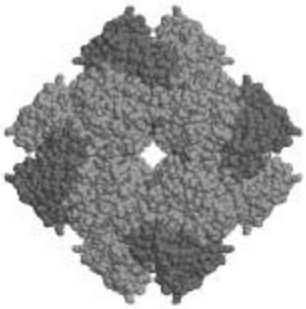
## The "Dark" Reaction: Carbon Fixation

**Requires Rubisco:**
**Ribulose-1,5-bisphosphate carboxylase**
**RuBPi + $CO_2$ 6 2 x 3PG**



**"dark" means light-indendent!**

**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)**

**$Mg^{2+}$ required in active and allosteric sites**

**Allosteric site lysine is bound to $CO_2$**

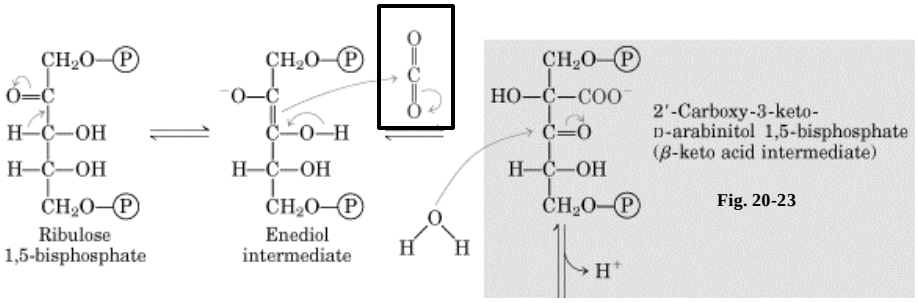
**lysine-$NH_2$ + $CO_2$ $\xrightarrow{Mg^{2+}}$ lysine-NH-$CO_2^-$ + $H^+$**

**(carbamate – nonsubstrate $CO_2$)**

## Mechanism of $CO_2$ Fixation: Rubisco Step 1

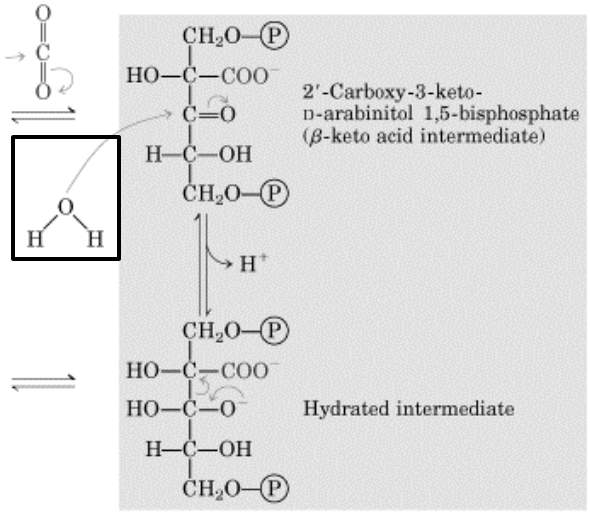
## Ribulose 1,5-Bisphosphate Carboxylase

## "rubisco"




**First Step: $CO_2$ adds to enediol form of RuBPi (not $HCO_2^-$ or lysine-NH-$CO_2^-$!)**

## Mechanism of $CO_2$ Fixation: Rubisco Step 2




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

Fig. 20-23

**$H_2O$ adds to b-keto intermediate**

## Mechanism of $CO_2$ Fixation: Rubisco Step 3

**Hydrated intermediate breaks down to 2 x 3PG**

****C3 plants** (nontropical)**

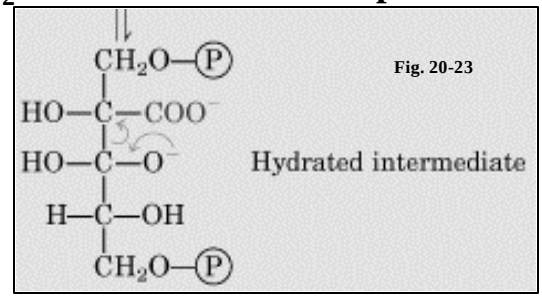
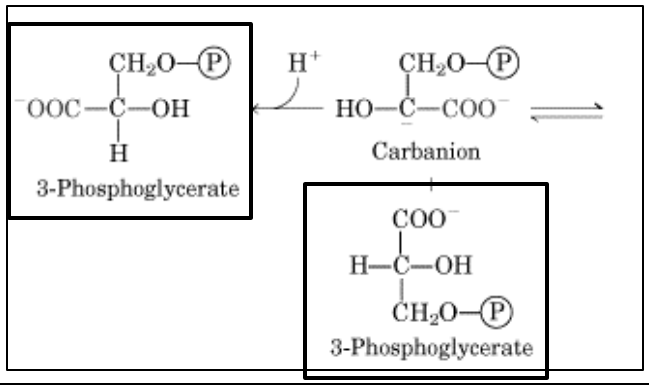



Fig. 20-23



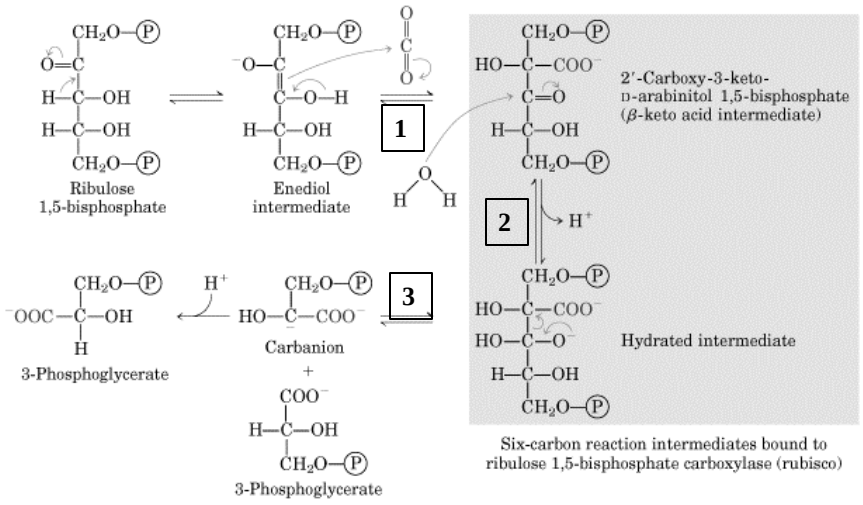

**All steps catalyzed by rubisco!**

**Enzyme makes up ~50% by wt total chloroplast protein**

**Most abundant protein in biosphere**

**$10^{11}$ tons $CO_2$ fixed per year!**

## Why is rubisco reaction energetically favorable?

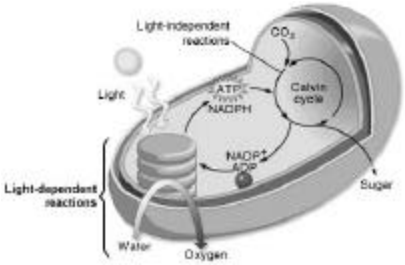



**RuBPi + $CO_2$ ⇌ 2 x 3PG  $\Delta G^{o'}$ = -35 kJ/mol**

**Step 1: highly unfavorable**

**Step 3: highly favorable**

## Regulation of Enzymatic Activity of Rubisco (stroma)




**Rubisco $pH_{max}$ ~ 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 ↑**

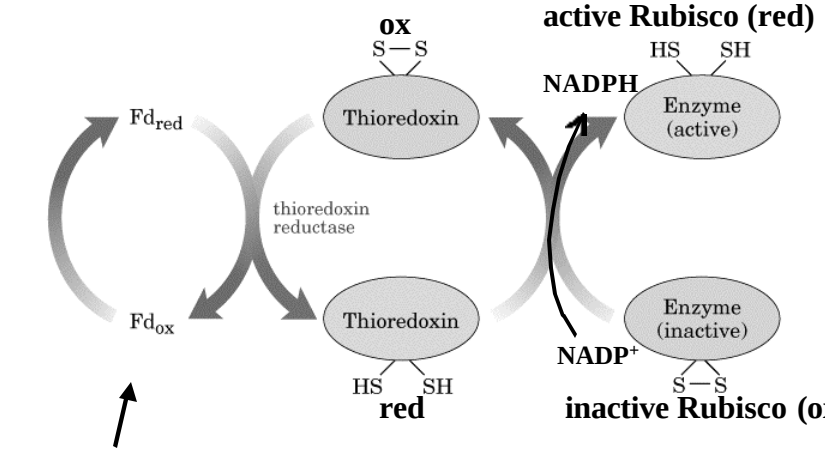
**$CO_2$ fixation does not take place in the dark!**

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

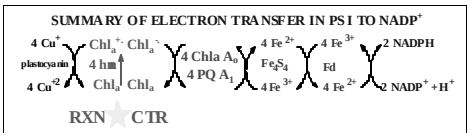
**Rubisco activity is controlled by a redox system that is dependent upon [NADPH]**

Fig. 20-36

## Redox regulation of Rubisco




**Where is this protein found?**



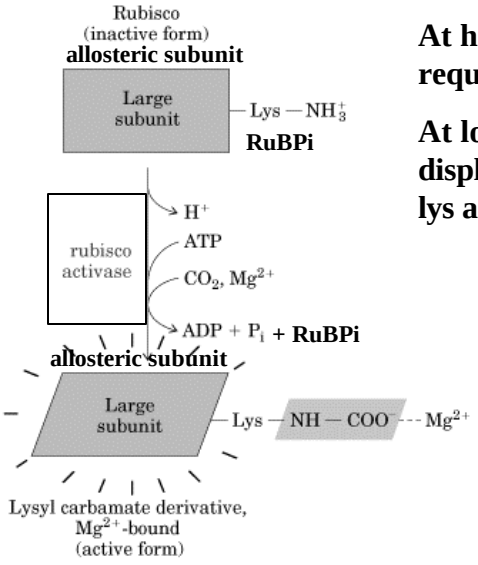

**What controls reduction of Fd?**

**hv + PSI**

**What glycolytic enzymes must be activated? deactivated?**

**FBPi phosphatase 8, PFK9**

## Allosteric regulation of Rubisco



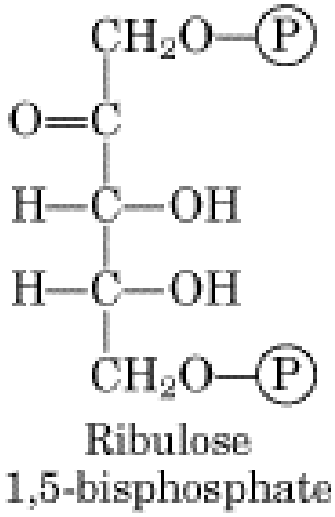

**At high $CO_2$, no ATP or activase is required.**

**At low $CO_2$, ATP required to help displace a RuBPi which blocks the lys and prevents carbamylation**

**RuBPi binds in both the active site and the allosteric site?**

Fig. 20-33

**From where comes the ribulose 1,5-bisphosphate (RuBPi)?**




Ribulose
1,5-bisphosphate

**Understanding of $C_5$ 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 ($C_5$) for RNA and cofactors (ATP, NAD, FAD, etc.).**

**Provides a breakdown pathway for $C_5$ sugars.**

**Provides a pathway to regenerate Ru-5-Pi ($C_5$) for carbon fixation.**

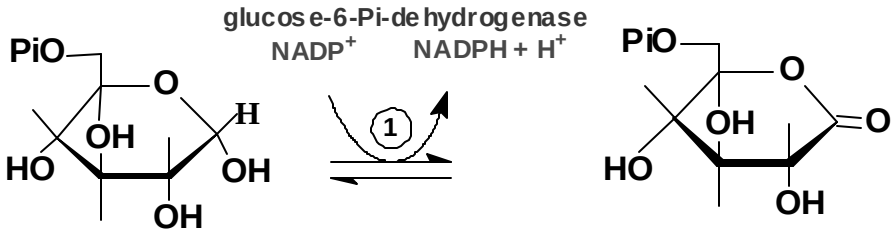
**regenerative (nonoxidative) portions**

**Ribulose 5-phosphate is produced in 3 steps!**

**Ribulose 1,5-bisphosphate is synthesized how?**

**Ru-5-Pi + ATP → Ru-1,5-BPi + ADP**

## Pentose-Pi pathway (oxidative): Step 1




glucose-6-Pi
$C_6$

6-Pi-glucono-d-lactone
$C_6$

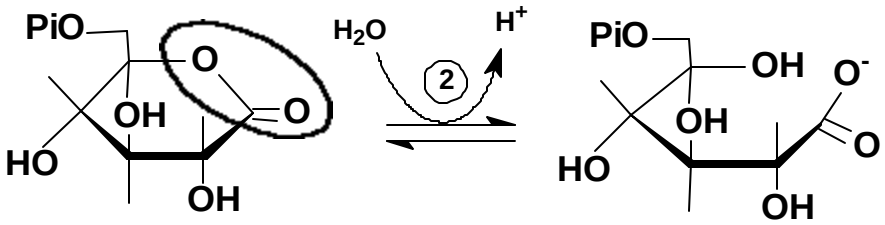
**Typical hydride transfer mechanism to $NADP^+$**

**What "H-" is transferred?**

**What is the source of G-6-Pi?**

**Comes from phosphorylation of glucose <u>and</u> from G-1-Pi from glycogen phosphorylase breakdown of glycogen using Pi**

## Pentose-Pi pathway (oxidative): Step 2



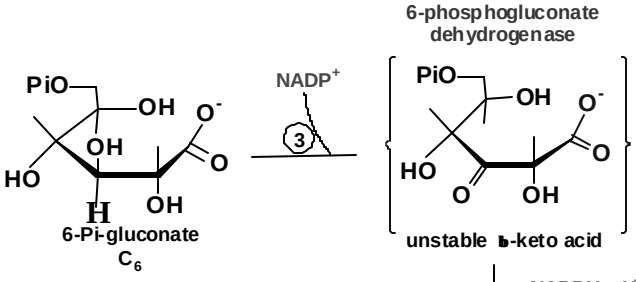

6-Pi-glucono-d-lactone
$C_6$

6-Pi-gluconate
$C_6$

**Typical hydrolysis of an ester bond.**

**Where is the ester?**

## Pentose-Pi pathway (oxidative): Step 3




**Typical hydride transfer mechanism to $NADP^+$**

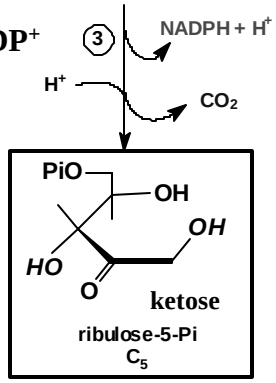
**What "H-" is transferred?**

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

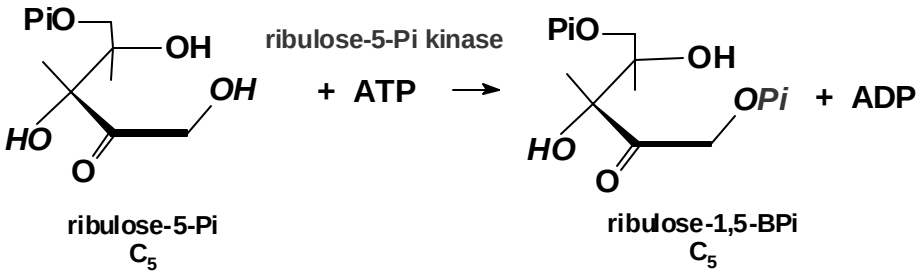
**CAC:**

**isocitrate dehydrogenase!**

**isocit + $NAD^+$ o a-KG + $CO_2$ + NADH**




## Phosphorylation of Ribulose-5-Pi to Bisphosphate



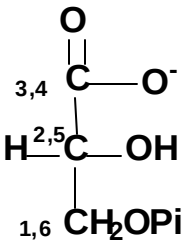
## A review of $C_6$ synthesis: Start with ?

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

$$C_5 + C_1 \rightarrow 2\ C_3$$

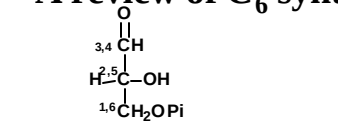
**How is $C_6$ made from $2C_3$?**

**Reverse of glycolysis starting from 3-phosphoglycerate!**

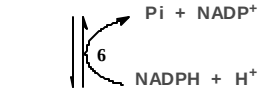


**3-phosphoglycerate (3PG)**

## A review of $C_6$ synthesis: Continue with ?

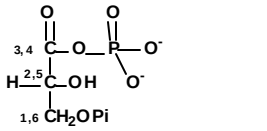


glyceraldehyde-3-phosphate (GAP)

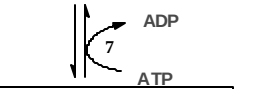


6 - glyceraldehyde-3-phosphate dehydrogenase

$\Delta G^{B}_{II}$ = + 6.3 kJ/mol



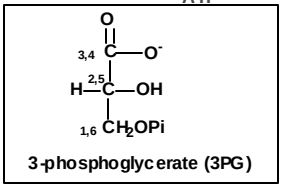
1,3-bisphosphoglycerate (1,3-BPG)



7 - phosphoglycerate kinase

$\Delta G^{B}_{II}$ = - 18.5 kJ/mol

*1st energy conservation step*



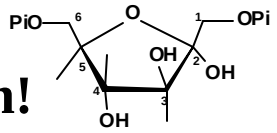
3-phosphoglycerate (3PG)

**7 Start here**

## A review of $C_6$ synthesis: End with ?

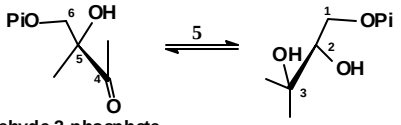
**$C_6$**

**Yeah!**




fructose-1,6-bisphosphate (F6P)

4




glyceraldehyde-3-phosphate (GAP) + dihydroxyacetone phosphate (DHAP)

4 - aldolase

$\Delta G^{\circ\prime}$ = + 24.0 kJ/mol

*Cleavage of $C_6$ into 2 x $C_3$ fragments*

5 - triosephosphate isomerase

$\Delta G^{\circ\prime}$ = - 7.6 kJ/mol

*Isomerization of $C_3$ to interconvert forms*

**For 2 $C_3$ → $C_6$, how many ATP required? NADPH?**

**Note: <u>1 ATP and 1 NADPH</u>**

**3PG ($C_3$)**

## How is $C_5$ regenerated?

**Rubisco reaction:**

**$C_5$ + $C_1$ → 2 $C_3$**

**$C_6$**

**Oops!**

**$C_5$ consumed**

**Multiply by 6**

**$6\ C_5$ + $6\ C_1$ → $12\ C_3$**

**30 6 36**

**10**

**2**

**?**

**$10\ C_3$**

**30**

**$2\ C_3$**

**6**

**$C_6$**

**$C_5$ regenerated using:**

1) **$C_2$ transfers (transketolase)**
2) **Condensations (transaldolase)!**

## $CO_2$ Fixation is called "Calvin Cycle"

$6\ C_5 + 6\ C_1 \rightarrow 12\ C_3$

30 6 36

10 2

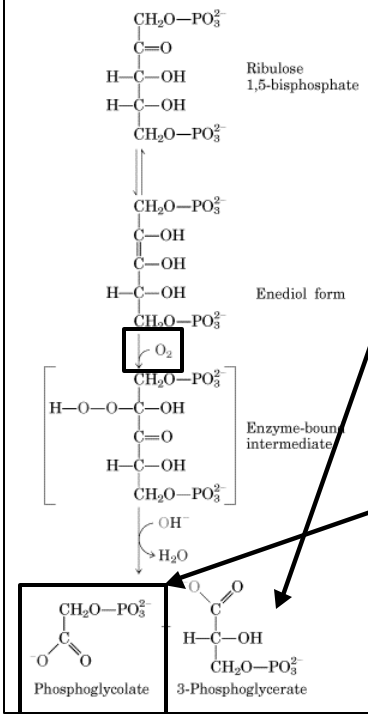
$10\ C_3$ (30) $2\ C_3$ (6) $C_6$

http://www.nobel.se/chemistry/laureates/1961/calvin-bio.html

## Photorespiration: Oxygenase Activity of Rubisco

$O_2$ competes with $CO_2$ for enol form of RuBPi

$K_m$ $O_2$ 350 ■M $K_m$ $CO_2$ 9 ■M




**Only 1 x 3PG formed**

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

Fig. 20-38

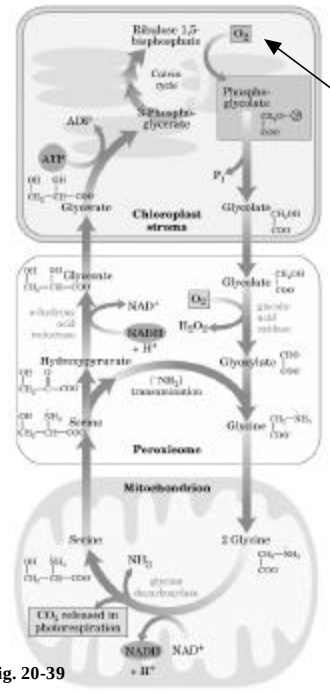



Fig. 20-39

**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 mM  $K_m$ $O_2$ 350 mM**

**Rubisco…**

**…most likely evolved before atmosphere had $O_2$!**

**…retains ability to react with $O_2$? Why?**

**Photorespiration…**
**…dominates if light levels are high → high $O_2$ production**
**…scavenges $O_2$ to help prevent oxidative damage to cells under intense light conditions**

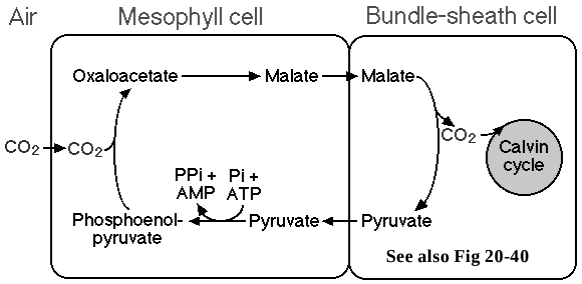
## $CO_2$ Fixation by C4 Plants (Tropical)

**Tropical grasses such as sugar cane use a different pathway for $CO_2$ fixation**

**Short-time $CO_2$ fixation with $^{14}CO_2$ leds to $^{14}C$ in C4 intermediates**

**$CO_2$ is fixed as follows: PEP + $CO_2$ → OXAL (PEP carboxylase)**

**Good summaries: http://methanogens.pdx.edu/boone/courses/BI336/BI336Lectures/200201/BI336Lec16.html**


**See also Fig 20-40**

**http://138.192.68.68/bio/Courses/biochem2/Photosynthesis/Photosynthesis2.html**

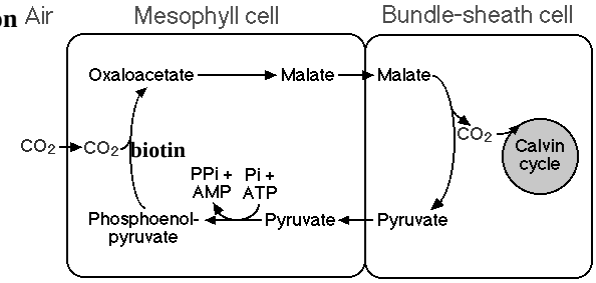
## Advantage of C4 Plants

**$CO_2$ fixation: photorespiration**

**Depends upon $CO_2$:$O_2$ ratio**

**Air has ~ 3 x $10^{-4}$ atm**

**(300 ppm) of $CO_2$**




**http://138.192.68.68/bio/Courses/biochem2/Photosynthesis/Photosynthesis2.html**

**C4 plants use $CO_2$ at $P_{CO2}$ down to 1 to 2 x $10^{-6}$ atm (1 to 2 ppm).**

**In C3 plants, $CO_2$ fixation stops when $P_{CO2}$ is 5 x $10^{-5}$ atm (50 ppm).**

**At 5 x $10^{-5}$ atm, $CO_2$ fixation rate = photorespiration rate.**

**However, plants living in hot climates need to conserve water, which requires them to use low $CO_2$ concentration (water is used in rubisco reaction!)**

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

**http://methanogens.pdx.edu/boone/courses/BI336/BI336Lectures/200201/BI336Lec16.html**

## Photoinhibition

**Photoinhibition:**

**Too much light can cause problems for photosynthetic cells**

**Destruction of RXN CTR occurs by side reactions of the $Chla^{+\cdot}$**

**The $P_{680}^{+\cdot}$ (reaction center of photosystem II) is strongly oxidizing**

**RXN CTR proteins are oxidatively damaged by $Chla^{+\cdot}$**

**At high hν intensity, RXN CTR proteins turn over rapidly**

**http://methanogens.pdx.edu/boone/courses/BI336/BI336Lectures/200201/BI336Lec16.html**