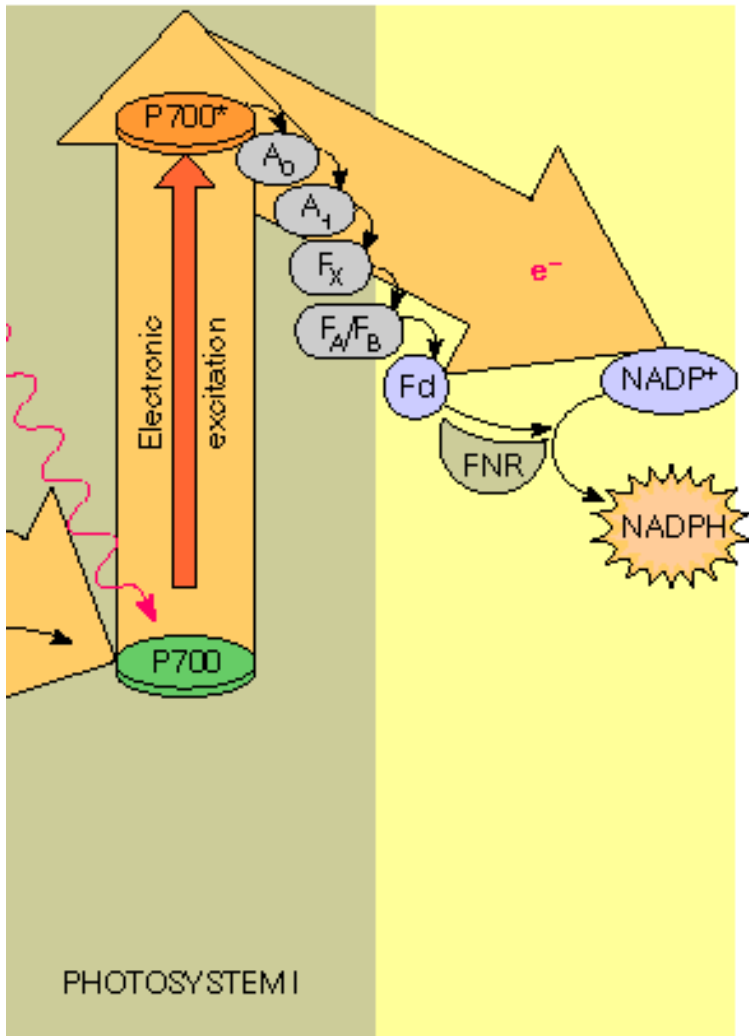


Complexes are named for longest effective wavelength.



Photosystem I P700

Electron transport to

Chlorophyll

Quinones

Iron Sulfur Clusters

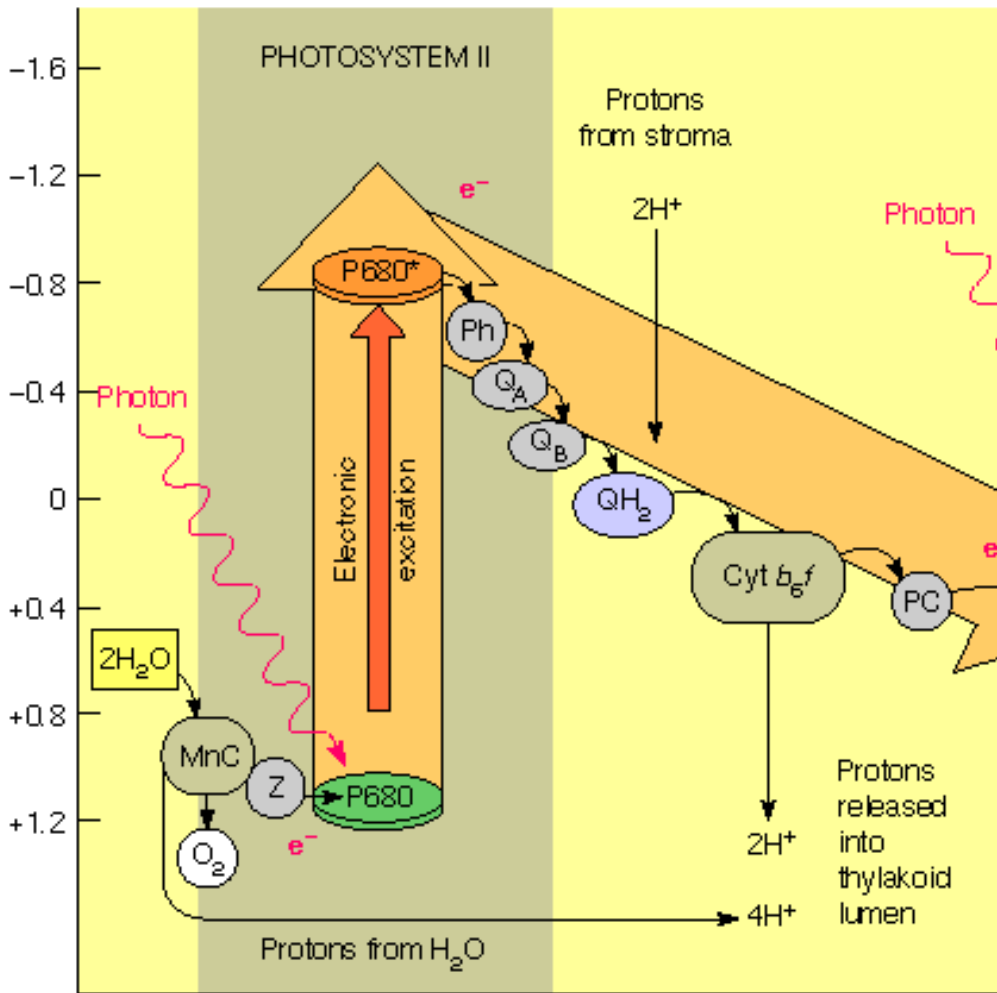
Ferredoxin

Ferredoxin NADP

Oxidoreductase

Protons are pumped

And NADPH is made.



Photosystem II

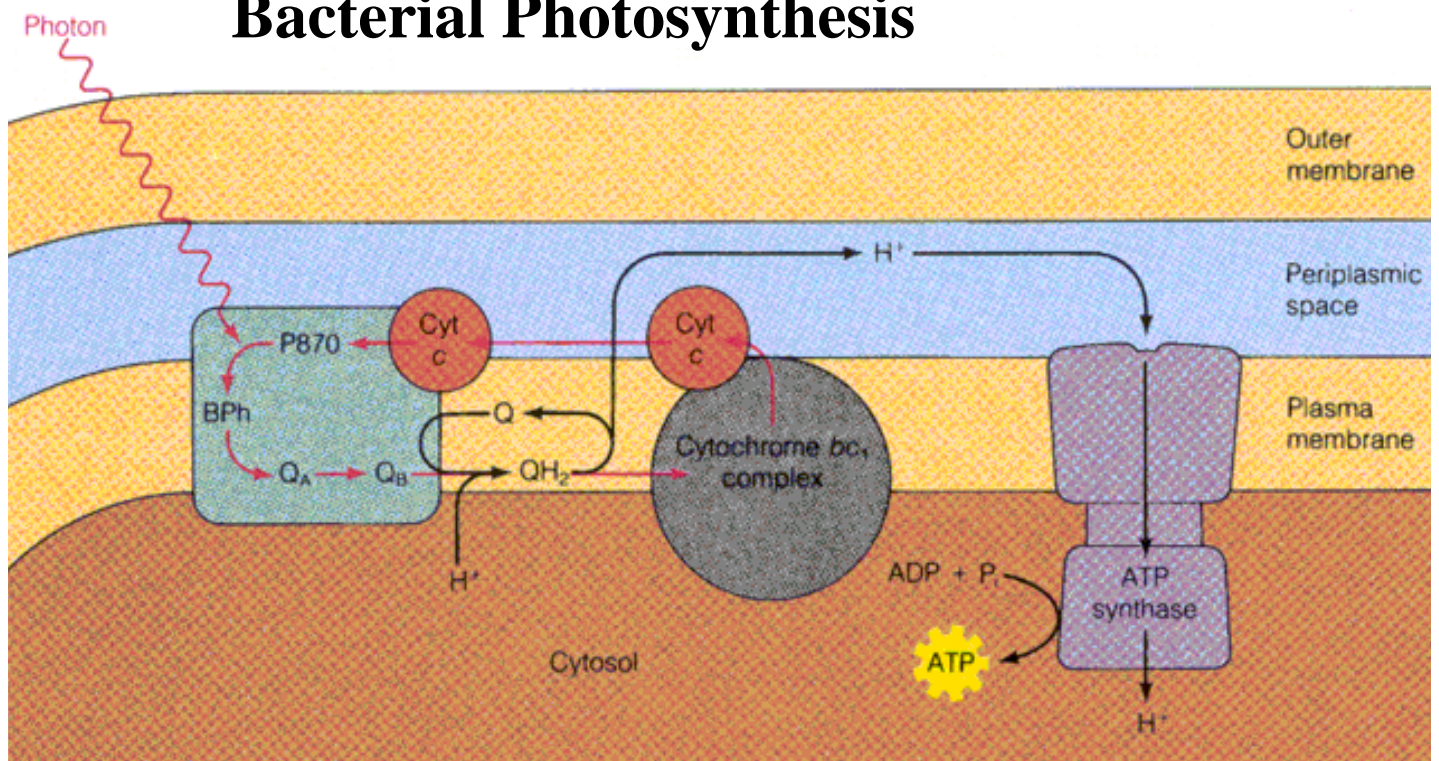
Excited
Electron is
Passed to

Phytins
Quinones
Cytochrome
Plastocyanin

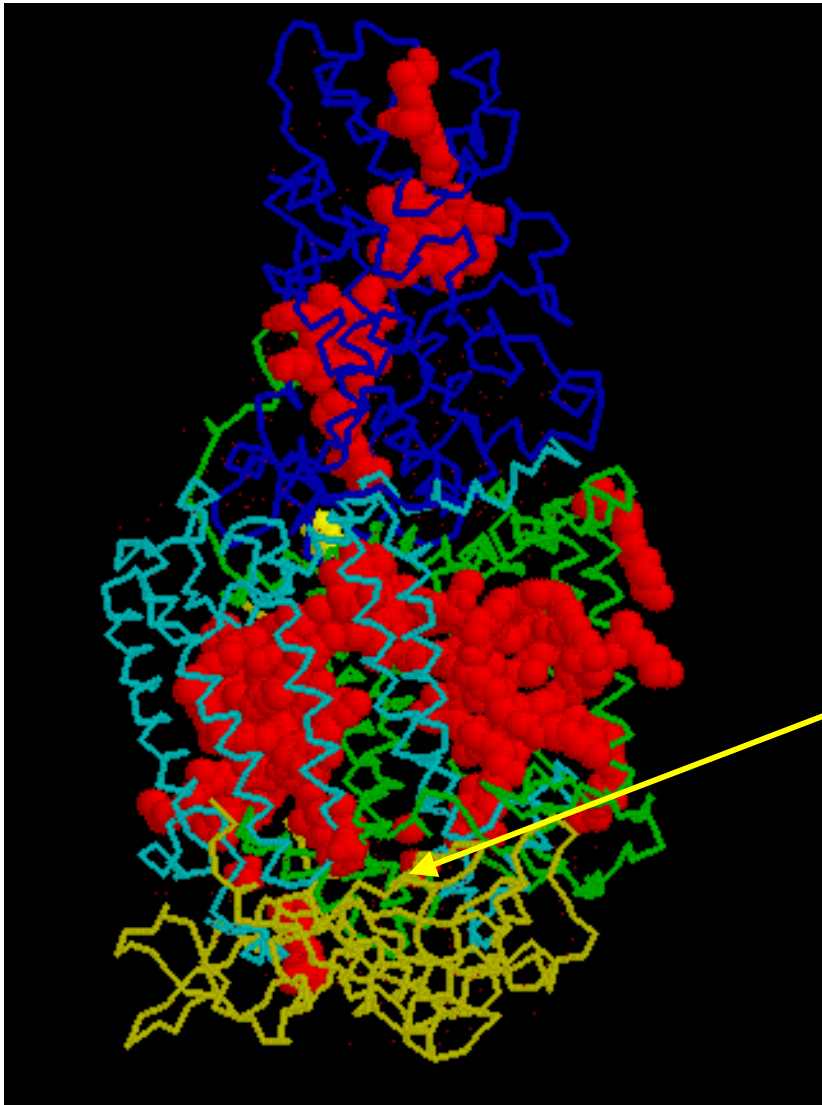
Protons are
Pumped.

Water supplies
Electron to P680

Bacterial Photosynthesis



Light is low energy-P870 not strong enough to oxidize water. Excited electron passed to Q. “hole” filled with electron from Cytochrome C. 100% efficient!

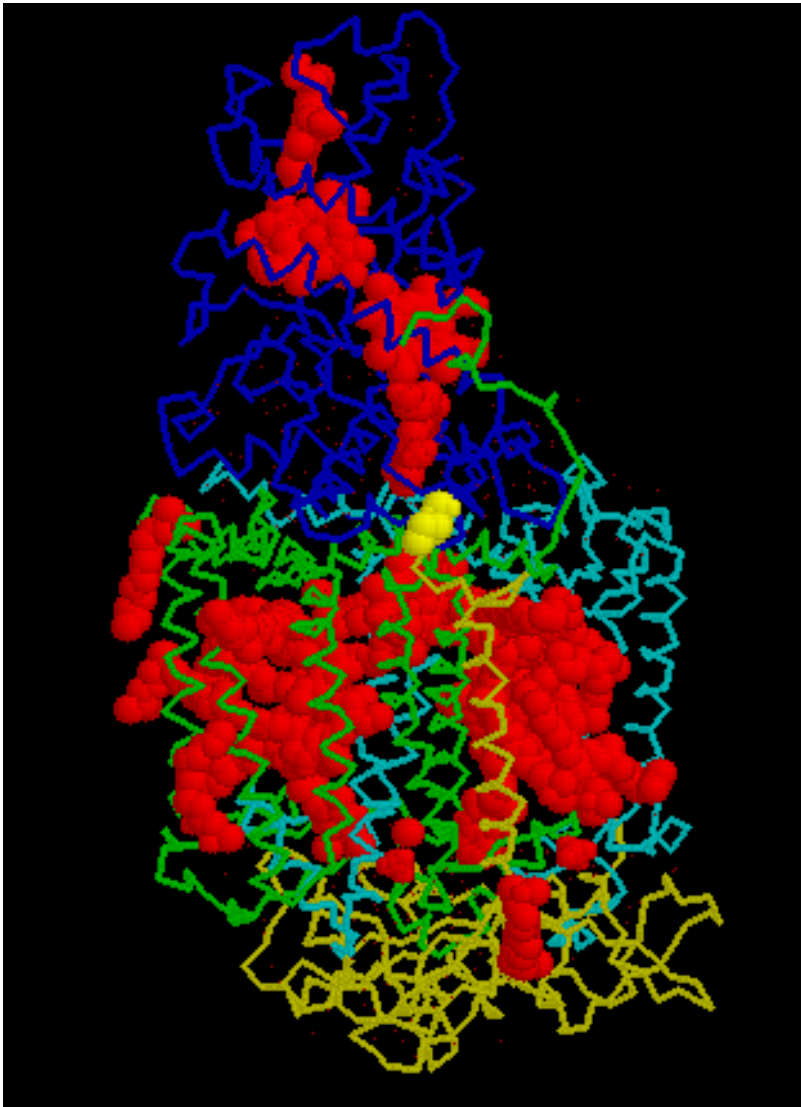


Bacterial Rxn Center

**Top--cytochrome
subunit (blue) (4 hemes)**

**Middle--membrane
Embedded protein
4 chlorophylls,
2 phytins
2 quinones
1 Fe**

**Bottom--
subunit H (yellow)**



Bacterial Rxn Center

(*Rhodobacter viridis*)

**Structure by
Deisenhofer and Michel
Nobel Prize 1988**

Net results

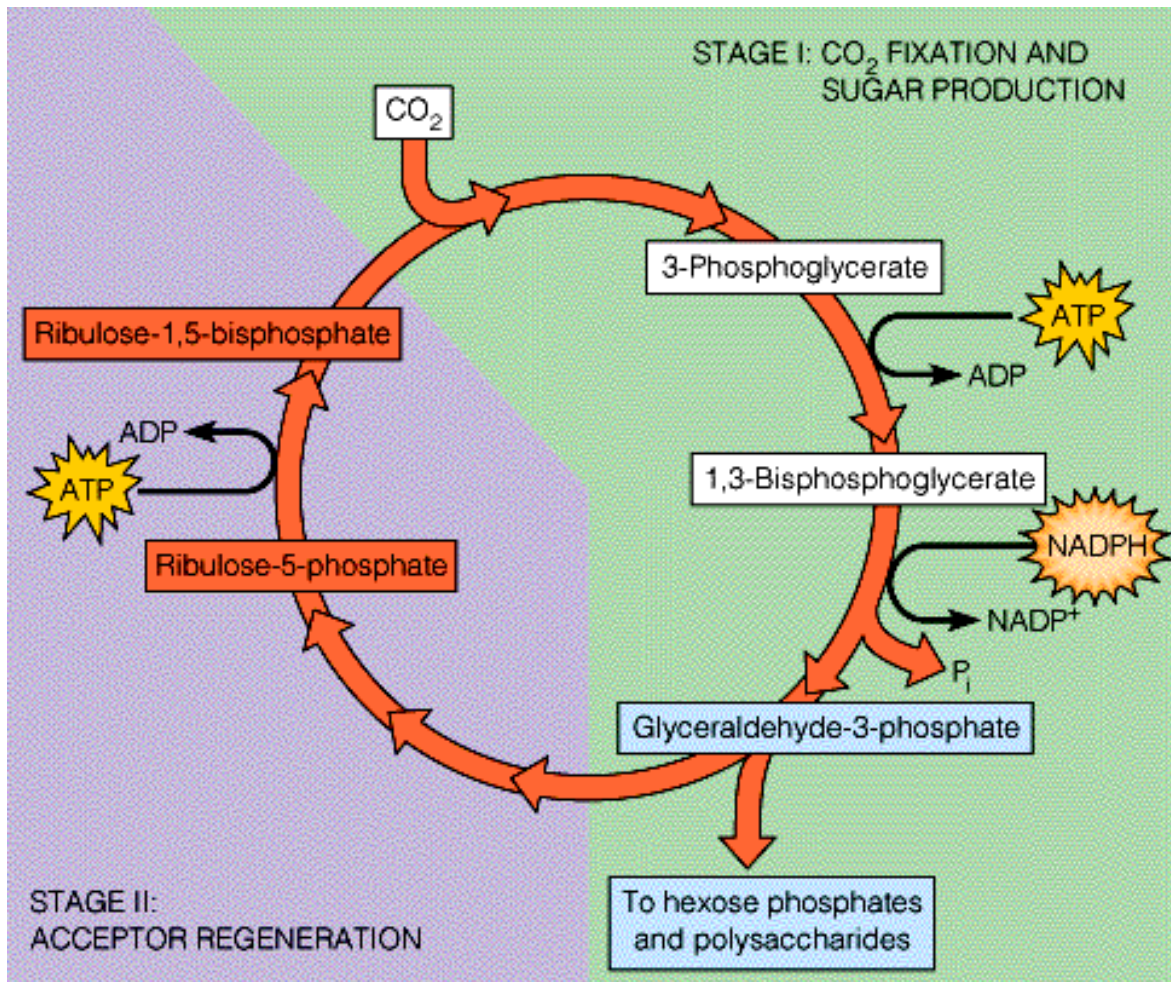
**No O₂ evolution
But does produce charge
Separation and proton
pumping.**

Photosynthesis vs Respiration

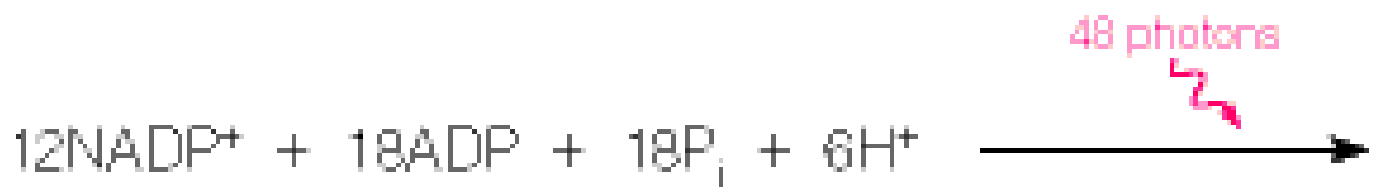
- **Electron transport**
- **From high to low potential**
- **Via series of membrane protein/cofactors**
- **Some mobile e carriers (PC)**
- **Proton gradients**
- **Similar ATP synthases**
- **Respiration reduced high potential electron**
- **Photon excites electron to high energy**
- **Proton gradients are opposite direction**

Practical Reasons to Study Photosynthesis

Artificial Photosynthesis
Solar Energy



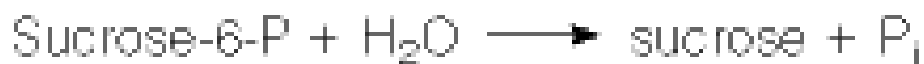
Net yield 48 photons/ glucose 30-40% efficient

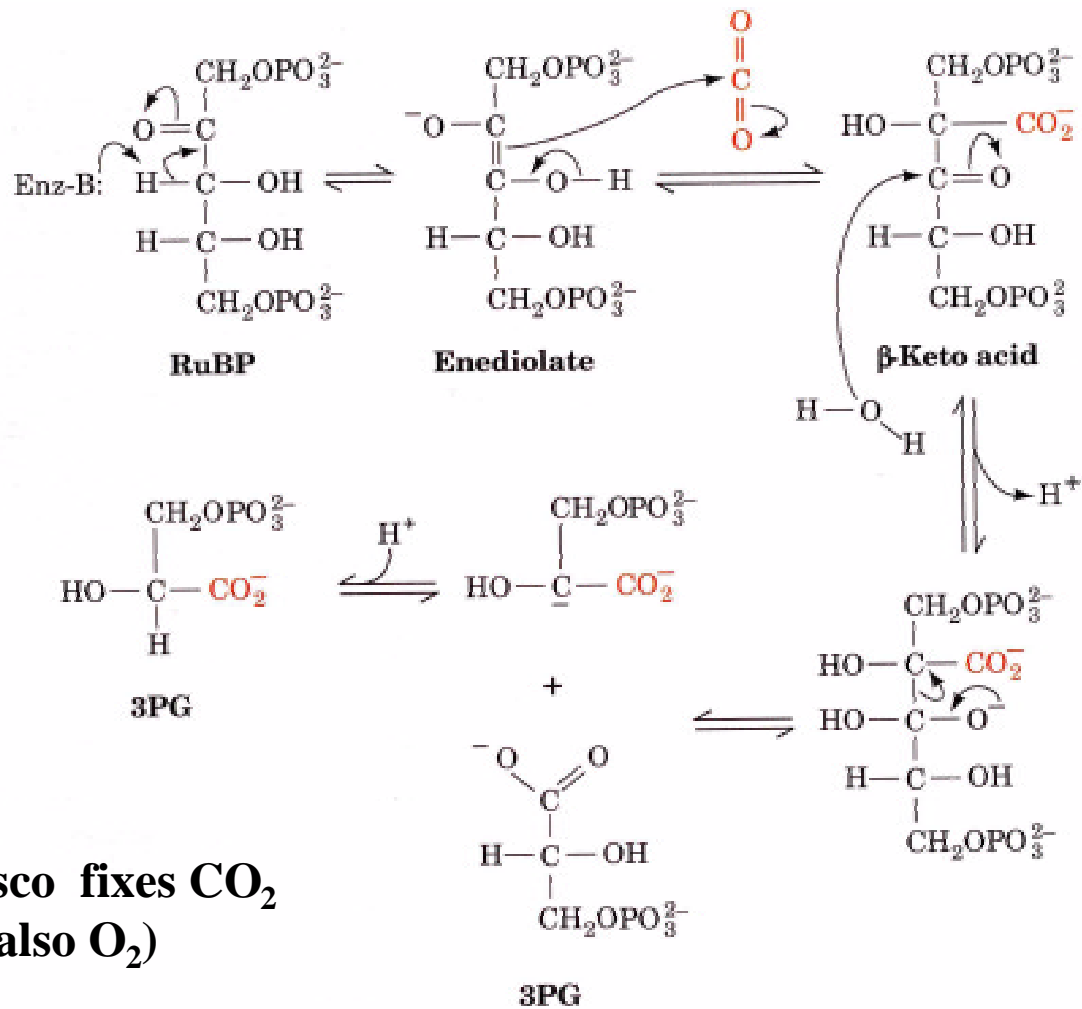


Amylose for storage

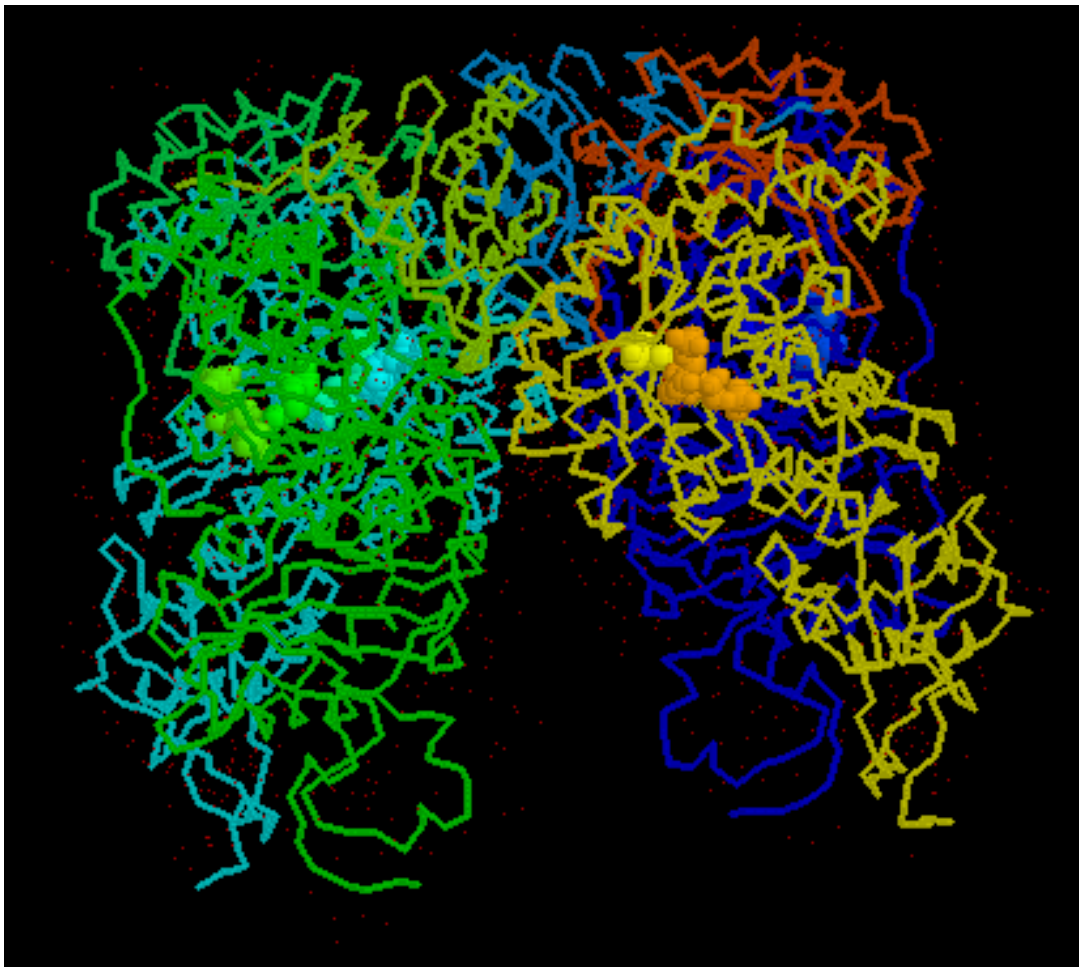


Sucrose for export



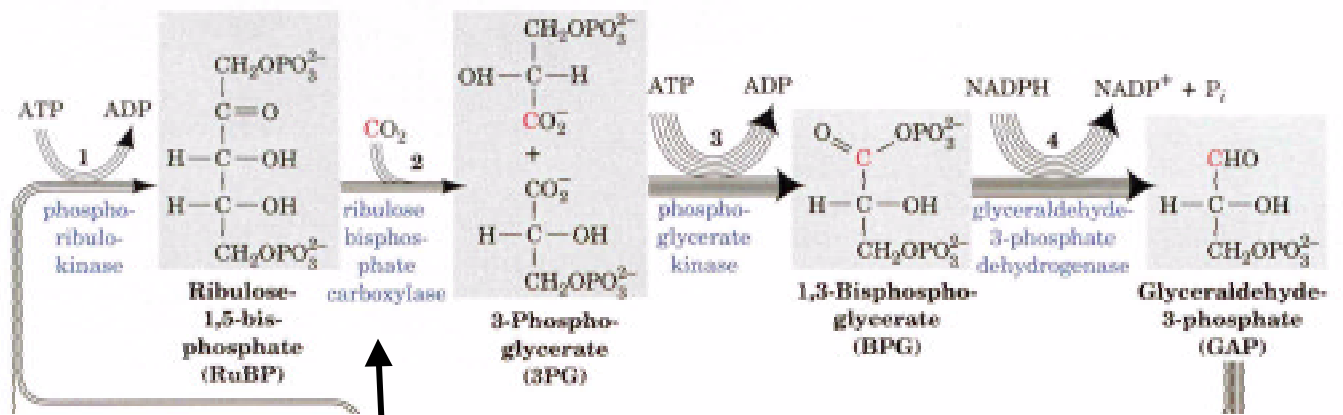


**Rubisco fixes CO_2
(and also O_2)**



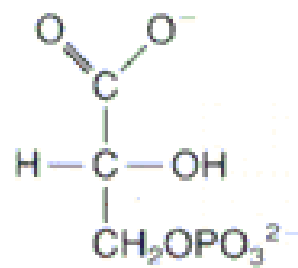
**Most
abundant
enzyme on
Earth.**

**But it's
lousy
(and
genetic
engineers
would like
to improve
it)**

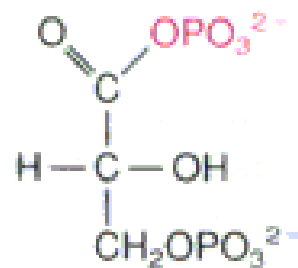
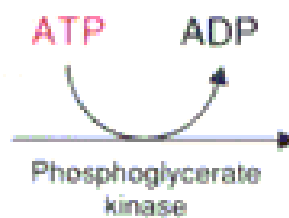


But K_m for O_2 is 10X larger

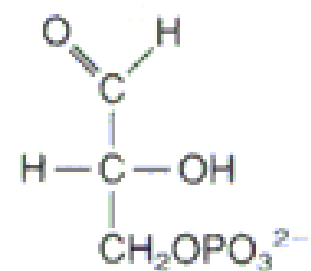
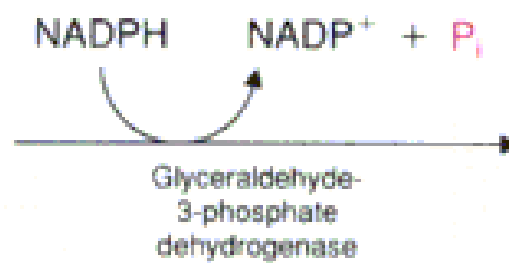
Photorespiration, production of CO_2 & ATP & Glyoxylate $\text{C}_2\text{O}_3\text{H}$.



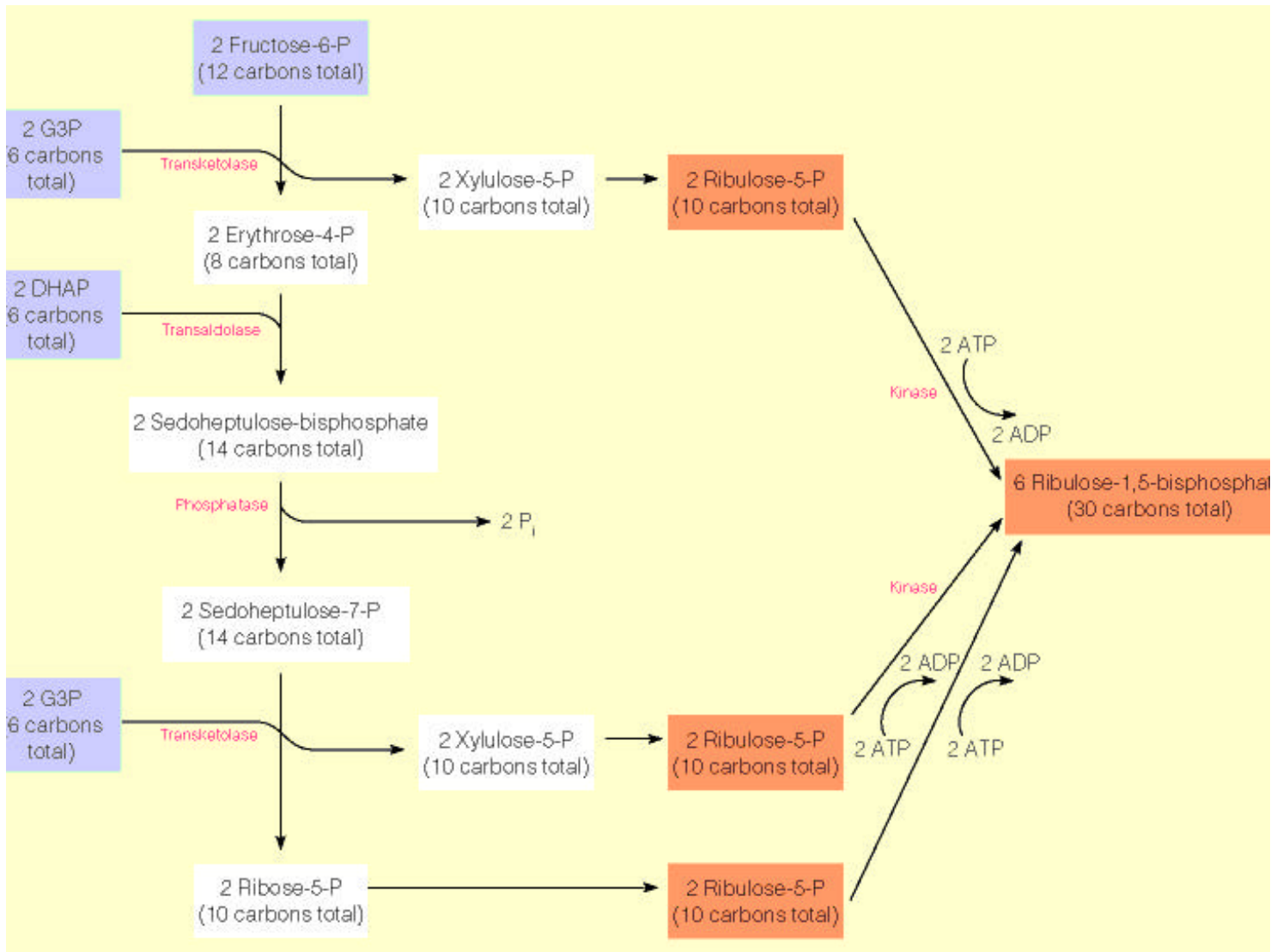
3-Phosphoglycerate

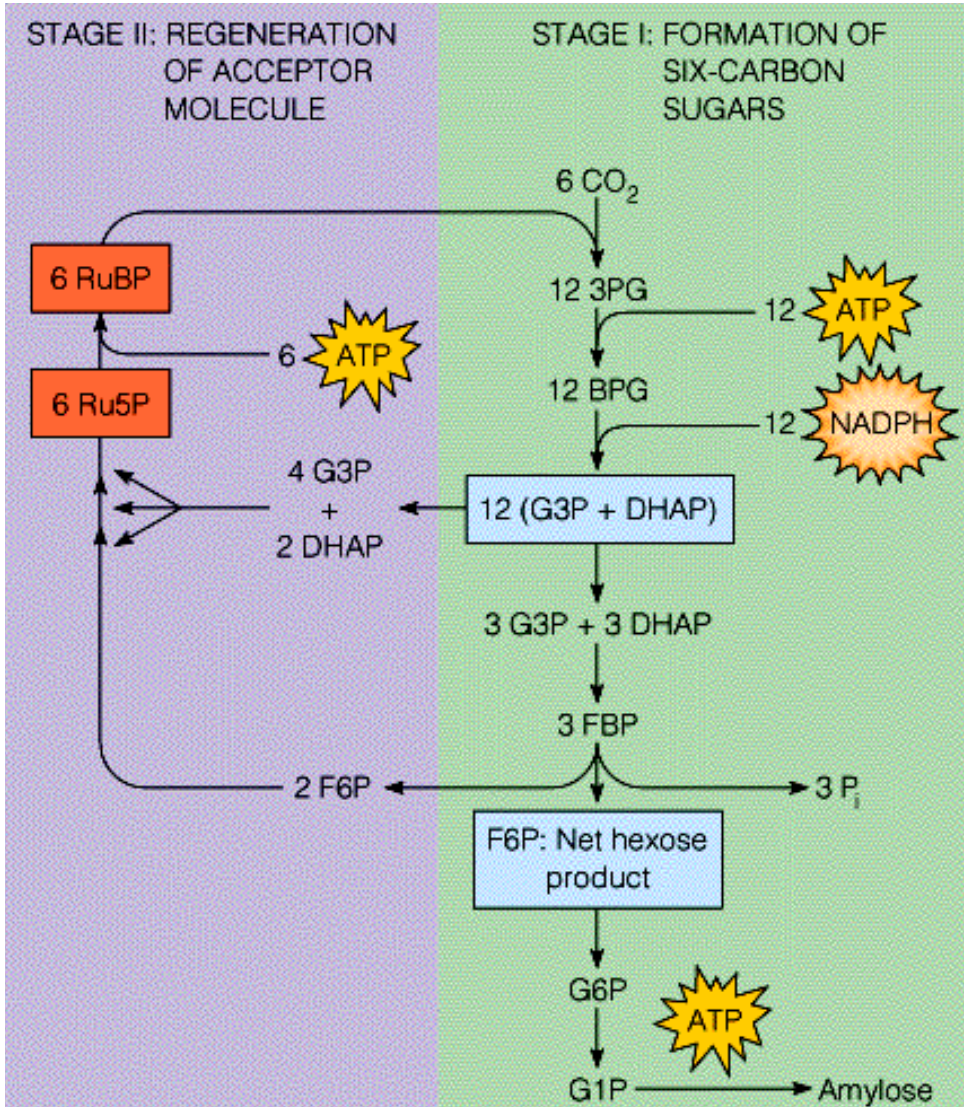


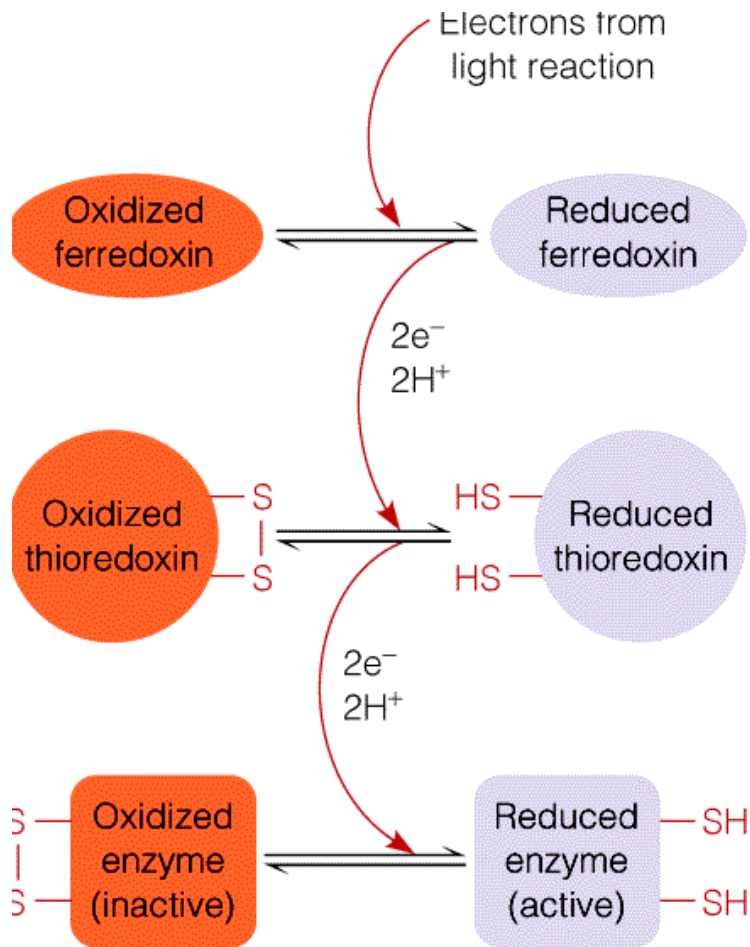
1,3-Bisphosphoglycerate



Glyceraldehyde-3-phosphate







Regulatory links Between Light and dark Reactions via Electrons/redox

Calvin cycle enzymes
activated/accelerate
by reduction
to SH.
“Dark” reactions work
better in the light.

Happy Earth Day!!--Check out the Garbage Lecture



This Week:

**One page abstracts due Monday
Rubisco article
Chap. 27 beginning.**

Wed/Thurs. your presentations

**Friday--Chapter 27 Paragraphs
(no HW) In class--evalutions.**

**Monday Exam #2 in class &
take home.**



General Regulation

Light associated changes in pH, Mg²⁺ .

Rubisco Regulation

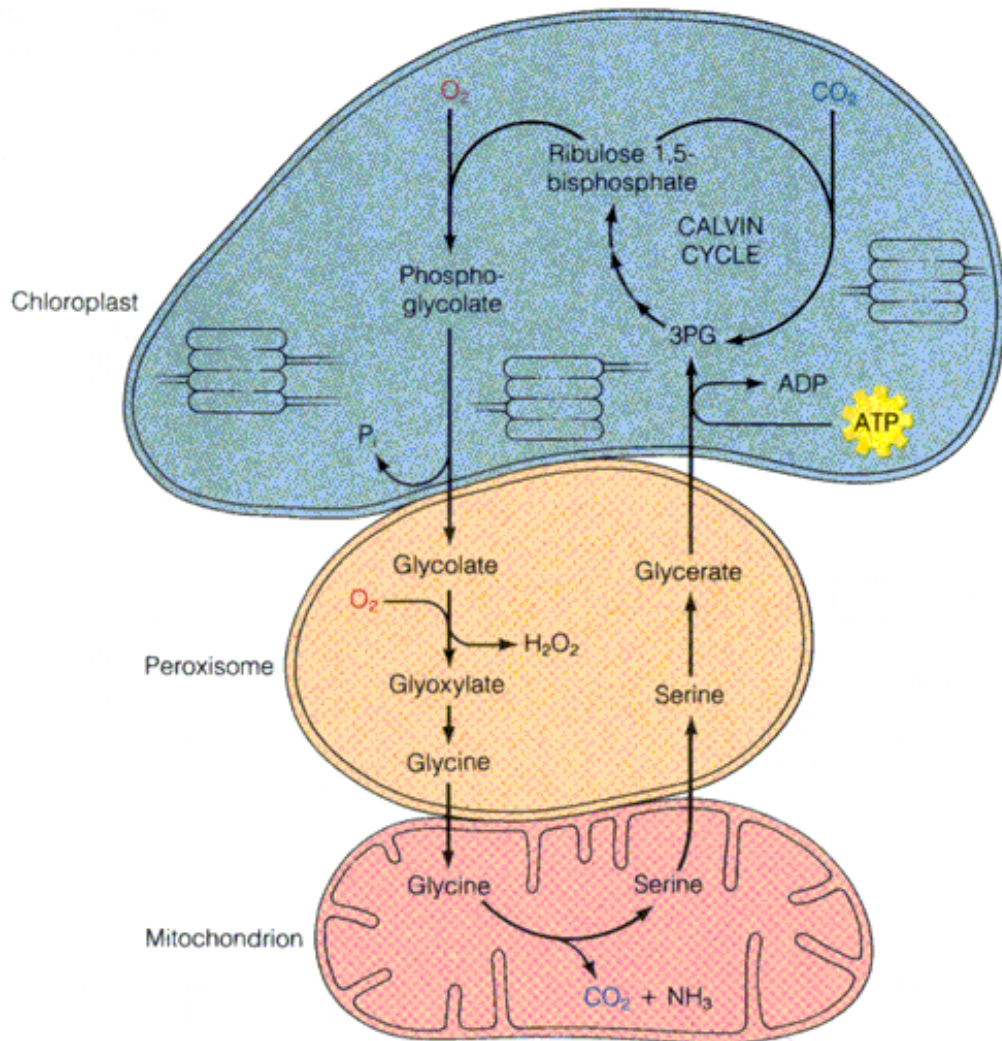
**Activated by CO₂ covalent modification
of lys near active site (carbamoylation)**

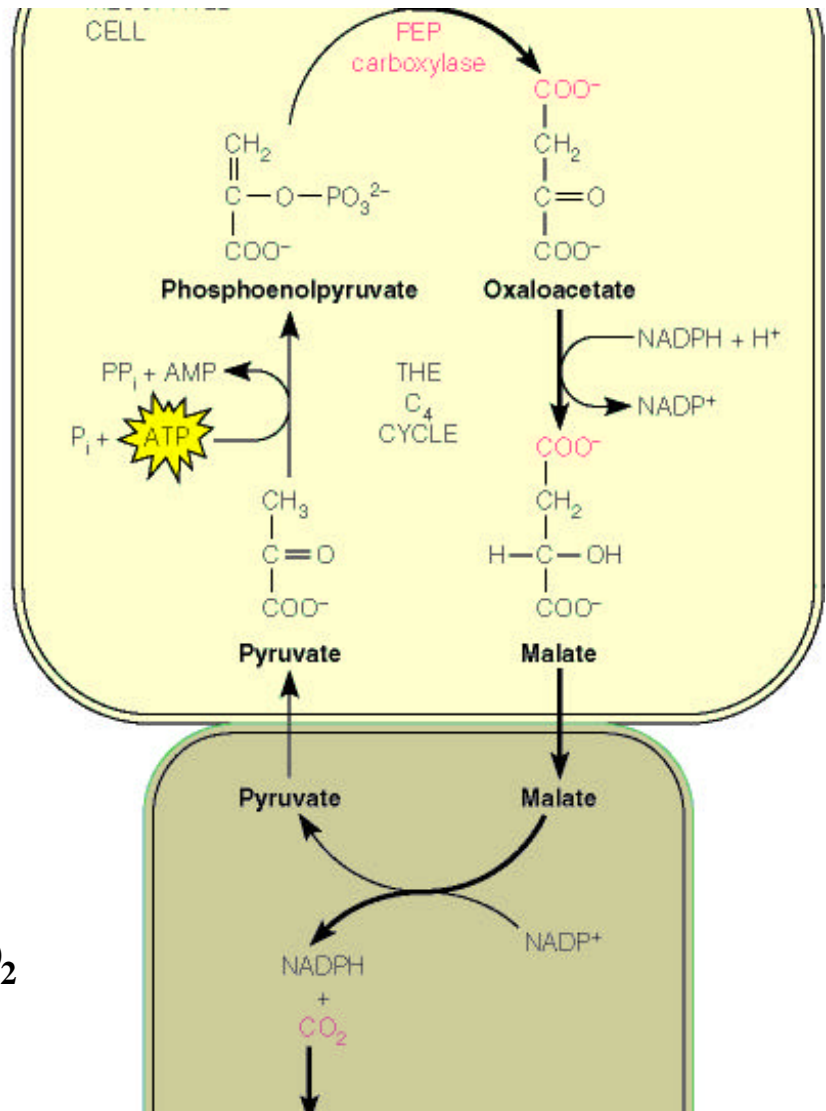
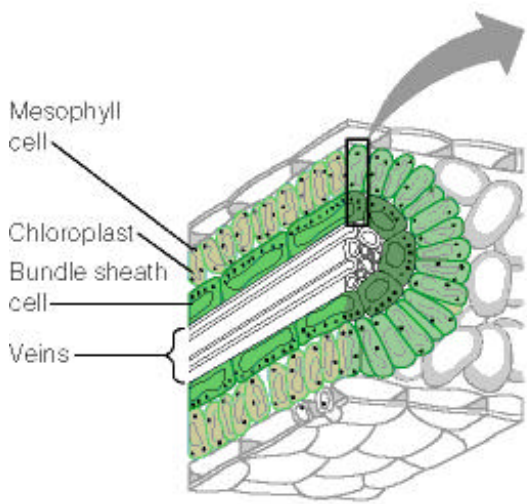
But substrate (RuBP) inhibits this activation

Activase forces ATP-dependent release of RuBP

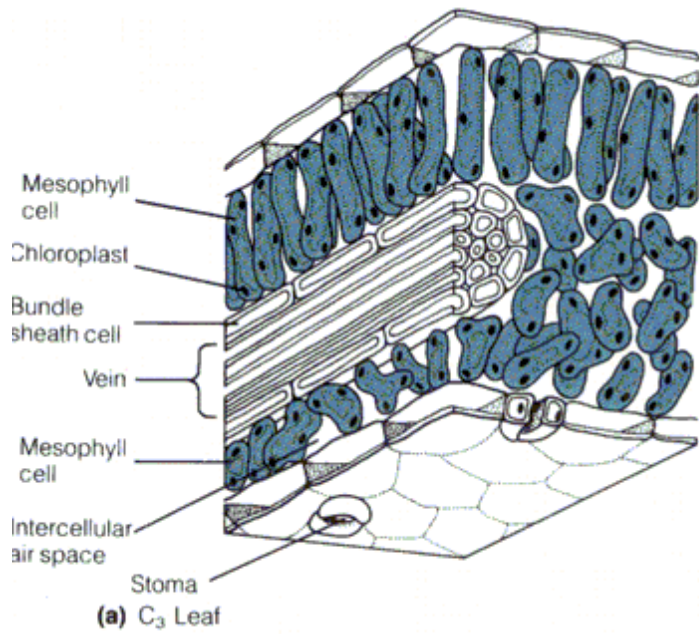
**Inhibitor--some plants make a natural TS inhibitor
“nocturnal” inhibitor that is broken down in the light.**

**All active site and regulatory sites are of interest to plant
genetic engineers.**

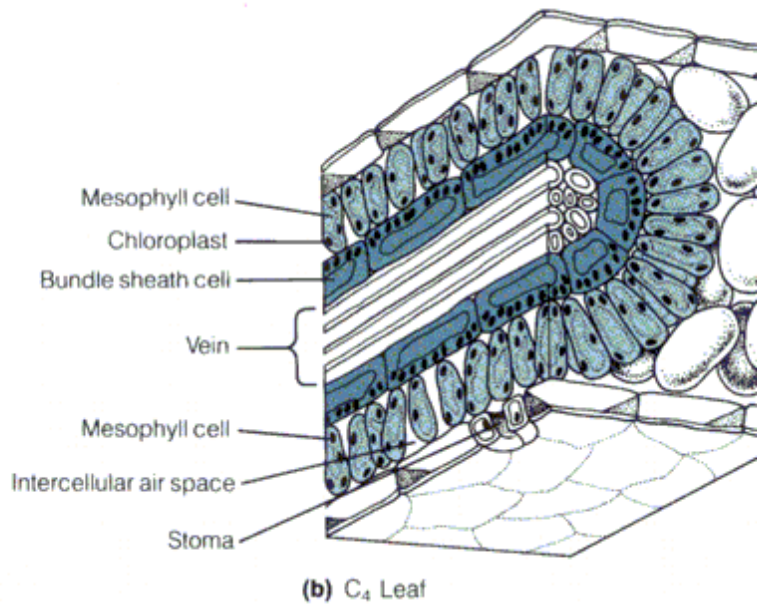




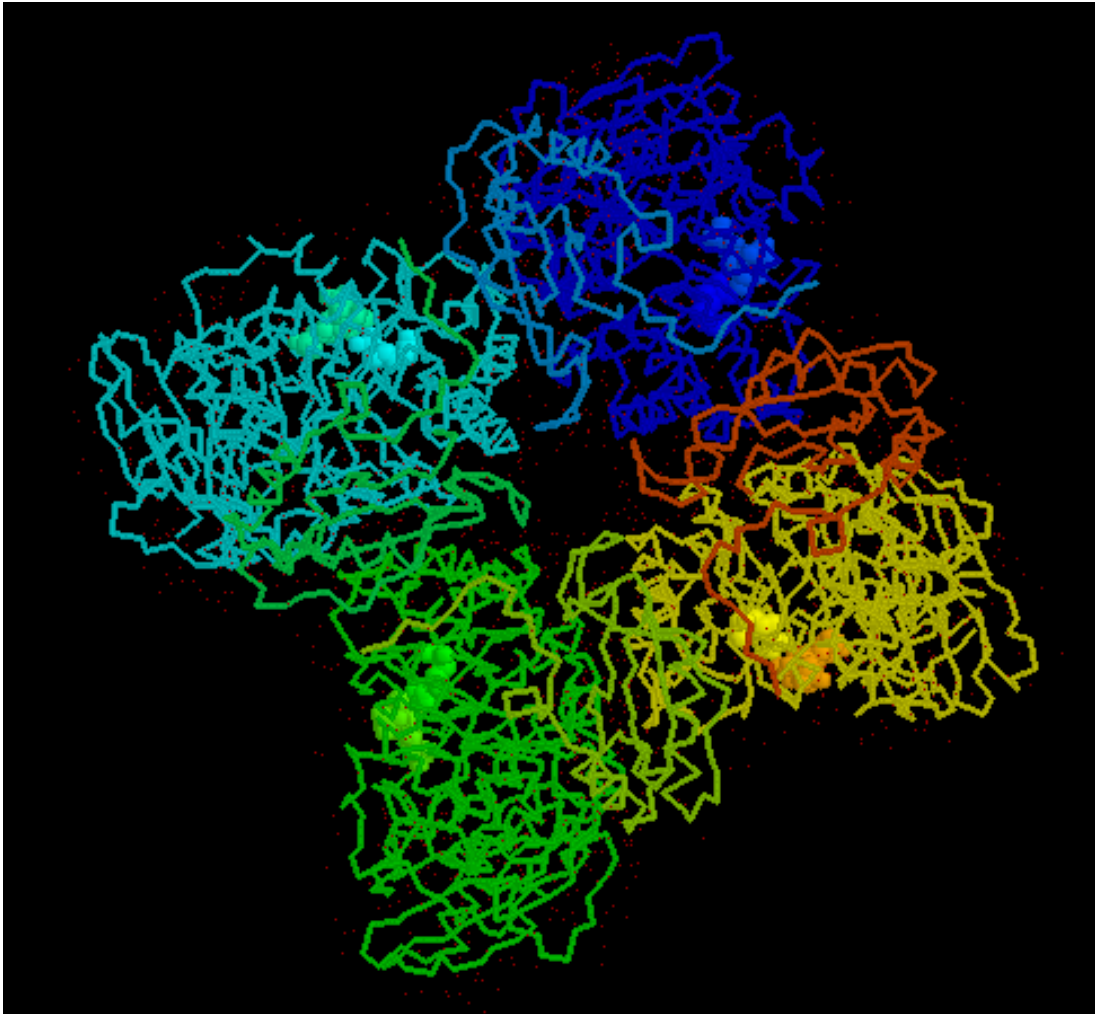
Tropical Plants
Small pores for
CO₂ and O₂
C4 cycle to “fix” CO₂



**Wheat
Sunflowers**



Corn

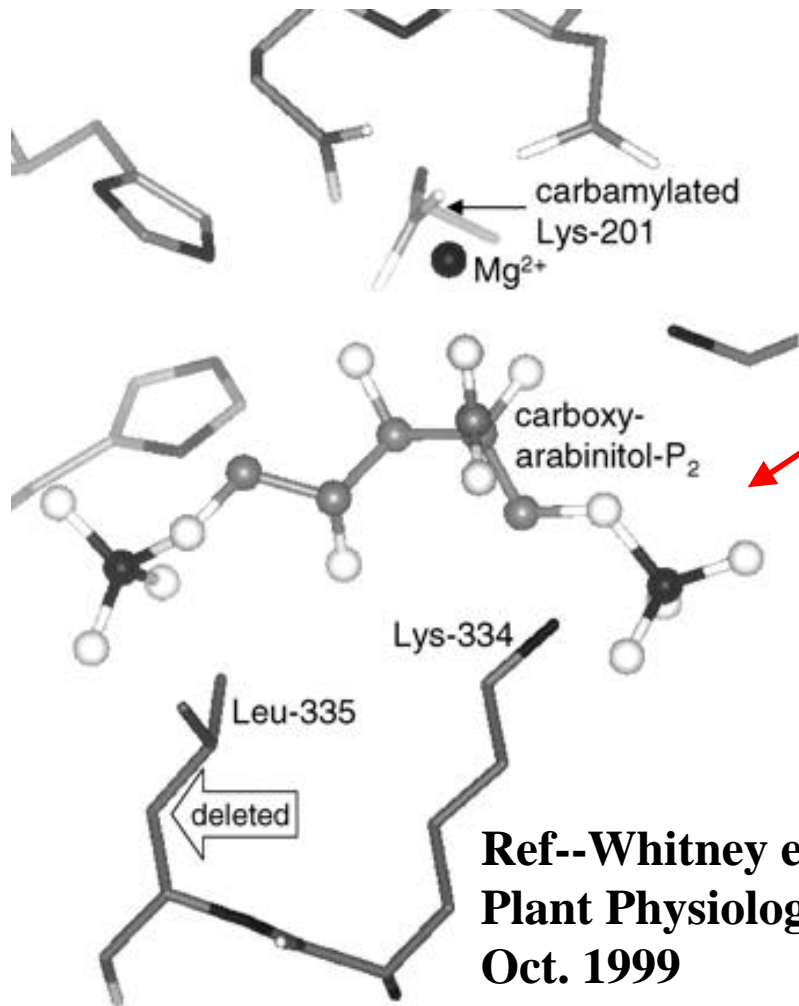


**“Rubisco”
Ribulose bis
Phosphate
carboxylase**

**Mg
Co-factors**

**8 catalytic
8 regulatory
Subunits**

**Poor CO₂/O₂
discrimination**

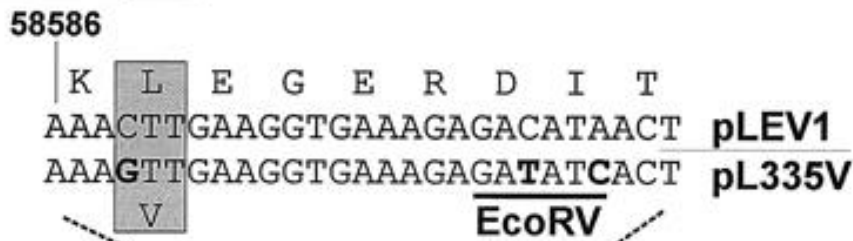


**Nocturnal Inhibitor
is a transition state
inhibitor.**

**Hypothesis--
Because N of Lys 334
Stabilizes new COO⁻,
mutation of Leu335 to Val
should alter N⁺ placement,
reducing catalysis and
CO₂/O₂ selectivity.**

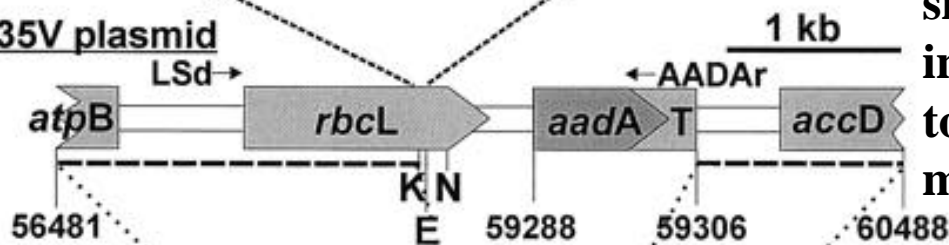
**Ref--Whitney et al
Plant Physiology
Oct. 1999
pp. 579-588.**

Mutated region



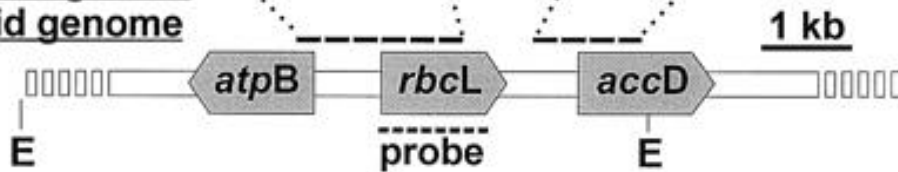
Large subunit
Gene is in
Chloroplast
(plastid)

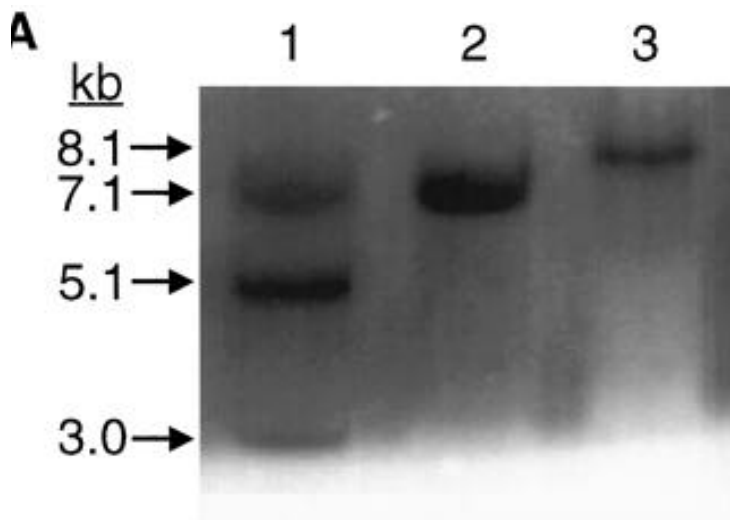
pL335V plasmid



A “silent” EcoRV
site was
introduced into
to detect
mutants.

**Target region of
plastid genome**

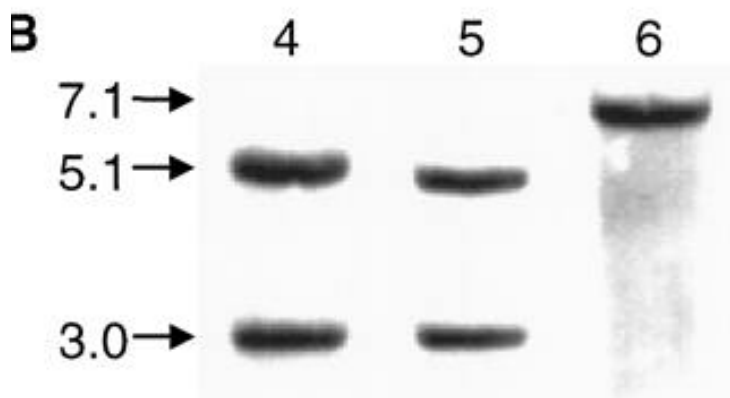




Lanes 2 & 6
Control no EcoRV site.

1 retains mutation.
3 has lost mutation.

4 & 5 are grown
From 1.



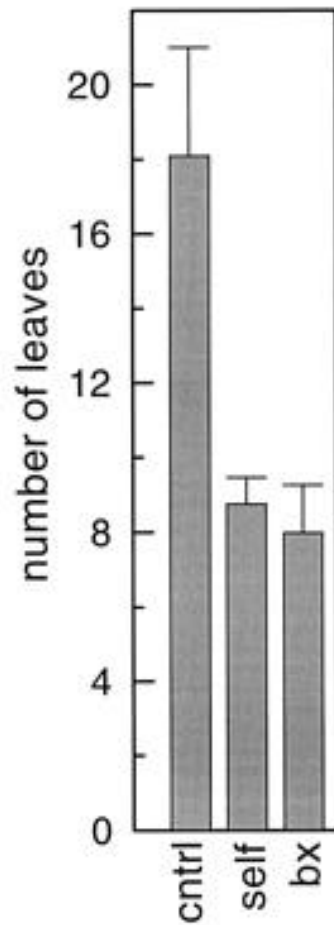
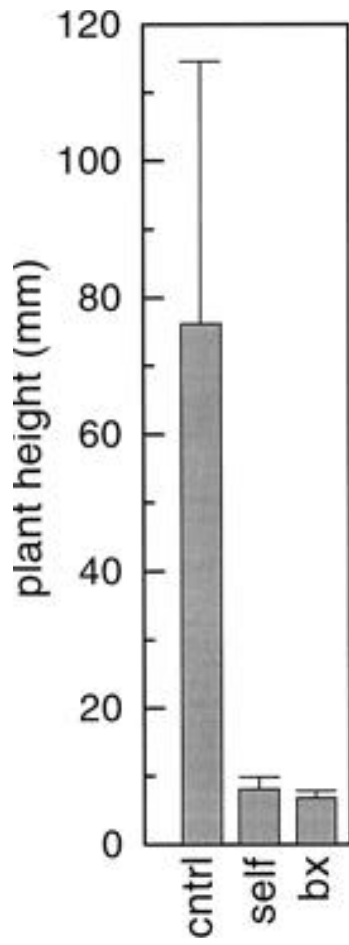
Only a small number of
transformant
stably bear mutant in
chloroplast gene.

DARN!

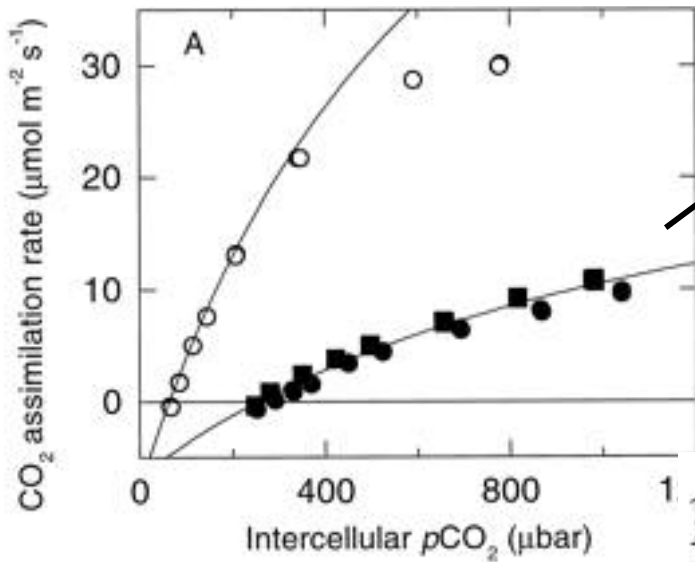


**Just one
Amino acid
Is changed!!!**

**But the mutant
does manage to
mature and flower.**



Mutated plants are quantitatively different.

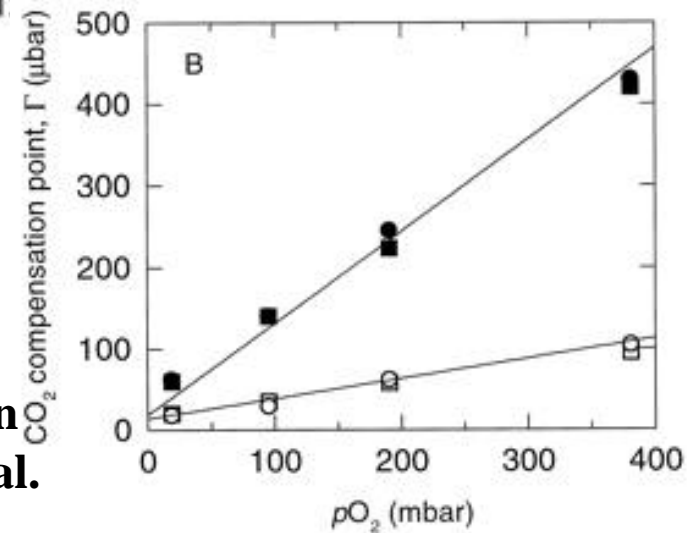


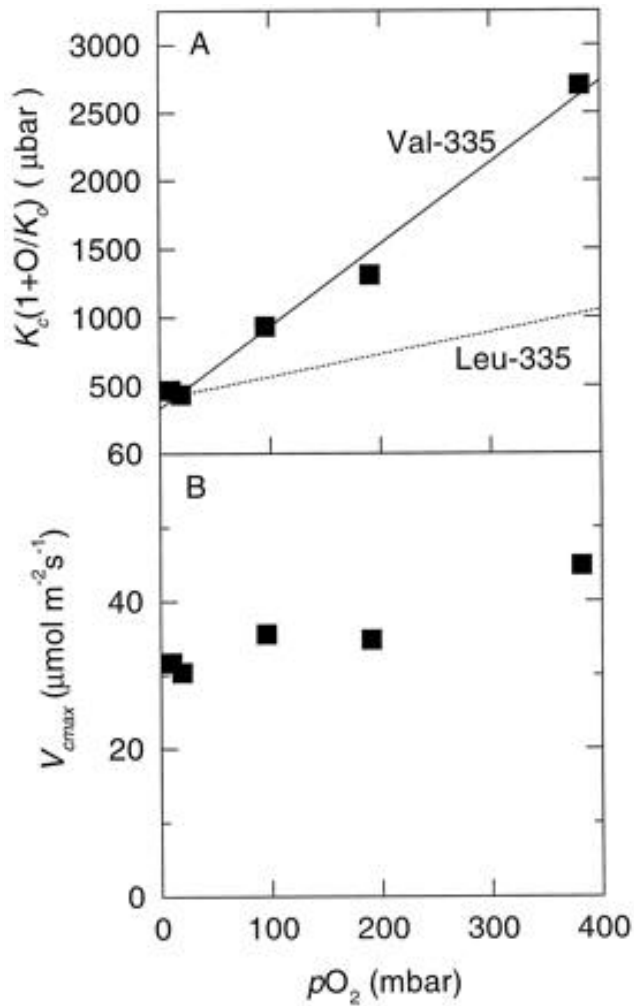
Physiological Measures

C intake barely sufficient
For growth.
But % Lys mod. is much
higher than WT.

- Mutants
- Use CO₂ less effectively

Compensation point=
[CO₂] at which photorespiration
and CO₂ incorporation are equal.
Mutant need CO₂ enrichment.





K_m (apparent) for mutant has steeper oxygen dependence than does wild type.

Mutants--rubisco limits photosynthesis.

Wild type--electron transport and RuBP production is limiting.

Rubisco Enzymology

	Wild Type L	Mutant Val
Rubisco umol	30.3±1.6	33.0 ±5.6
% carbamoylat	48.1 ± 5.6	89.9 ±5.4
V max /sec	3.43	.81
Km CO ₂ (uM)	10.7 ± .6	5.1 ±.8
Ki O ₂ (uM)	295 ± 71	48.9 ± 11.5
SelectivityC/O	81.1 ± 1.6	20.1 ± 1.5

Conclusion--Rubisco Genetic Engineering

**1)Alteration of the chloroplast genome is possible
(but hard)**

**2)The re-engineered Rubisco has altered properties.
Changes in the enzyme are visible in the plant.**

The Challenge--

Find mutations to improve Rubisco.