The “Dark” Reaction: Carbon Fixation

Requires Rubisco:
Ribulose-1,5-bisphosphate carboxylase
RuBPi + CO₂ → 2 x 3PG

MW = 550,000 g/mol
Subunits
eight small (14,000 g/mol)
eight large (53,000 g/mol)
4 mM in stroma (250 mg/mL)
Mg²⁺ required in active and allosteric sites

Allosteric site lysine is bound to CO₂

lysine-NH₂ + CO₂ + Mg²⁺ → lysine-NH-CO₂⁻ + H⁺
(carbamate – nonsubstrate CO₂)

“dark” means light-independent!

Mechanism of CO₂ Fixation: Rubisco Step 1

Ribulose 1,5-Bisphosphate Carboxylase

“rubisco”

First Step: CO₂ adds to enediol form of RuBPi
(not HCO₂⁻ or lysine-NH-CO₂⁻)

Fig. 20-23
**Mechanism of CO₂ Fixation: Rubisco Step 2**

H₂O adds to β-keto intermediate

Six-carbon reaction intermediates bound to ribulose 1,5-bisphosphate carboxylase (rubisco)

Fig. 20-23

**Mechanism of CO₂ Fixation: Rubisco Step 3**

Hydrated intermediate breaks down to 2 x 3PG

**C₃ plants** (nontropical)

All steps catalyzed by rubisco!

Enzyme makes up ~50% by wt total chloroplast protein

Most abundant protein in biosphere

10¹¹ tons CO₂ fixed per year!
Why is rubisco reaction energetically favorable?

\[
\text{RuBPi} + \text{CO}_2 \rightarrow 2 \times 3\text{PG} \quad \Delta G^o = -35 \text{ kJ/mol}
\]

Step 1: highly unfavorable
Step 3: highly favorable

Regulation of Enzymatic Activity of Rubisco (stroma)

Rubisco pH\(_{\text{max}}\) ~ 8 (H\(^+\) pumped into lumen from stroma)

With hv, as H\(^+\) pumped into lumen, Mg\(^{2+}\) pumped out into stroma

As hv, NADPH, ATP, [Mg\(^{2+}\) H\(^+\)], Rubisco activity.

CO\(_2\) fixation does not take place in the dark!

At night, glycolysis and oxidative phosphorylation meet energy needs of the plant.

Rubisco activity is controlled by a redox system that is dependent upon [NADPH]
Redox regulation of Rubisco

**Active Rubisco (red)**

- Oxidized form: $\text{NADPH}$
- Reduced form: $\text{Enzyme (active)}$  
  - $\text{HS} \quad \text{SH}$
  - $\text{Thioredoxin}$
  - $\text{Fd}_{\text{red}}$

**Inactive Rubisco (ox)**

- Oxidized form: $\text{NADP}^+$
- Reduced form: $\text{Enzyme (inactive)}$  
  - $\text{HS} \quad \text{SH}$
  - $\text{Thioredoxin}$
  - $\text{Fd}_{\text{ox}}$

Where is this protein found?

- $\text{4 Fe}^{2+}$
- $\text{SUMMARY OF ELECTRON TRANSFER IN PSI TO NADPH}^+$
  - $\text{Chl}_a$ - $\text{Chl}_b$
  - $\text{h}_{\text{v}} + \text{PSI}$
  - $\text{NADPH}$

What controls reduction of Fd?

- $\text{hv} + \text{PSI}$
- What glycolytic enzymes must be activated? deactivated?
  - $\text{FBPi phosphatase}$
  - $\text{PFK}$

Allosteric regulation of Rubisco

At high $\text{CO}_2$, no ATP or activase is required.

At low $\text{CO}_2$, ATP required to help displace a $\text{RuBPi}$ which blocks the lys and prevents carbamylation

$\text{RuBPi}$ binds in both the active site and the allosteric site?
From where comes the ribulose 1,5-bisphosphate (RuBPi)?

Understanding of C₅ sugar synthesis requires a knowledge of how the pentose phosphate pathway works for carbon fixation!

Pentose-Pi pathway: Pentose Pi “Shunt”

Functions:

- Oxidative portion
  - Produces NADPH for biosynthesis (fatty acids, steroids).
  - Produces Ribose (C₅) for RNA and cofactors (ATP, NAD, FAD, etc.).

- Regenerative (nonoxidative) portions
  - Provides a breakdown pathway for C₅ sugars.
  - Provides a pathway to regenerate Ru-5-Pi (C₅) for carbon fixation.

Ribulose 5-phosphate is produced in 3 steps!

- Ribulose 1,5-bisphosphate is synthesized how?

\[ \text{Ru-5-Pi} + \text{ATP} \rightarrow \text{Ru-1,5-BPi} + \text{ADP} \]
Pentose-Pi pathway (oxidative): Step 1

**glucose-6-Pi-dehydrogenase**

\[ \text{NADP}^+ \rightarrow \text{NADPH + H}^+ \]

**Typical hydride transfer mechanism to NADP⁺**

What “H-” is transferred?

What is the source of G-6-Pi?

Comes from phosphorylation of glucose and from G-1-Pi from glycogen phosphorylase breakdown of glycogen using Pi

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Pentose-Pi pathway (oxidative): Step 2

**lactonase**

**Typical hydrolysis of an ester bond.**

Where is the ester?
Pentose-Pi pathway (oxidative): Step 3

Typical hydride transfer mechanism to NADP⁺

What “H-” is transferred?

In what other pathway was a β-keto acid formed? Name of enzyme?

CAC:
isocitrate dehydrogenase!

isocit + NAD⁺ $\int$ α-KG + CO₂ + NADH

Phosphorylation of Ribulose-5-Pi to Bisphosphate

ribulose-5-Pi kinase

ribulose-5-Pi kinase

$\frac{\text{PiO}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$

$\frac{\text{C}_5}{\text{C}_5}$

$\frac{\text{PiO}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{Pi}}{\text{Pi}}$

$\frac{\text{C}_5}{\text{C}_5}$

$\frac{\text{PiO}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{OH}}{\text{OH}}$ $\frac{\text{Pi}}{\text{Pi}}$

$\frac{\text{C}_5}{\text{C}_5}$
A review of $C_6$ synthesis: Start with?

Using "$C_x$" notation, summarize the rubisco reaction:

$$C_5 + C_1 \rightarrow 2 C_3$$

How is $C_6$ made from $2C_3$?

Reverse of glycolysis starting from 3-phosphoglycerate!

$$3\text{-phosphoglycerate (3PG)}$$

A review of $C_6$ synthesis: Continue with?

$$6\text{-glyceraldehyde-3-phosphate dehydrogenase}$$

$$\Delta G = + 6.3 \text{ kJ/mol}$$

$$7\text{-phosphoglycerate kinase}$$

$$\Delta G = - 18.5 \text{ kJ/mol}$$

1st energy conservation step

7 Start here
A review of C₆ synthesis: End with ?

C₆

Yeah!

C₆ synthesis

4 - aldolase

\[ \Delta G^{\circ} = 24.0 \text{ kJ/mol} \]

Cleavage of C₆ into 2 x C₃ fragments

5 - triosephosphate isomerase

\[ \Delta G^{\circ} = -7.6 \text{ kJ/mol} \]

Isomerization of C₃ to interconvert forms

fructose-1,6-bisphosphate (F6P) + dihydroxyacetone phosphate (DHAP)

For 2 C₃ → C₆, how many ATP required? NADPH?

Note: 1 ATP and 1 NADPH

3PG (C₃)

How is C₅ regenerated?

Rubisco reaction:

\[ \text{C₅} + \text{C₁} \rightarrow 2 \text{C₃} \]

Multiply by 6

\[ 6 \text{C₅} + 6 \text{C₁} \rightarrow 12 \text{C₃} \]

Oops! C₅ consumed

\[ 10 \text{C₃} \rightarrow 2 \text{C₆} \]

C₅ regenerated using:

1) C₂ transfers (transketolase)

2) Condensations (transaldolase)!
CO₂ Fixation is called "Calvin Cycle"

\[ 6 \text{C}_5 \text{O}_6 + 6 \text{C}_1 \rightarrow 12 \text{C}_3 \]

Photorespiration:

Oxygenase Activity of Rubisco

\[ \text{O}_2 \text{ competes with CO}_2 \text{ for enol form of RuBPi} \]

\[ K_m \text{O}_2 \text{ 350 } \mu\text{M} \quad K_m \text{CO}_2 \text{ 9 } \mu\text{M} \]

Only 1 x 3PG formed

Other product, Pi-glycolate, is salvaged through a complex series of reactions involving both peroxizomes and mitochondria!
Salvage Pathway of Pi-glycolate

Photorespiration is a wasteful process, and is especially problematic at higher temperatures (above 28°C).

At 25°C, the carboxylase activity is four times the oxygenase activity.

Function of Photorespiration? Oxygenase Activity of Rubisco?

K_m CO_2 9 μM \quad \text{K_m O}_2 \ 350 \ \mu\text{M}

Rubisco...
...most likely evolved before atmosphere had O_2!
...retains ability to react with O_2? Why?

Photorespiration...
...dominates if light levels are high \rightarrow high O_2 production
...scavenges O_2 to help prevent oxidative damage to cells under intense light conditions.
**CO₂ Fixation by C4 Plants (Tropical)**

Tropical grasses such as sugar cane use a different pathway for CO₂ fixation. Short-time CO₂ fixation with ¹⁴CO₂ leads to ¹⁴C in C4 intermediates. CO₂ is fixed as follows: PEP + CO₂ → OXAL (PEP carboxylase).


**Advantage of C4 Plants**

CO₂ fixation: photorespiration

Depends upon CO₂:O₂ ratio

Air has ~ 3 x 10⁻⁴ atm (300 ppm) of CO₂

C4 plants use CO₂ at P_{CO₂} down to 1 to 2 x 10⁻⁴ atm (1 to 2 ppm).

In C3 plants, CO₂ fixation stops when P_{CO₂} is 5 x 10⁻⁵ atm (50 ppm).

At 5 x 10⁻⁵ atm, CO₂ fixation rate = photorespiration rate.

However, plants living in hot climates need to conserve water, which requires them to use low CO₂ concentration (water is used in rubisco reaction!)

The disadvantage of C4 plants is extra ATP used and a more complex pathway.

Photoinhibition

Photoinhibition:

Too much light can cause problems for photosynthetic cells

Destruction of RXN CTR occurs by side reactions of the Chla\(^+\).

The P\(_{680}^+\) (reaction center of photosystem II) is strongly oxidizing

RXN CTR proteins are oxidatively damaged by Chla\(^+\).

At high hv intensity, RXN CTR proteins turn over rapidly

http://methanogens.pdx.edu/boone/courses/B1336/B1336Lecures/200201/B1336Lec16.html