BB 422/622

ANABOLISM I: Carbohydrates

PHOTOSYNTHESIS OUTLINE:

Overview of Photosynthesis
Key experiments:
- Light causes oxygen, which is from water splitting (Hill)
- NADPH made (Ochoa)
- Separate from carbohydrate biosynthesis (Rubin & Kamen)

Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes-"red-drop expt."
  - Reaction center
  - Photosystems (PS)
    - PSII – oxygen from water splitting
  - (Cytochrome b_6f Complex)
  - Photosystems (PS) (cont.)
    - PSI – NADPH

Proton Motive Force – ATP
Overview of light reactions
Carbon Assimilation – Calvin Cycle
- Stage One – Rubisco
  - Carboxylase Know Mechanism
  - Oxygenase
  - Glycolate cycle
- Stage Two – making sugar
- Stage Three - remaking Ru $1,5P_2$

Overview and regulation
Calvin cycle connections to biosyn.
C4 versus C3 plants
Kornberg cycle - glyoxylate

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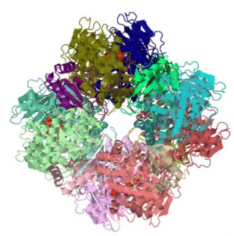
Photosynthesis

First Stage of Calvin Cycle

CO_2 Fixation Is Catalyzed by Rubisco



- Most plentiful, most important, enzyme on Earth (4 tons/yr)
  - **Low turnover # of k_{cat} = 3 s^{-1} at 25 C°**
  - **means a LOT of the enzyme needed!**
  - **50% of plant enzymes are rubisco.**
- Large (560 KDa; $\alpha_8\beta_8$)(L=56 kDa, S=14 kDa); Mg^{2+}–dependent enzyme; activated by CO_2-carbamylation
- pH dependence is sharp, optimal activity at pH=8
- Catalyzes the reaction:
  - ribulose 1,5-bisphosphate + CO_2 → **2** 3-phosphoglycerate
  - $\Delta G°'$ = –35.1 kcal/mol

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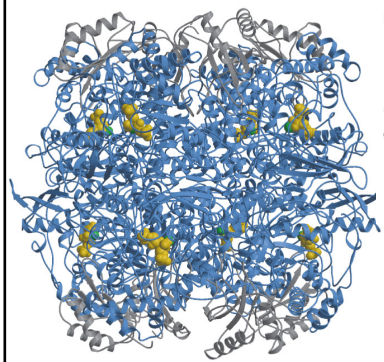
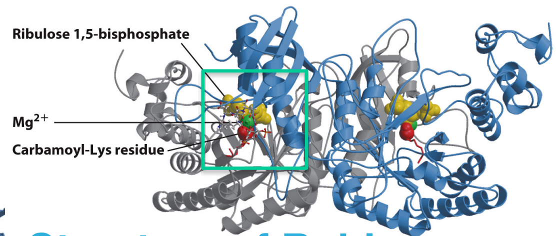
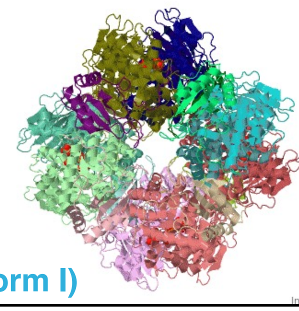
Photosynthesis

First Stage of Calvin Cycle





Structure of Rubisco (Form II)



There are two distinct forms of rubisco. Form I is found in vascular plants, algae, and cyanobacteria; form II is confined to certain photosynthetic bacteria.

Structure of Rubisco (Form I)

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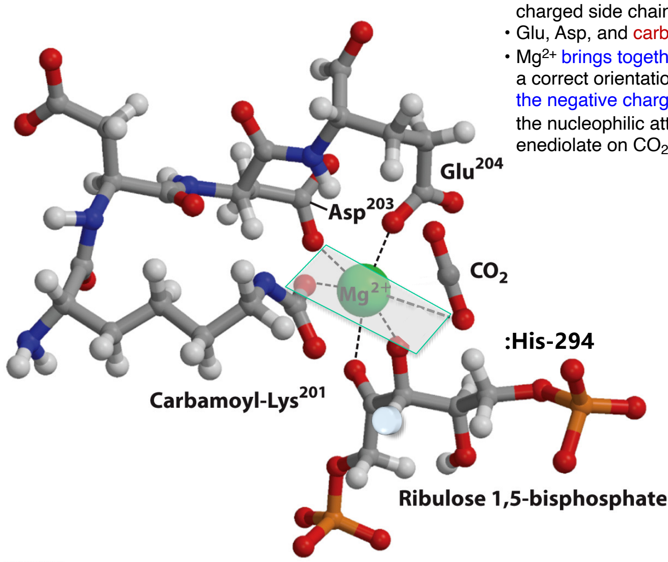
Photosynthesis

Role of Mg^{2+}

- Notice that Mg^{2+} is held by negatively charged side chains of:
- Glu, Asp, and carbamoylated Lys
- Mg^{2+} brings together the reactants in a correct orientation and stabilizes the negative charge that forms upon the nucleophilic attack by the enediolate on CO_2.



- Rubisco is inactive until Lys^{201} is carbamoylated by CO_2. But, in the apo-enzyme Lys^{201} inaccessible.
- ***Rubisco activase*** (an enzyme sometimes triggered by light) changes Rubisco conformation, in an ATP-dependent manner, to expose Lys^{201}.

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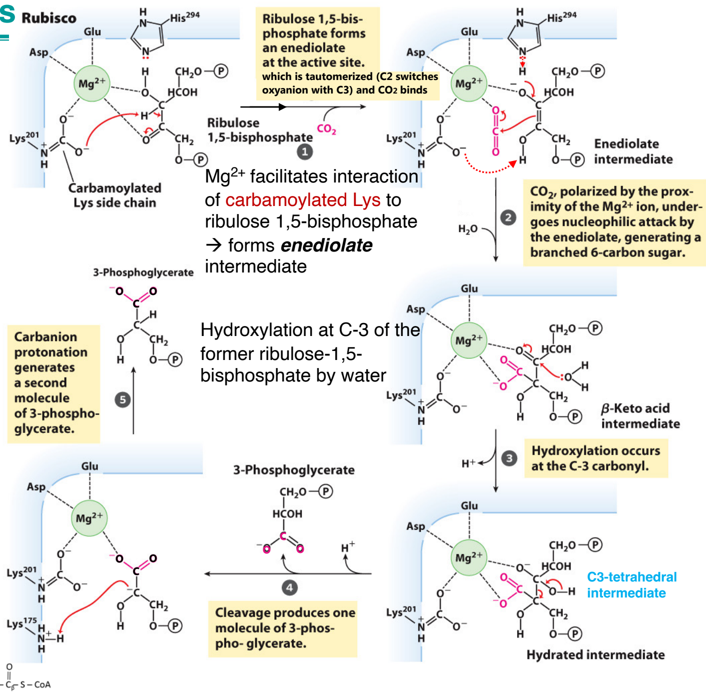
Photosynthesis

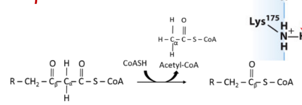
Rubisco Carboxylase Mechanism—Overview



Mg^{2+} facilitates interaction of carbamoylated Lys to ribulose 1,5-bisphosphate → forms ***enediolate*** intermediate

Hydroxylation at C-3 of the former ribulose-1,5-bisphosphate by water

Recall another C-C cleavage of a β-keto acid?



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Photosynthesis

First Stage of Calvin Cycle

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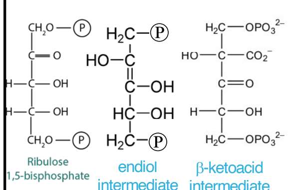
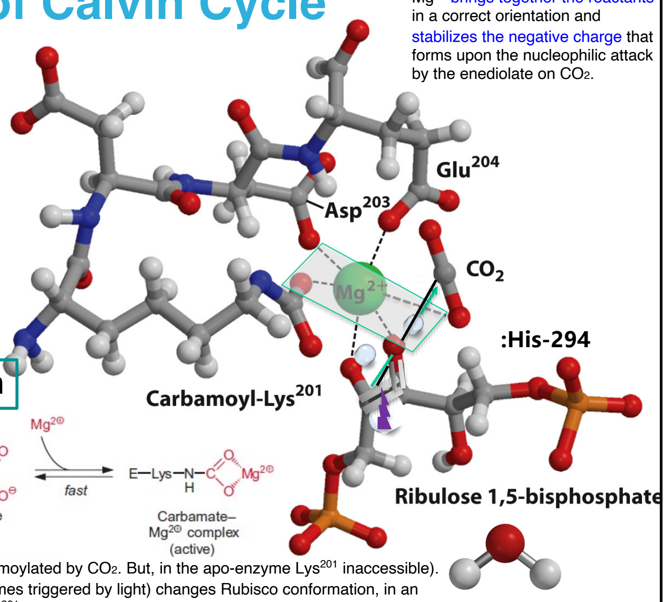
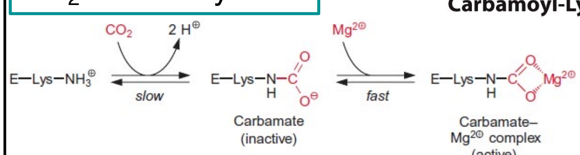




CO₂ carbamoylation



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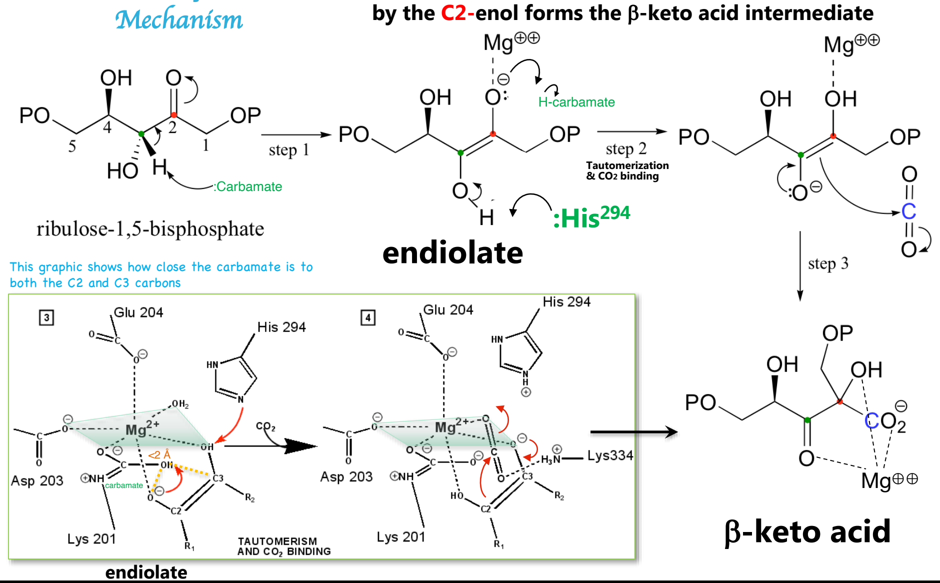
RUBISCO
(Ribulose 1,5-bisphosphate carboxylase/oxygenase)
Carboxylase
Mechanism

- **The first catalytic intermediate is the endiolate formation**
- **Not covered in text or class is the tautomerization to convert the C2-endiolate to the C3-endiolate**
- **Immediately after CO_2 binding, the nucleophilic attack by the C2-enol forms the β-keto acid intermediate**



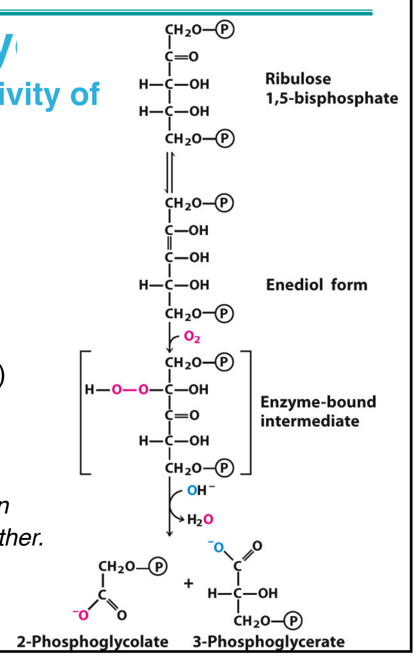
Photosynthesis

First Stage of Calvin Cy

Oxygenase Activity of Rubisco

- O_2 competes with CO_2 for the active site.
  - ~1 in every 3 or 4 turnovers, O_2 binds
- The reactive nucleophile in the rubisco reaction is the electron-rich enediol form of ribulose 1,5-bisphosphate.
- The nucleophile adds to O_2 to form 3-phosphoglycerate (same as in Calvin cycle) and ***2-phosphoglycolate (2-PG)***.
  - *–2-PG is metabolically difficult.*
  - *–Salvaging its Carbons requires energy*
  - *–As it is produced in appreciable amounts, an elaborate pathway has been cobbled together.*

This process is called PHOTO-RESPIRATION



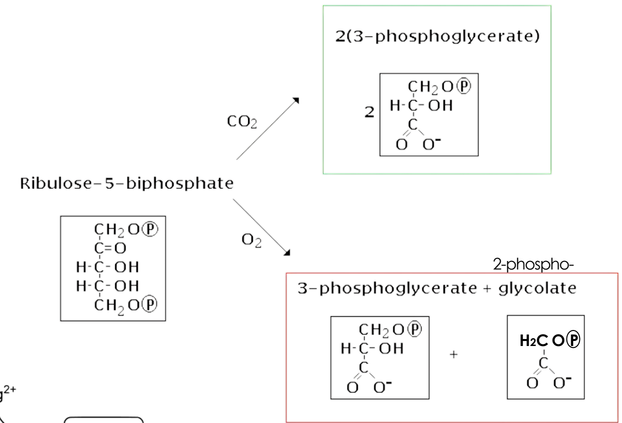
RUBISCO

(Ribulose 1,5-bisphosphate carboxylase/oxygenase)

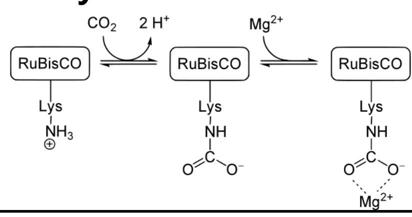
Carboxylase *versus* Oxygenase

Mechanism

Here are the two overall reactions RUBISCO catalyzes



Both RUBISCO reactions require activation by carbamoylation



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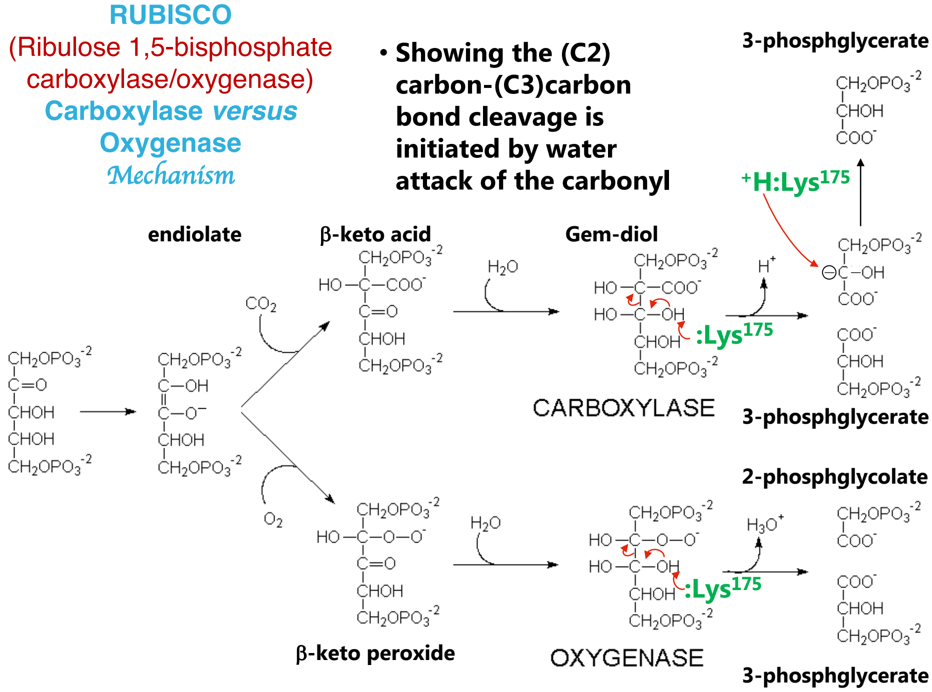
RUBISCO

(Ribulose 1,5-bisphosphate carboxylase/oxygenase)

Carboxylase *versus* Oxygenase

Mechanism

- **Showing the (C2) carbon-(C3)carbon bond cleavage is initiated by water attack of the carbonyl**



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Photorespiration

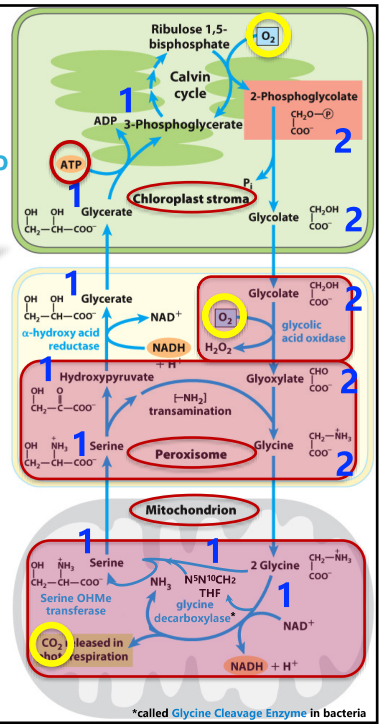
First Stage of Calvin Cycle

Dealing with the oxygenase activity of rubisco

The Glycolate Pathway*

- Complex ATP-consuming process for the recovery of C_2 fragments from **photorespiration**. 1st Glycolate is made.
- Uses three organelles
- Loss of C as CO_2 by mitochondrial decarboxylation of glycine (see Pyr family)
- **Two 2-PGs are converted to Ser + CO_2.**
- The Ser is cycled back to the chloroplast to generate one 3-PGA.
- Whole cycle costs an ATP per **1** 3-PGA converted back

*Don't confuse with the glyOXYlate Cycle



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Photosynthesis

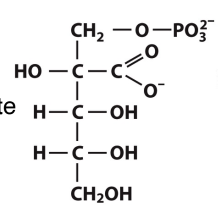
First Stage of Calvin Cycle

Regulation of RUBISCO activity

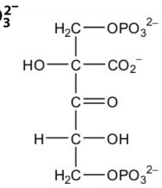
Rubisco is Inhibited by Several Criteria:

1. **pH**
   - In the stroma the pH is ~8 when light is on
   - pH decreases in the dark; rubisco inactive at low pH
2. **CO_2** (for the carbamoylation of Lys-210; also better at higher pH)
3. **NADPH** (indirectly through rubisco activase)
4. **a "Noctural" Inhibitor**

- ***2-carboxyarabinitol 1-phosphate*** inhibits carbamoylated Rubisco.
  - transition state analog of β-keto acid intermediate
  - synthesized in the dark in some plants



2-Carboxyarabinitol 1-phosphate



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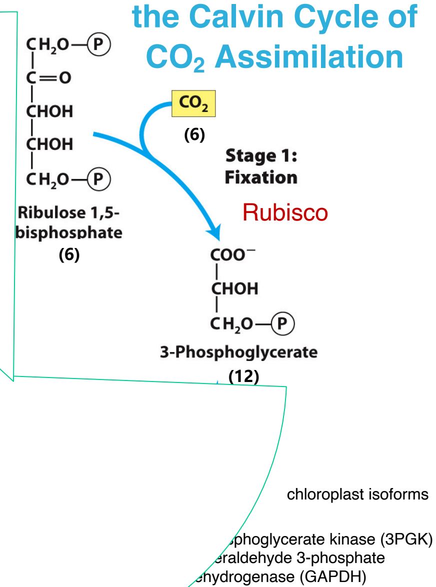
Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation

Second Stage of Calvin Cycle



Overall: 6 CO_2 + 12 NADPH + 1… …aldehyde 3-phosphate (GA3P) + 4 H^+ + 12 NADP… …P_i

*Ribulose 1,5-bisphosphate carboxylase/oxygenase (also RuBisCo)

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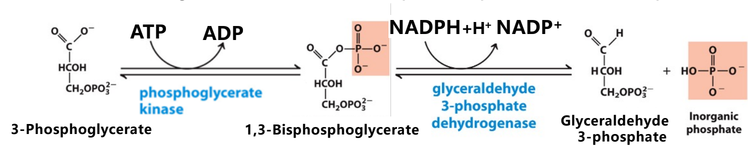
Photosynthesis

Second Stage of Calvin Cycle: making Glucose

3-PGA Reduced to GA3P

Twelve 3-phosphoglycerate + 12 ATP + 12 NADPH + 12 H^+ → 12 glyceraldehyde 3-phosphate + 12 ADP + 12 $NADP^+$ + 12 P_i

- Requires most of the ATP and NADPH from photosynthesis at ratio of 12:12
- Reverse of the key reactions in glycolysis (except NADPH used rather than NADH)
  - uses chloroplast isozymes of 3-phosphoglycerate kinase (3-PGK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
- Driven forward by high concentration of NADPH and ATP in the chloroplast stroma; and the pulling of GA3P and P_i by the cycle and ATP synthase



$\Delta G^{\circ\prime}$ = +5.5 kcal/mol —1.8 kcal/mol +3.7 kcal/mol

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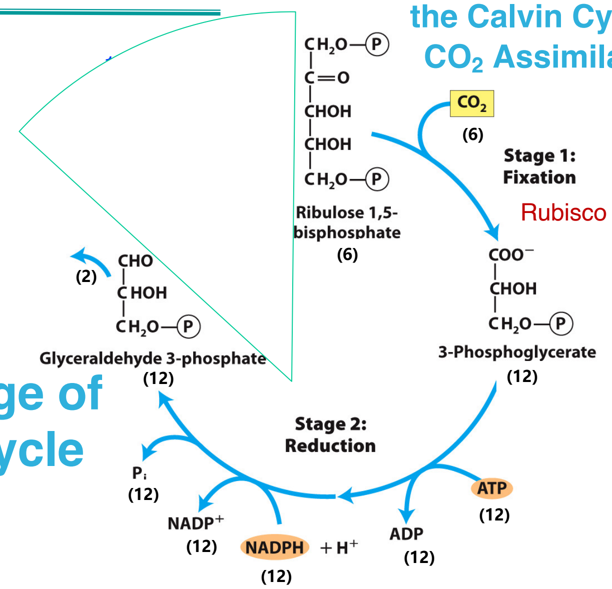
Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation

Third Stage of Calvin Cycle



Overall: 6 CO_2 + 12 NADPH + 10 H_2O + 18 ATP → 2 glyceraldehyde 3-phosphate (GA3P) + 4 H^+ + 12 $NADP^+$ + 18 ADP + 16 P_i

*Ribulose 1,5-bisphosphate carboxylase/oxygenase (also RuBisCo)

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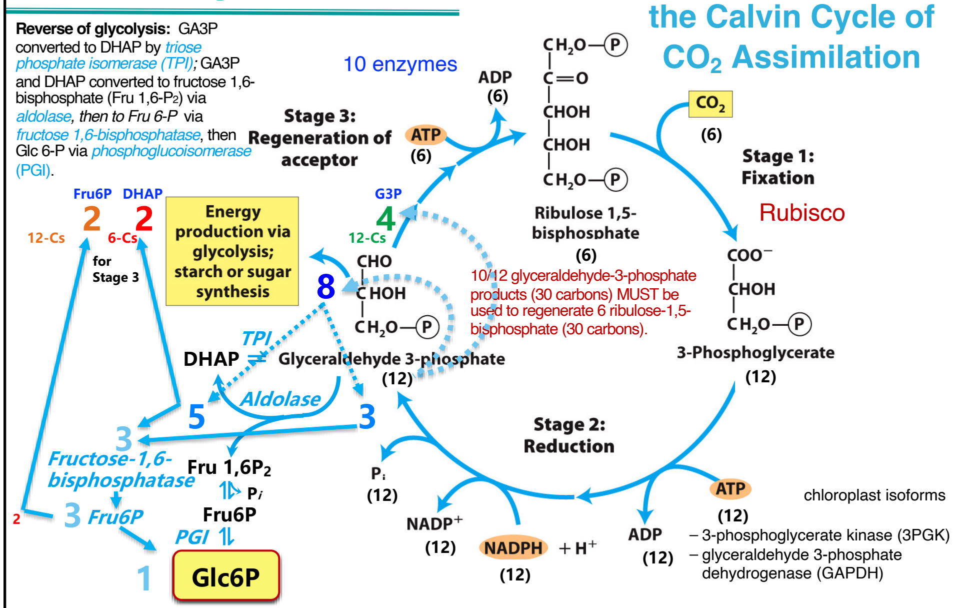
Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation



- 12 glyceraldehyde-3-phosphate are produced during the Calvin cycle in the assimilation of 6 carbons into Glc.
- 2/12 glyceraldehyde-3-phosphate products are pulled out of the cycle and used for Glc6P, then to other carbos.

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Photosynthesis

Third Stage of Calvin Cycle

Stage 3: Regeneration of Ru 1,5-P_2

1. We have 3-carbon compounds and need 5-carbon compounds: put 2 together to make 6 (stop at Fru6P), then can pull 2 carbons from 6 (leaving 4) and plus another 3-carbon compound make a 5-carbon sugar.

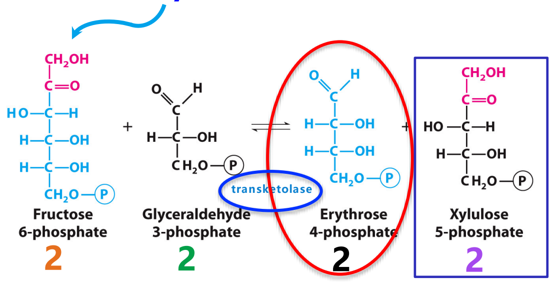
DO THIS TWICE.

We have 8GA3P for making 3 hexoses and 2 DHAP, and 4 GA3P left

The 2 Fru6P, plus 2/4 more GA3P to make 2 5-carbon sugars (Xyl5P).

From 4/8 GA3P*

12 → use 6 GA3P (4+2)



4 GA3P → 2 Fru 6-P
2 GA3P → 2 Xyl 5-P
+2 Ery 4-P

2 GA3P left

*the other 4 go to make Glc(1) and DHAP(2) →use 6 GA3P

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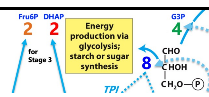
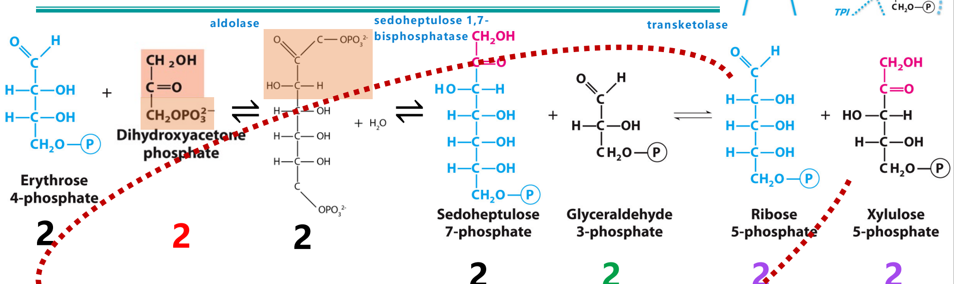
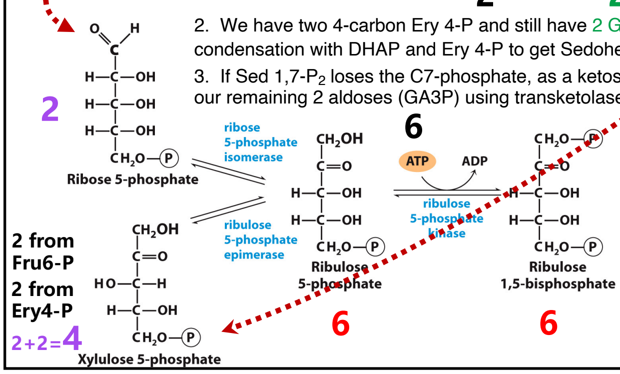
Stage 3 Photosynthesis





2. We have two 4-carbon Ery 4-P and still have 2 GA3Ps and 2DHAP. Do aldol condensation with DHAP and Ery 4-P to get Sedoheptulose 1,7-bisphosphate (Sed 1,7-P_2).

3. If Sed 1,7-P_2 loses the C7-phosphate, as a ketose, we can pull 2 carbons off and put on our remaining 2 aldoses (GA3P) using transketolase as we did with Fru 6P.



Sedoheptulose 1,7-bisphosphate dephosphorylated to sedoheptulose 7-phosphate (S7P) via *sedoheptulose 1,7-bisphosphatase*

- This enzyme is unique to plants.

Finally, RuP phosphorylated to ribulose 1,5-bisphosphate by *phosphoribulokinase*

- This enzyme is also unique to plants and regulated by NADPH

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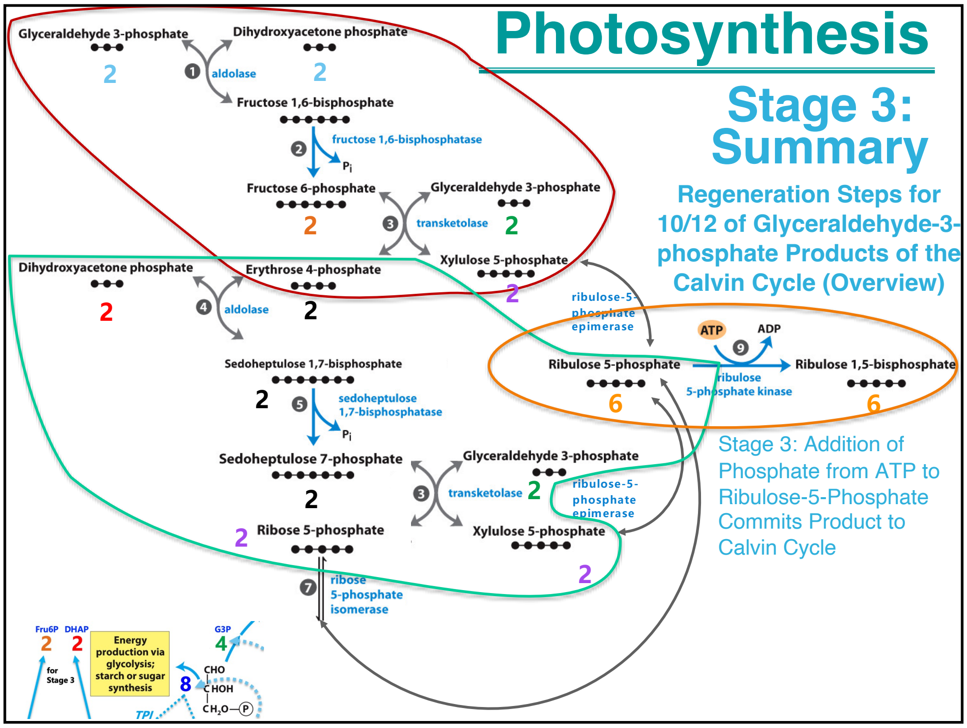
Photosynthesis

Stage 3: Summary

Regeneration Steps for 10/12 of Glyceraldehyde-3-phosphate Products of the Calvin Cycle (Overview)



Stage 3: Addition of Phosphate from ATP to Ribulose-5-Phosphate Commits Product to Calvin Cycle

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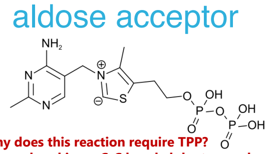
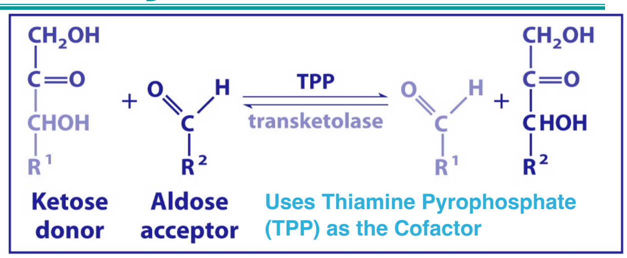
Photosynthesis

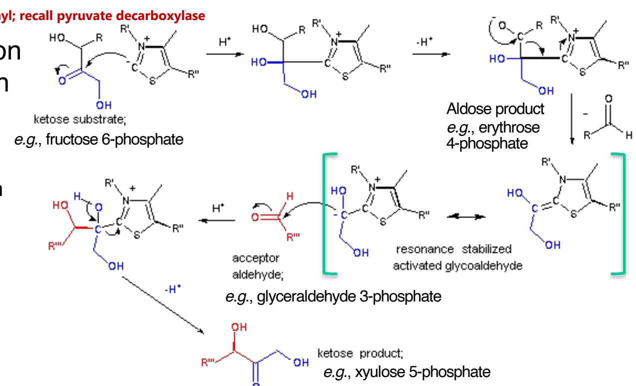
Transketolase: exchange of 2C from ketose to an aldose acceptor





Why does this reaction require TPP?
We are breaking a C-C bond alpha to a carbonyl; recall pyruvate decarboxylase

- Contains thiazolium anion for nucleophilic attack on carbonyls of ketose
- Also used by:
  - pyruvate dehydrogenase in acetyl CoA formation
  - pyruvate decarboxylase in ethanol metabolism
  - α-ketoglutarate dehydrogenase in CAC
  - transketolase in pentose phosphate pathway



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