BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Fermentations
Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
- Electron transport
- Chemiosmotic theory: Phosphorylation

Exam-2 material

Fat Catabolism
Fatty acid Catabolism
- Mobilization from tissues (mostly adipose)
- Activation of fatty acids
- Transport; carnitine
- Oxidation: b-oxidation, 4 steps:
  - Saturated FA
  - Unsaturated FA
  - Odd-chain FA
  - Ketone Bodies

Protein Catabolism
- Digestion, lysosome, Ubiquitin-Proteosome

Amino-Acid Degradation
- Dealing with the nitrogen
  - Urea Cycle
- Dealing with the carbon
  - Seven Families
    - One-carbon (1-C) metabolism; THF, SAM
    - PLP uses
- Convergence with Fatty acid-odd chain

Nucleic Acid & Nucleotide Degradation
- Nucleic Acids
- Nucleotides
  - Salvage pathway
  - Degradation of purines
  - Degradation of pyrimidines

Exam-3 material

ANABOLISM I: Carbohydrates

PHOTOSYNTHESIS:

Overview of Photosynthesis
Key experiments:
Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes–"red-drop experiment"
  - Reaction center
  - Photosystems (PS)
    - PSII – oxygen from water splitting
    - PSI – NADPH

Proton Motive Force – ATP
Overview of light reactions
Carbon Assimilation – Calvin Cycle
- Stage One – **Rubisco**
  - Carboxylase
  - Oxygenase

  } Know mechanism
  - Glycolate cycle
- Stage Two – making sugar
- Stage Three – remaking Ru 1,5P_2

Overview and regulation
Calvin cycle connections to biosynthesis
C4 versus C3 plants
Kornberg cycle – gly**ox**ylate

Photorespiration

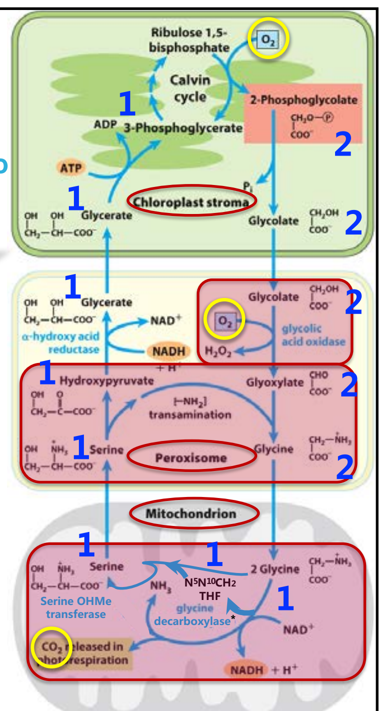
First Stage of Calvin Cycle

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



Photosynthesis

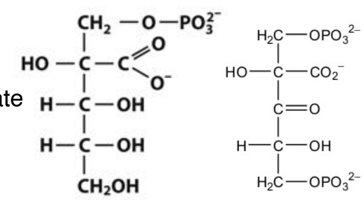
First Stage of Calvin Cycle

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**

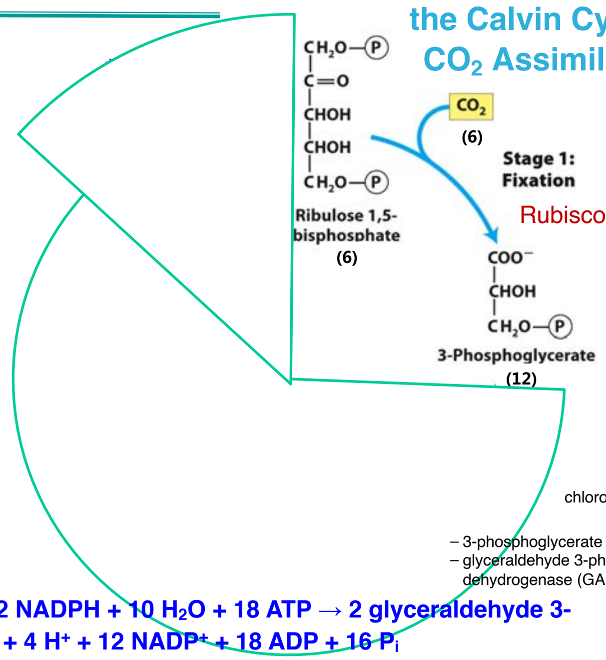
- ***2-carboxyarabinitol 1-phosphate*** inhibits carbamoylated Rubisco.
  - transition state analog of β-keto acid intermediate
  - synthesized in the dark in some plants



2-Carboxyarabinitol 1-phosphate

Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation



chloroplast isoforms

– 3-phosphoglycerate kinase (3PGK)
– glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

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

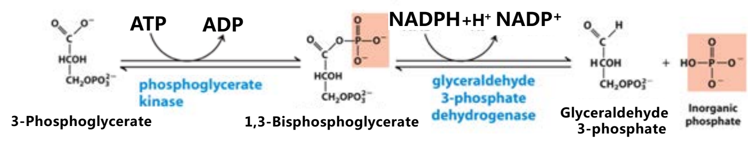
Photosynthesis

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

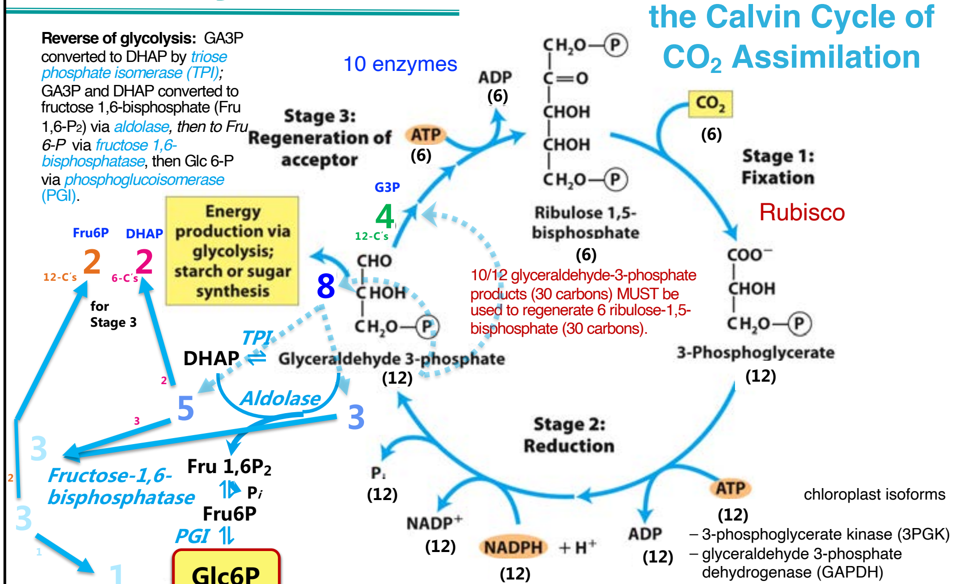
- Requires most of the ATP and NADPH from photosynthesis at ratio of 12:12
- Reverse of the key reactions in glycolysis (except NADPH used rather than NADH)
  - uses chloroplast isozymes of 3-phosphoglycerate kinase (3-PGK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
- Driven forward by high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



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

Photosynthesis

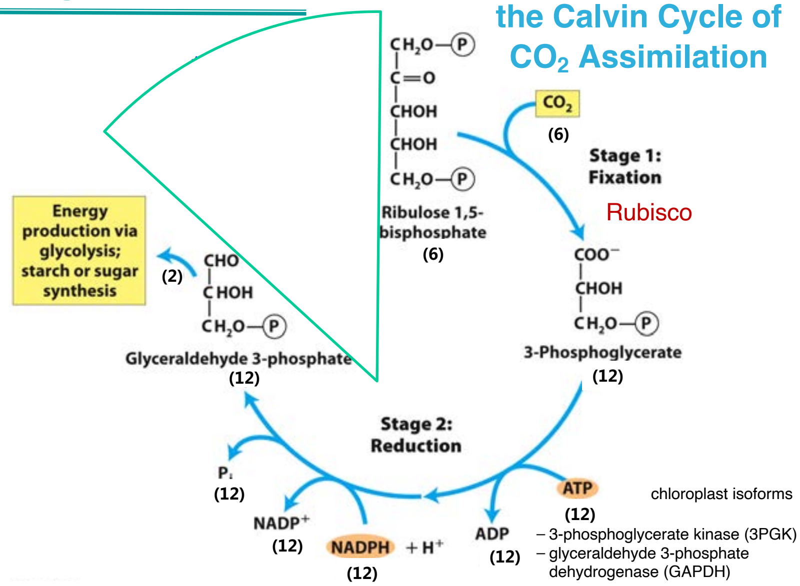
The Three Stages of the Calvin Cycle of CO_2 Assimilation



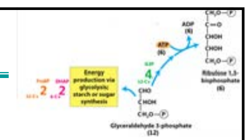
- 12 glyceraldehyde-3-phosphate are produced during the Calvin cycle in the assimilation of 6 carbons into Glc.
- 2/12 glyceraldehyde-3-phosphate products are pulled out of the cycle and used for Glc6P, then to other carbos.

Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation



Photosynthesis



Third Stage of Calvin Cycle

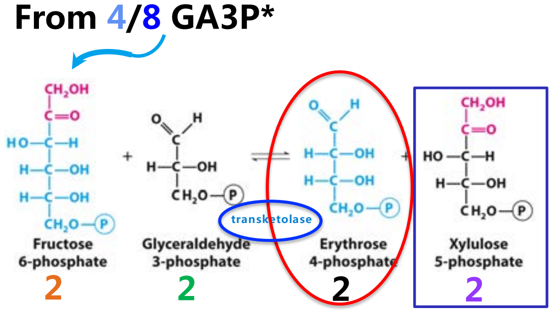
Stage 3: Regeneration of Ru 1,5-P_2

1. We have 3- and 6-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 with another 3 carbon compound make a 5-carbon sugar.

DO THIS TWICE.

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

From 4/8 GA3P*



10→use 6 GA3P

4 GA3P → 2 Fru 6-P

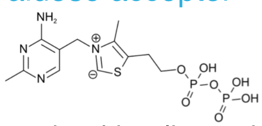
2 GA3P → 2 Xyl 5-P

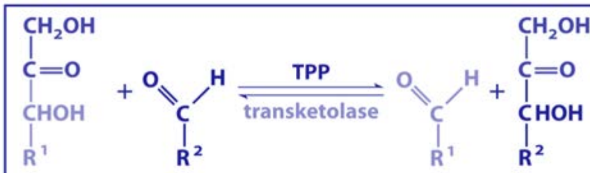
+2 Ery 4-P

***the other 4 go to make Glc(1) and DHAP(2)**

Mechanism Photosynthesis

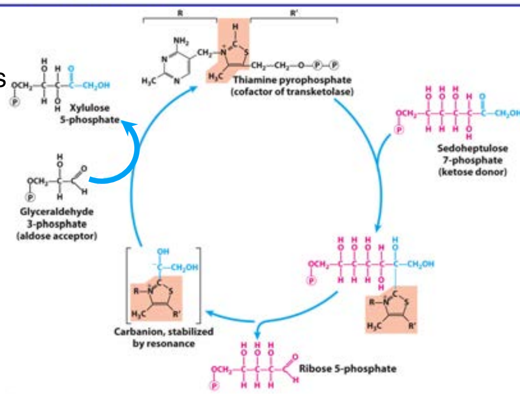
Transketolase: exchange of 2C from ketose to an aldose acceptor



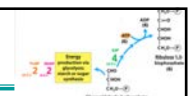


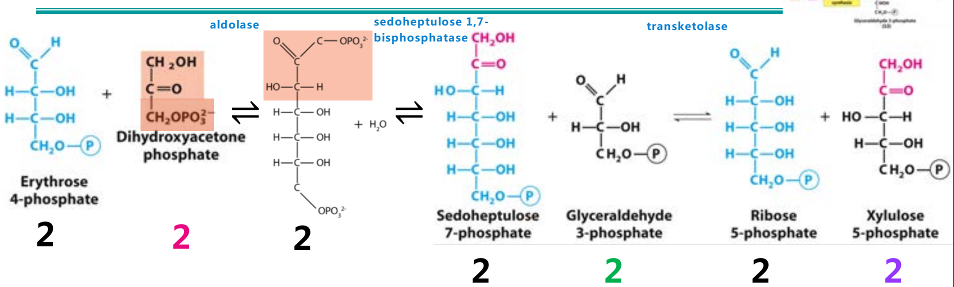
Uses Thiamine Pyrophosphate (TPP) as the Cofactor

- 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
  - Branched-chain amino acid dehydrogenase
  - transketolase in pentose phosphate pathway

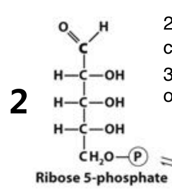


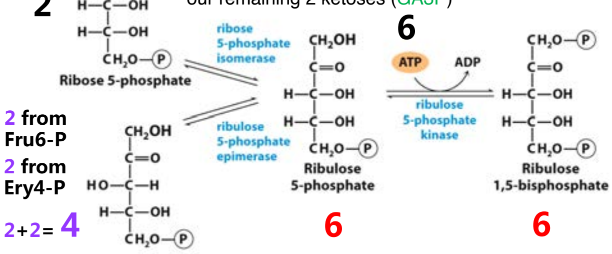
Stage 3 Photosynthesis





2. We have two 4-carbon Ery 4-P and still have 2 GA3P and 2 DHAPs. Do aldol condensation with DHAP 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 ketoses (GA3P)





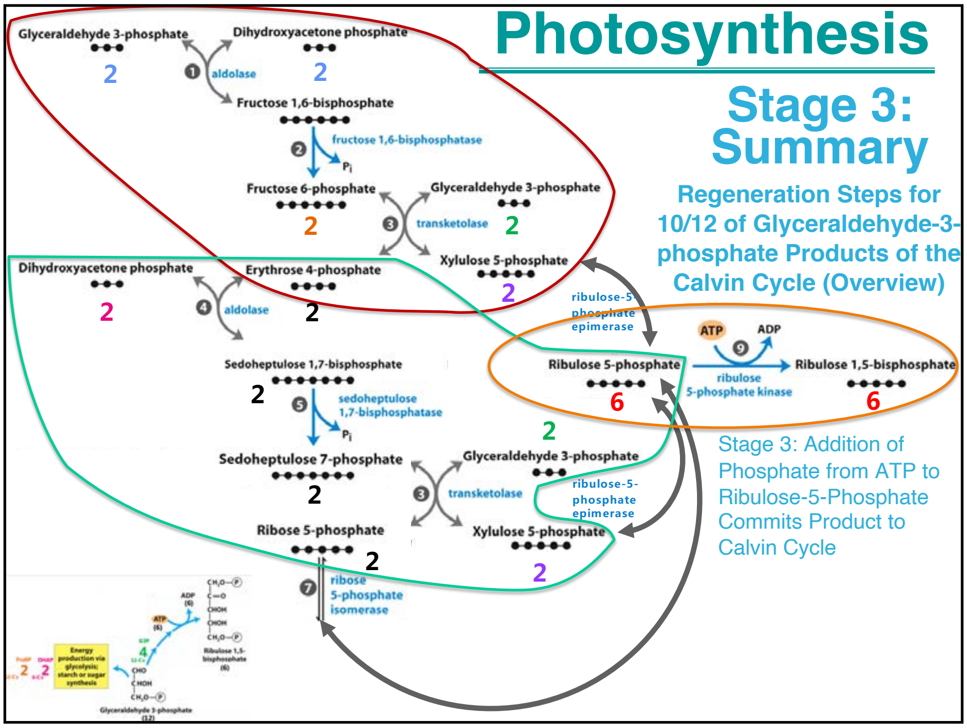
Sedoheptulose 1,7-bisphosphate dephosphorylated to sedoheptulose 7-phosphate (S7P) via *sedoheptulose 1,7-bisphosphatase*
- This enzyme is unique to plants.

Finally, RuP phosphorylated to ribulose 1,5-bisphosphate by *phosphoribulokinase*
- This enzyme is also unique to plants and regulated by NADPH

Photosynthesis

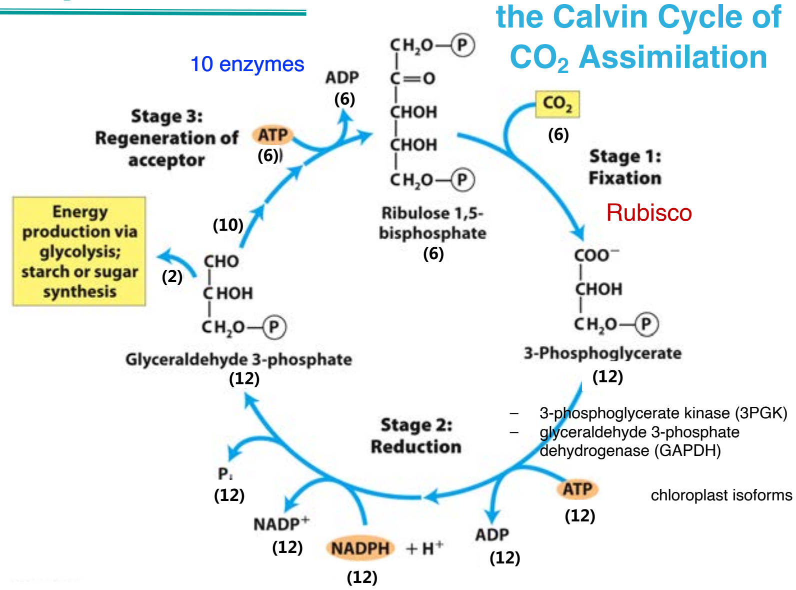
Stage 3: Summary

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



Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation



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

Photosynthesis

Stoichiometry and Energetics of CO_2 Assimilation in the Calvin Cycle & NET Reaction for Photosynthesis:

$6\ CO_2 + 12\ NADPH + 12\ H^+ + 12\ H_2O + 18\ ATP \rightarrow 1\ C_6H_{12}O_6 + 2\ H^+ + 12\ NADP^+ + 18\ ADP + 18\ P_i + 18\ H^+$

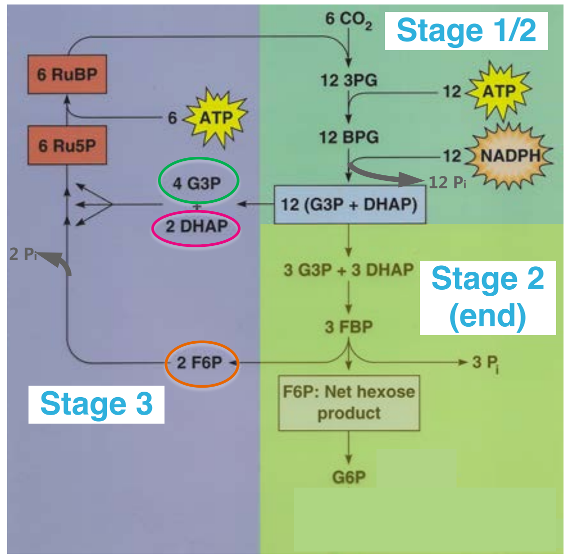
This is 3:2 ATP:NADPH! (from 8 photons)
For every NADPH, 4 photons needed
∴ need 12x4=48 photons to make 1 Glc

Stage 1/2
6Ru5P→12GA3P
$\Delta G^{\circ\prime}$ = –56 kcal/mol

Stage 2 (end)
2GA3P→1Glc
$\Delta G^{\circ\prime}$ = –10 kcal/mol

Stage 3
10GA3P→ 6Ru5P
$\Delta G^{\circ\prime}$ = –38 kJ/mol

NET: –104/6 = –17.3 per CO_2

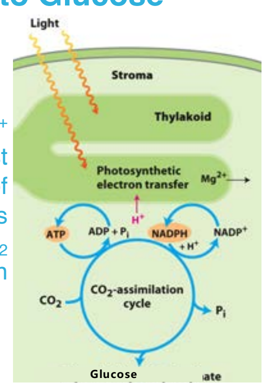


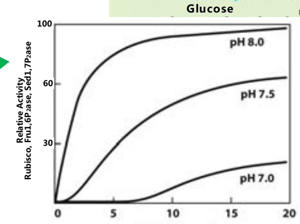
Photosynthesis

Photosynthesis: Control by Light and CO_2 to Glucose

- The assembly of one molecule of glucose requires the capture of roughly 48 photons.
  - H^+ move from the stroma to the thylakoid
  - creates alkaline conditions in the stroma
- Accompanied by Mg^{2+} transport from thylakoid to stroma
- Enzyme for photosynthesis and CO_2 assimilation more active in the alkaline & high [Mg^{2+}] conditions of the stroma
- This is a source of coordinated regulation by several enzymes:
  - Target enzymes regulated by NADPH, Mg^{2+}, and pH, or all three are:
    - Rubisco
    - fructose 1,6-bisphosphatase
    - seduloheptose 1,7-bisphosphatase
    - glyceraldehyde 3-phosphate dehydrogenase
    - ribulose 5-phosphate kinase (phosphoribulokinase)

How H^+ and Mg^{2+} Movement Assist in Activation of Photosynthesis and CO_2 Assimilation





How are these regulated by NADPH?

Photosynthesis

Photosynthesis: Control from Light and CO_2 to Glucose

Target enzymes regulated by Fd:NADPH:

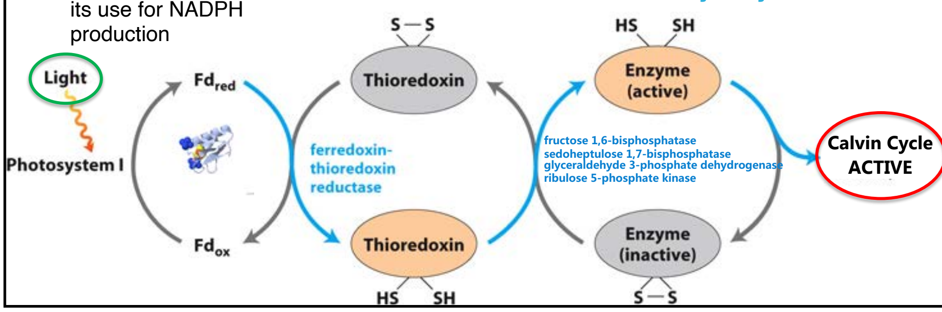
- fructose 1,6-bisphosphatase
- seduloheptose 1,7-bisphosphatase
- glyceraldehyde 3-phosphate dehydrogenase
- ribulose 5-phosphate kinase (phosphoribulokinase)
- Rubisco activase

If oxidized (Cys residues in Cys-Cys disulfide form) → enzymes are inactive.

In light, photosystem I sends e^- to *ferredoxin,* which sends them to **thioredoxin**, which donates them to disulfide bonds to reduce them to free Cys.

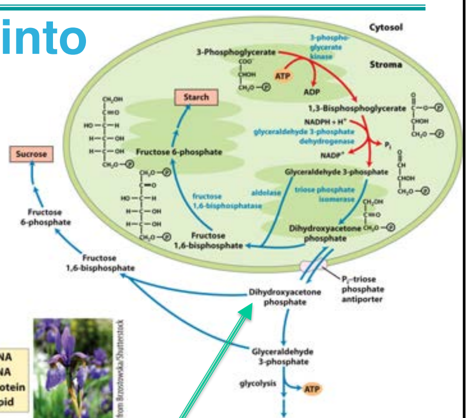
Excess reduced F_d is only available after its use for NADPH production

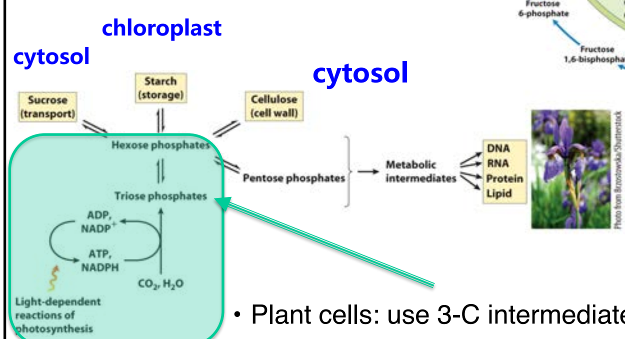
Light Activation of FOUR Calvin Cycle Enzymes via Electron-Driven Reduction of Cys-Cys Crosslinks



Photosynthesis

Assimilation of CO_2 into Biomass





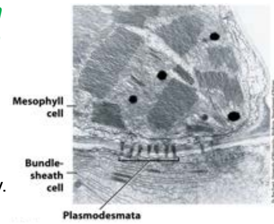
- Plant cells: use 3-C intermediates for further synthesis
  - Glyceraldehyde 3-phosphate (G3P) is the most important one.
  - made from CO_2, H_2O, plus ATP and NADPH from photosynthesis

Photosynthesis

The C_4 Pathway

C_4 versus C_3 Plants; Benefits of C_4 Plants: Heat and Drought Resistance

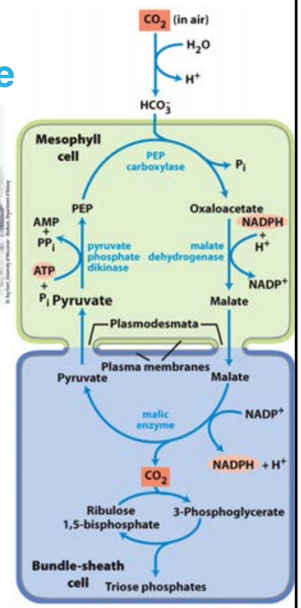
- *C_4 plants (tropical, hot climates) have an earlier step, in different cells, that isolate Rubisco from the air.*
  - In heat, the Rubisco's oxidase is favored.
  - C_4 plants spatially separate CO_2 fixation from rubisco activity, resulting in less reaction of rubisco with oxygen and avoidance of the costly glycolate pathway.



- Physical separation of reactions:
  - CO_2 is captured into ***oxaloacetate*** in mesophyll cells of the leaf.
  - Oxaloacetate then passes into bundle-sheath cells where CO_2 is released for Rubisco.

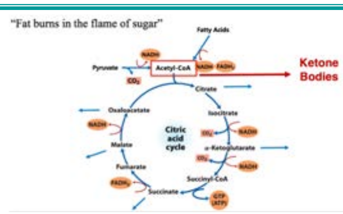
- The C_4 pathway has a higher energy cost than the glycolate cycle on a stoichiometric basis, but its all about the ratio of carboxylase:oxidase. This pathway has overall increased efficiency in heat.

- Another pathway to avoid photorespiration was first discovered in *Crassulacae (**C**rassulacean **A**cid **M**etabolism (**CAM**))* in high, dry conditions
  - Stomata open/close; the CO_2 from C4 fixation is stored as malate in **vacuoles**.



Photosynthesis

Recall in animals:

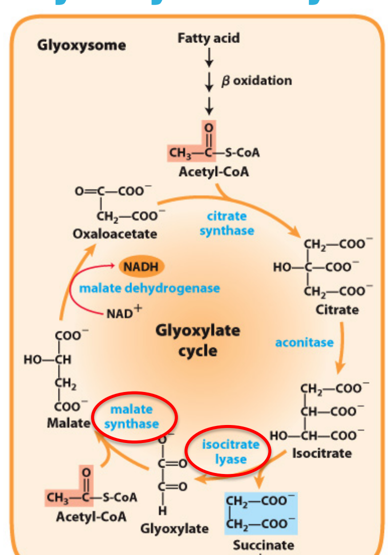


Kornberg Cycle

Plants Use Fats and Proteins for Carbohydrate Synthesis:

- In the TCA cycle, in the **glyoxysome**, instead of burning isocitrate, it short circuits TCA, taking isocitrate directly to succinate
- The result is the glyoxylate intermediate
- Re-cycle this glyoxylate by making malate from more acetyl CoA in a similar reaction as citrate synthase

GlyOXylate Cycle



We'll come back to this later............

Photosynthesis: Carbon Fixation Summary

We learned that:

- ATP and NADPH from photosynthesis are needed in order to assimilate CO_2 into carbohydrates by Rubisco
- This key enzyme of the Calvin cycle fixes carbon dioxide as well as oxygen.
- assimilations of six CO_2 molecules via the Calvin cycle lead to the formation of one molecule of glucose for use in anabolic reactions
- enzymes of Calvin Cycle have common regulation mechanisms via pH, Mg^{2+}, and/or NADPH (F_d)
- low selectivity of rubisco causes a wasteful incorporation of molecular oxygen in C_3 plants. C_4 and CAM plants have evolved separate methods for reducing this waste.
- plants can convert acetyl-CoA into carbohydrates via the Kornberg Cycle (glyoxylate cycle)