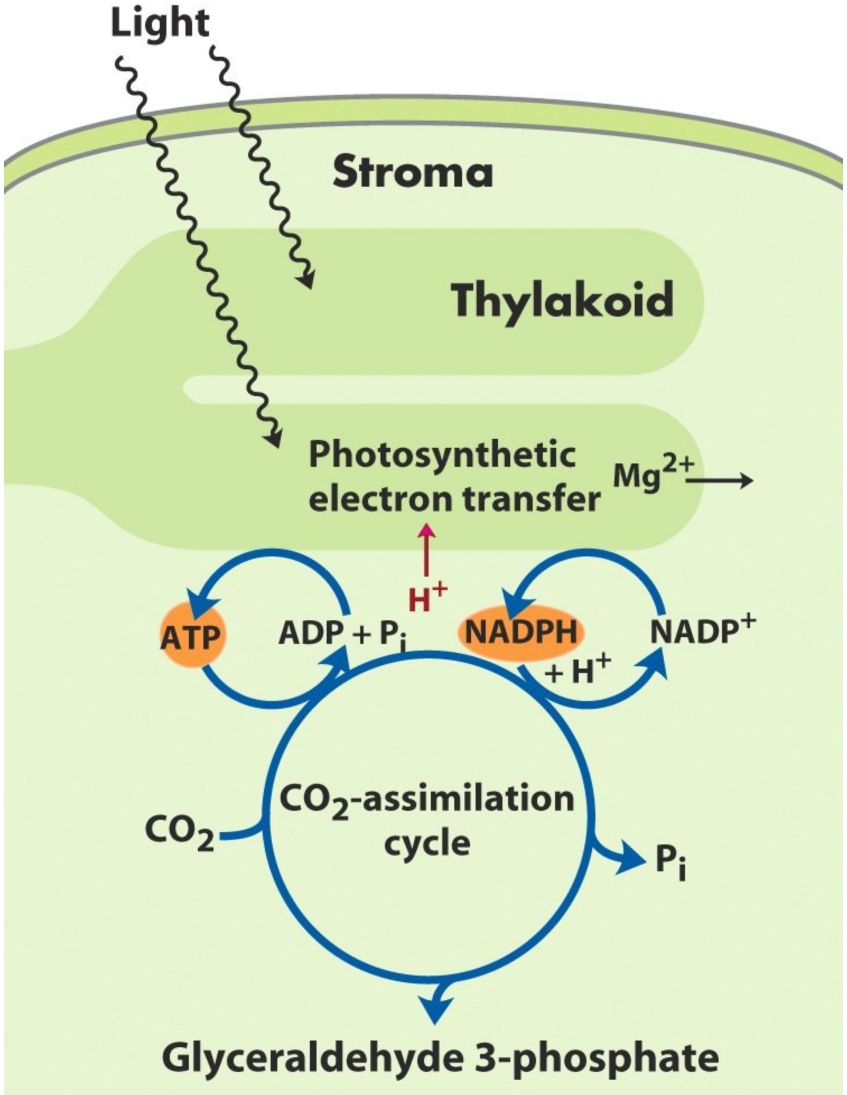
→ glycolytic enzymes



Dark Reaction Revisited: Light Regulated?



Light Regulation of Dark Reaction



Illumination of chloroplasts leads to
→ transport of H^+ into the thylakoid
→ results in Mg^{2+} transport to the stroma

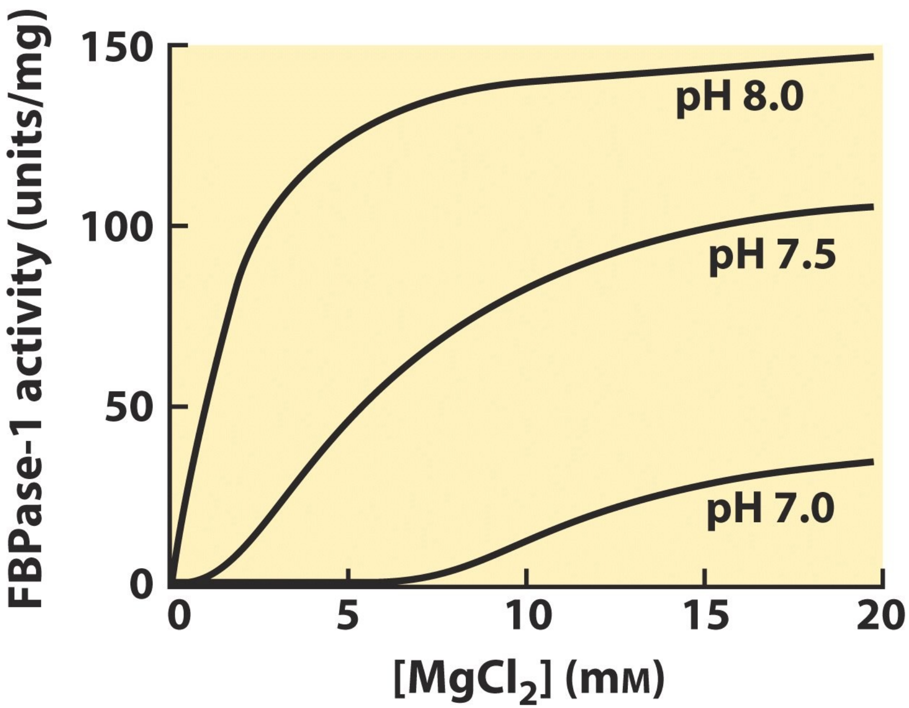
→ increase in [NADPH]

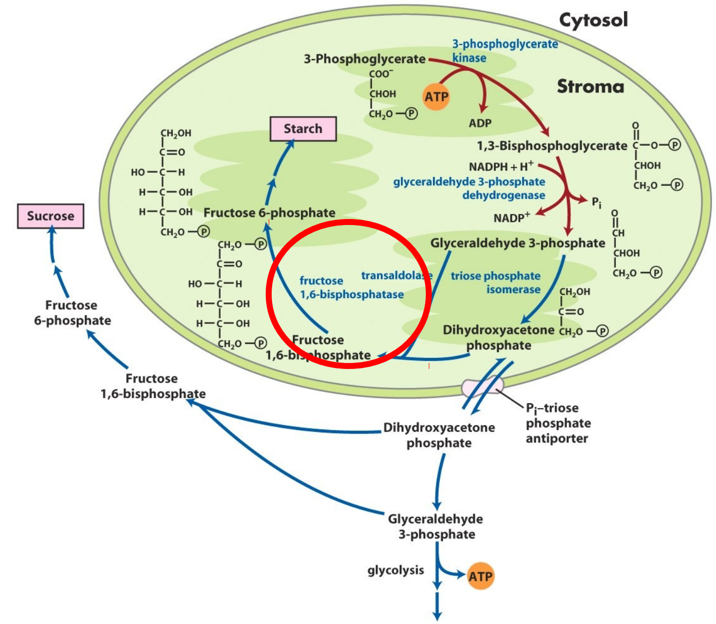
These signals are used to regulate stromal enzymes.

RUBISCO activation (carbamoylysine) is faster at alkaline pH.

High Mg^{2+} concentration favors formation of the active RUBISCO complex.

(more) Light Regulation of The Dark Reactions





Fructose 1,6-bisphosphatase requires Mg^{2+} and is very dependent on pH.

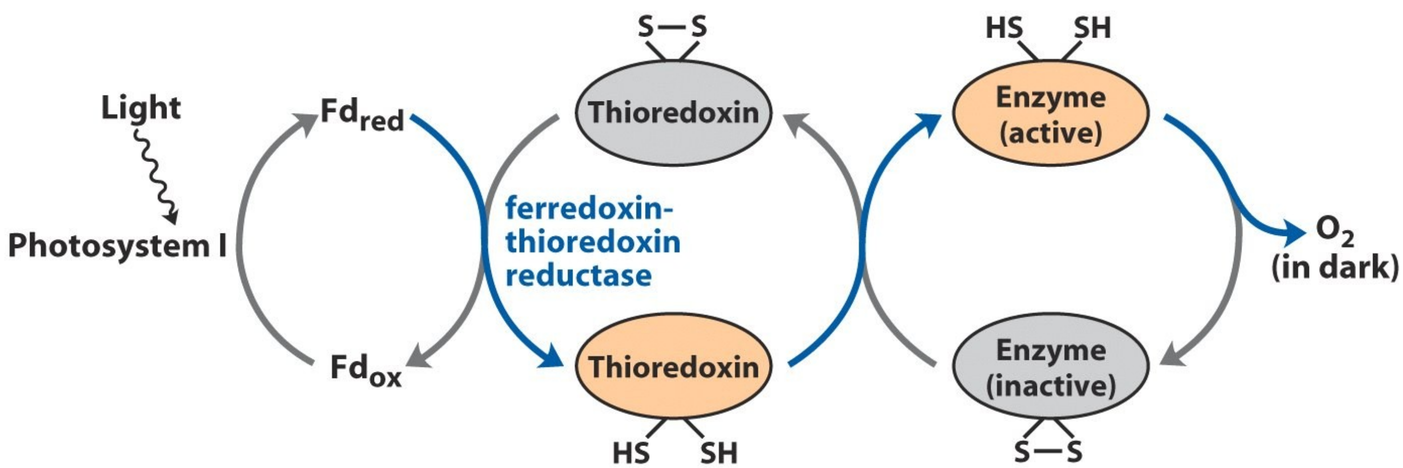
→ its activity increases more the 100 fold when pH and [Mg^{2+}] rise

Note: Calvin cyle (Dark Reaction) is more active in the presence of light

(more) Light Regulation of The Dark Reactions

Light reactions also result in electron flow through ferredoxin to thioredox (ferredoxin-thioredoxin reductase.)

→ thioredoxin activates the carbon assimilation reactions.



Reduction of disulfide activates multiple Calvin cycle enzymes:

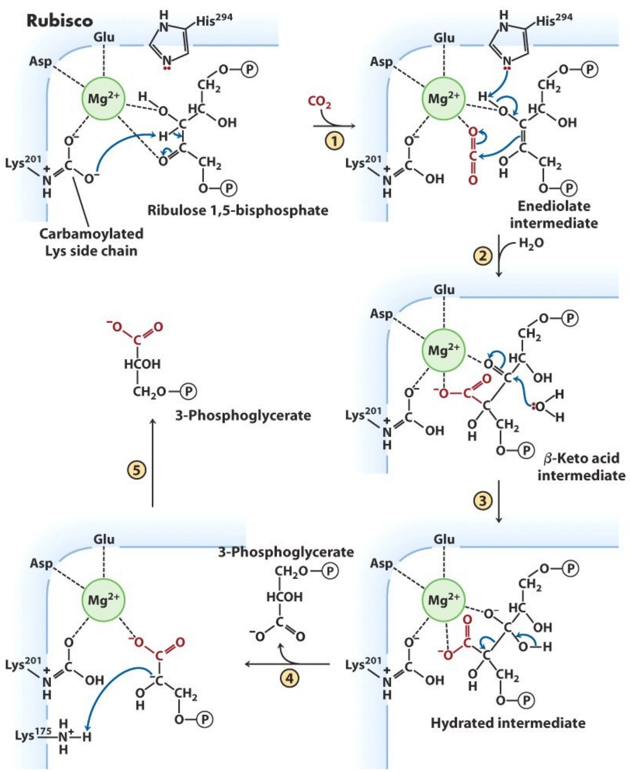
- **→ seduheptulose 1,7 bisphosphatase**
- **→ fructose 1,6 bisphosphatase**
- **→ ribulose 5-phosphate kinase**
- **→ glyceraldehyde 3-phosphate dehydrogenase**

Photorespiration

RUBISCO is not absolutely specific for CO_2 as a substrate.

→ **molecular O_2 competes with CO_2**

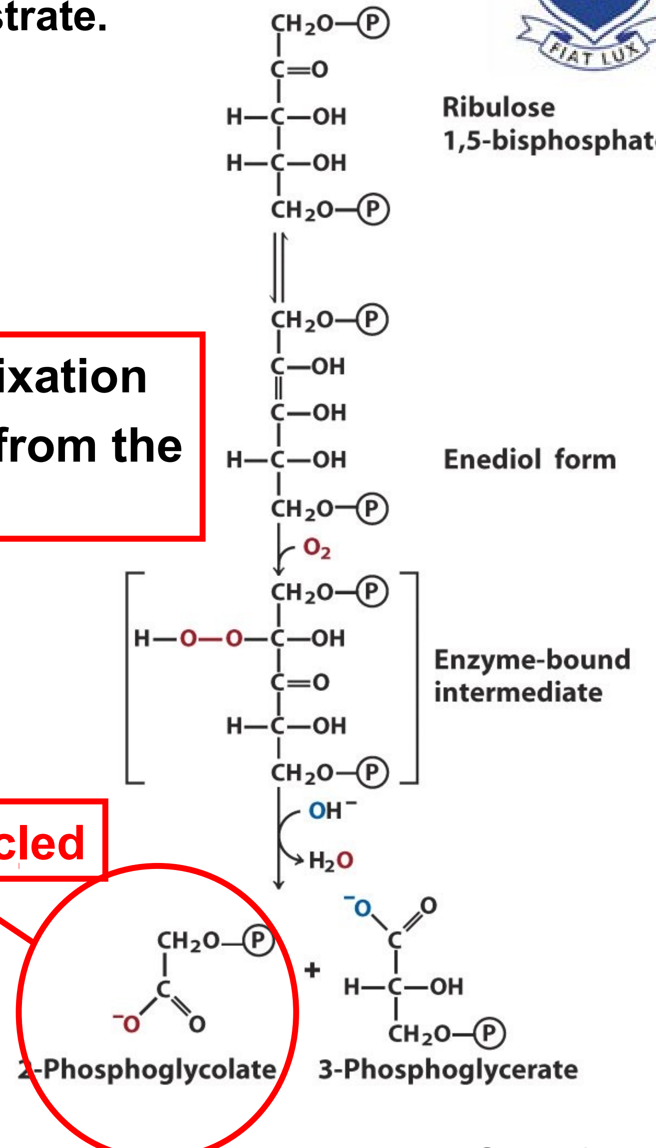
→ **about 30% of the reactions consume O_2**



Result of O_2 fixation

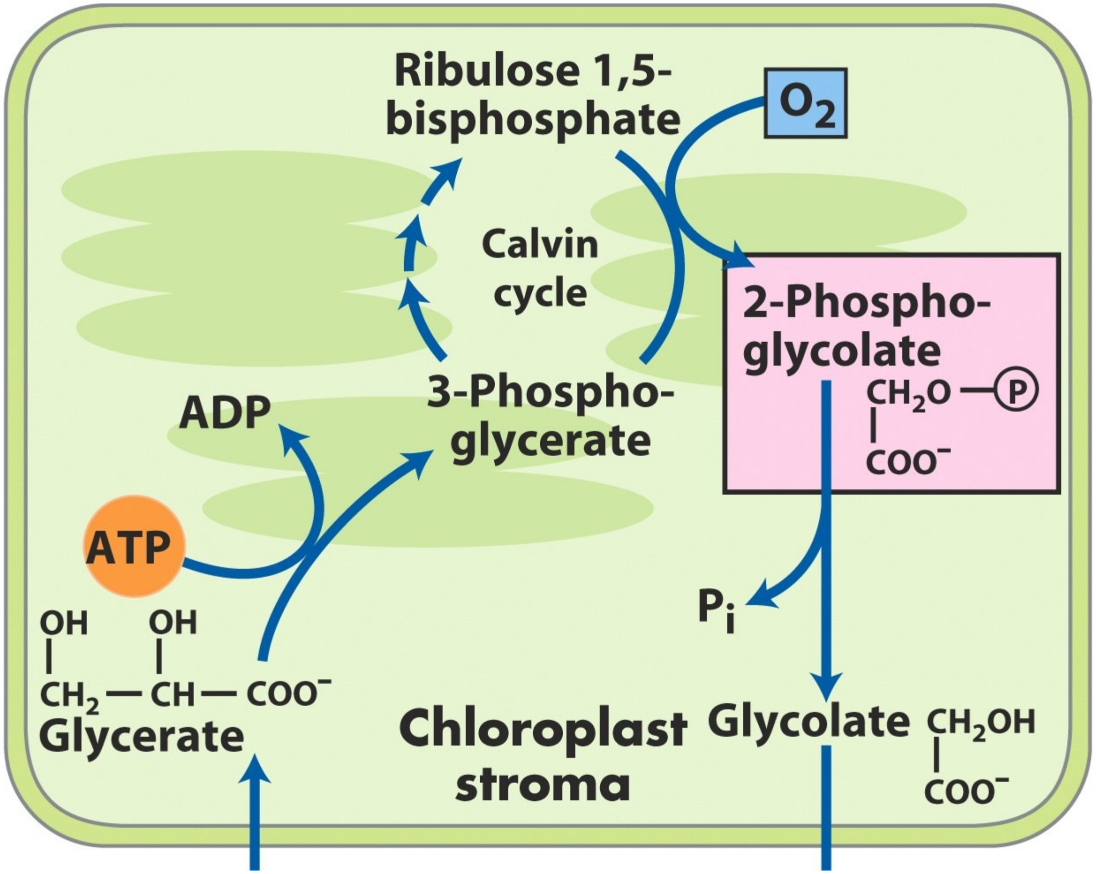
Loss of 2C's from the Calvin-cycle

Has to be recycled



The Salvage of Phosphoglycolate

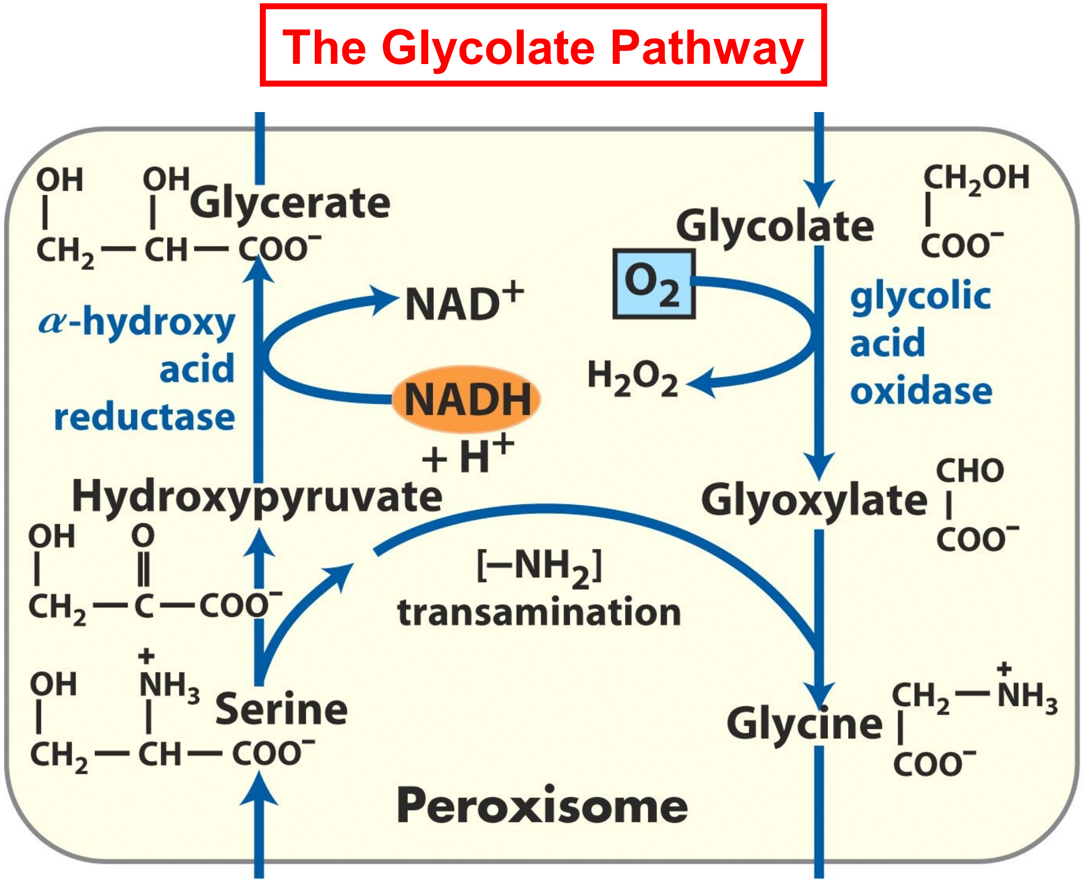
The Glycolate Pathway



In chloroplasts a phosphatase converts 2-phosphoglycolate to glycolate.

→ exported to peroxisome

The Salvage of Phosphoglycolate



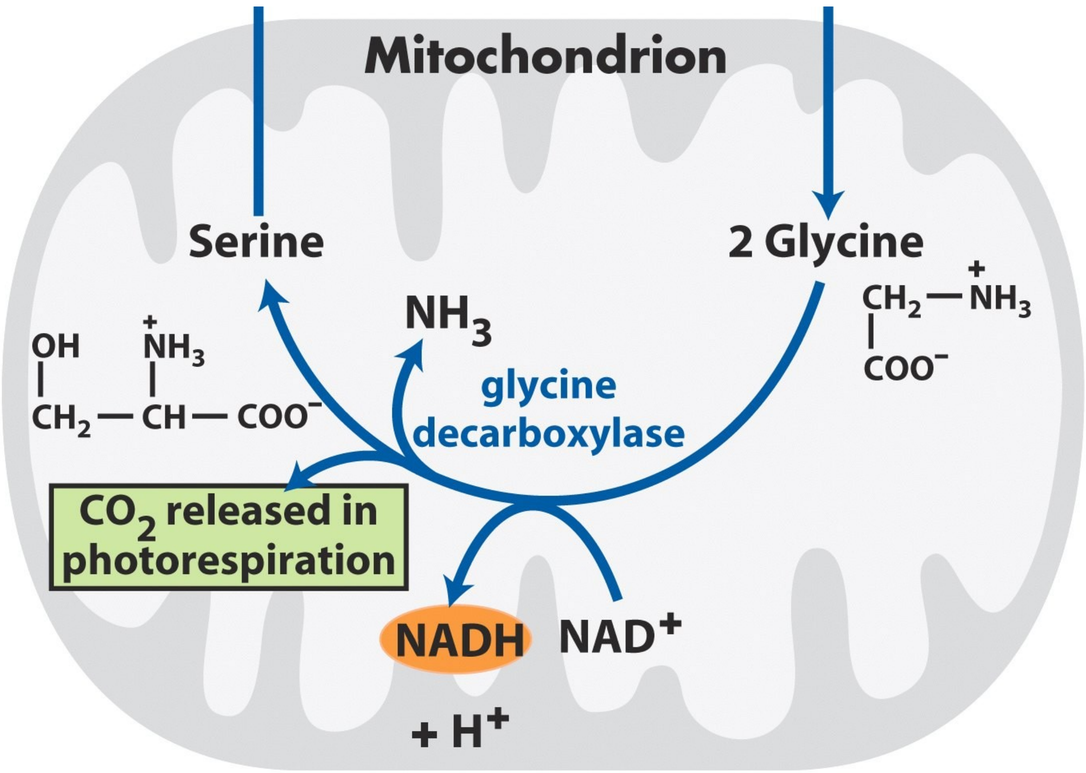
Glycolate is oxidized to glyoxylate.

Glyoxylate undergoes transamination to glycine → exported to mitochondria

Peroxide formed is deactivated by peroxidases in the peroxysome.

The Salvage of Phosphoglycolate

The Glycolate Pathway

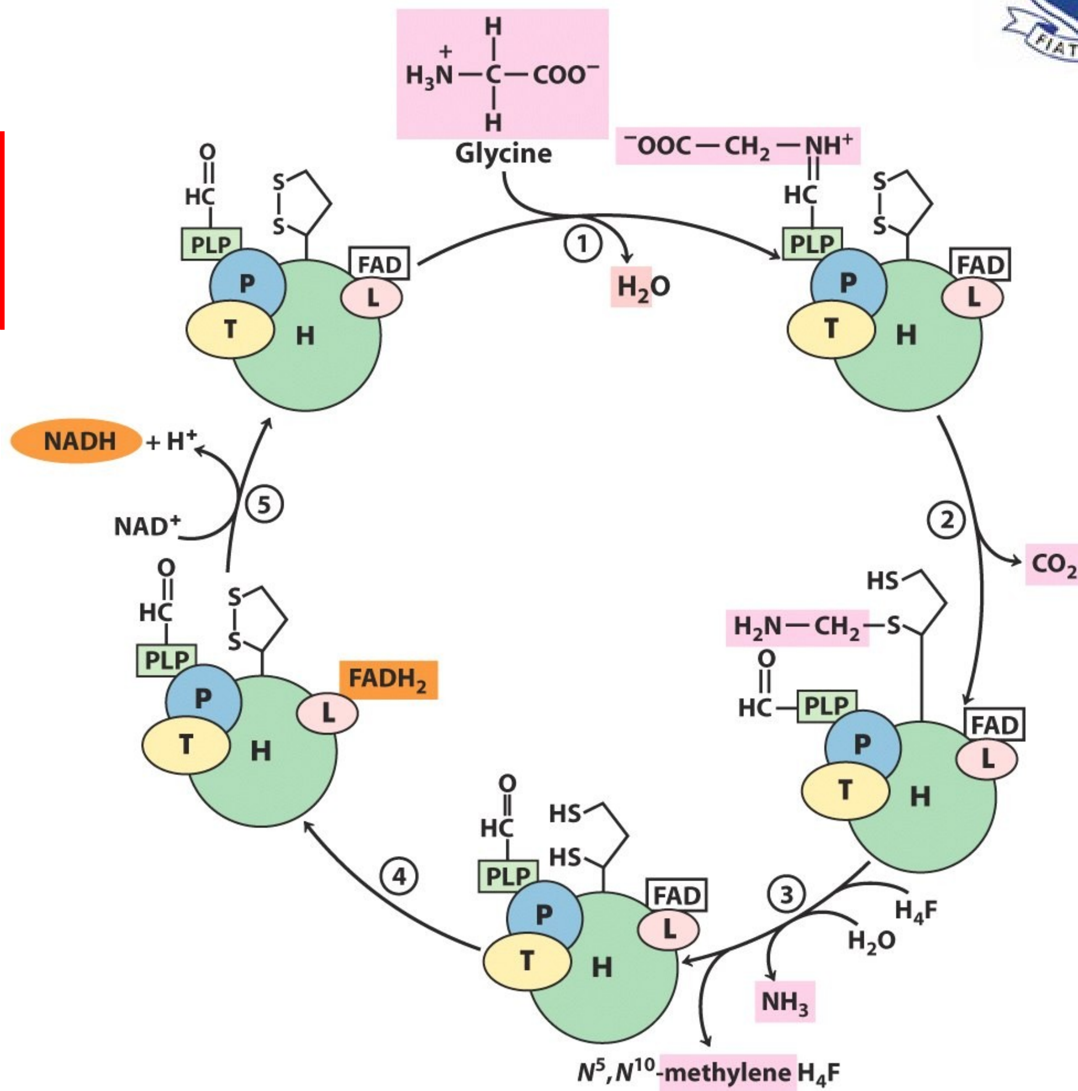


Two glycine molecules form Serine and CO_2

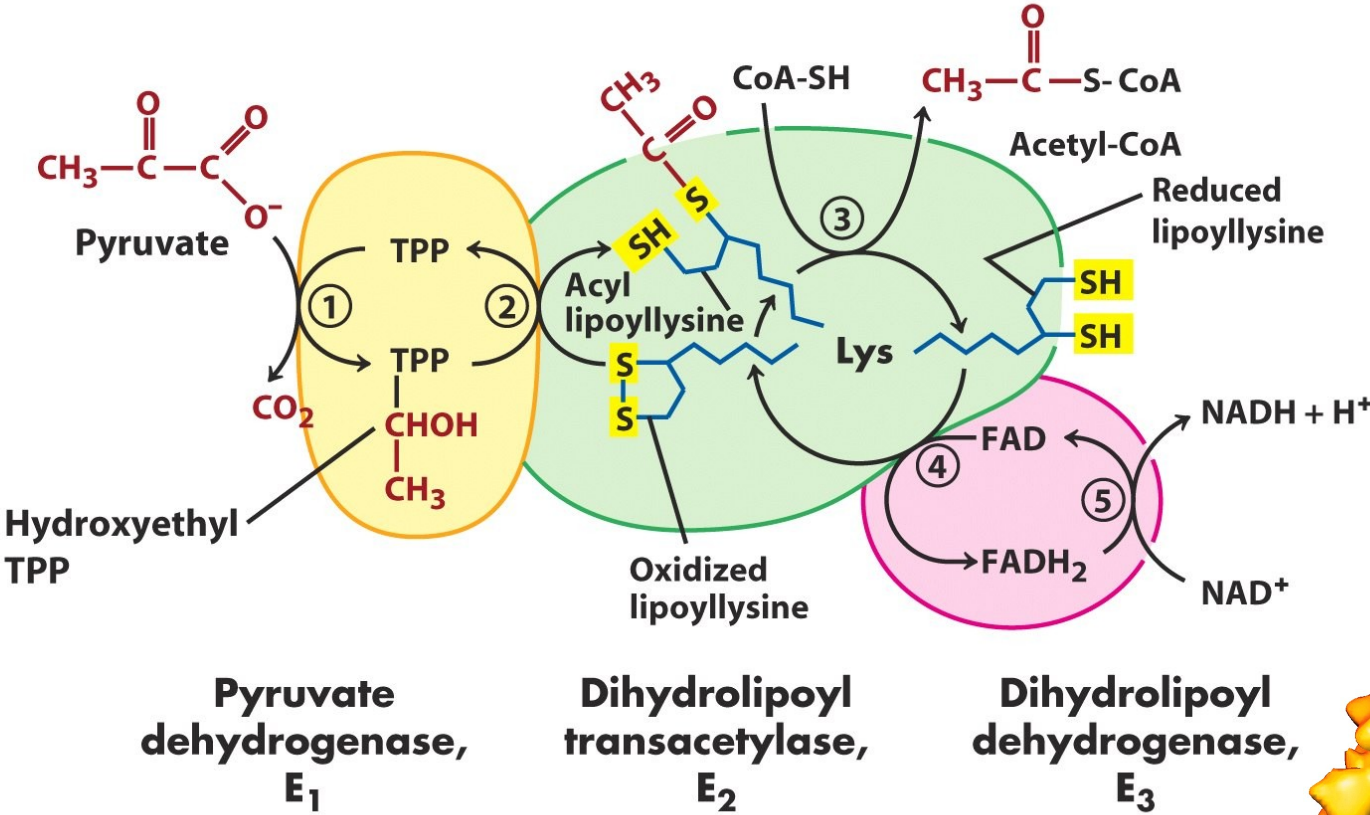
→ Glycine decarboxylase

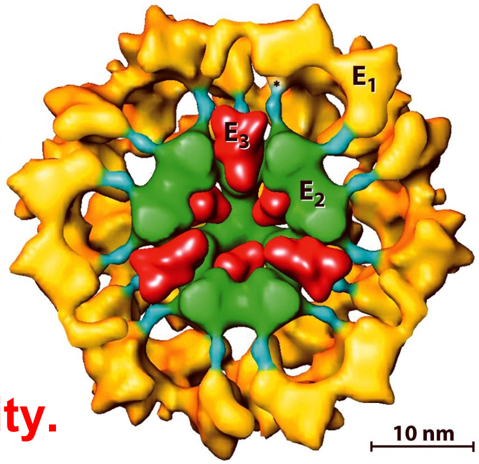
Glycine Decarboxylase

Have you seen something similar before?
→ where?



Pyruvate Dehydrogenase Complex





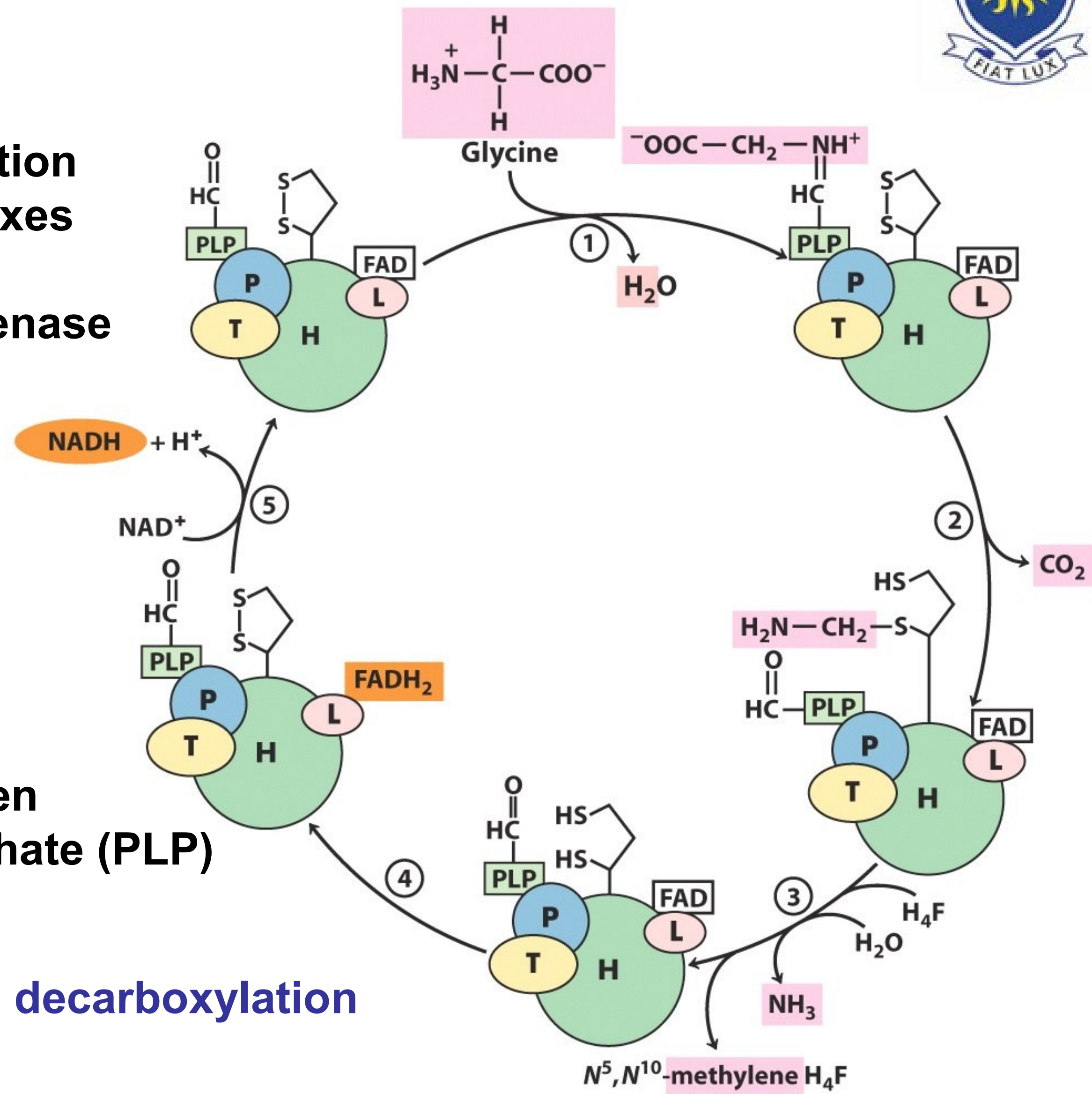
Oxidative decarboxylation of pyruvate to acetyl-CoA.

Step 1 is rate limiting and responsible for substrate specificity.

Glycine Decarboxylase

Similar in structure and function to two mitochondrial complexes
→ pyruvate dehydrogenase
→ α-ketoglutarate dehydrogenase

Glycine decarboxylase:
4 subunits P,H,T & L
→ stoichiometry $P_4H_{27}T_9L_2$



Step 1
Schiff base formation between glycine and pyridoxal phosphate (PLP)

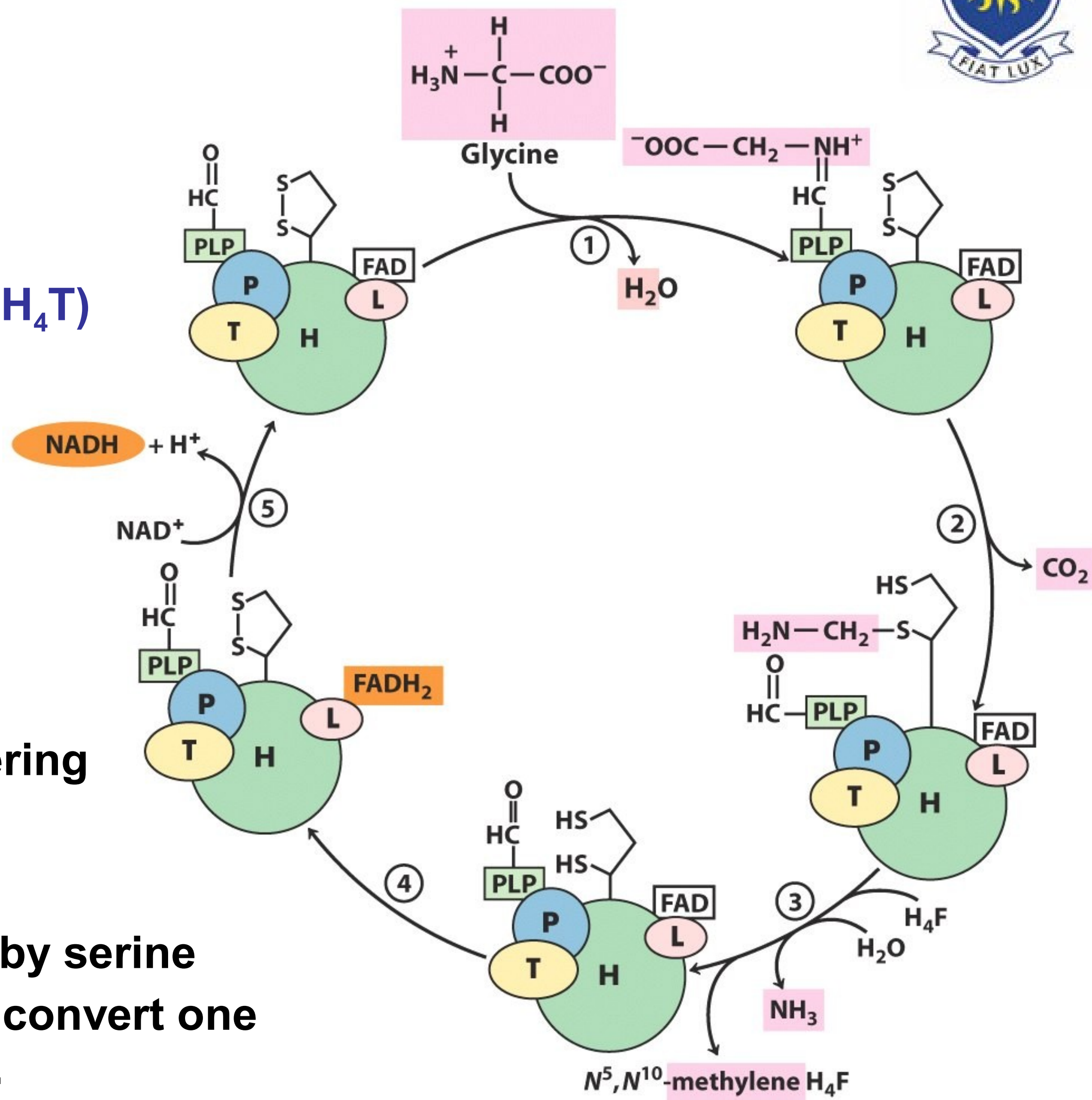
Step 2
Protein P catalyzes oxidative decarboxylation of glycine

Glycine Decarboxylase

Step 3
Protein T releases NH_3 from the methylamine moiety
→ cofactor: tetrahydrofolate (H_4T)

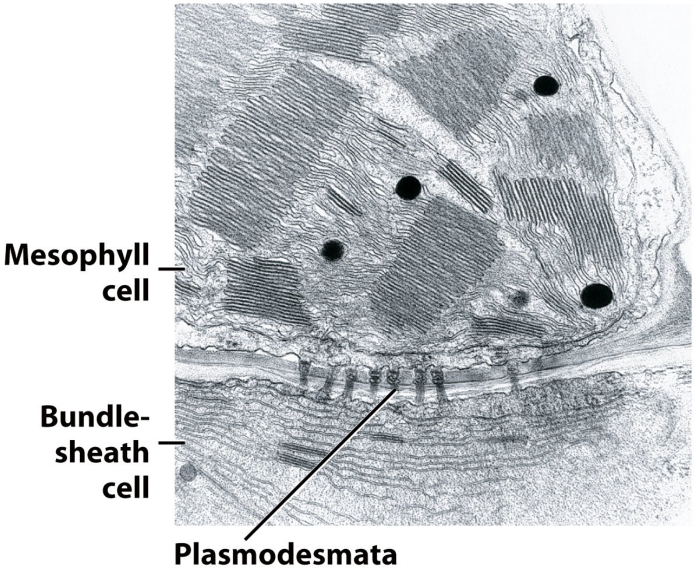
Step 4
Protein L oxidized the two SH-groups of lipoic acid.

Step 5
FAD is regenerated by transfering electrons ton NADH.

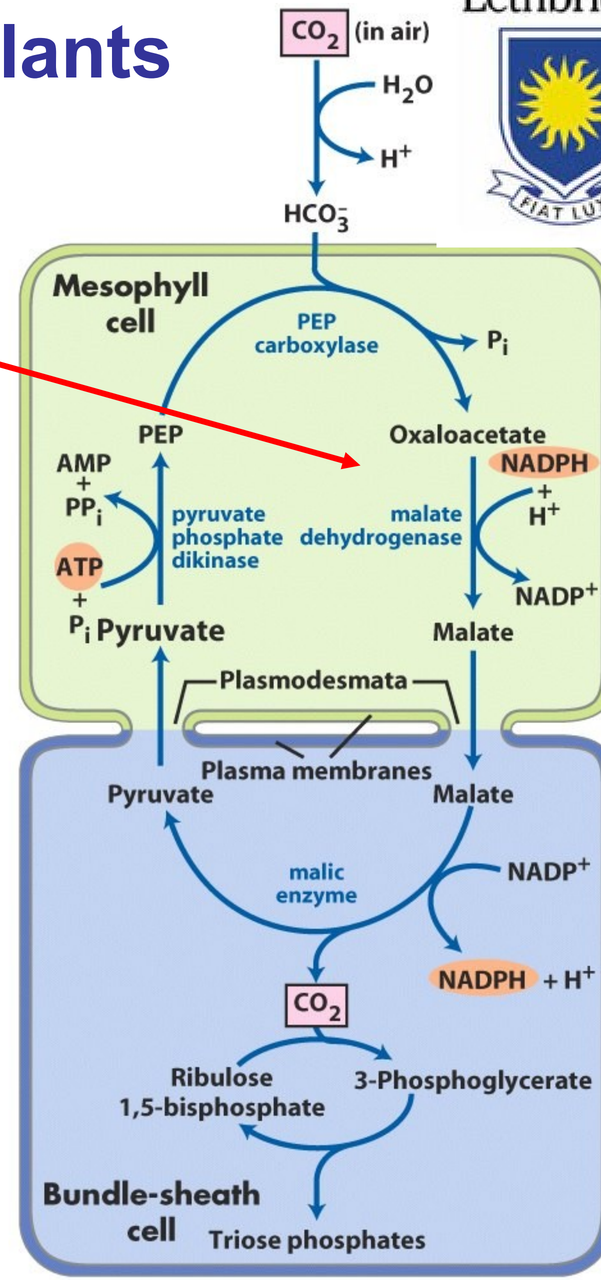


N^5,N^{10}-methylene H_4F is used by serine hydroxymethyltransferase to convert one molecule of glycine to serine.

CO_2 Fixation in C_4 Plants



C_4 component



Avoiding the wasteful photorespiration some plants have evolved a separate "C_4 pathway" → specialized cells

In mesophyll cells CO_2 is fixed as HCO_3^- by PEP carboxylase.

CO_2 is split off by malic enzyme in the bundle-sheath cell → high local concentration → less O_2 misincorporation

CO_2 Fixation in C_4 Plants

C_4 pathway has a greater energy cost than the C_3 pathway.

→ 5 ATP per CO_2 vs. 3 ATP in C_3

But at higher temperatures the affinity of RUBISCO for CO_2 decreases.

→ at about 28 to 30°C the gain in efficiency is overcompensated by the elimination of photorespiration.

→C_4 plants outgrow most C_3 plants during summer.

Some plants, native to very hot & dry environments separate CO_2 trapping and fixation over time. CO_2 is trapped and stored as malate in the night. During day stomata close and CO_2 is released by malic enzyme → assimilation via RUBISCO