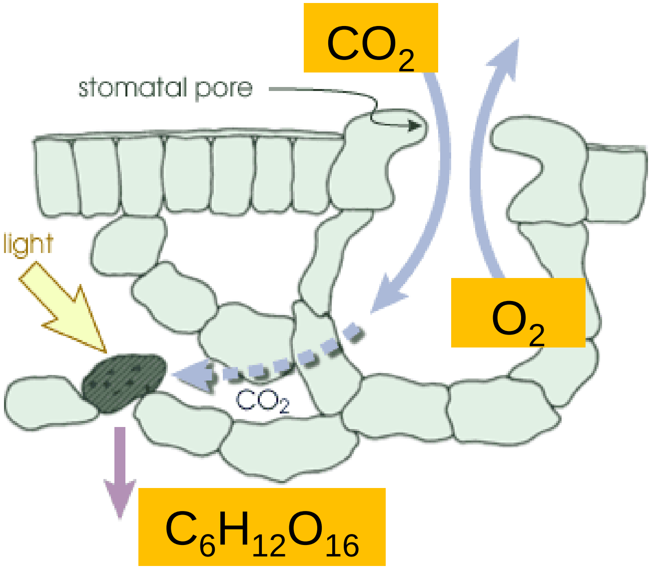
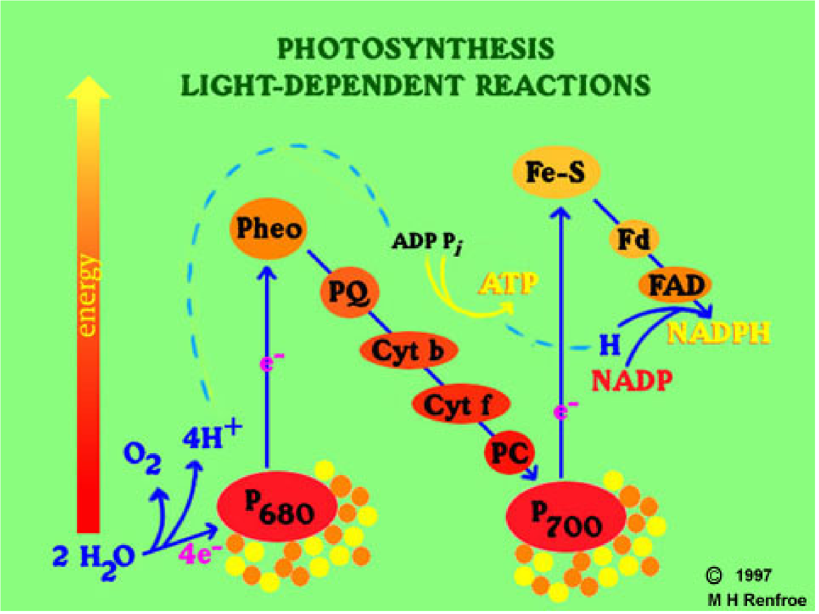
Photosynthesis

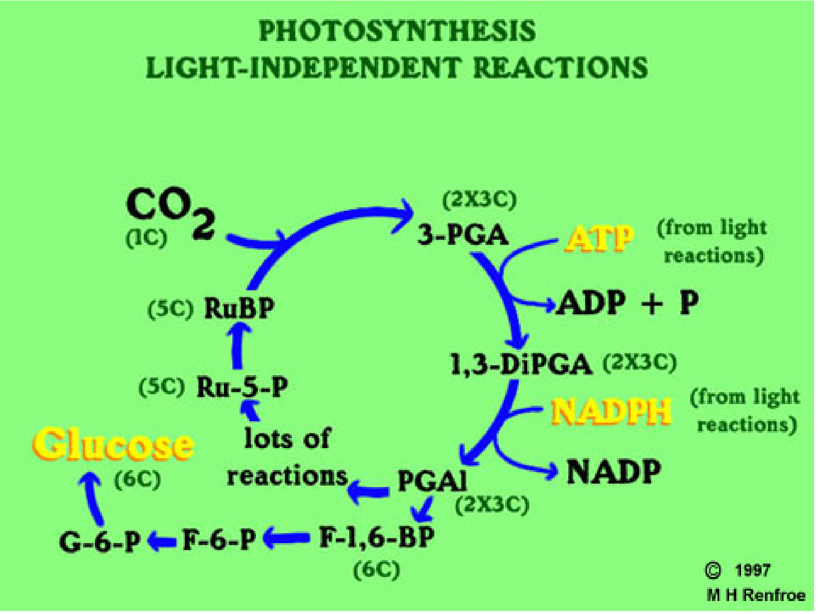
1. **Introduction and evolution**
2. **Light reactions at the thylakoid membrane**
3. **Dark reactions: C3 photosynthesis and photorespiration**
4. **C4 Photosynthesis – CAM plants**
5. **N and S metabolism**
6. **Plastid gene expression**



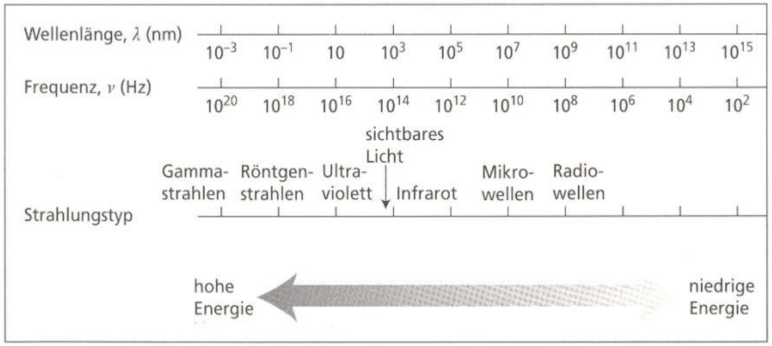
$$6\,CO_2 + 6\,H_2O \Rightarrow C_6H_{12}O_6 + 6\,O_2$$

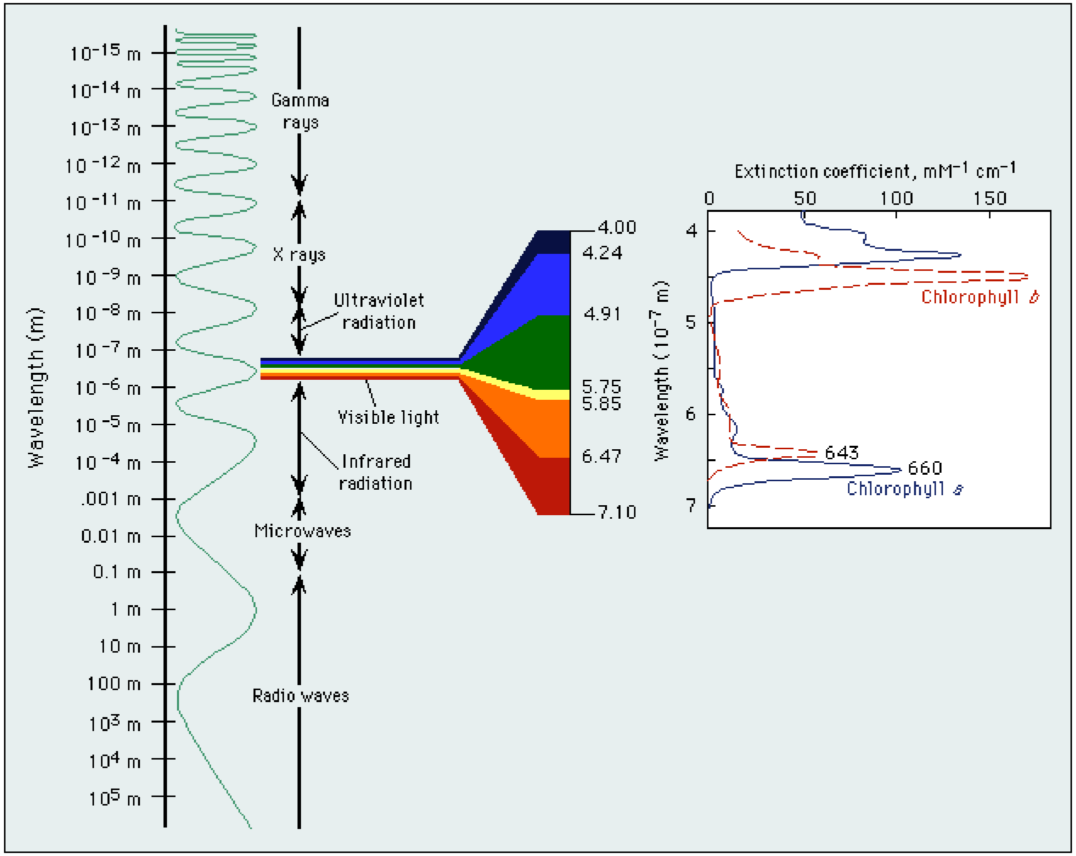
1. Introduction and evolution





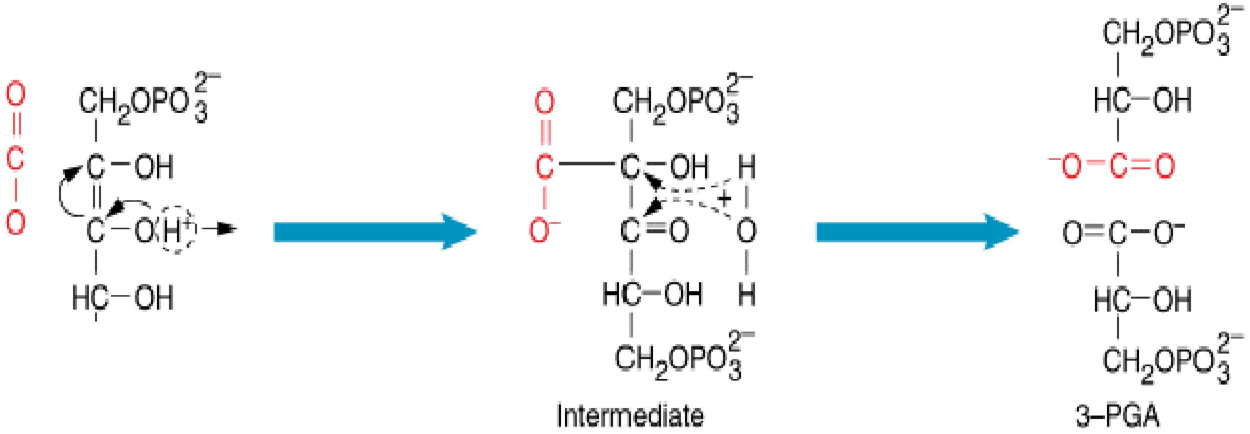
Electro-magnetic irradiance and sunlight

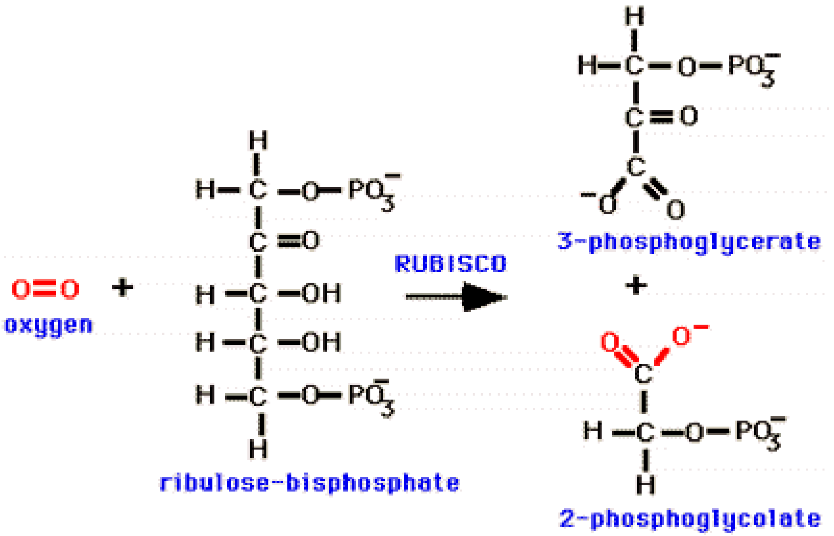




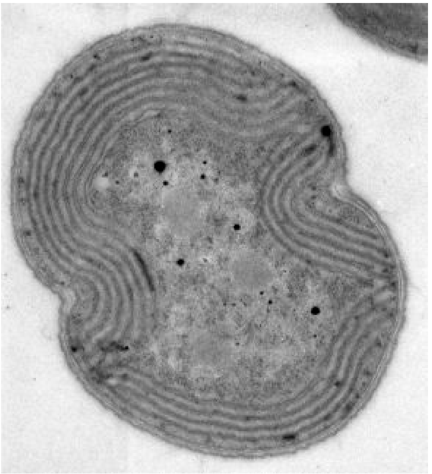
CO_2 and O_2 fixation by Rubisco

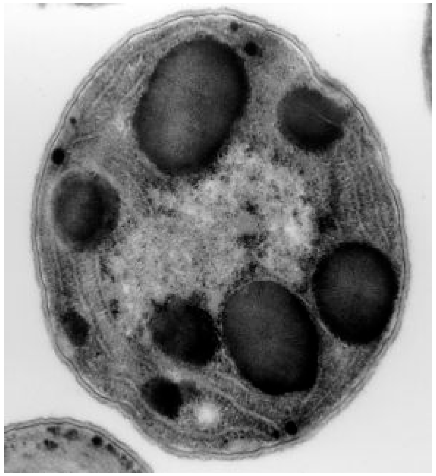
- Development of C4 photosynthesis

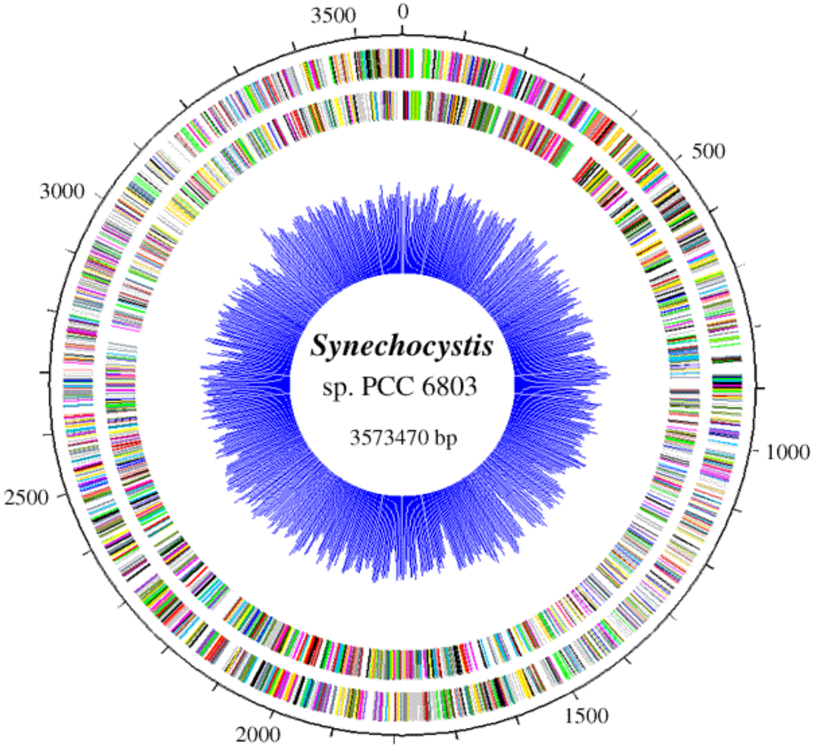




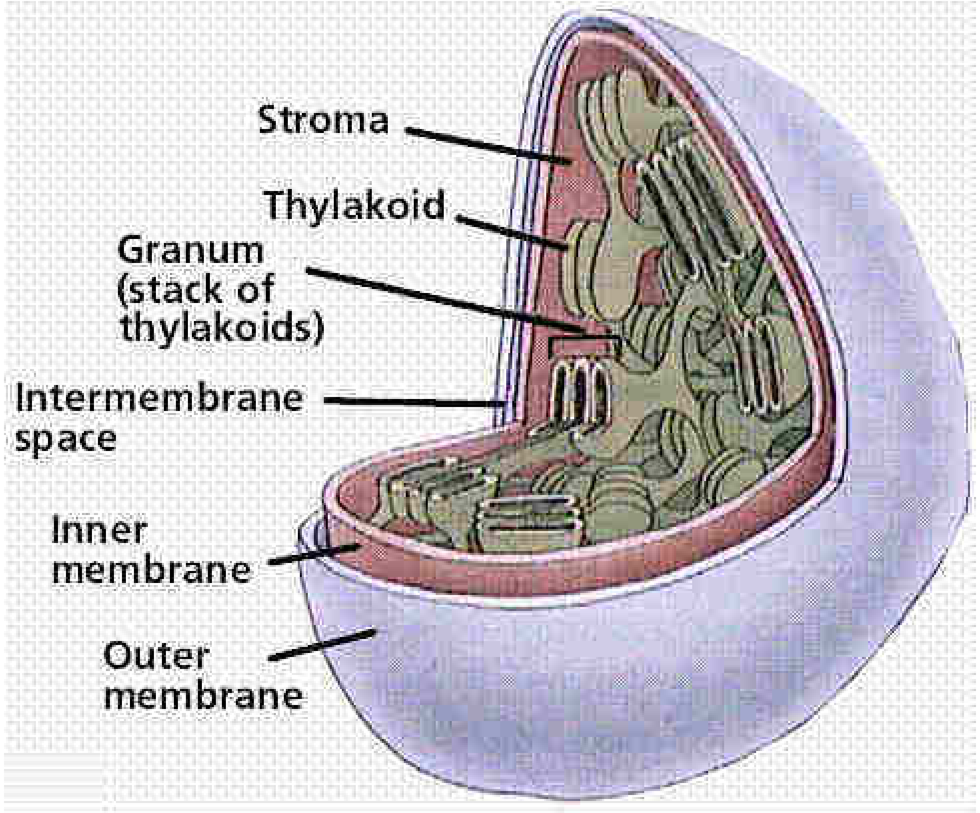
Oxygenic photosynthesis was established in Cyanobacteria

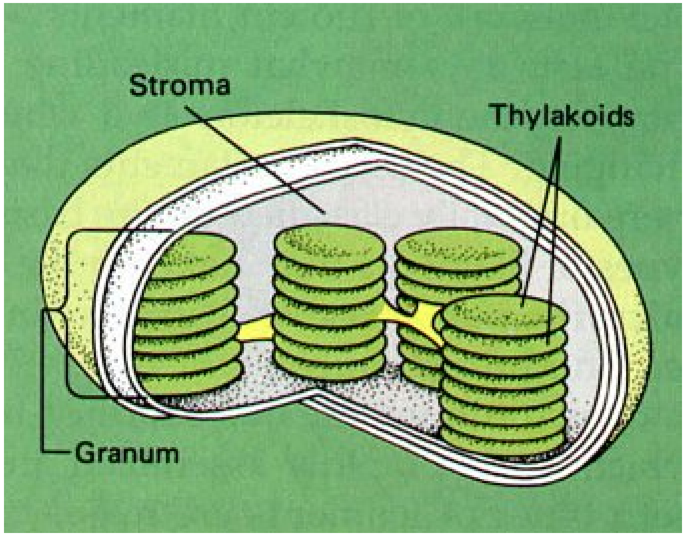


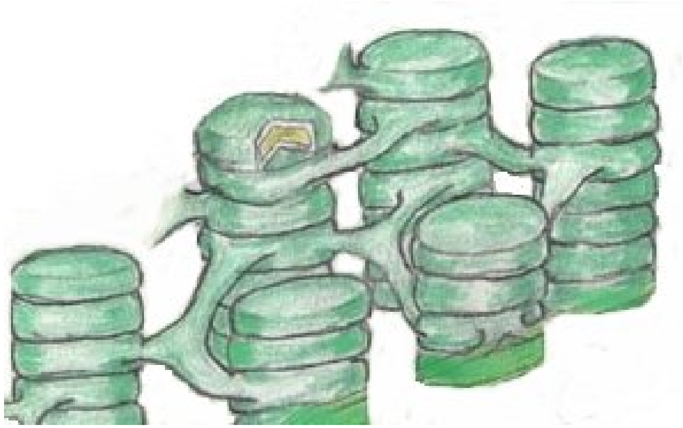




Plastids derive from endosymbiosis







Localisation of the photosynthetic complexes

- 100 chloroplasts/cell

- chloroplasts have two membranes with different origin
(lipids, proteins)

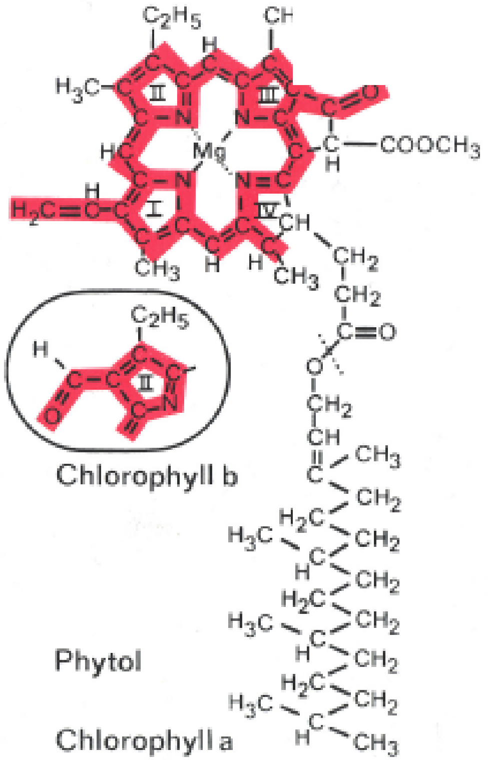
- thylakoids derive from inner (procaryotic) membrane
- grana- and stroma thylakoids

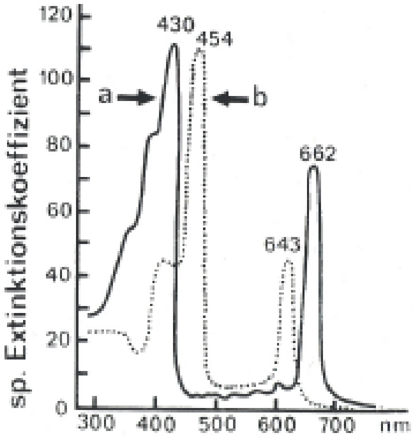
-new compartment: lumen *vs*. stroma

- different transport processes for proteins into the two plastid compartments

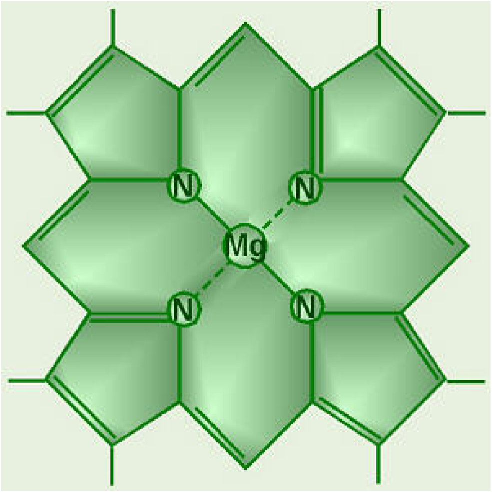
-photosynthesis in thylakoid membranes

Pigments: **chlorophyll**, carotinoid, phycobilin





Chlorophyll a+b (2,3:1)



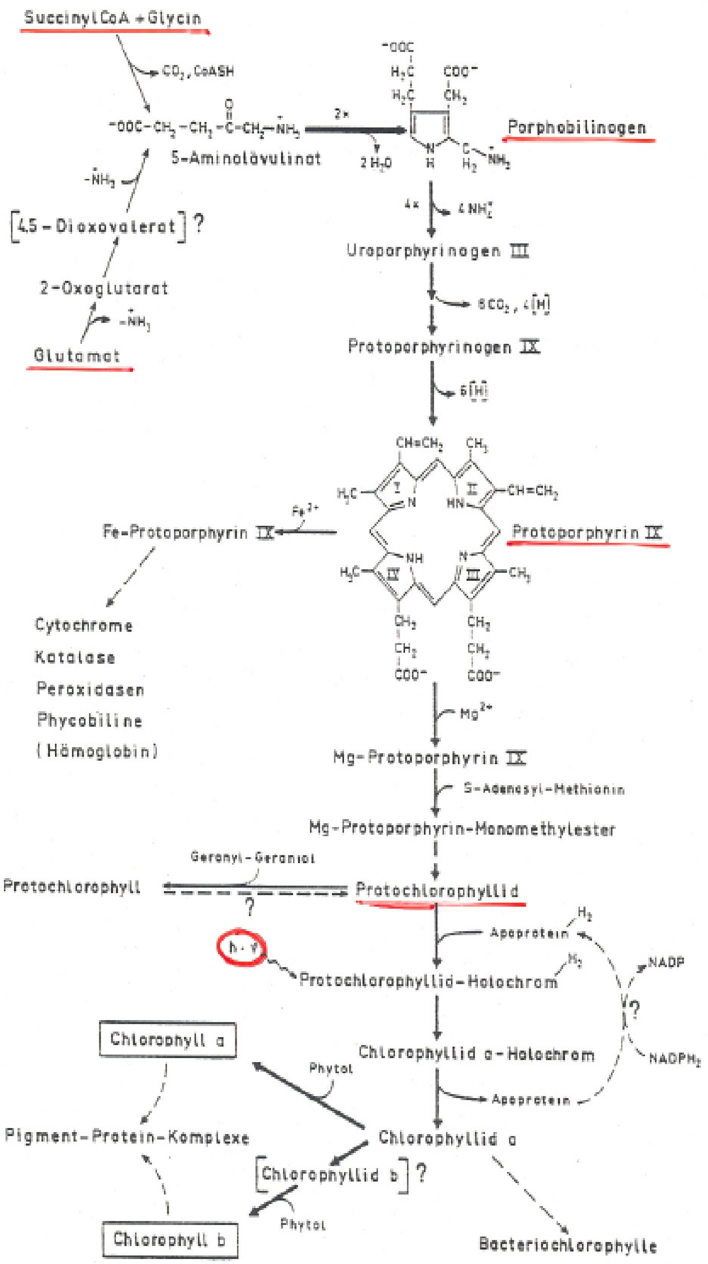
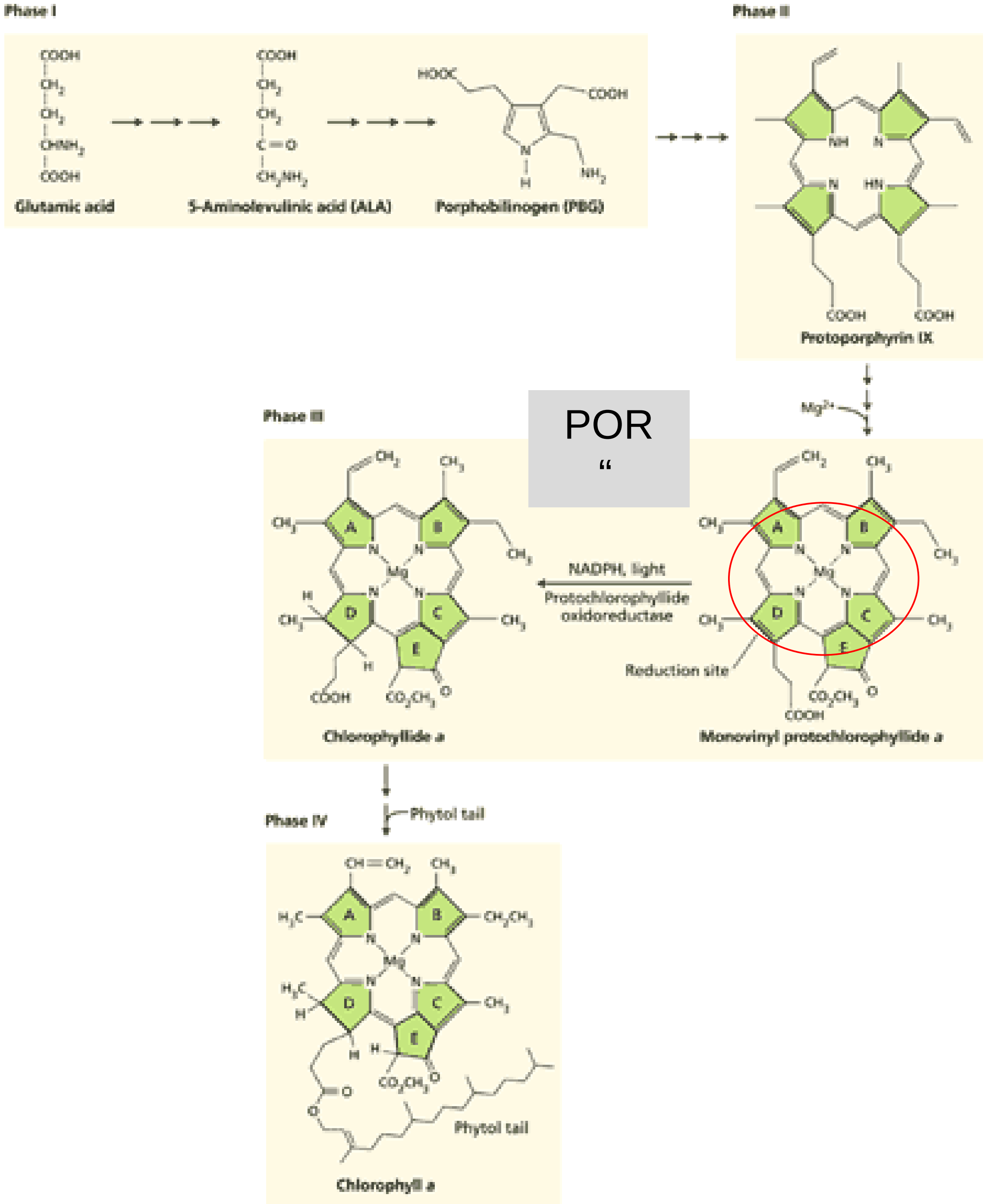


Abb. 246

Biosynthesis of chlorophylls

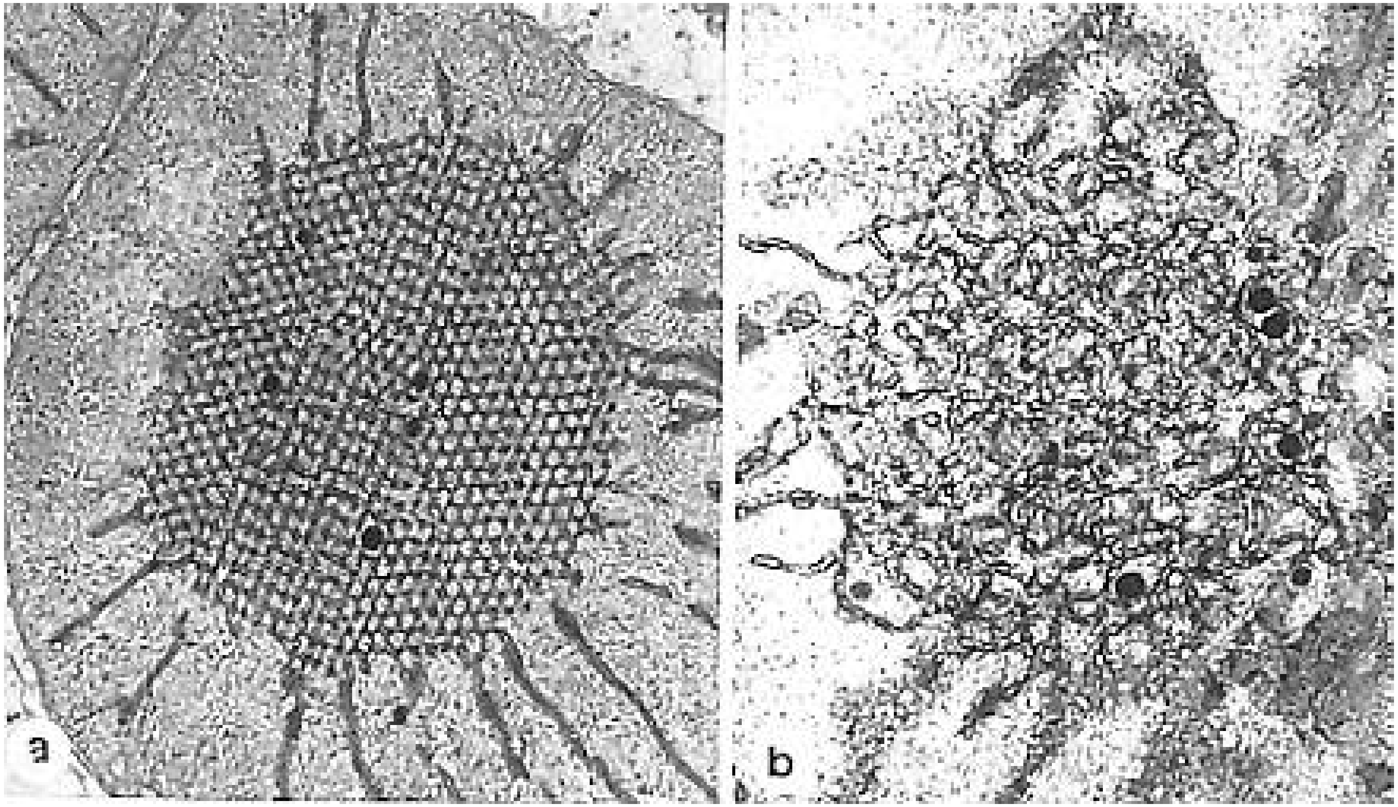
- starts with glutamate
- porphyrin
- tetrapyrrole



POR
“

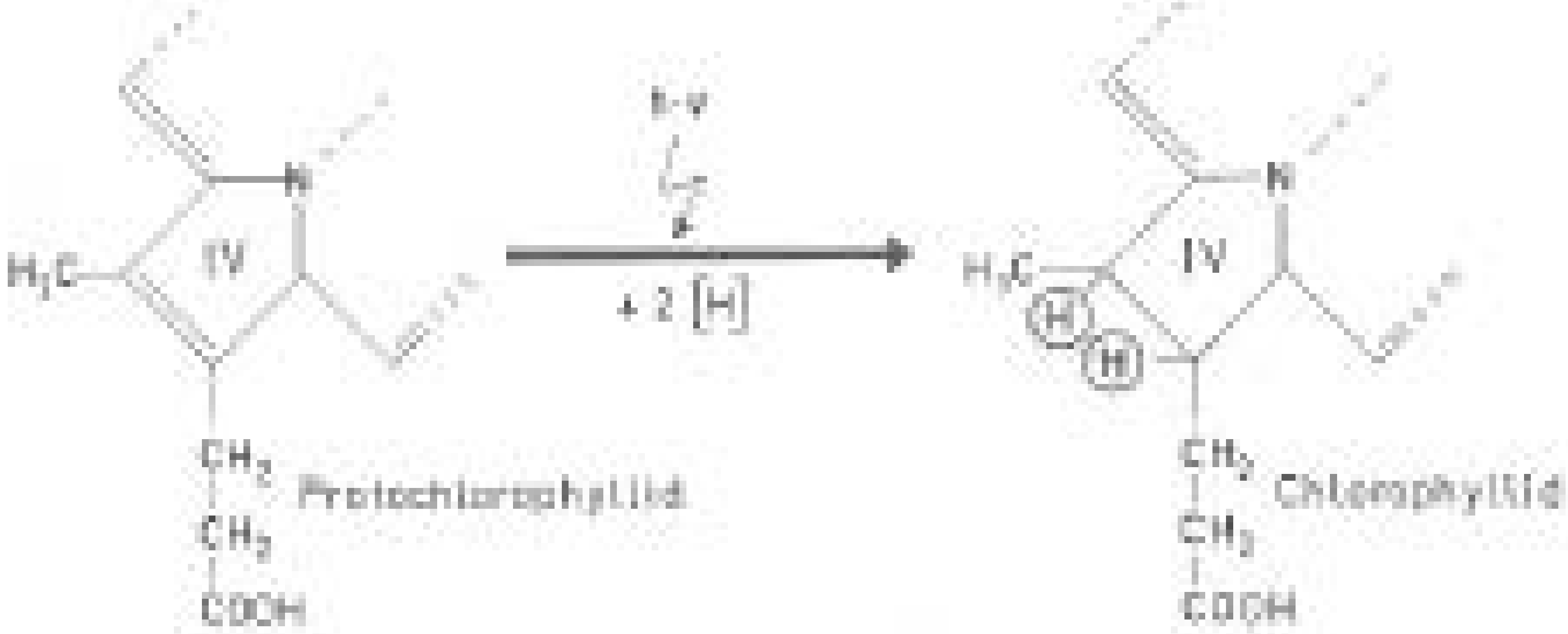
POR is light-dependent

In the dark: Protochlid accumulates and forms prolamellar body

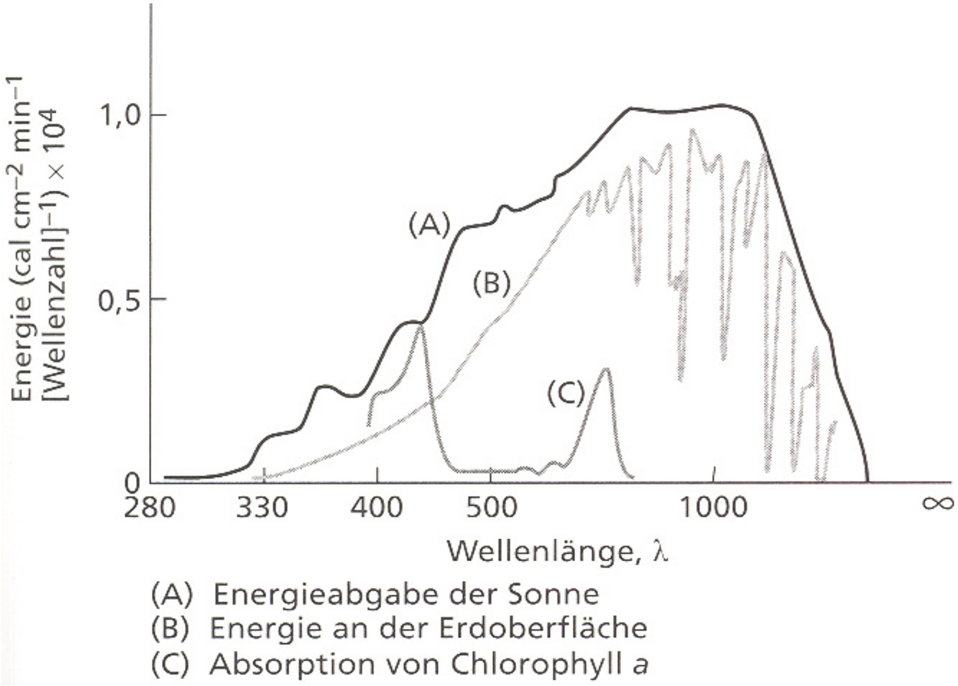


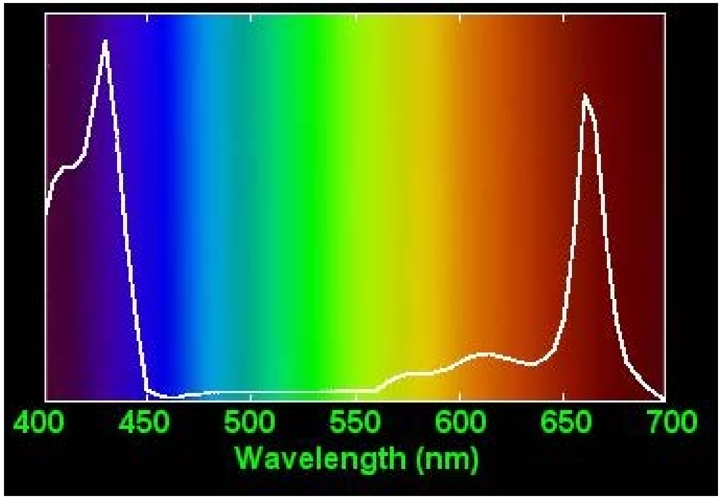
dark

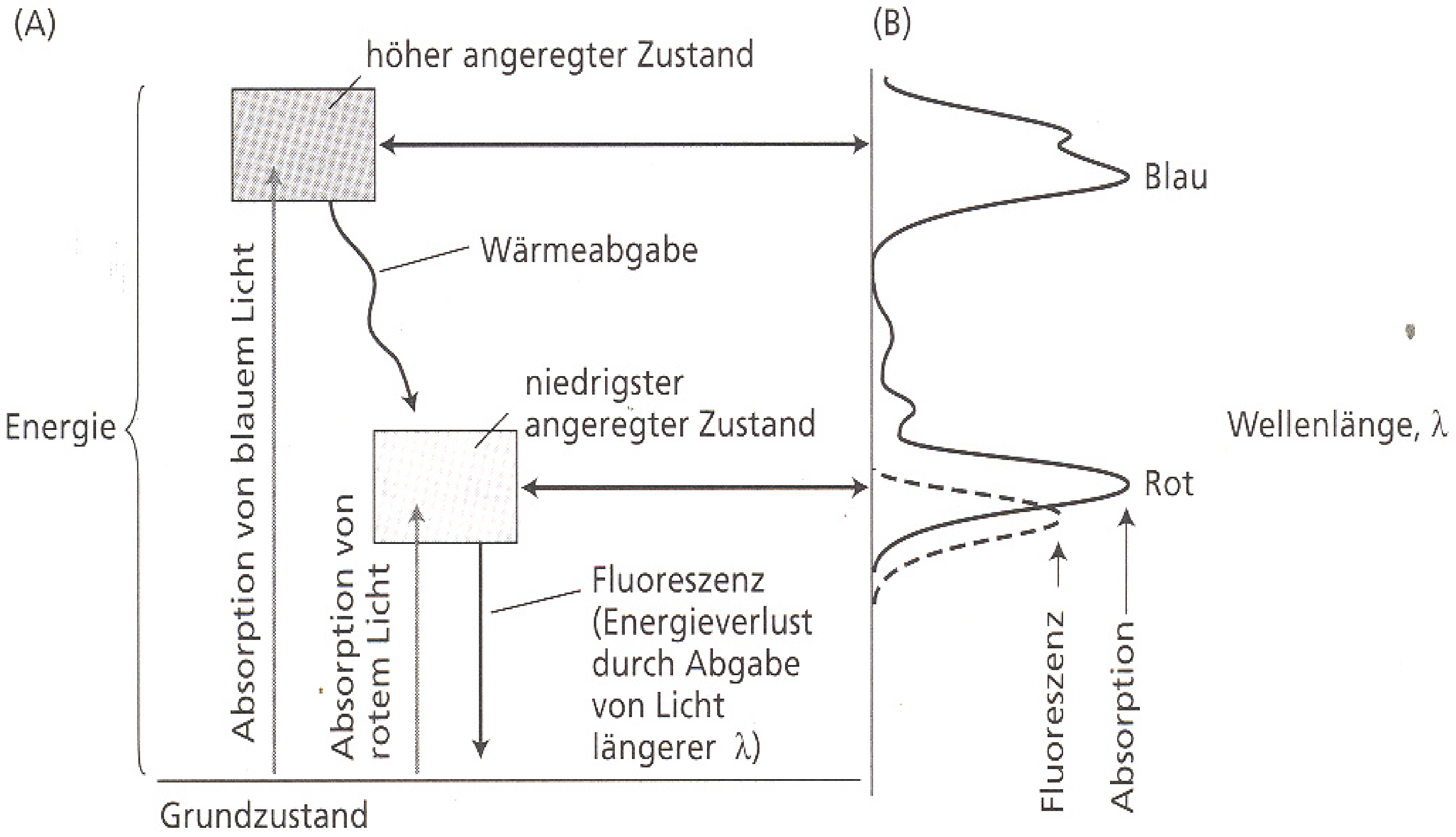
2 h illumination


H₃C
IV
N
CH₂
CH₂
COOH
Protochlorophyllid
h·ν
+ 2 [H]
H₃C
H
H
IV
N
CH₂
CH₂
COOH
Chlorophyllid

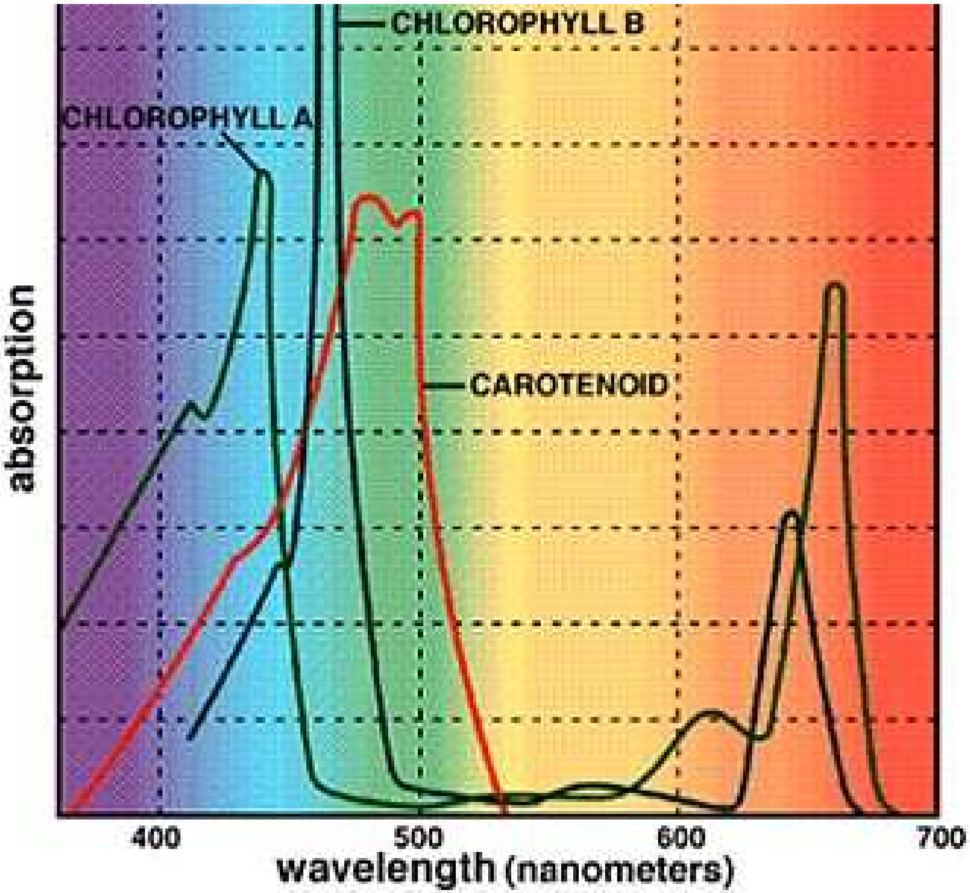
Chl absorbs visible light



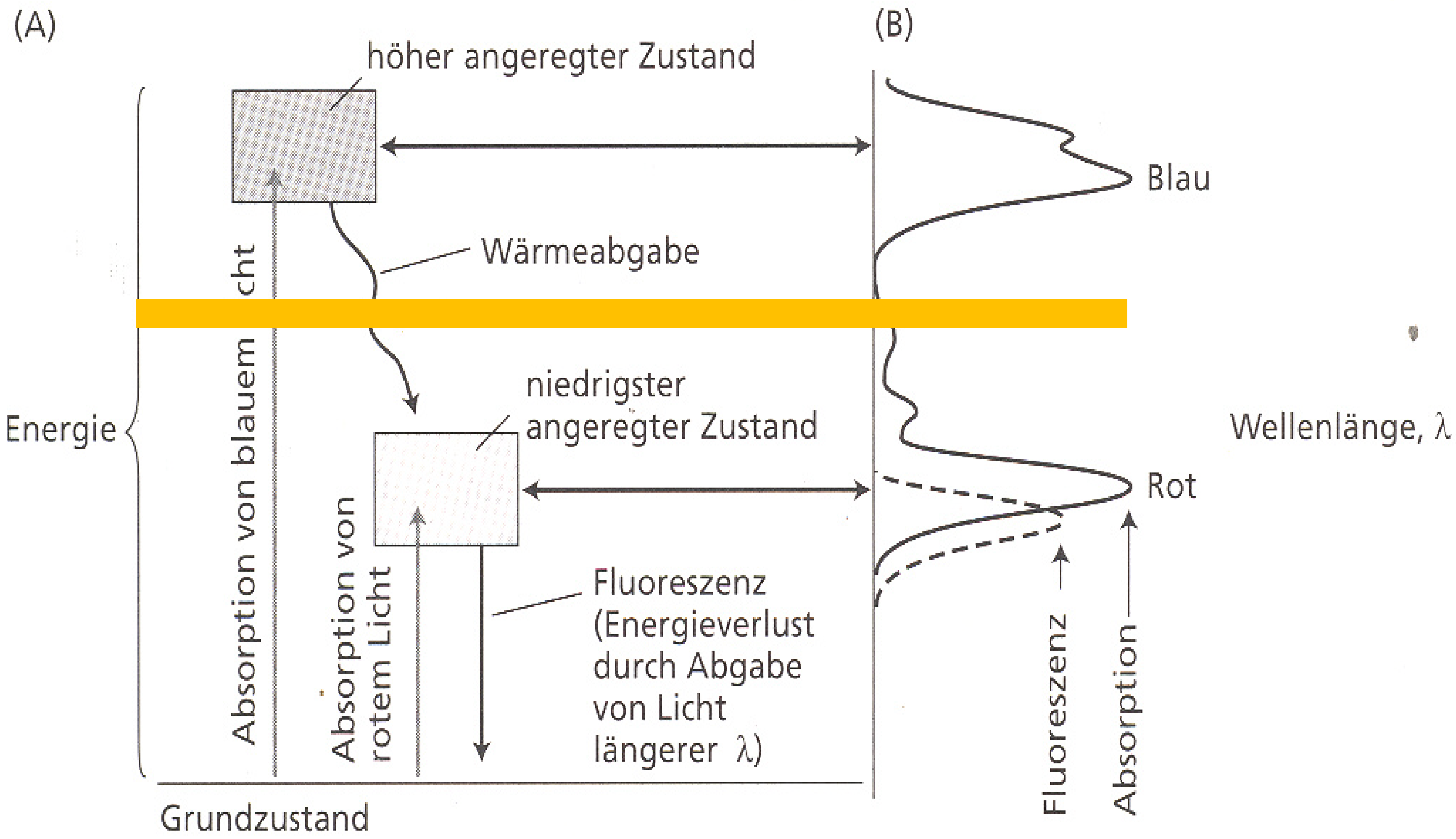



(A)
(B)
höher angeregter Zustand
Blau
Wärmeabgabe
Energie
Absorption von blauem Licht
niedrigster angeregter Zustand
Wellenlänge, λ
Rot
Absorption von rotem Licht
Fluoreszenz (Energieverlust durch Abgabe von Licht längerer λ)
Fluoreszenz
Absorption
Grundzustand

Pigments: chlorophyll, **carotinoid**, phycobilin



Light absorption




For Healthy Skin, Eyes & Immunity*
Mixed Carotenoids
10,000 IU
Beta Carotene
With 5 Natural Carotenoids from Algae
Antioxidant Vitamin Supplement
SOFTGELS
NATURE'S LIFE

C_2 Acetyl CoA

↓

C_5 Isopentyl pyrophosphate

↓

C_{10} Geranyl pyrophosphate

↓

C_{15} Farnesyl pyrophosphate

↓

C_{20} Geranylgeranyl pyrophosphate → Phytol → Chlorophyll

↓

C_{40} Phytoene

↓

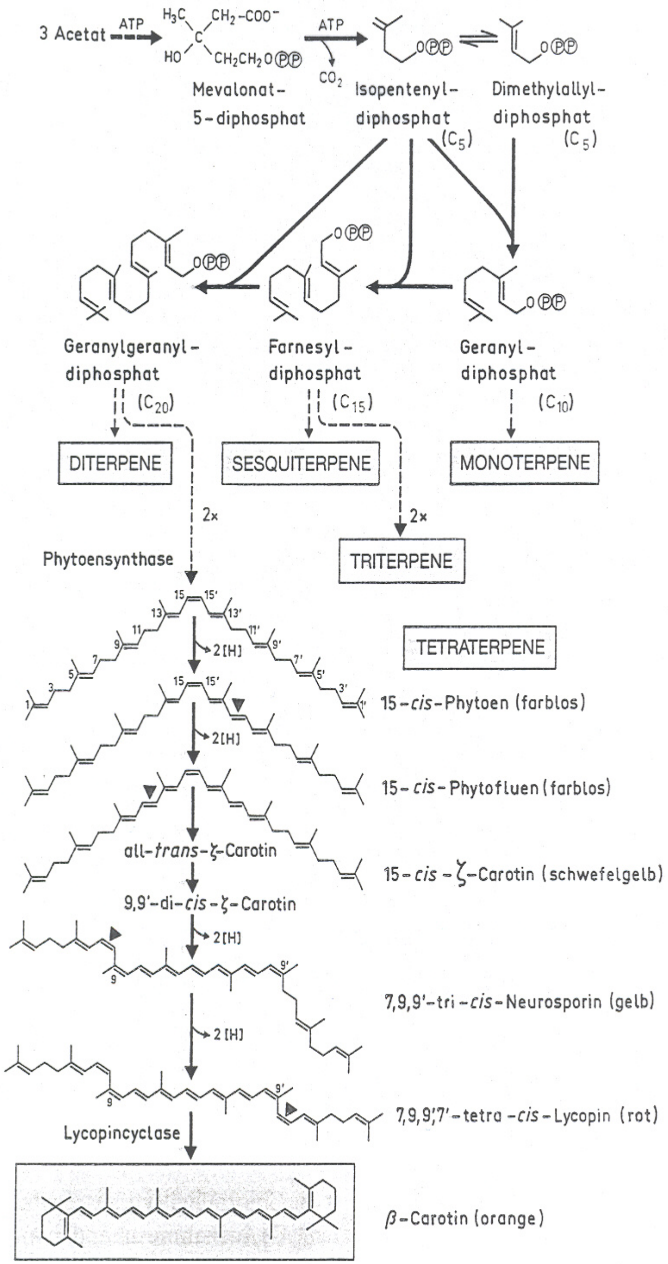
C_{40} Lycopene

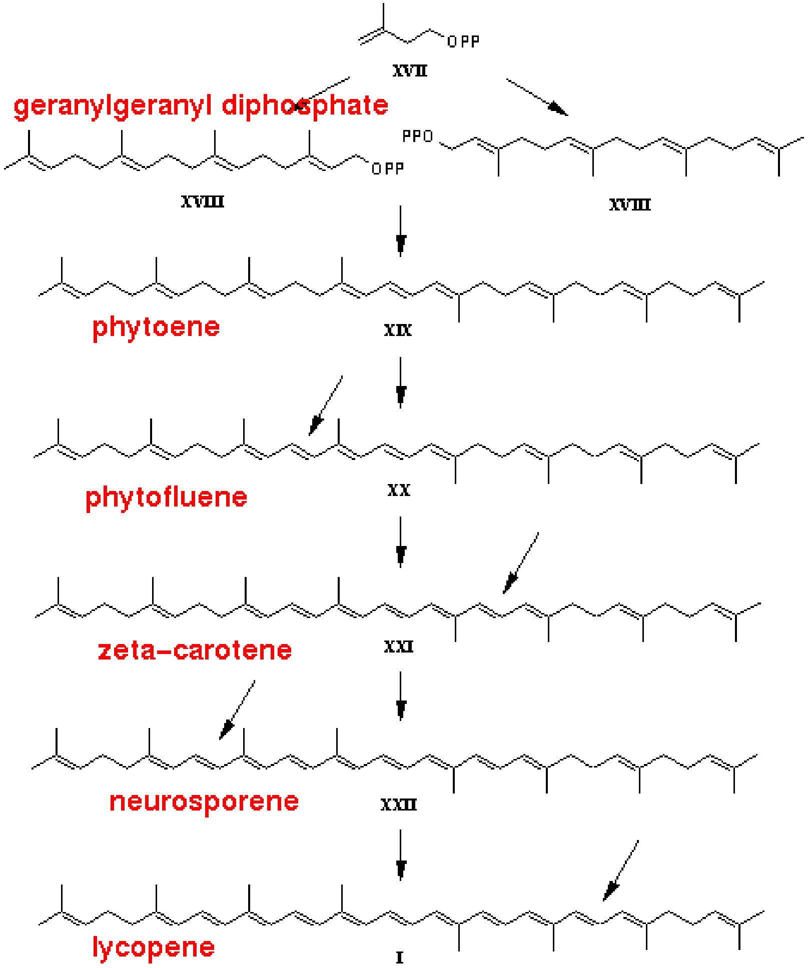
↓ cyclase

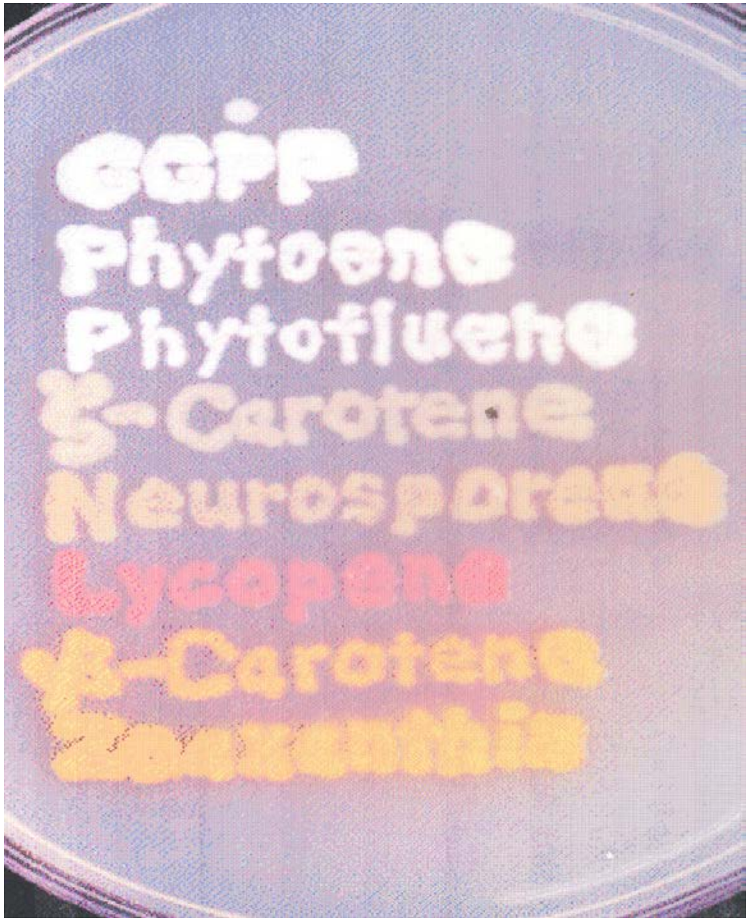
C_{40} Carotenes

↓ hydroxylase

C_{40} Xanthophylls
(lutein)
(zeaxanthin)



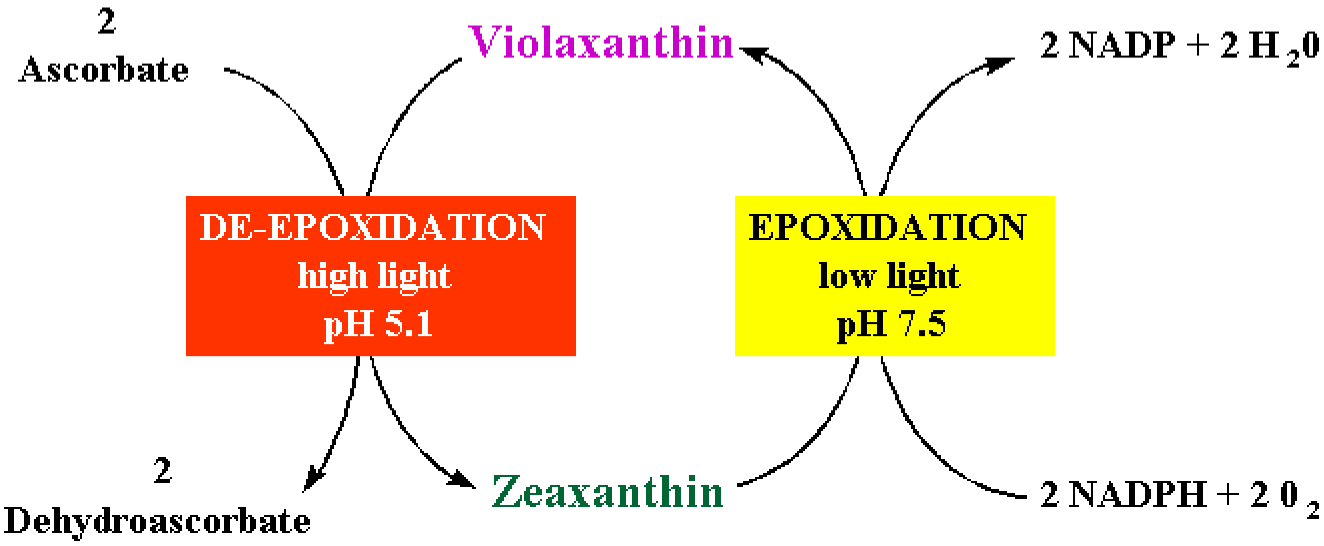

OPP
XVII
geranylgeranyl diphosphate
PPO
OPP
XVIII
XVIII
phytoene
XIX
phytofluene
XX
zeta–carotene
XXI
neurosporene
XXII
lycopene
I


GGPP
Phytoene
Phytofluene
ζ-Carotene
Neurosporene
Lycopene
β-Carotene
Zeaxanthin

Carotenoids have two functions:

- **light absorbance**
- **protection against excess light**

Energy dissipation by xanthophylls



2 Ascorbate

Violaxanthin

2 NADP + 2 $H_2 0$

DE-EPOXIDATION
high light
pH 5.1

EPOXIDATION
low light
pH 7.5

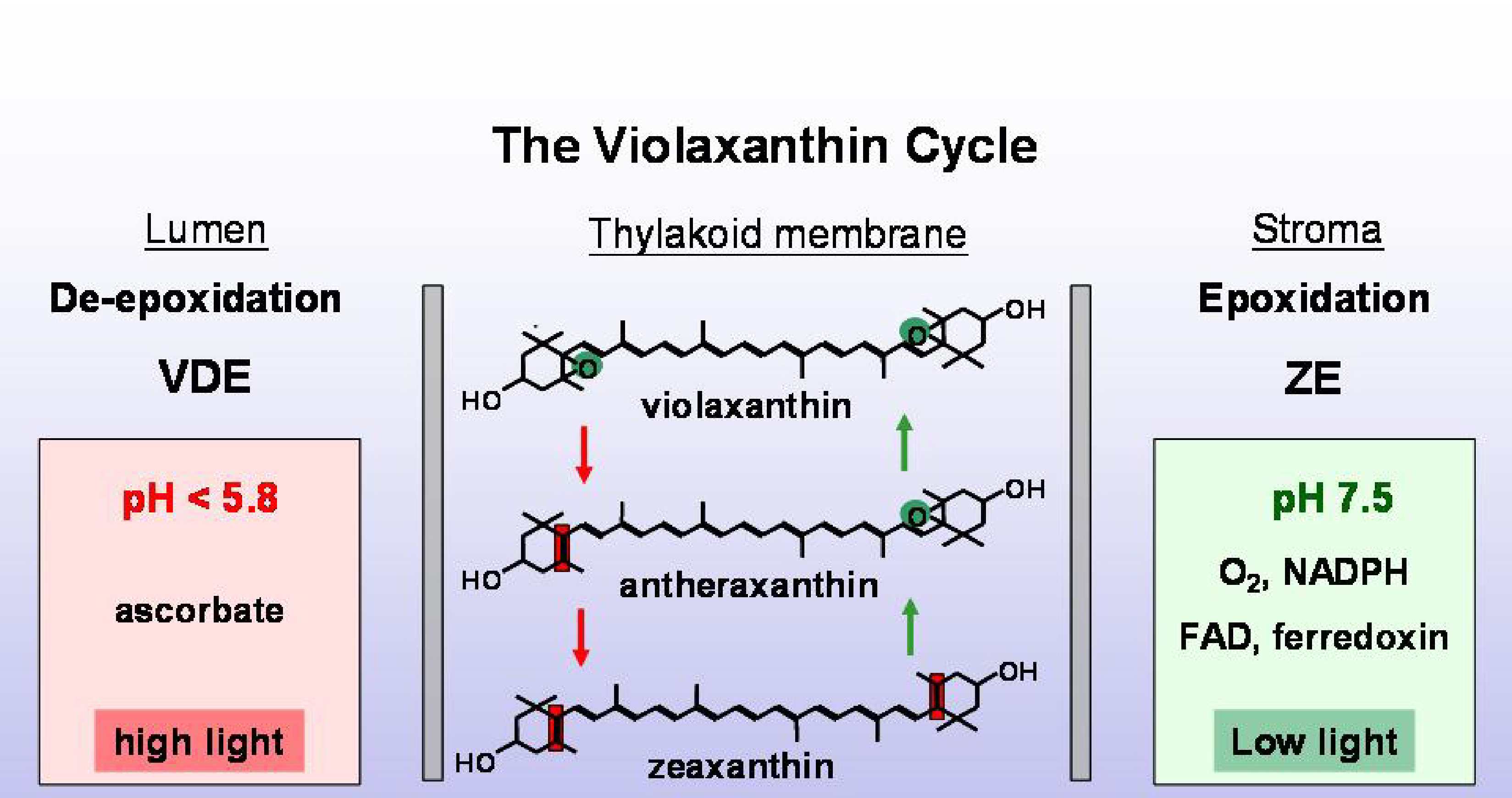
2 Dehydroascorbate

Zeaxanthin

2 NADPH + 2 0_2

Low light = Violaxanthin is present in and around PSII

High light = Zeaxanthin synthesis in and around PSII



The Violaxanthin Cycle

Lumen

De-epoxidation

VDE

pH < 5.8

ascorbate

high light

Thylakoid membrane

violaxanthin

antheraxanthin

zeaxanthin

Stroma

Epoxidation

ZE

pH 7.5

O_2, NADPH

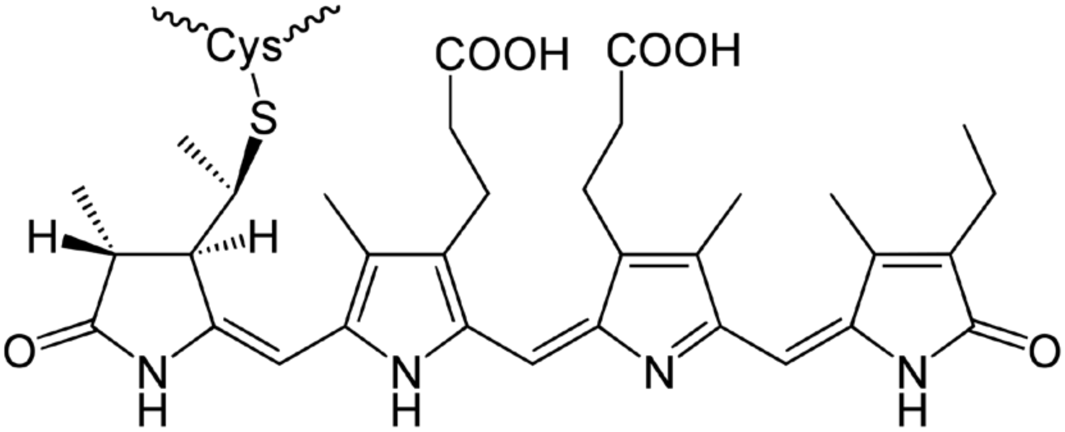
FAD, ferredoxin

Low light

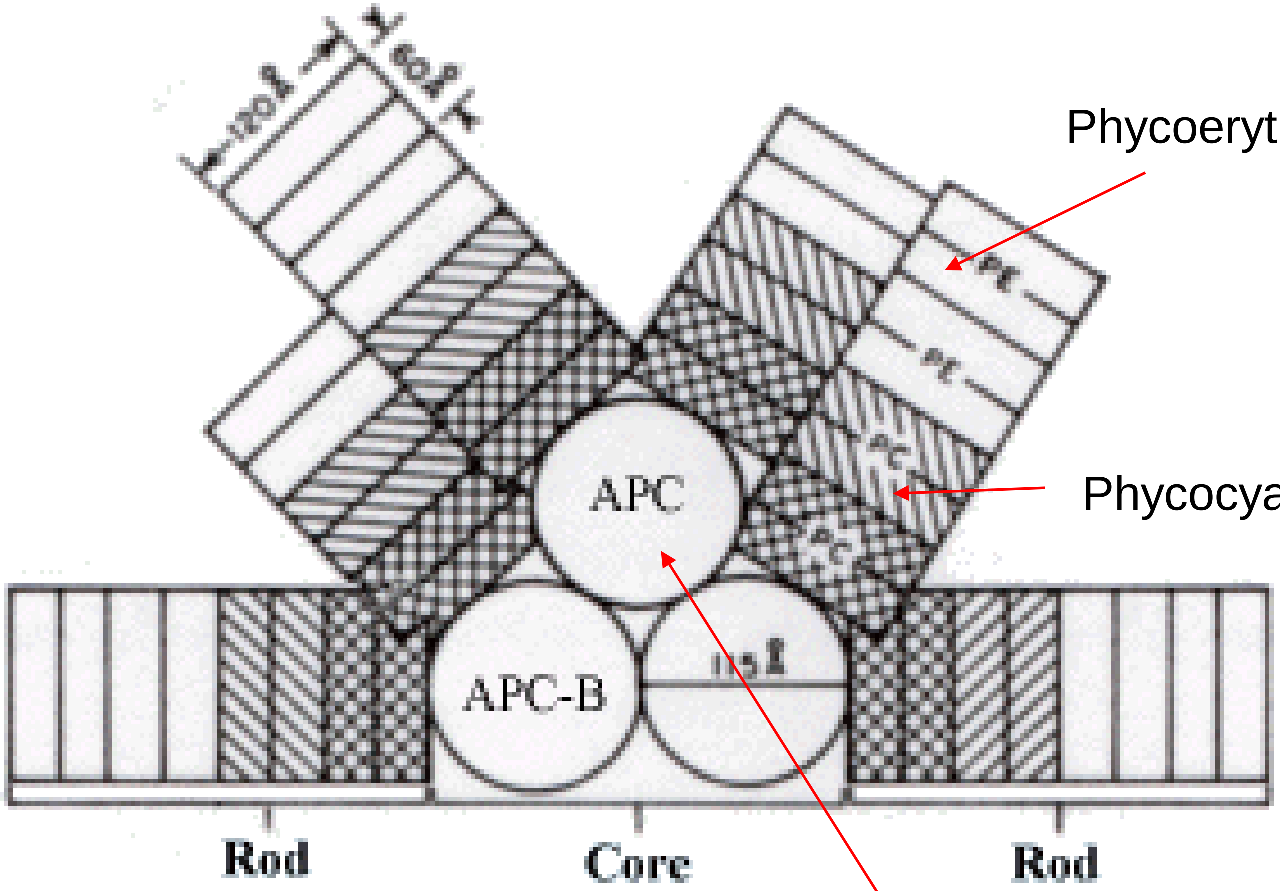
In response to high light, plants have evolved photo protection mechanisms to dissipate the excess absorbed light energy and thus avoid damages to the photosynthetic apparatus. One of the mechanisms is through transfer of the absorbed energy from **chlorophyll a to xanthophyll pigment zeaxanthin since excited zeaxanthin decays to the ground level much more rapidly than excited chlorophyll a. Under excessive light condition, violaxanthin is converted to zeaxanthin in the xanthophyll cycle, and thus accelerates the energy dissipation from excited chlorophyll a to zeaxanthin.**

Pigments: chlorophyll, carotinoid, **phycobilin**

phycoerythrin
phycocyanin
allophycocyanin



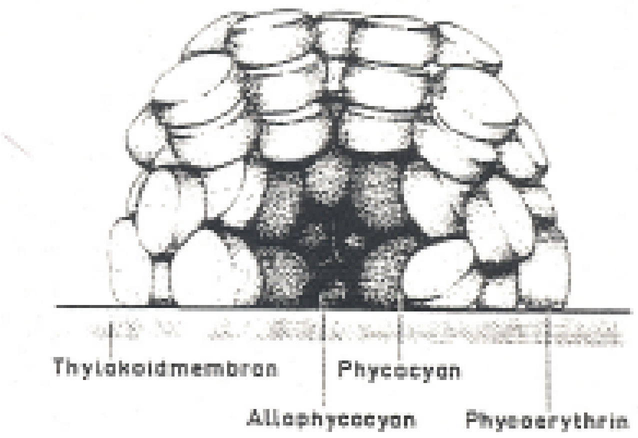
Phycocyanin: chromophor is bound to the protein
Open chain tetrapyrol system



Phycoerythrin, 545 nm

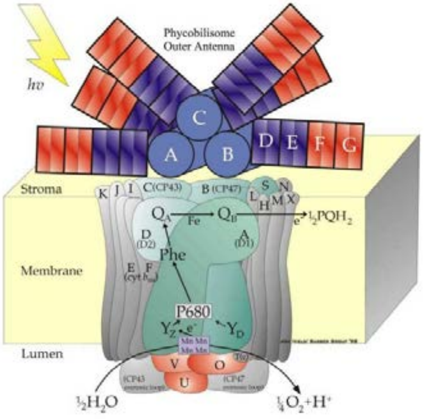
Phycocyanin, 615 nm

Allophycocyanin, 650 nm

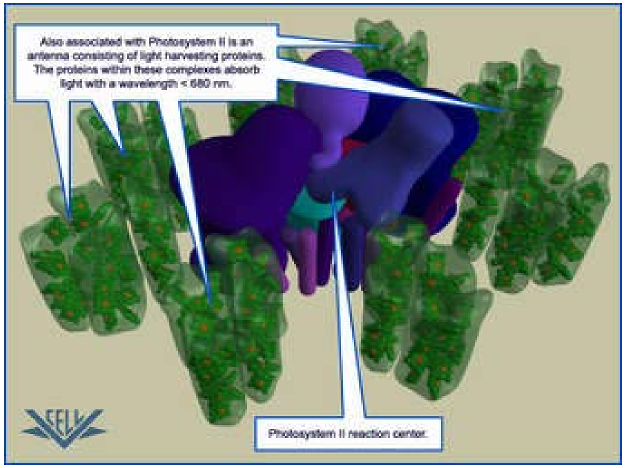


Organisation of light harvesting complexes in cyanobacteria and higher plants

cyanobacteria	higher plants



Cyanobacterial Photosystem II supercomplex



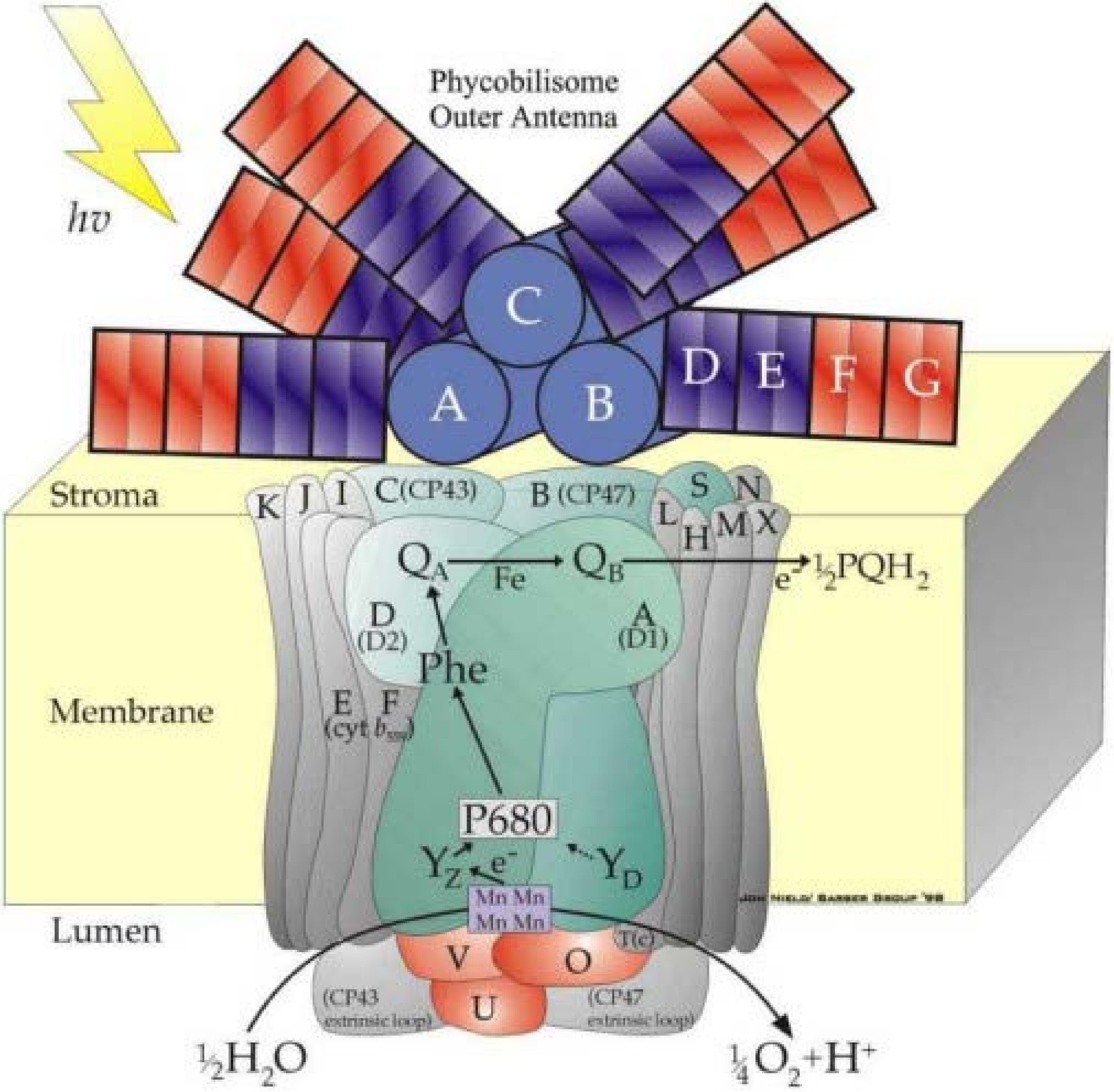
Also associated with Photosystem II is an antenna consisting of light harvesting proteins. The proteins within these complexes absorb light with a wavelength < 680 nm.

Photosystem II reaction center.

Antenna with **phycobilisomes**
ON the thylakoid membrane

Antenna of **higher plants**
IN the thylkoid membrane

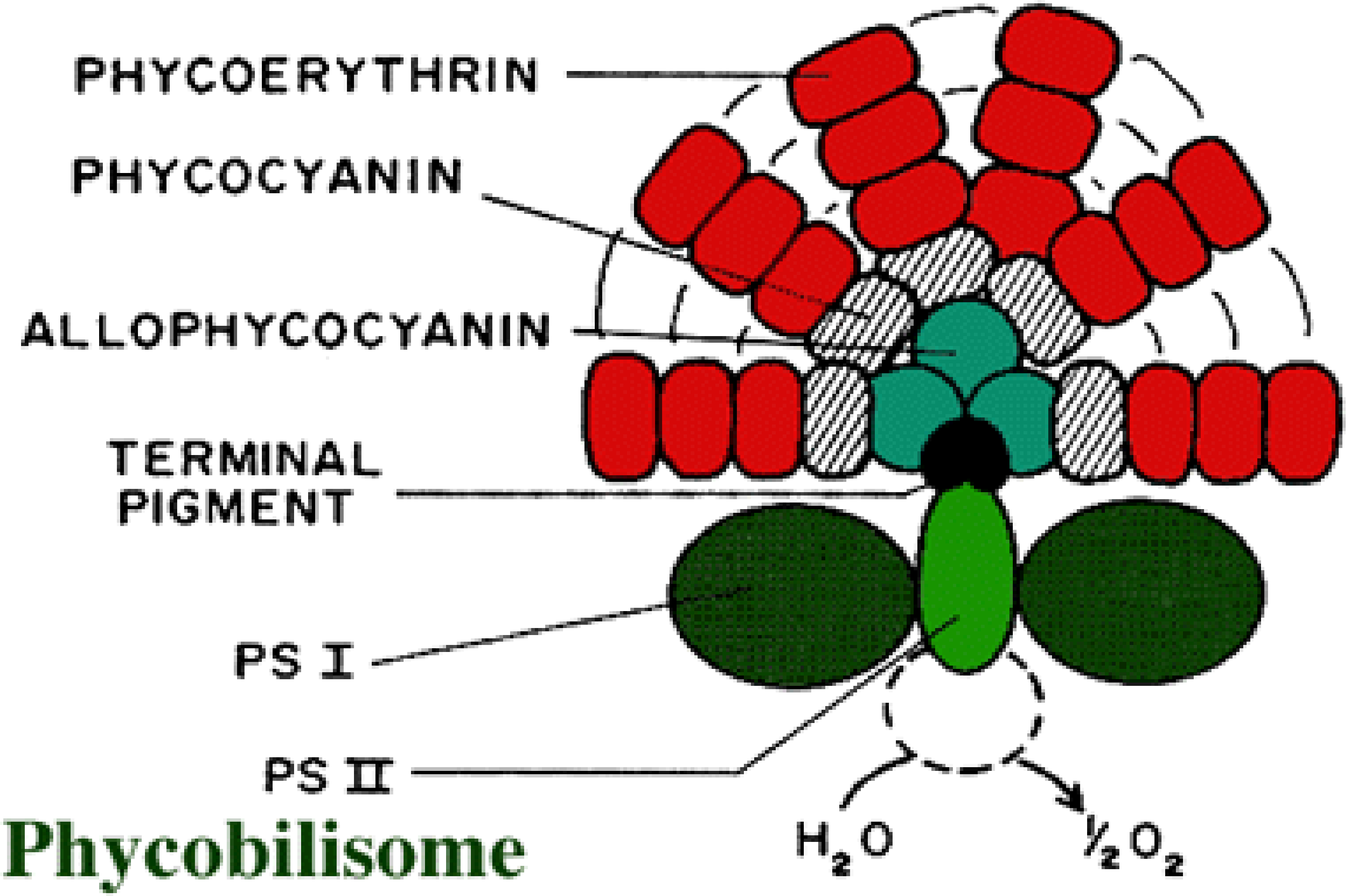
Cyanobacteria



Chromatic Adaptation

Fremyella diplosiphon

Cyanobacteria



Phycobilisome

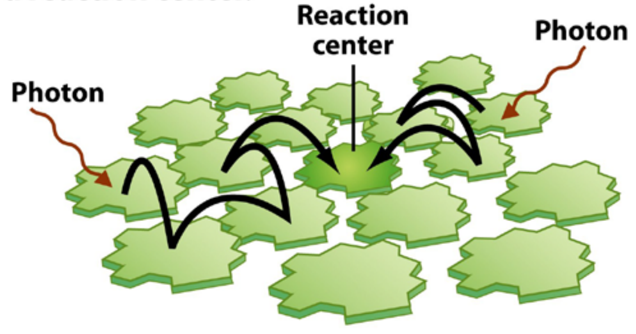
Light harvesting complexes

Higher plants

Antenna harvest light energy and transfer it to the reaction centers of the photosystems II or I

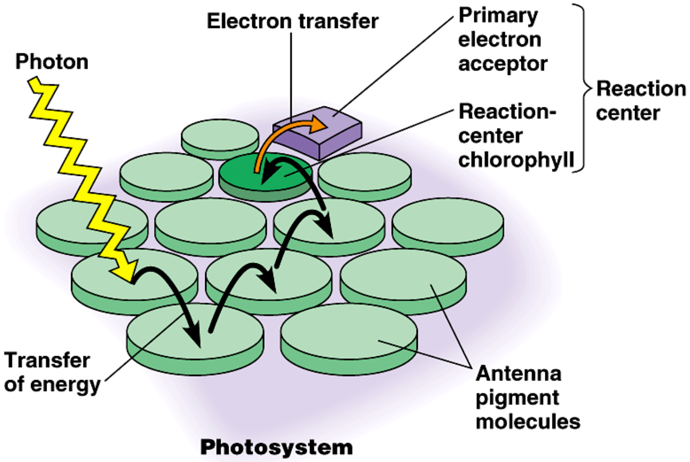
Pigments in antenne:
<u>Energy</u> transfer

Pigment (P680 or P700) in the photosystems
Photochemical work

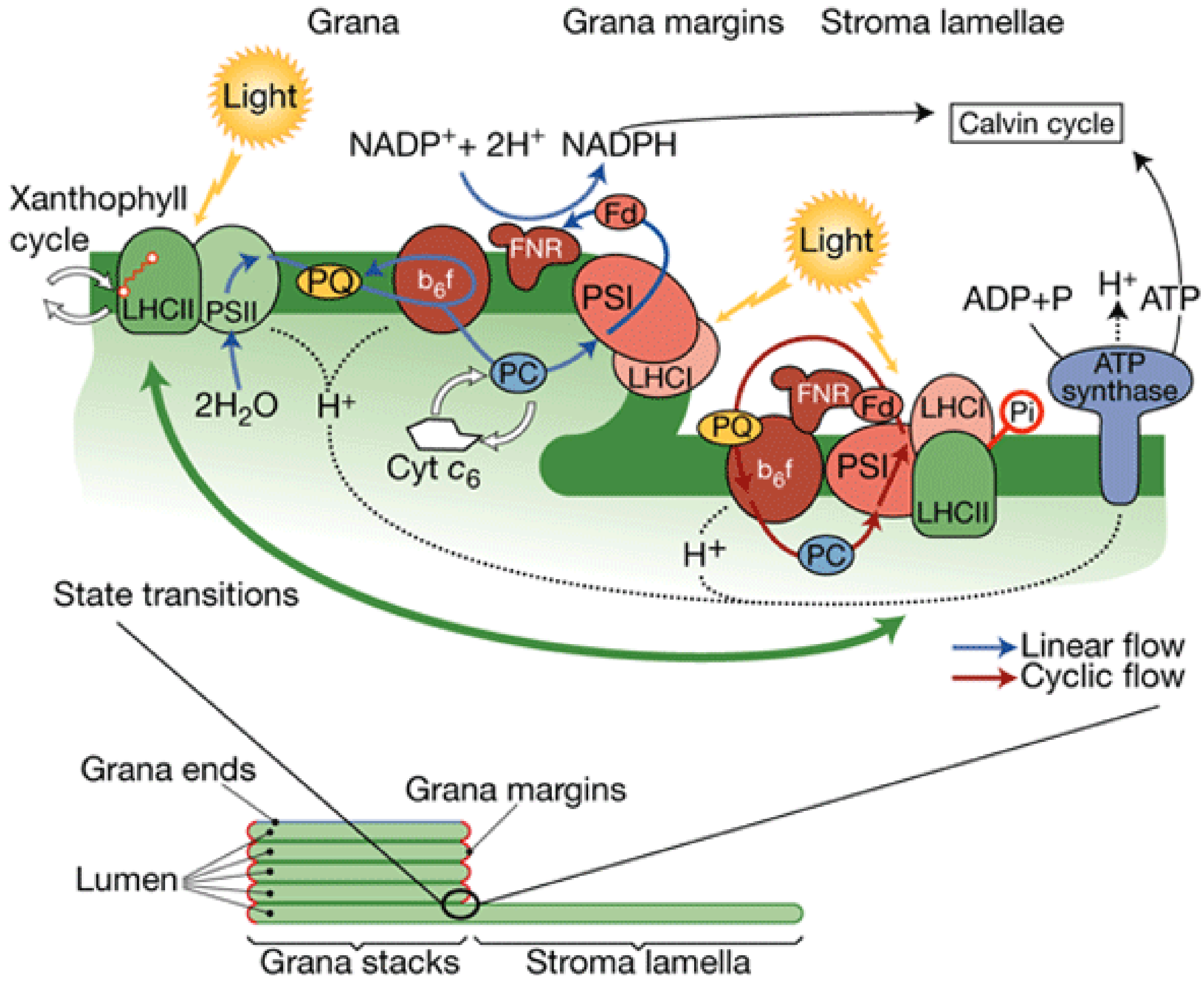

Chlorophyll molecules transmit energy from excited electrons in the antenna complex to a reaction center.

Chlorophyll molecules in antenna complex

Figure 10-12a Biological Science, 2/e



Most of the chlorophylls and carotenoids are organized in light-harvesting complexes of the photosystems I and II



Chl a and b (and carotenoids) not covalently bound to light-harvesting proteins of **antenna**

Stable antenna for the photosystems I and II

Mobile antenna migrates between photosystems

Pigments are also bound to the two photosystems

-inner antenna

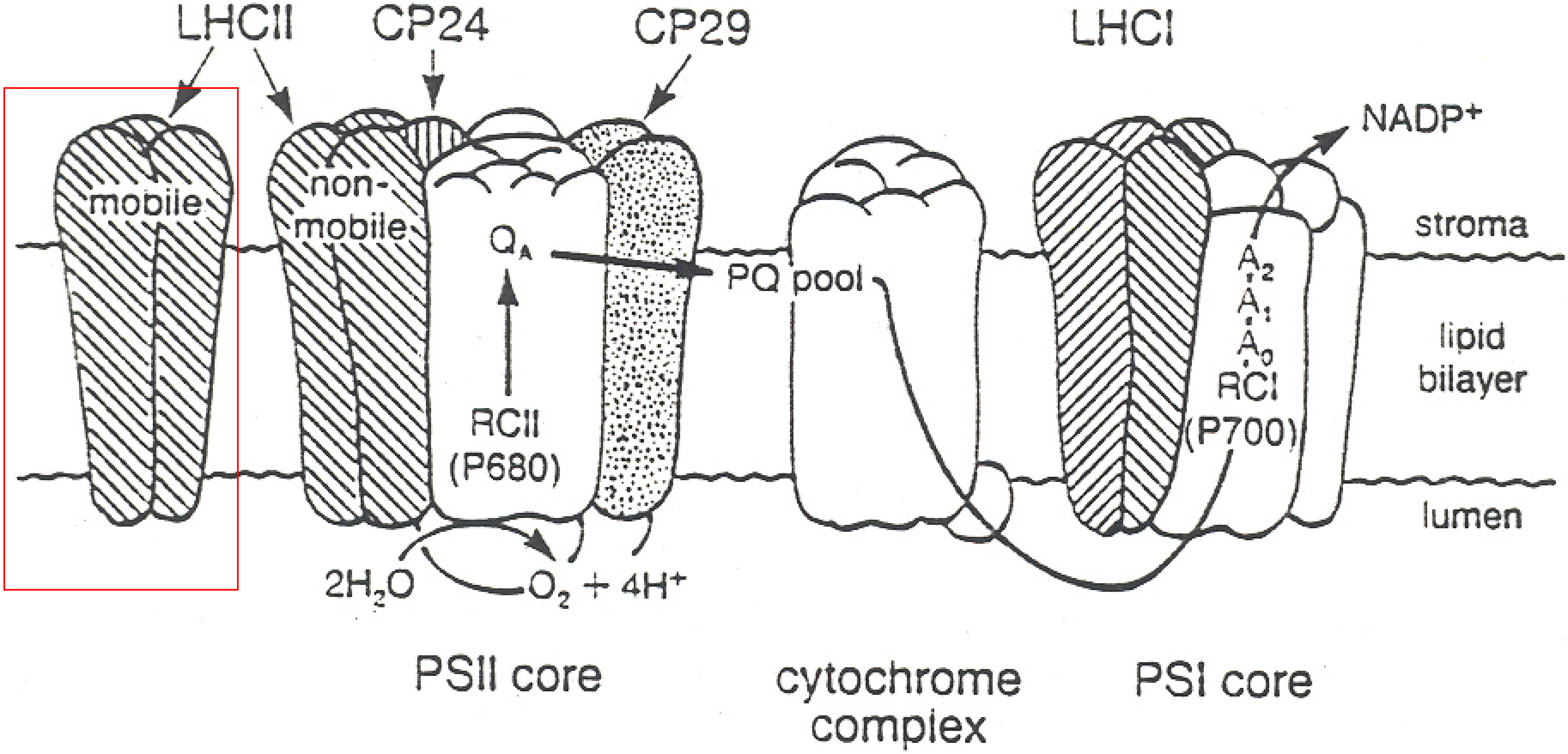
- reaction center pigmnents

-Light harvesting proteins are encoded by **multigene families**

-Specificity for the two photosystems

-Phyologenetic analyses

-Early light-induced proteins (**ELIPs**)

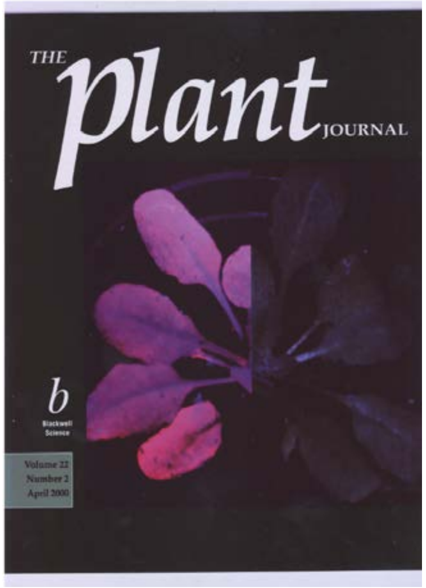


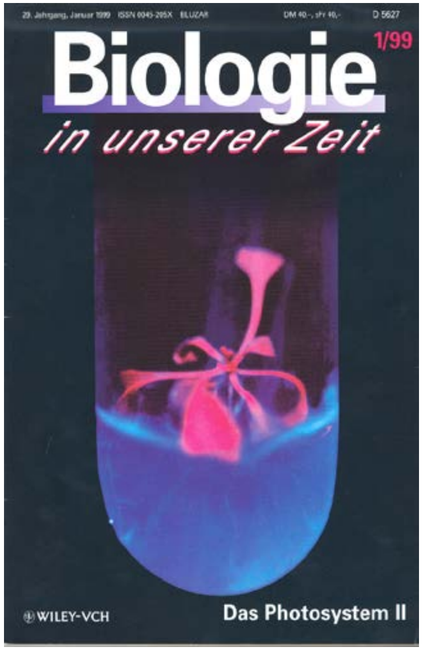
Mobile antenna

Non-mobile antenna

Both contain Chl, car, and LHCPs

LHCPs evolved from ELIPs





Mutants in photosynthesis

Light is absorbed by antenna

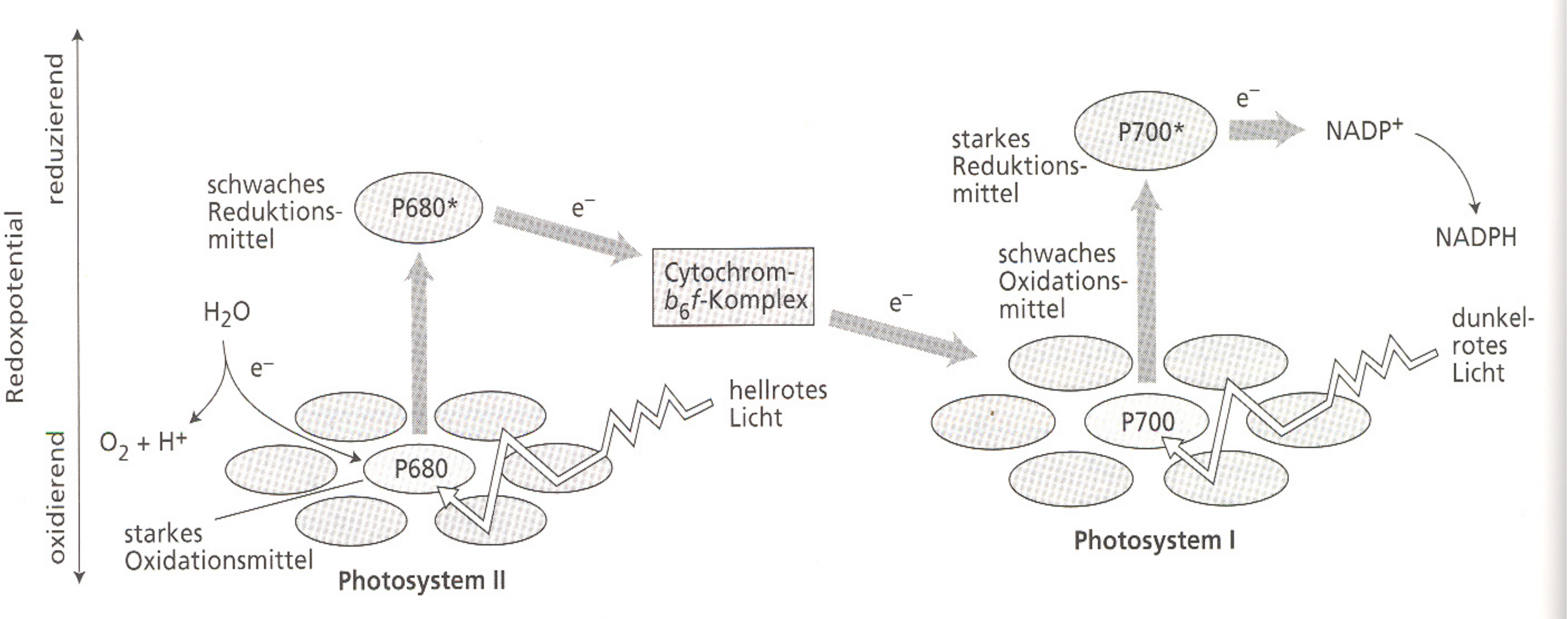
and emited as fluorescence

hcf (*high chlorophyll fluorescence*) phenotype

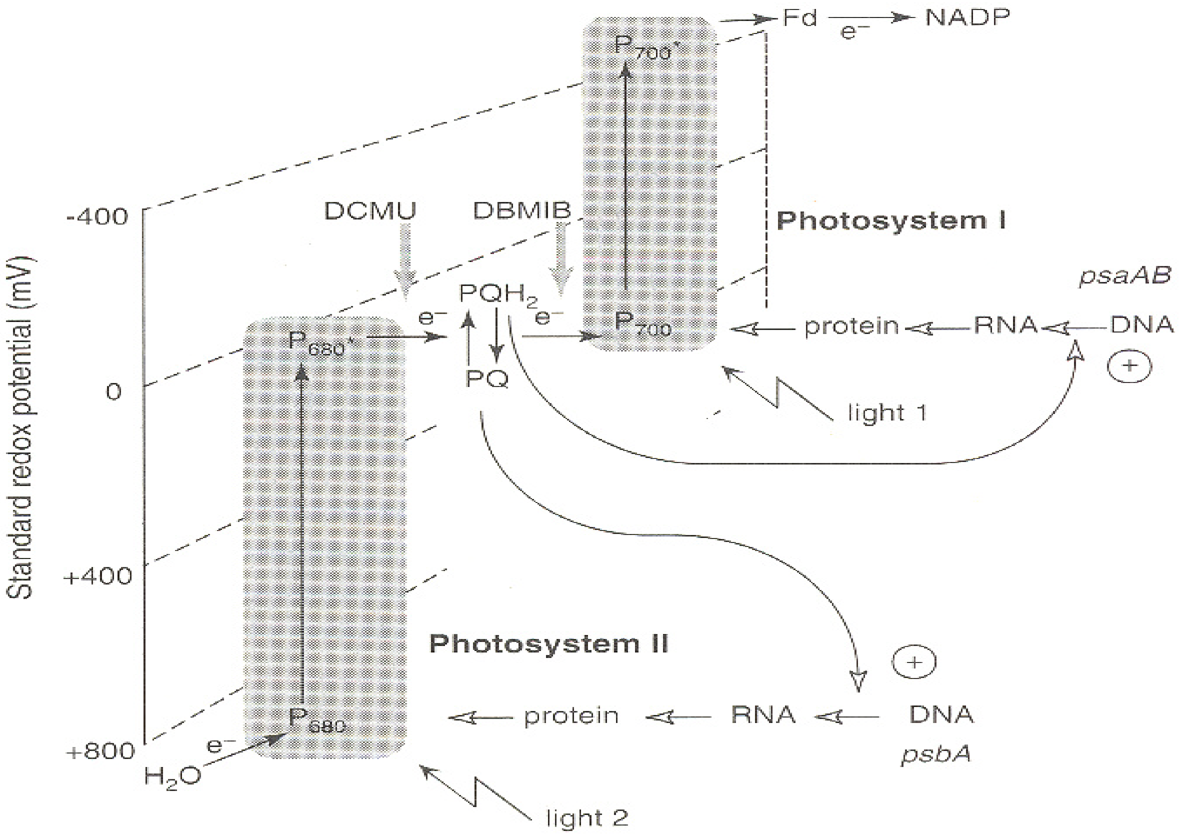
Pigments in PSII and PSI reaction centers are excited by different wavelengths

P_{680}, P_{700}

Generation of redox signals



Redox signals regulate plastid and nuclear gene expression

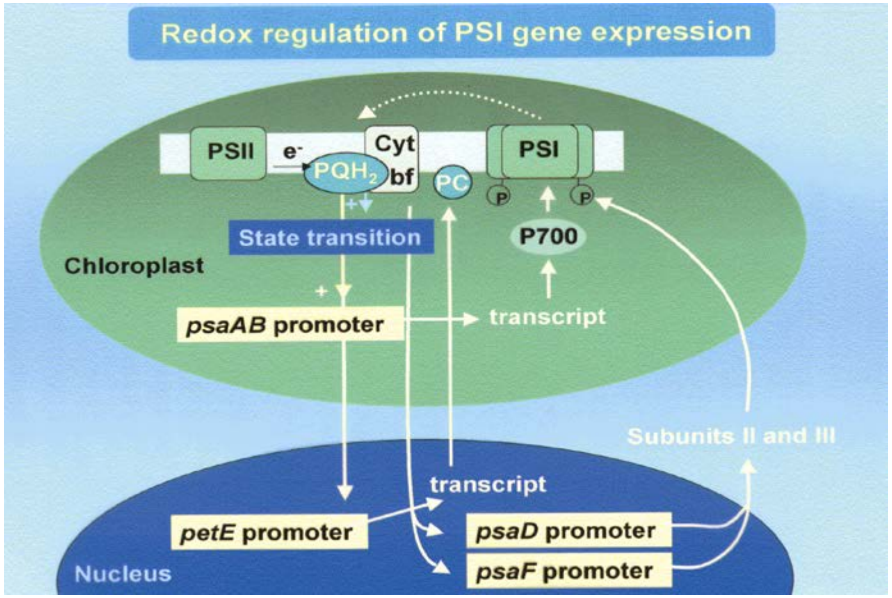


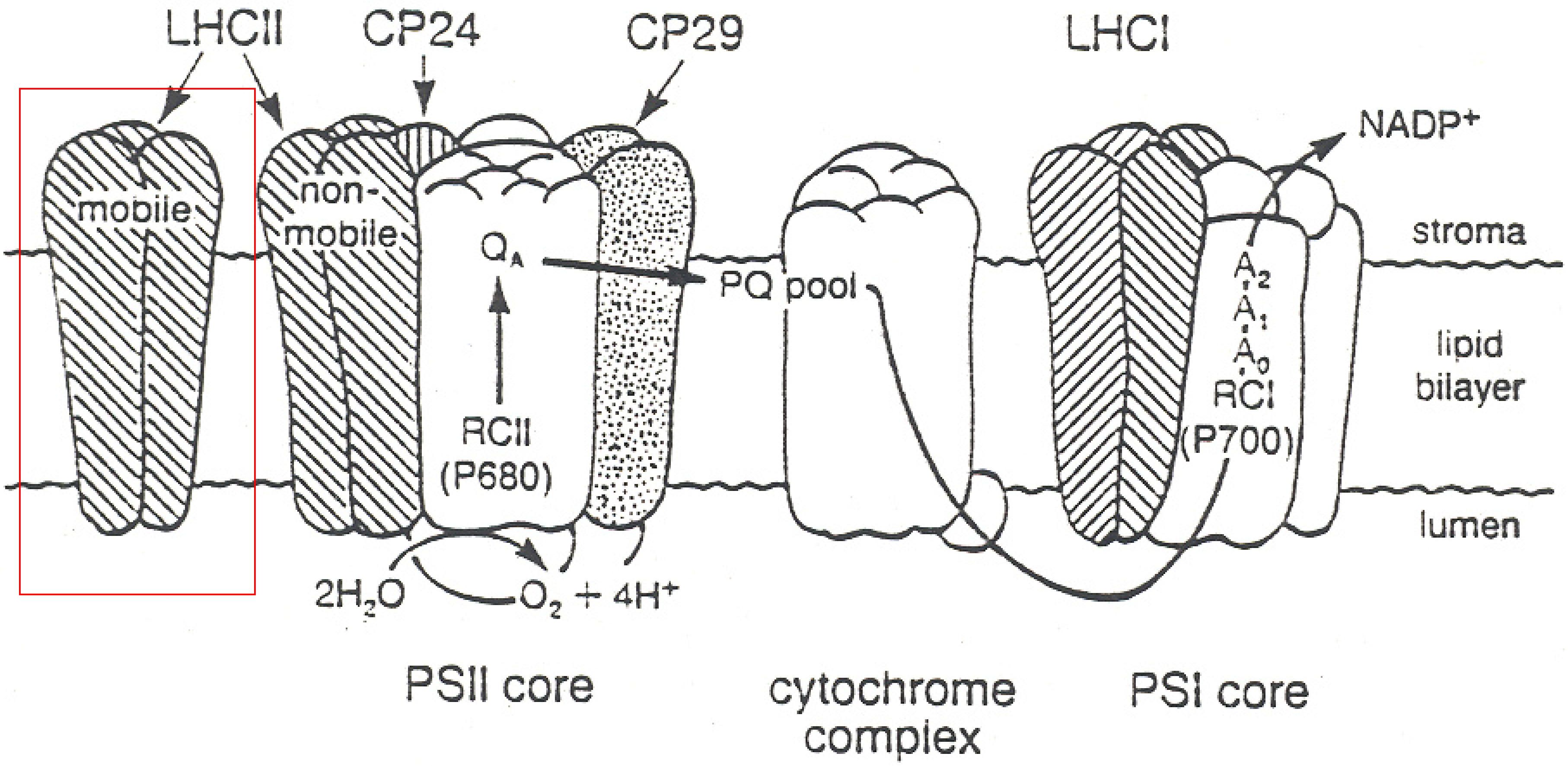
Adaptation to unbalanced excitation of PSII and PSI

(1) – state transition (fast): relocation of mobile antenna

(2) – change in plastid gene expression: genes for limiting PS complex are upregulated

(3) – change in nuclear gene expression





More PSII excitation > PSI activity is limiting

➢ Phosphorylation of mobile antenna

➢ migration to PS I

2. Light reactions at the thylakoid membrane

The four photosynthetic complexes are evolutionary conserved from cyanobacteria to higher plants

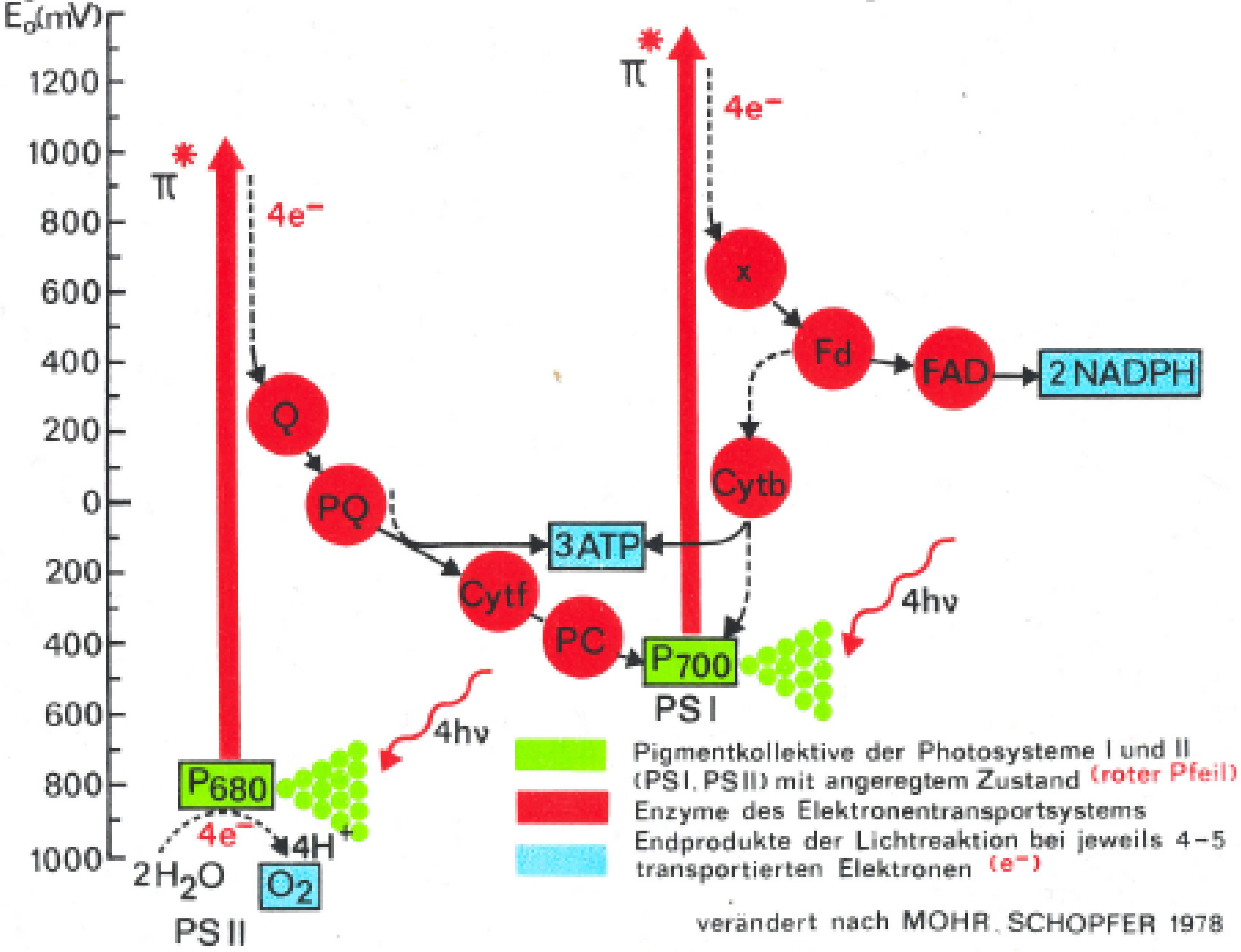
Photosystem II

Cytochrom $_{b6/f}$-complex

Photosystem I

ATP-synthase

Lichtreaktion der Photosynthese

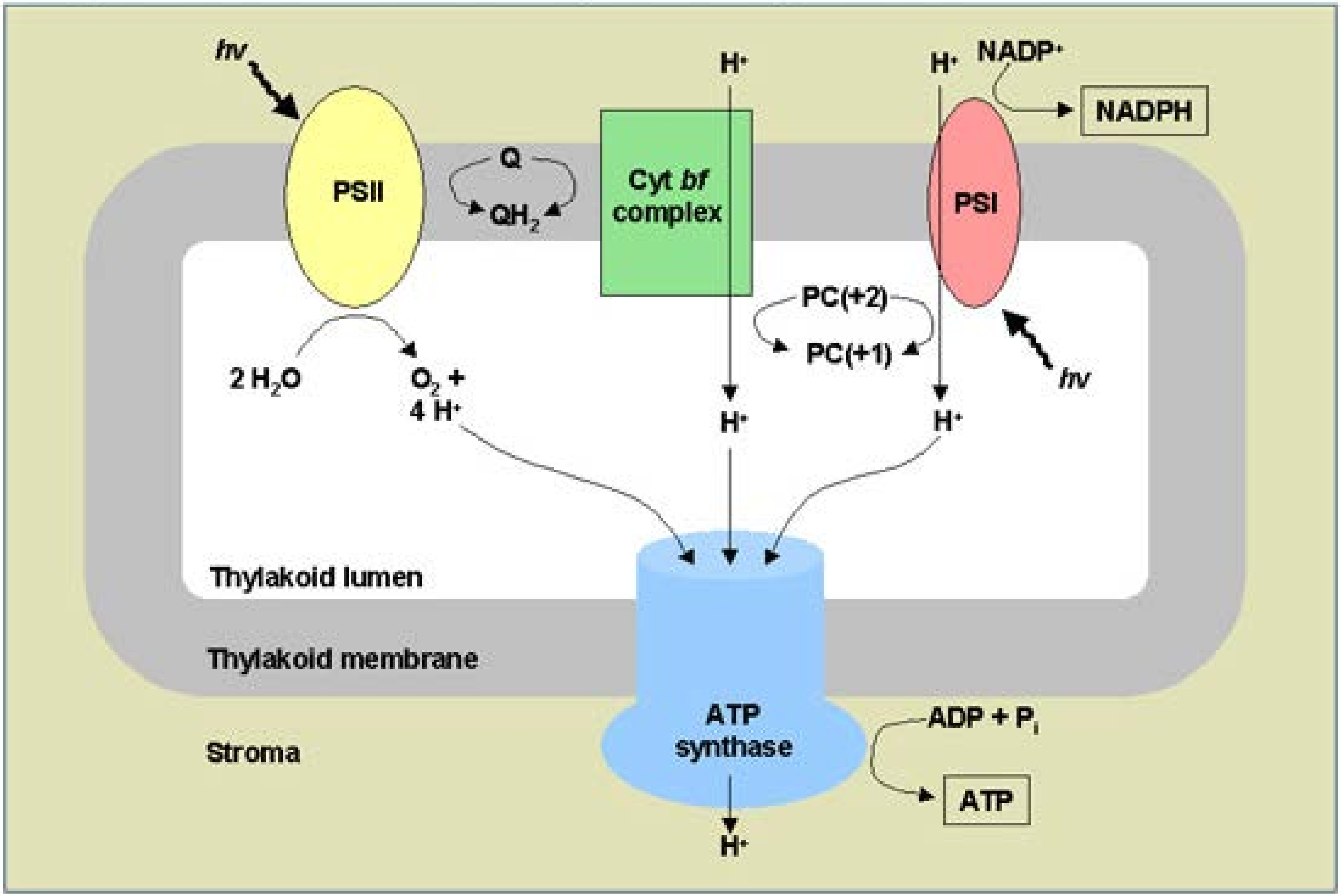


🟩 Pigmentkollektive der Photosysteme I und II (PS I, PS II) mit angeregtem Zustand (roter Pfeil)

🟥 Enzyme des Elektronentransportsystems

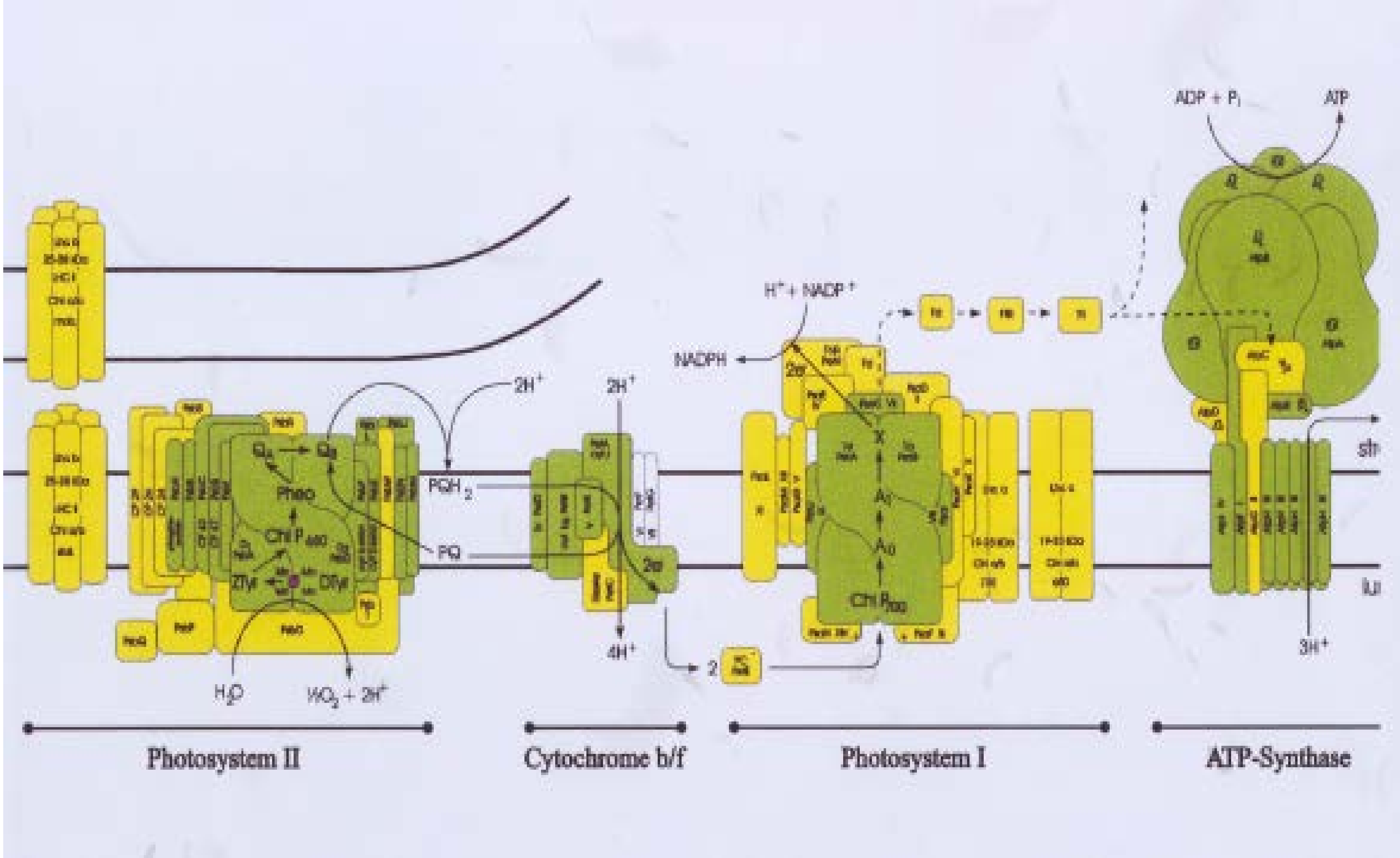
🟦 Endprodukte der Lichtreaktion bei jeweils 4–5 transportierten Elektronen (e⁻)

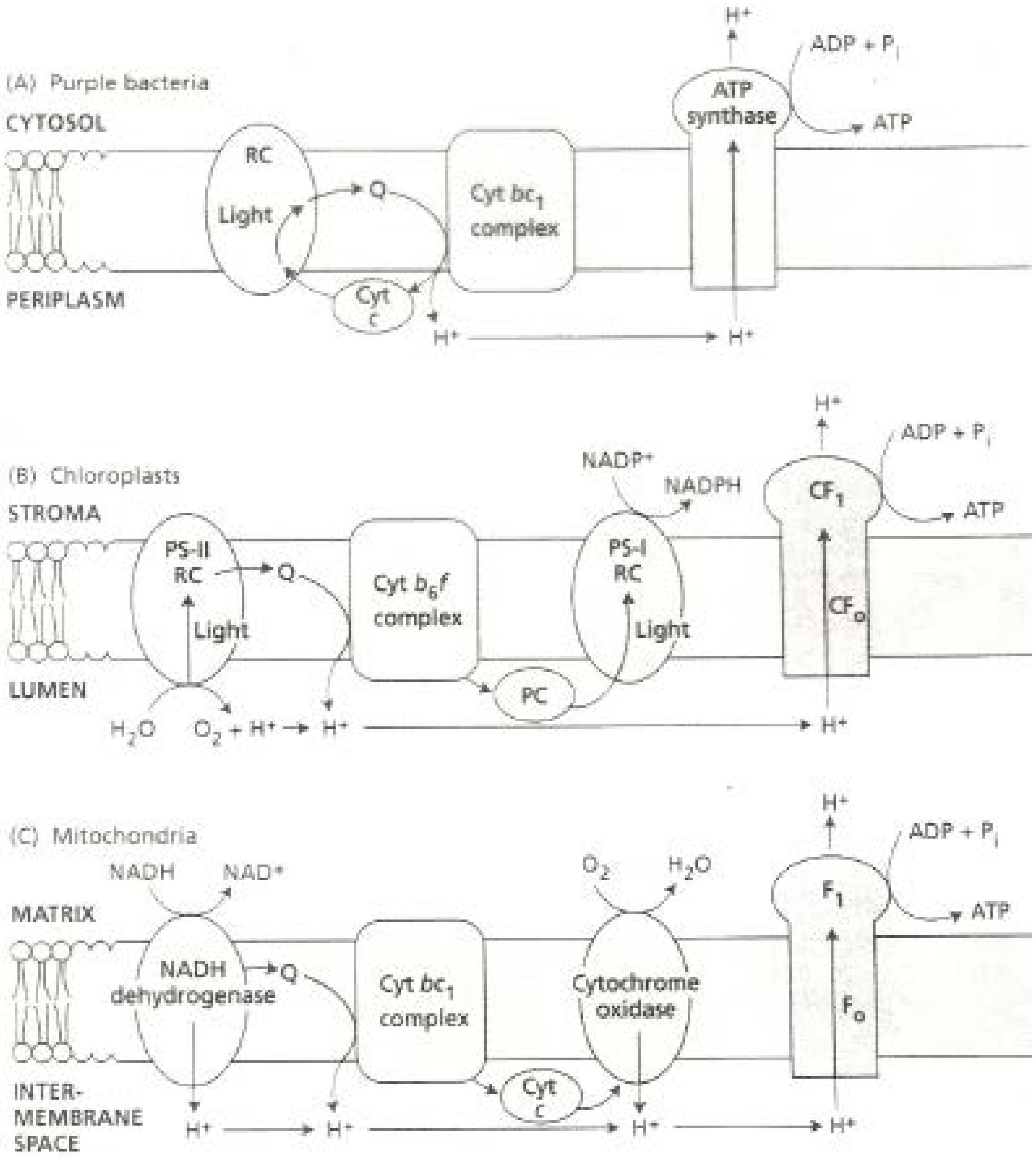
verändert nach MOHR, SCHOPFER 1978



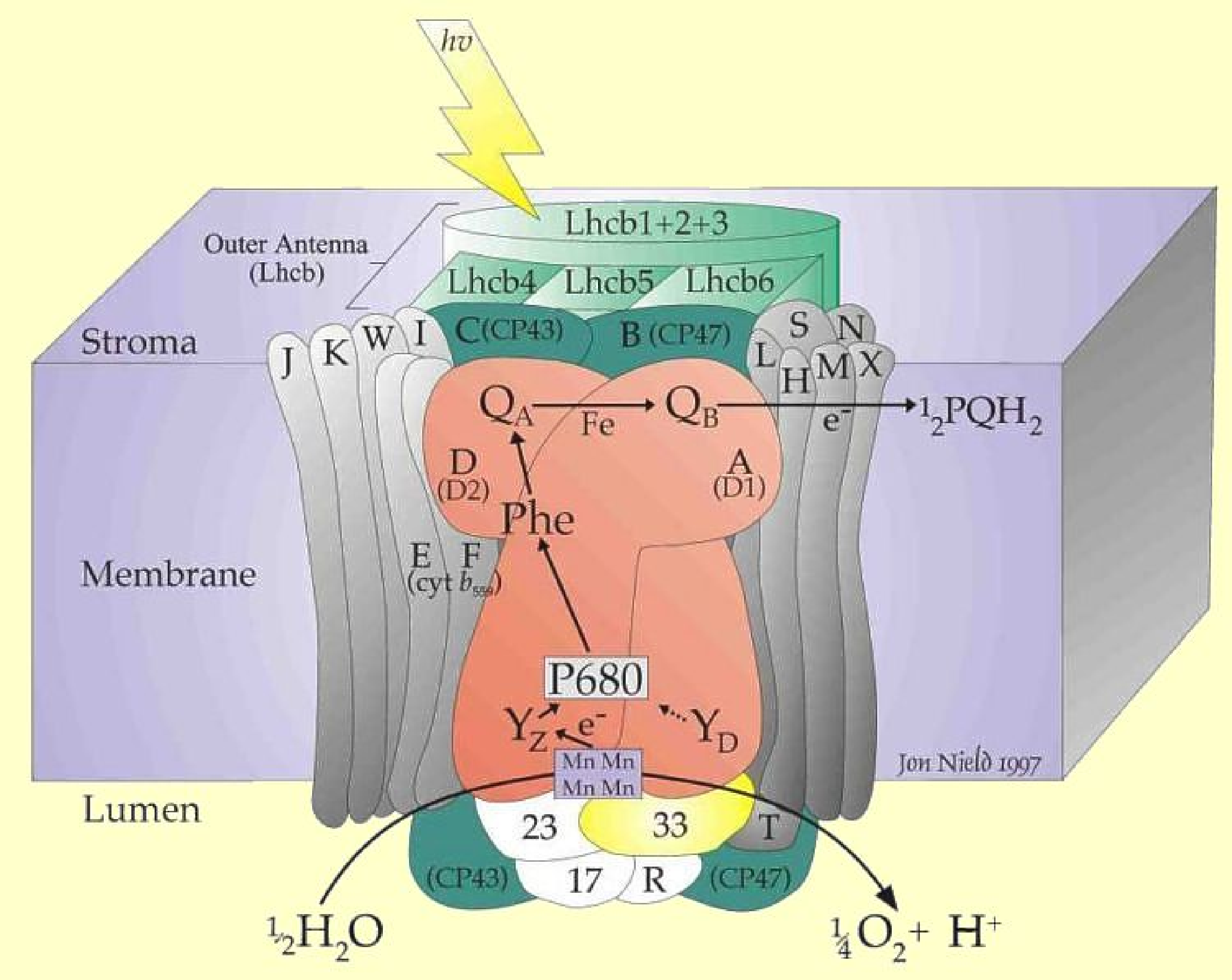
hν
PSII
Q
QH_2
Cyt *bf* complex
H^+
H^+
$NADP^+$
NADPH
PSI
PC(+2)
PC(+1)
2 H_2O
O_2 + 4 H^+
H^+
H^+
hν
Thylakoid lumen
Thylakoid membrane
Stroma
ATP synthase
ADP + P_i
ATP
H^+

In eukaryotic organism: thylakoid proteins are of dual genetic origin



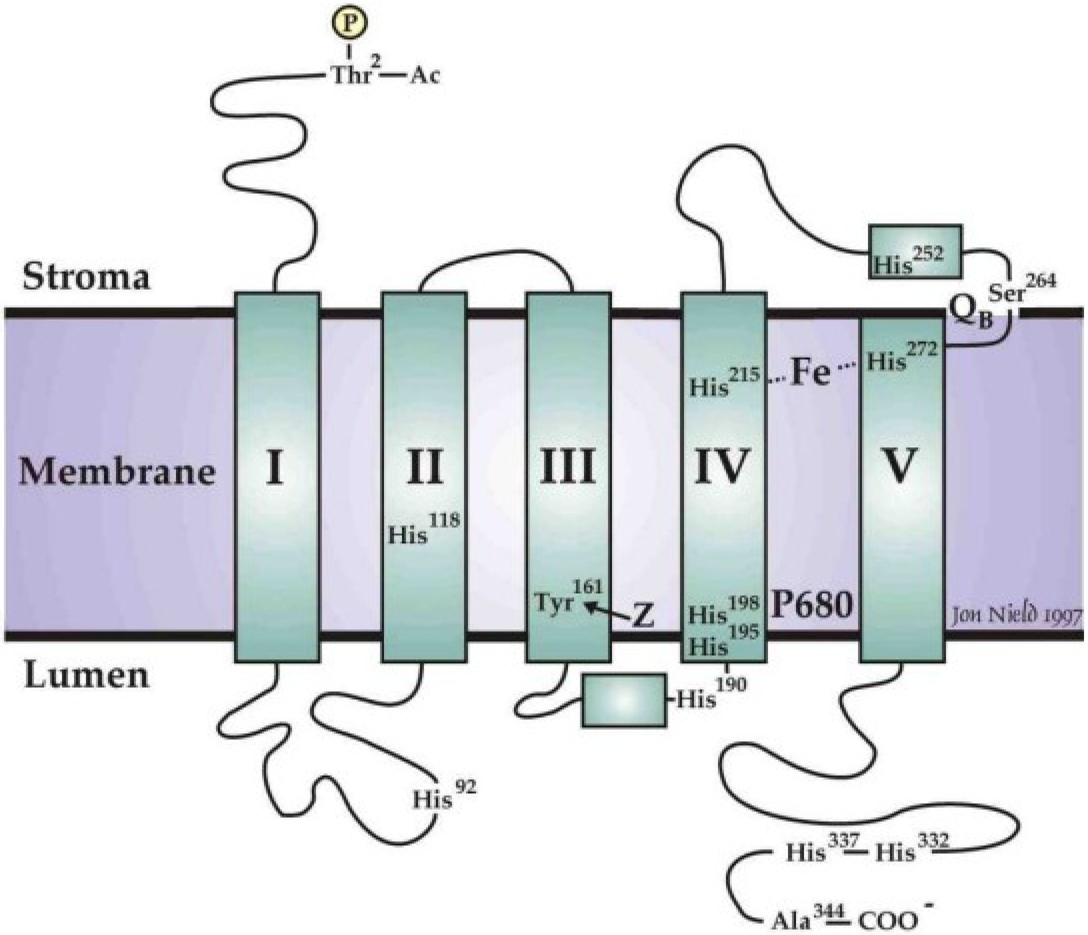

(A) Purple bacteria
CYTOSOL
PERIPLASM
RC
Light
Q
Cyt bc_1 complex
Cyt c
H^+
H^+
ATP synthase
H^+
ADP + P_i
ATP
(B) Chloroplasts
STROMA
LUMEN
PS-II RC
Light
Q
Cyt b_6f complex
PC
PS-I RC
Light
NADP⁺
NADPH
CF_1
CF_o
H^+
ADP + P_i
ATP
H_2O
$O_2 + H^+$
H^+
H^+
(C) Mitochondria
MATRIX
INTER-MEMBRANE SPACE
NADH
NAD⁺
NADH dehydrogenase
Q
Cyt bc_1 complex
Cyt c
O_2
H_2O
Cytochrome oxidase
F_1
F_o
H^+
ADP + P_i
ATP
H^+
H^+
H^+
H^+

Photosystem II

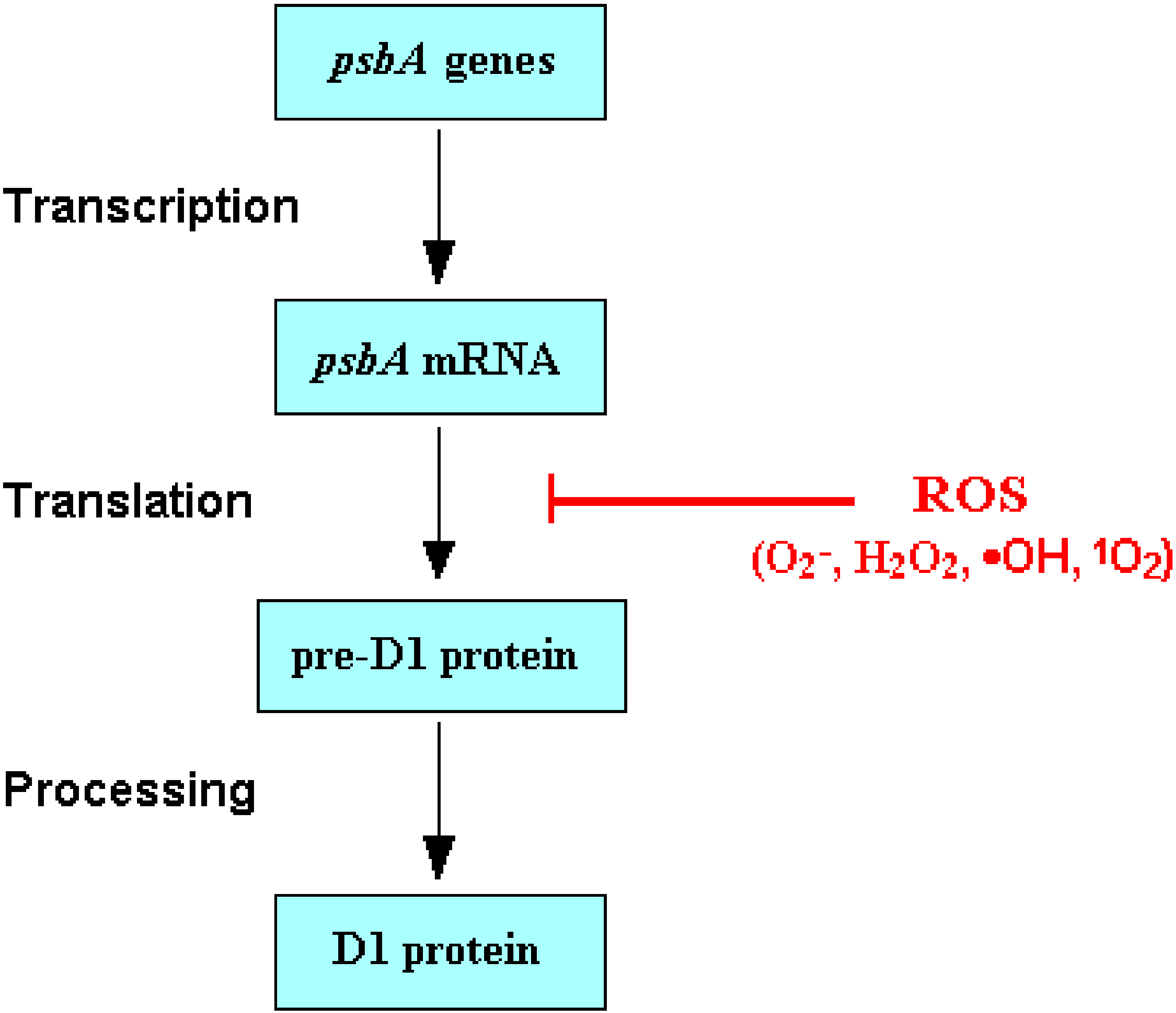


Yz = Tyr $_{161}$

D1 PsbA



The predicted membrane folding pattern of the mature spinach D1 protein. Roman numerals I-V indicate the membrane-spanning helices. Two minor helices, on the stromal and lumenal sides, are also indicated in green. The putative positions of the bound cofactors and histidine residues on the proposed transmembrane helices are shown. Sequence data were obtained from the SWISSPROT database.



psbA genes

Transcription

psbA mRNA

Translation

ROS

(O_2^-, H_2O_2, •OH, $^1O_2$)

pre-D1 protein

Processing

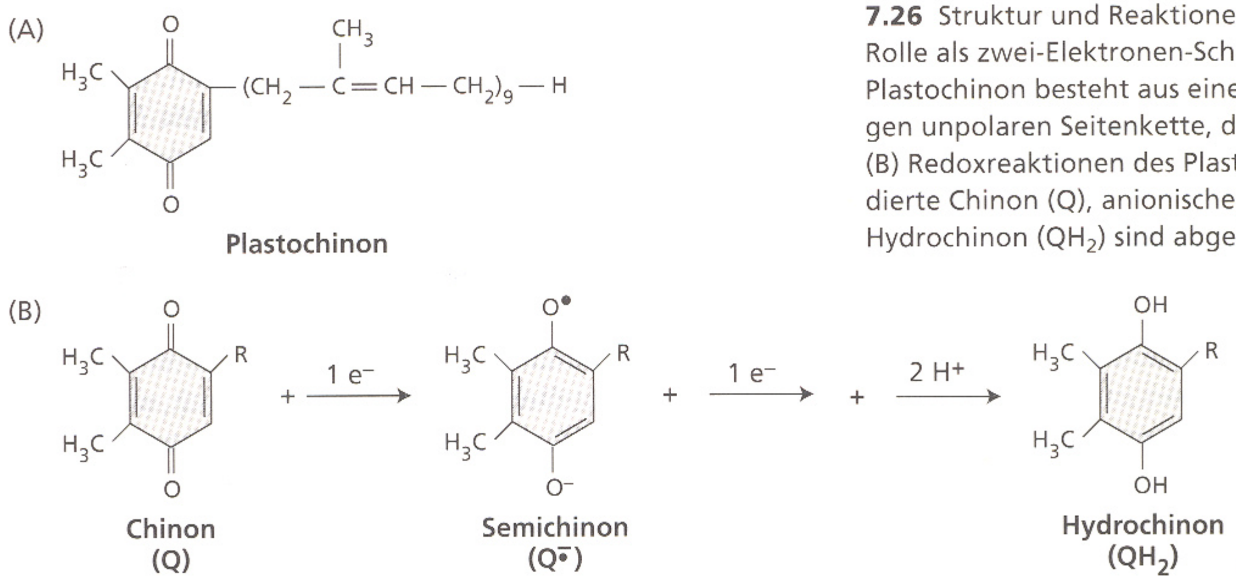
D1 protein

D1 protein

- high *turnover*
- de novo synthesis in the stroma thylakoids during insertion into membrane
    - co-factor bindung
    - *translational arrest* when no chlorophyll is available
    - transport to grana thylakoids
    - association with antenna
- high light: photodestruction

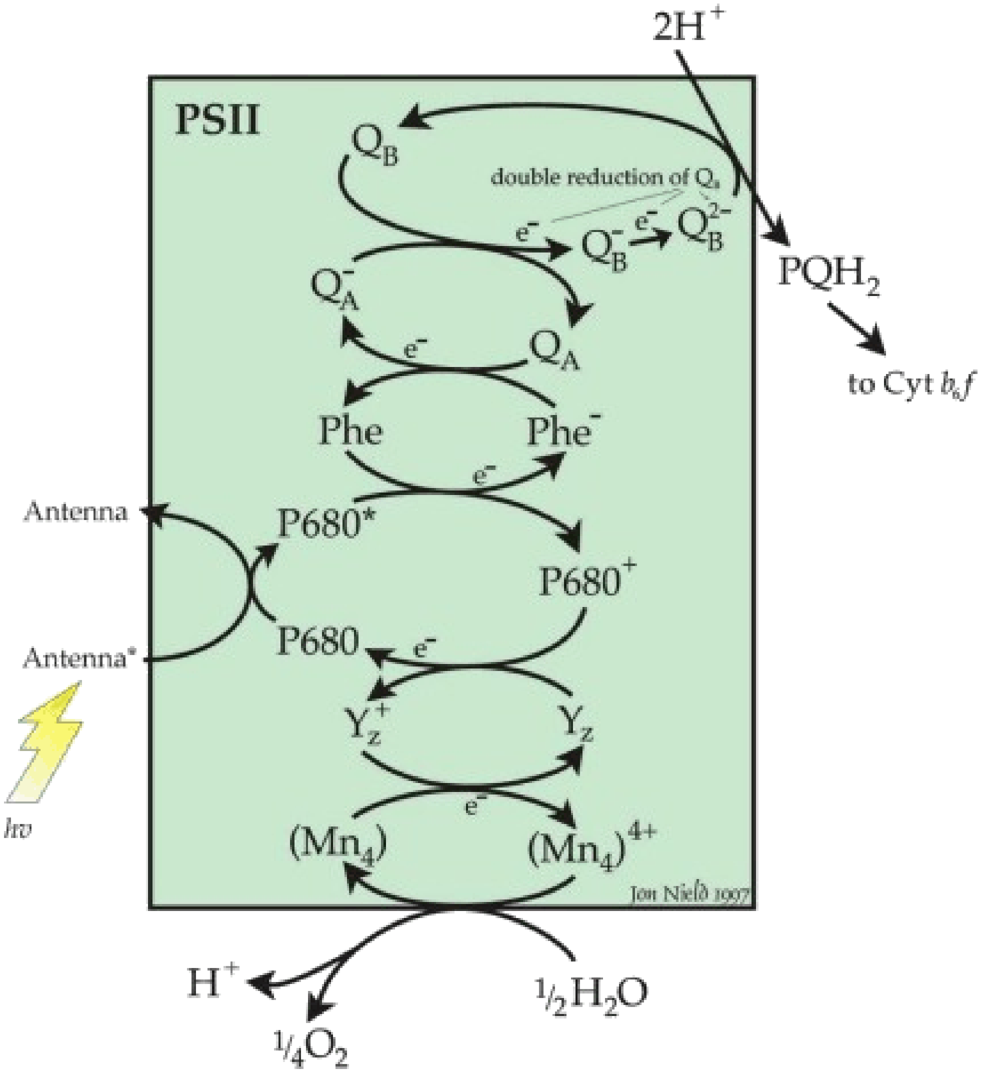
- D2: similar regulation

Two electron transporters: Q_A and Q_B

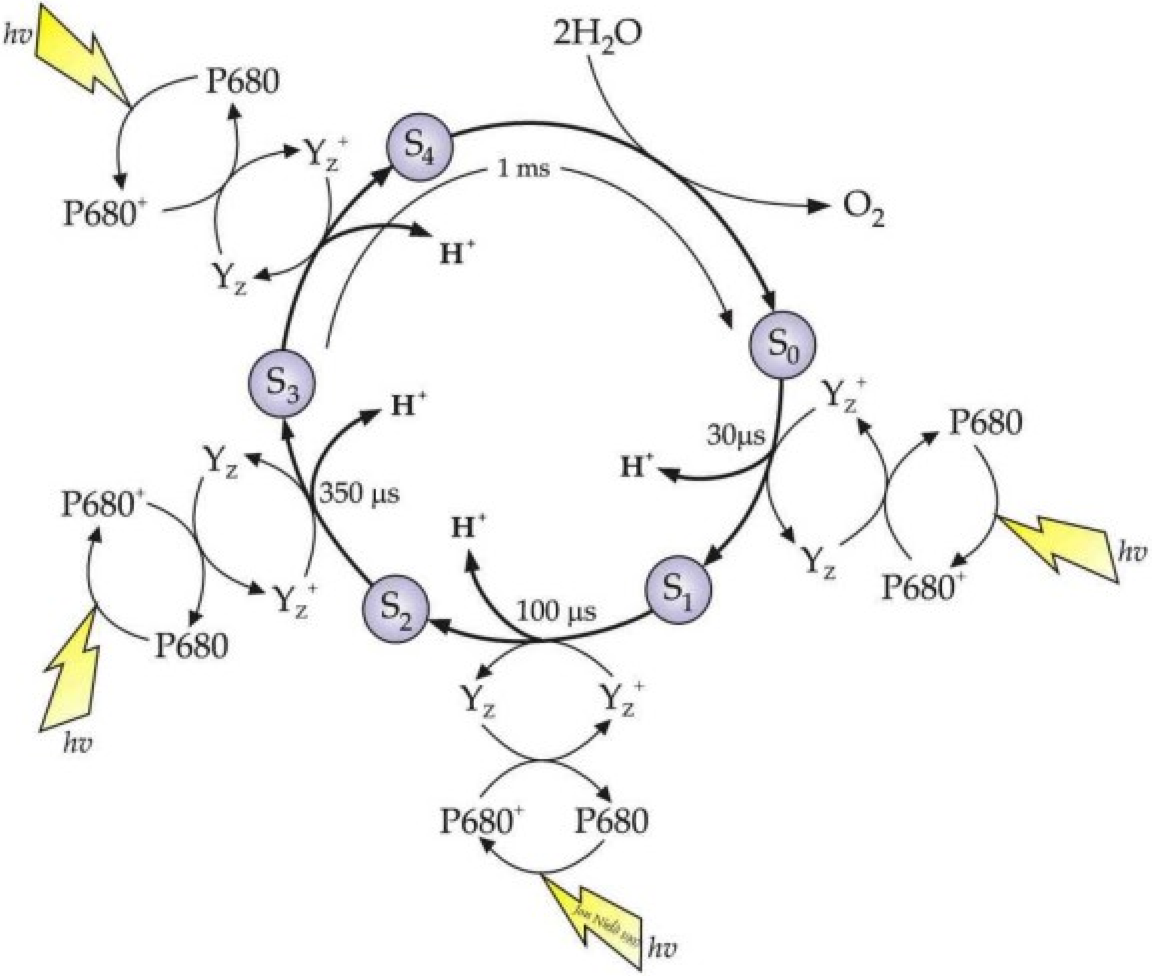


7.26 Struktur und Reaktionen des Plastochinons und seine Rolle als zwei-Elektronen-Schleuse im Photosystem II. (A) Das Plastochinon besteht aus einem Chinon-Kopfteil und einer langen unpolaren Seitenkette, die es in der Membran verankert. (B) Redoxreaktionen des Plastochinons. Das vollständig oxidierte Chinon (Q), anionisches Semichinon ($Q^{\bar{\bullet}}$) und reduziertes Hydrochinon (QH_2) sind abgebildet. R steht für die Seitenkette.

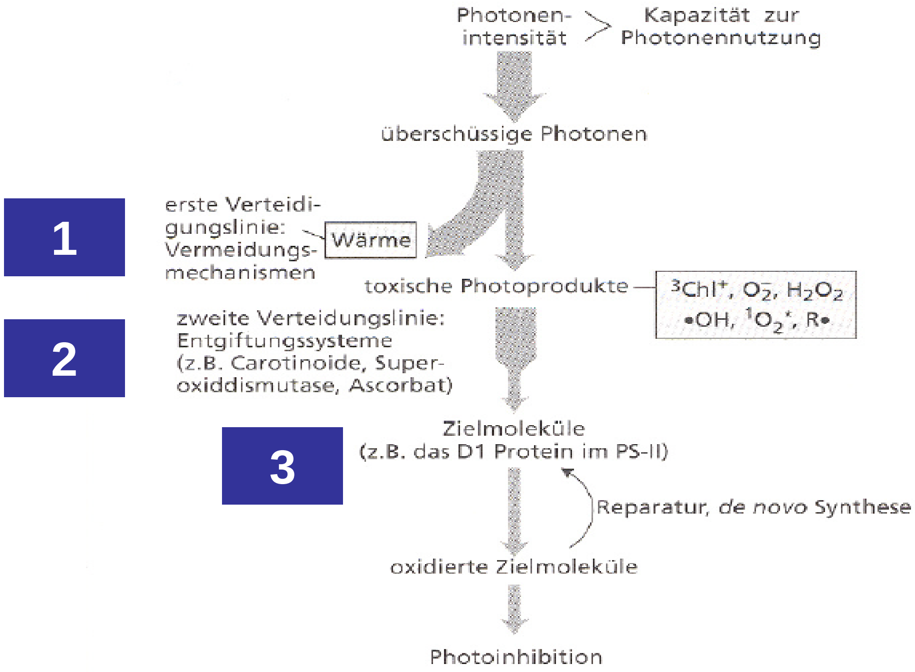
The Mn cluster splits water and is responsible for O_2 evolution



Schematic diagram showing the association of the main redox components within the PSII reaction centre. The substrate water, binds to a manganese (Mn) cluster attached to the lumenal surface of PSII. The arrows indicate the electron transport pathway from water via the Mn cluster, Y_Z (D1-Tyr161), P680, Pheophytin, Q_A and the double reduction of Q_B. The minimum complex needed for high rates of oxygen evolution requires the 33 kDa extrinsic protein and CP47/CP43 proteins to be present (see section 1.5.2 and Fig. 1.4).

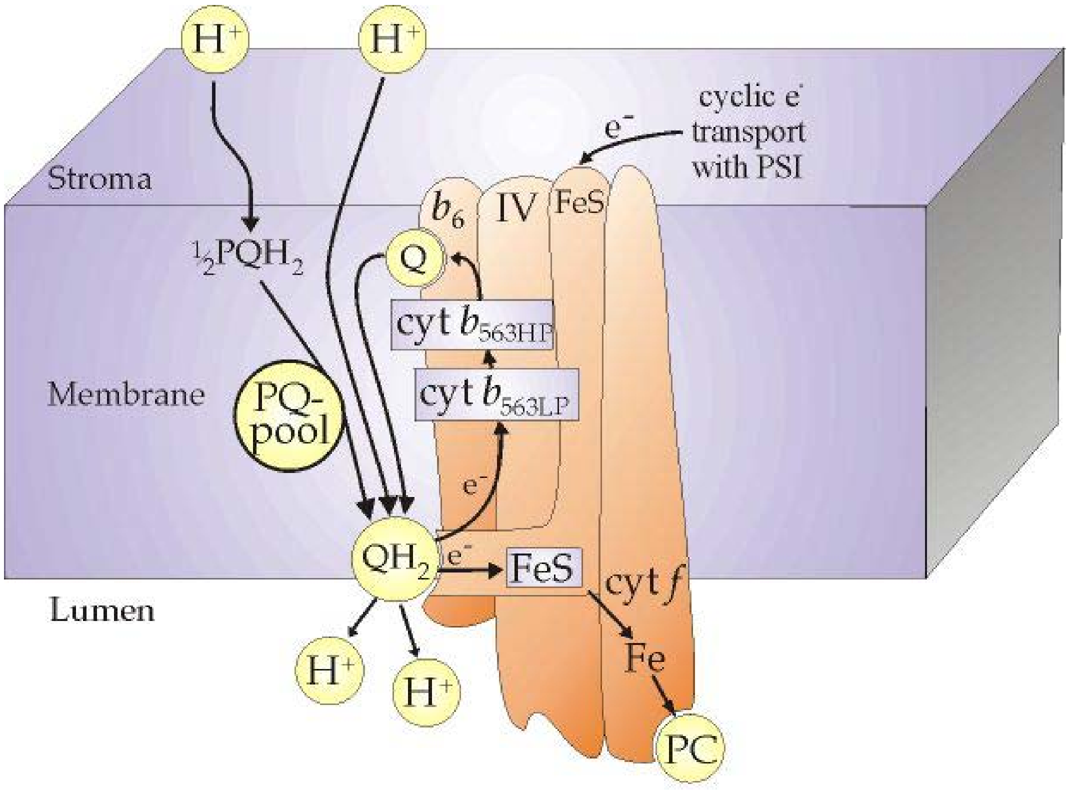


The S-state cycle for the oxygen evolving reactions of PSII (Kok *et al.*, 1970). Diagram adapted from Rutherford, (1989).



7.33 Gesamtübersicht über die Regulation der Lichtabsorption, den Lichtschutz und die Reparatur von Lichtschäden. Der Schutz vor Lichtschäden erfolgt auf mehreren Ebenen. Die erste Verteidigungslinie ist die Vermeidung von Schäden durch die Umwandlung überschüssiger Anregungsenergie in Wärme. Wenn dieser Mechanismus nicht ausreicht und toxische Photoprodukte gebildet werden (der Triplett-Zustand des Chlorophylls [$^3Chl^*$], Superoxid [O_2^-], Singulettsauerstoff [$^1O_2^*$], Wasserstoffperoxid [H_2O_2] und Hydroxylradikale [$\bullet OH$]), können diese durch eine Vielzahl von Entgiftungssystemen (Carotinoide, Superoxiddismutase, Ascorbat) vernichtet werden. Wenn auch diese zweite Verteidigungslinie nicht ausreicht, können die reaktiven Photoprodukte bestimmte besonders empfindliche Moleküle zerstören. Vor allem das D1-Protein des PS-II ist betroffen. Die Schädigung führt zur Photoinhibition. Das D1-Protein wird dann aus dem Reaktionszentrum des PS-II entfernt und abgebaut. Ein neu synthetisiertes D1-Protein wird in das PS-II-Reaktionszentrum eingebaut und so die Funktionalität wieder hergestellt. (Nach Asada 1996)

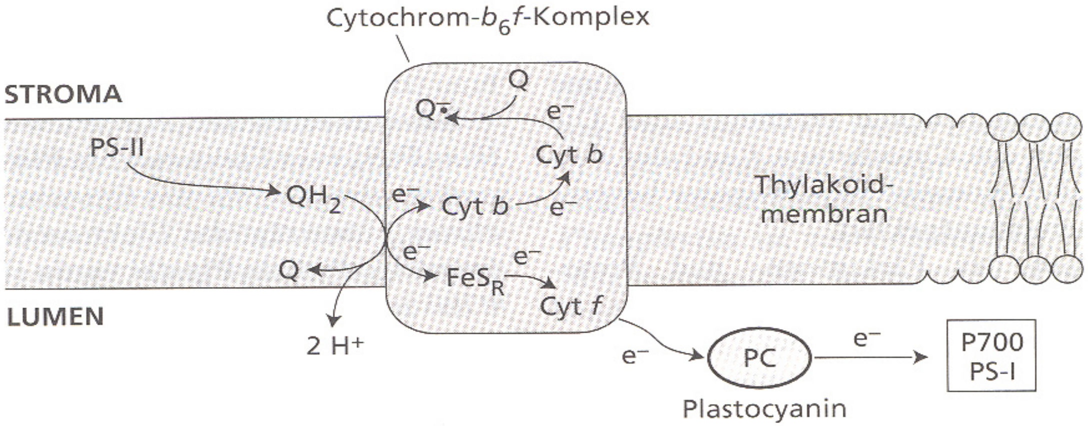
Cytochrome b_6/*f*-complex



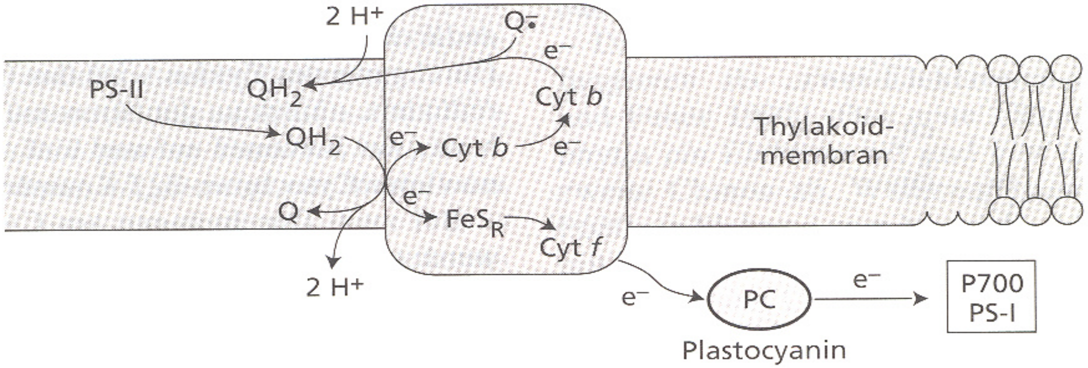
Three main features:

i) Plastoquinol-plastocyanin oxido-reductase
ii) Pumps $2H^+$ from stroma to lumen via the Q-cycle
iii) Participates in cyclic e^- transport from PSI

(A) Oxidation des ersten QH_2



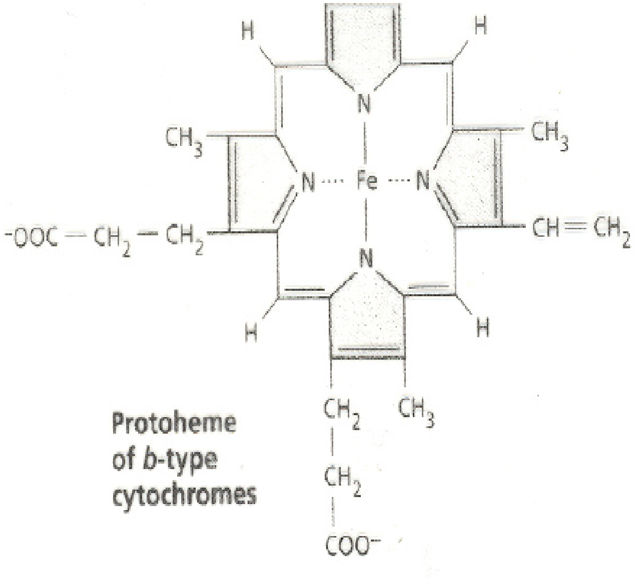
(B) Oxidation des zweiten QH_2



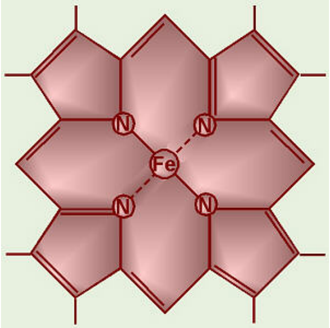
7.28 Mechanismus des Elektronen- und Protonentransfers im Cytochrom-b_6f-Komplex. Dieser Komplex enthält zwei *b*-Typ-Cytochrome (Cyt *b*), ein *c*-Typ-Cytochrom (Cyt *c*, aus historischen Gründen als Cytochrom *f* bezeichnet), ein Rieske Fe-S-Protein (FeS_R), und zwei Stellen, an denen Oxidation oder Reduktion des Chinons erfolgt. (A) Ein am PS-II (siehe Abb. 7.26) gebildetes Plastohydrochinon(QH_2)-Molekül wird in der Nähe der Lumenseite des Komplexes oxidiert. Dabei überträgt es jeweils ein Elektron an das Rieske-Fe-S-Protein und an eines der beiden *b*-Typ-Cytochrome. Gleichzeitig werden zwei Protonen in das Lumen abgegeben. Das an FeS_R abgegebene Elektron gelangt über Cytochrom *f* (Cyt *f*) zu Plastocyanin (PC). PC reduziert im PS-I das P700. Das reduzierte *b*-Typ-Cytochrom überträgt ein Elektron auf das andere *b*-Typ-Cytochrom, und dieses reduziert das Chinon (Q) zum Semichinon ($Q^{\bullet-}$) (siehe Abb. 7.26). (B) Ein zweites QH_2 wird oxidiert, wobei ein Elektron von FeS_R zum PC und weiter zum P700 gelangt. Das zweite Elektron wird über die beiden *b*-Typ-Cytochrome auf das Semichinon übertragen und reduziert es zum Plastohydrochinon. Dabei nimmt es aus dem Stroma zwei Protonen auf. Insgesamt werden für jeweils zwei Elektronen, die zum P700 gelangen, vier Protonen durch die Membran transportiert.

2 Hämgruppen vom b-Typ

(Cytochrom b)



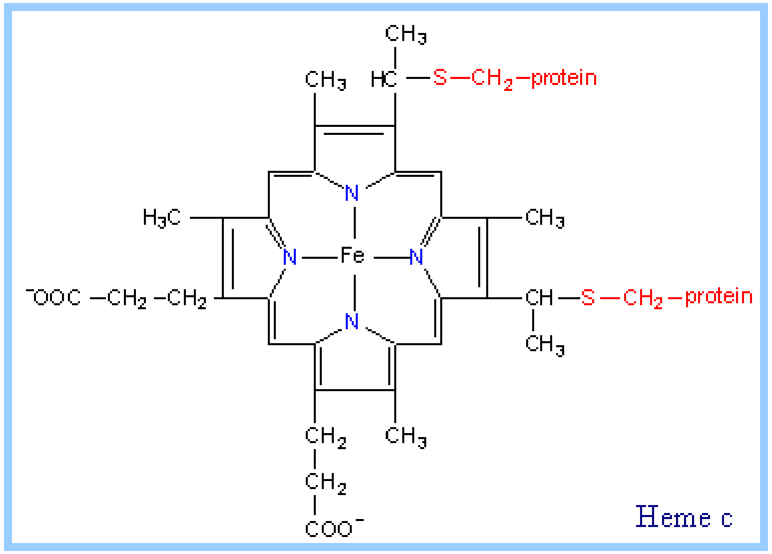
Protoheme of *b*-type cytochromes



1 Hämgruppe vom

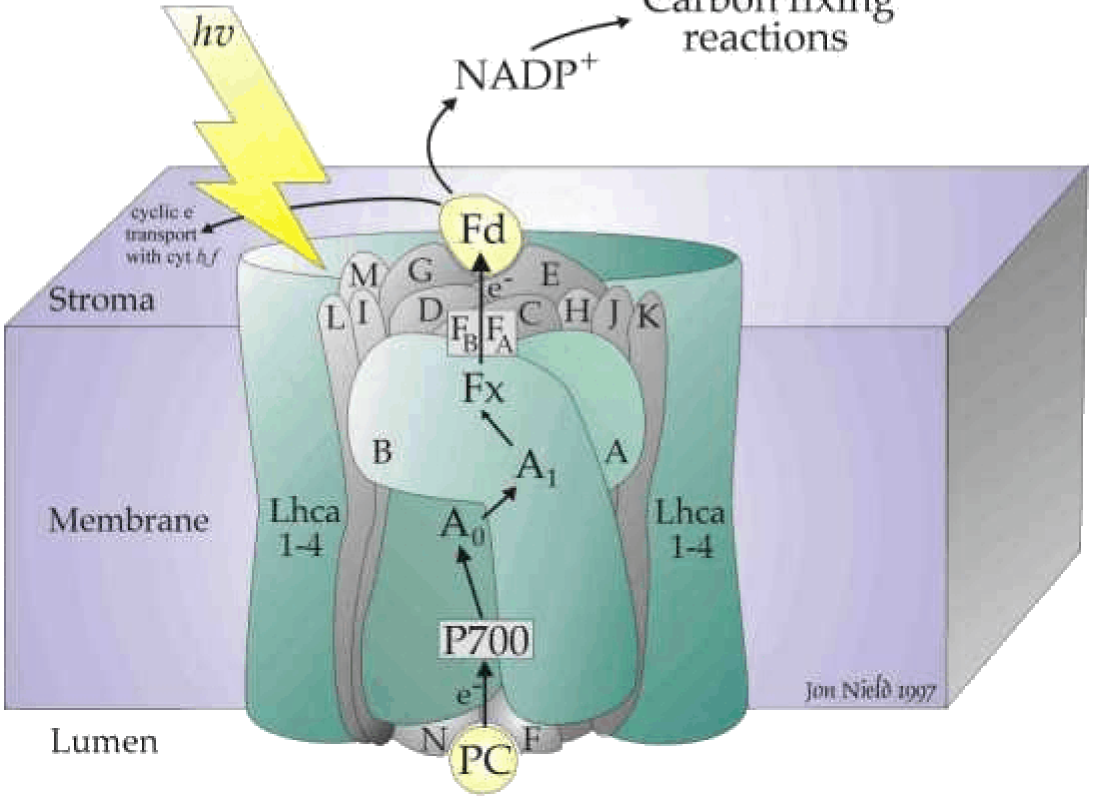
c-Typ

(Cytochrom f)



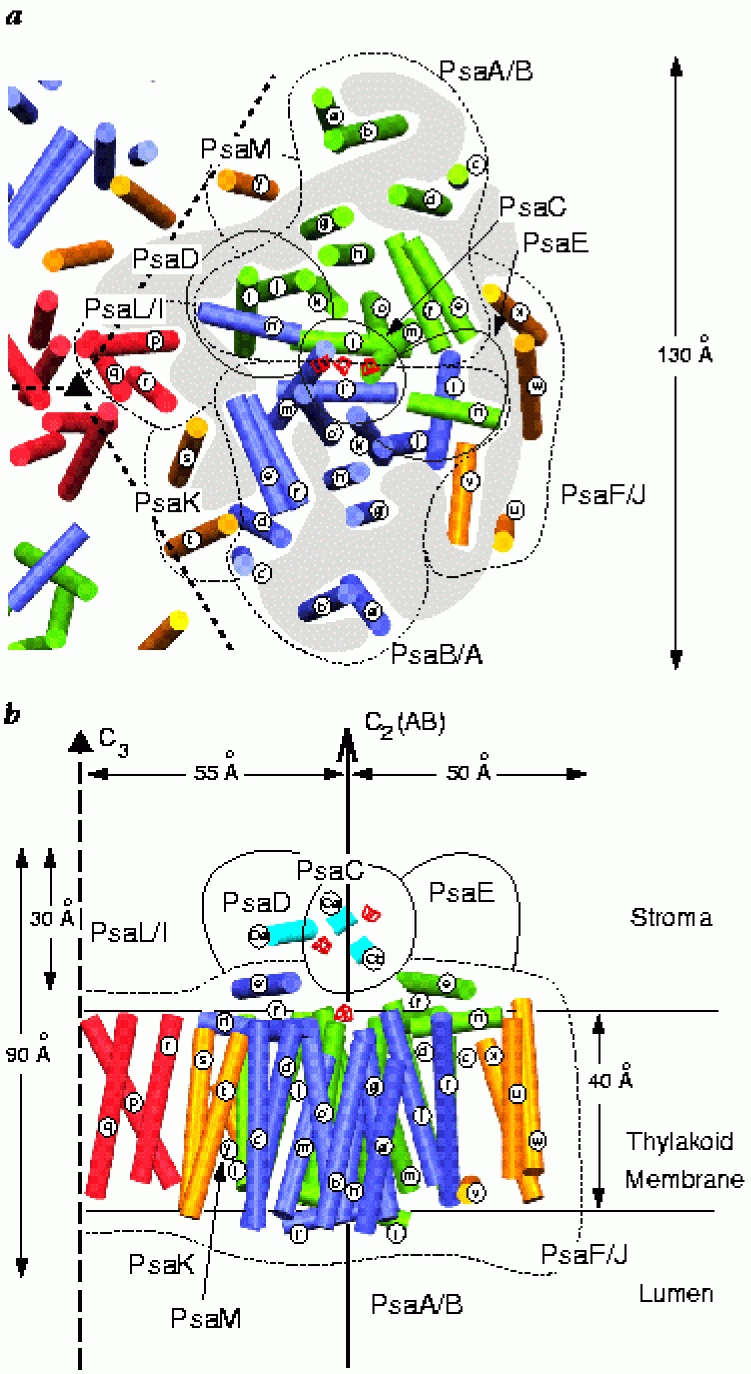
Heme c

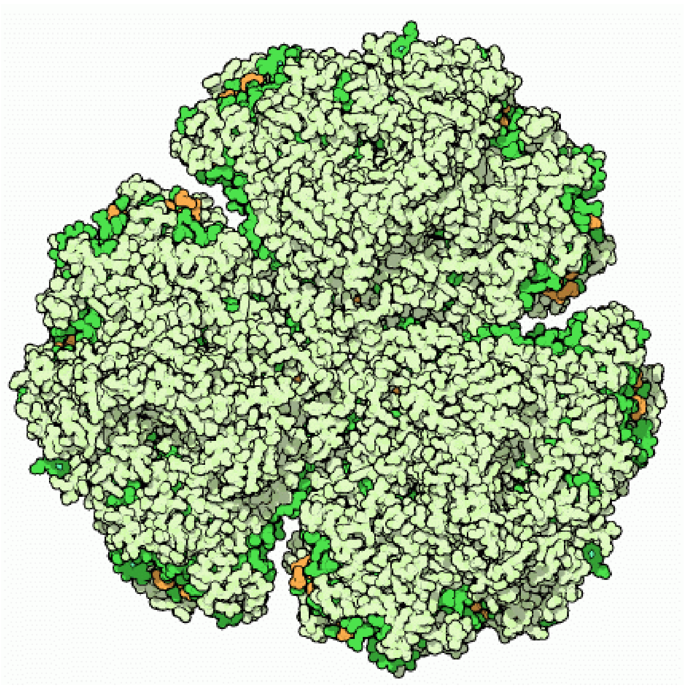
Photosystem I

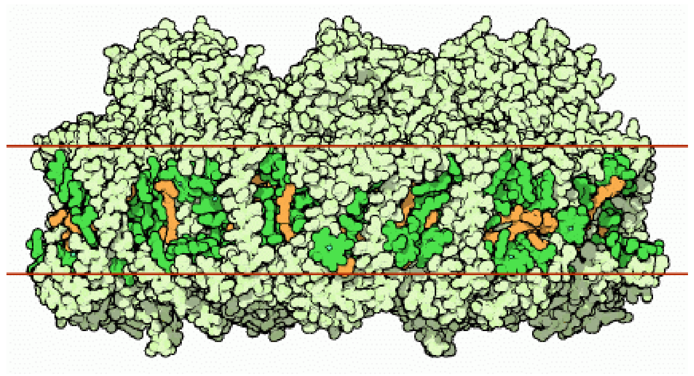


Three main features:

i) Multi-subunit complex > 18
ii) Plastocyanin-ferredoxin oxido-reductase
iii) Participates in cyclic e^- transport with cyt $b_6 f$
in addition to reducing NADP


a
PsaA/B
PsaM
PsaC
PsaE
PsaD
PsaL/I
PsaK
PsaF/J
PsaB/A
130 Å
b
C_3
C_2 (AB)
55 Å
50 Å
30 Å
90 Å
PsaL/I
PsaD
PsaC
PsaE
Stroma
40 Å
Thylakoid Membrane
PsaK
PsaM
PsaA/B
PsaF/J
Lumen





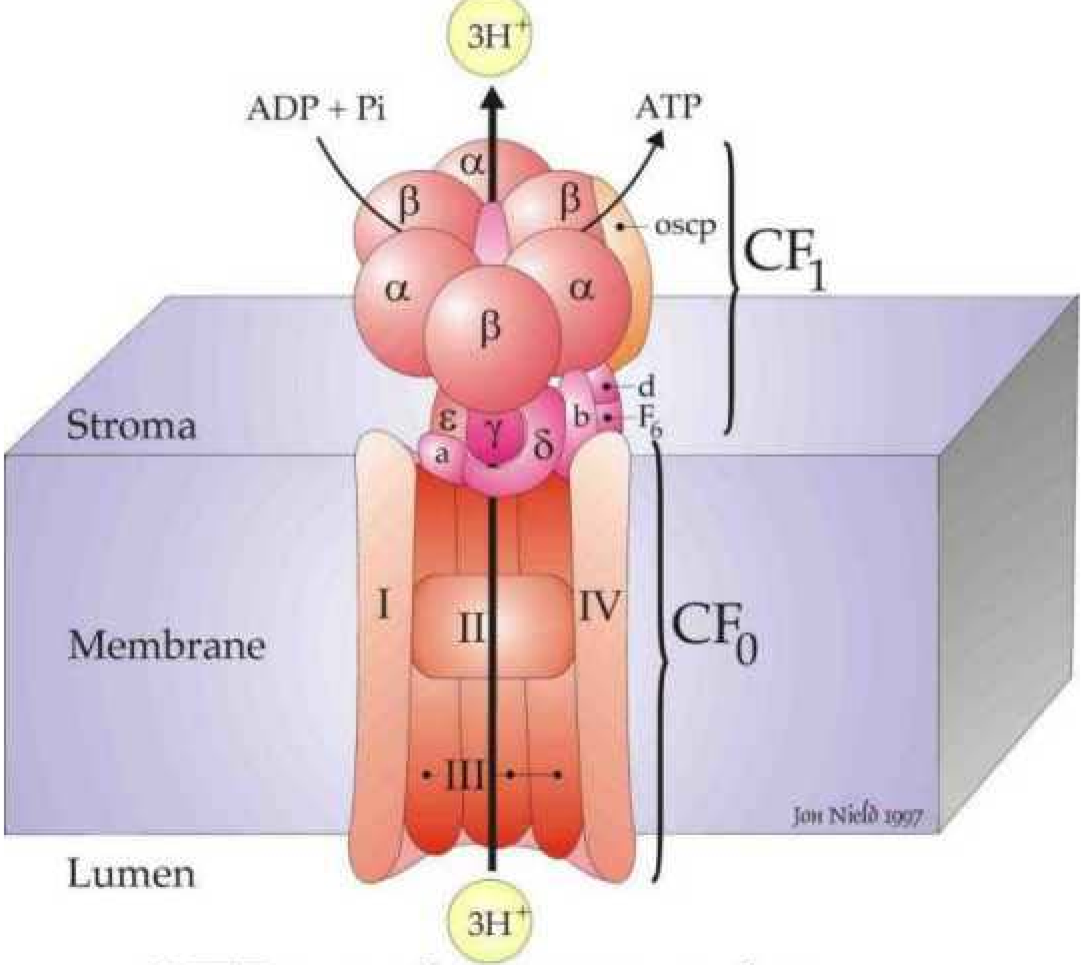
Cyclic electron transport

- electrons from PSI to cytochrome-b_6f-complex, back to P_{700}

- no oxygen evolution, no NADPH synthesis

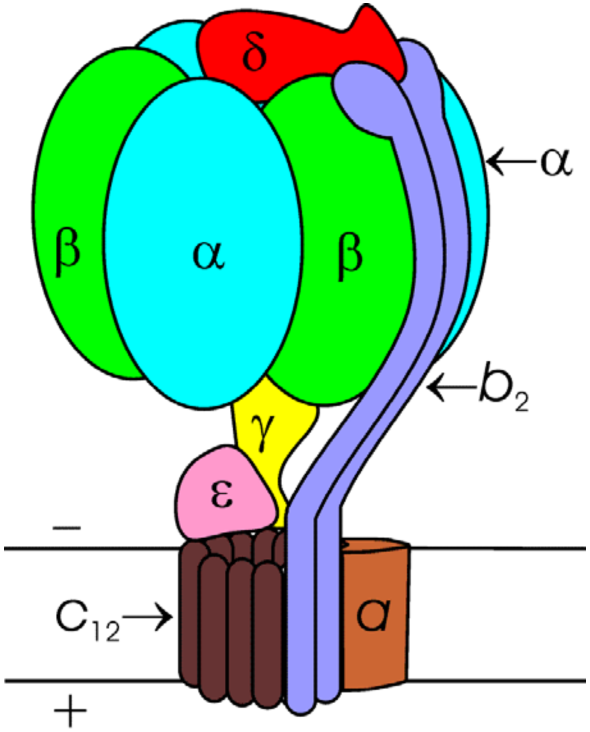
- generation of proton gradient

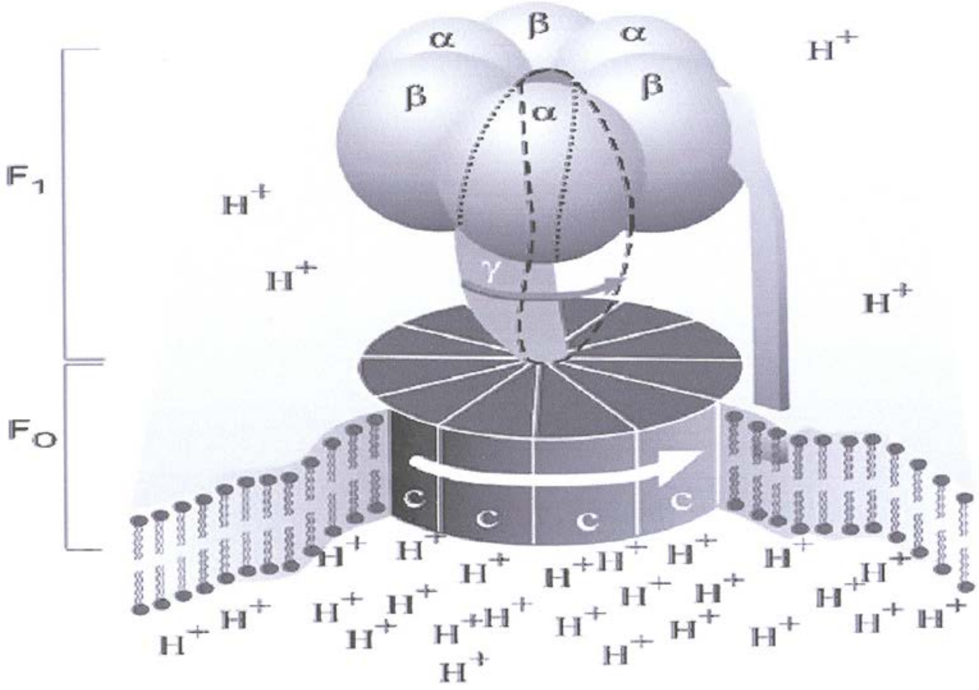
ATP synthase

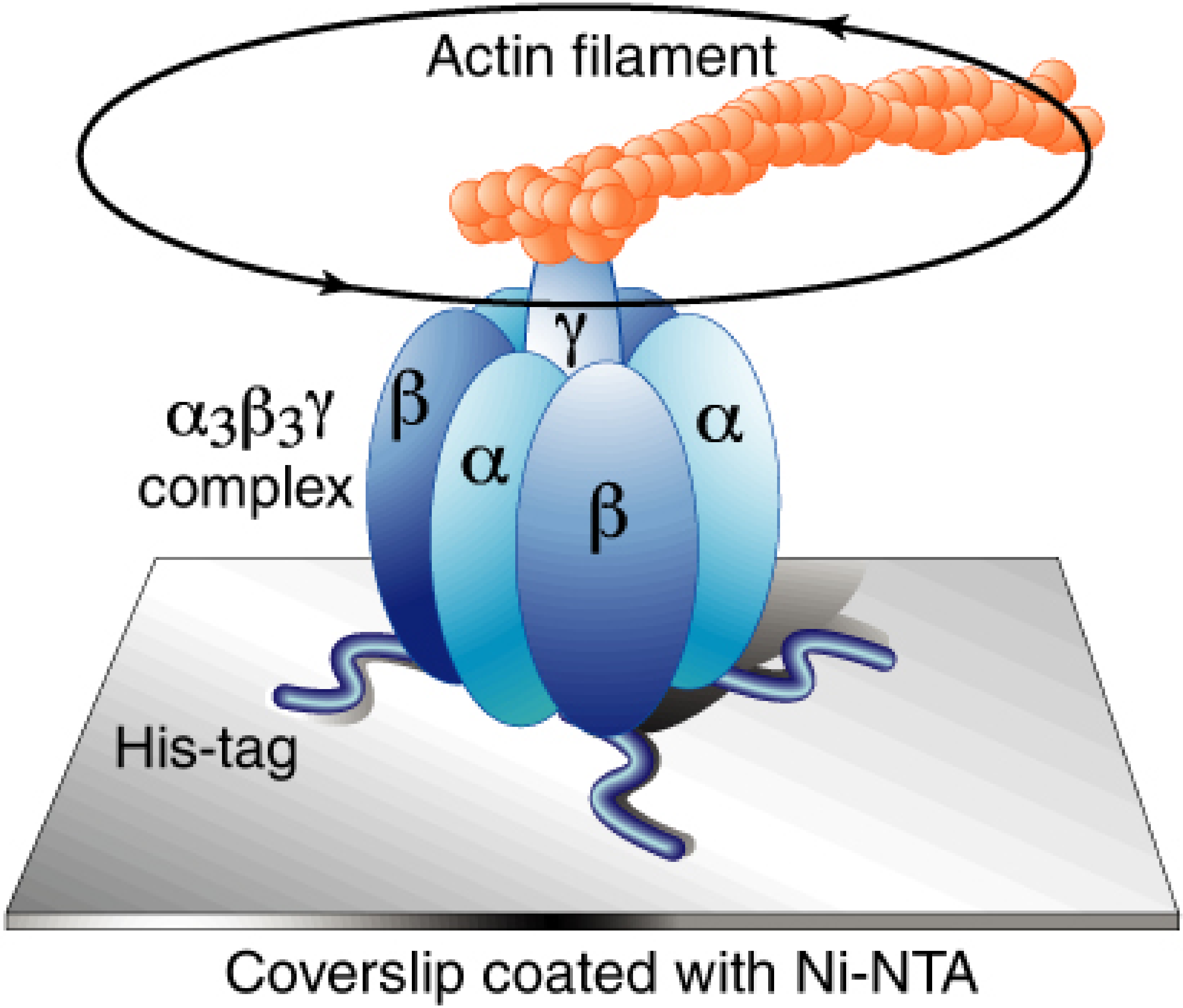


Main feature:

Translocation of $3H^+$ from the lumen to the stroma leading to the conversion of one ADP to one ATP


δ
β
α
β
α
b2
γ
ε
−
C12
a
+


F1
Fo
α
β
α
β
α
β
γ
c
c
c
c
H+



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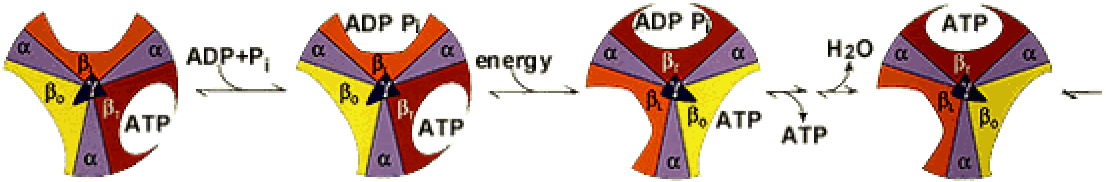
Paul D. Boyer and **John E. Walker** have shown how the enzyme ATP synthase makes ATP. ATP synthase is found in chloroplast and mitochondrial membranes and in the cytoplasmic membrane of bacteria. A difference in hydrogen ion concentration across the membrane drives the enzyme to synthesise ATP.

"The Binding Change Mechanism"

Using chemical methods Paul Boyer proposed that ATP synthase is like a cylinder with alternating alpha and beta subunits. An asymmetrical gamma subunit in the middle of the cylinder causes changes in the structure of the beta subunits when it rotates (100 r.p.s.). He termed these structures open (beta$_o$), loose (beta$_L$) and tight (beta$_T$).

Four stages in ATP synthesis

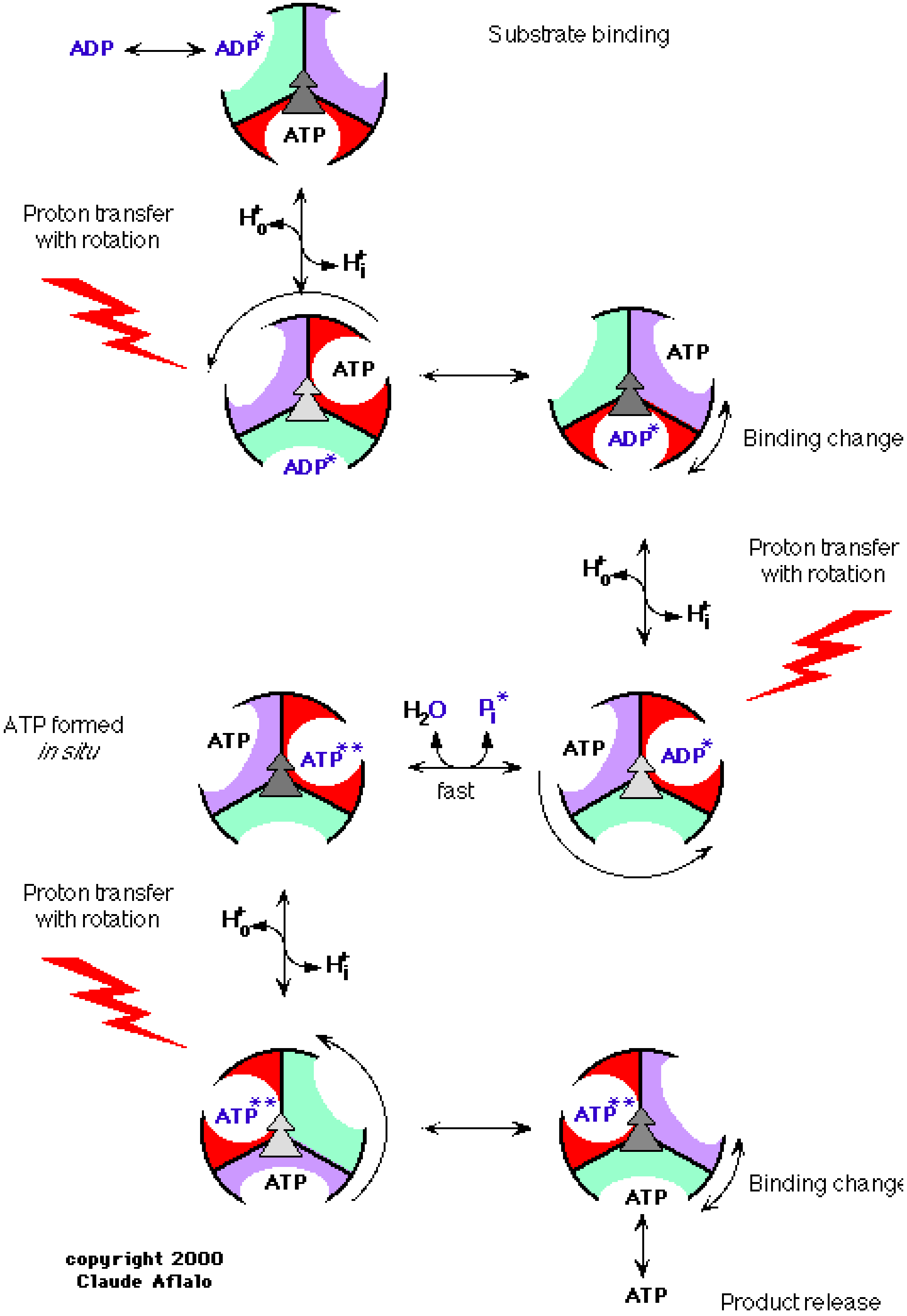


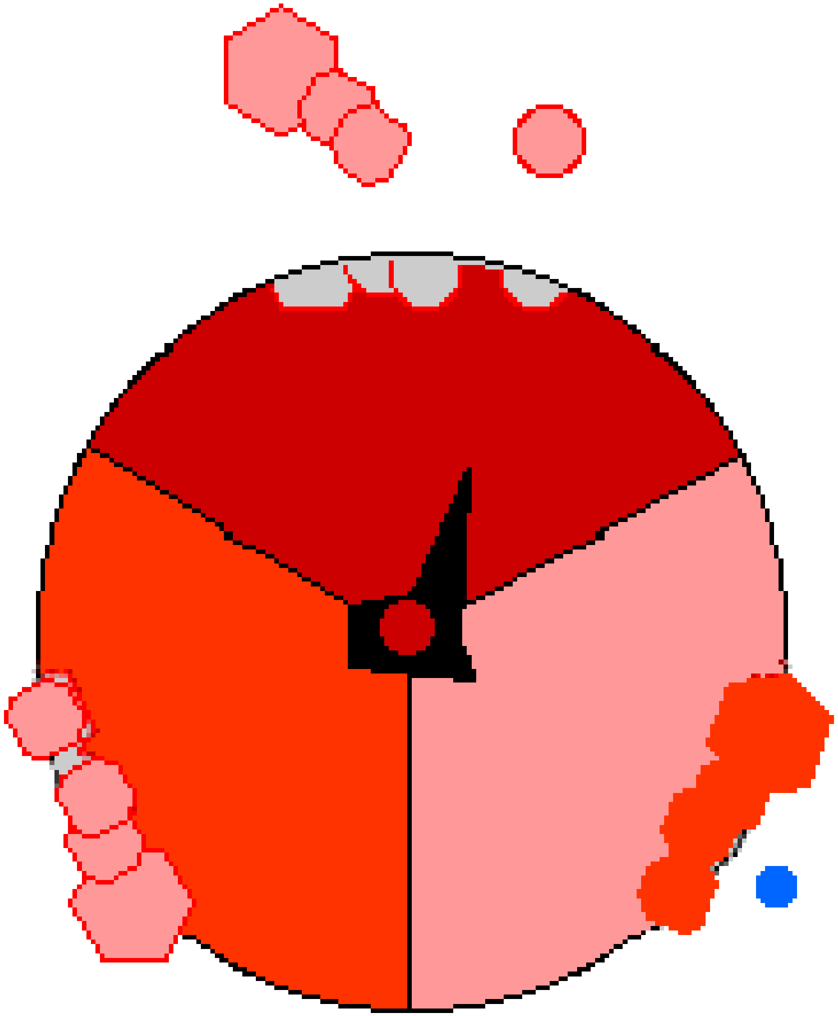
A complete ATP molecule is bound to β_T.

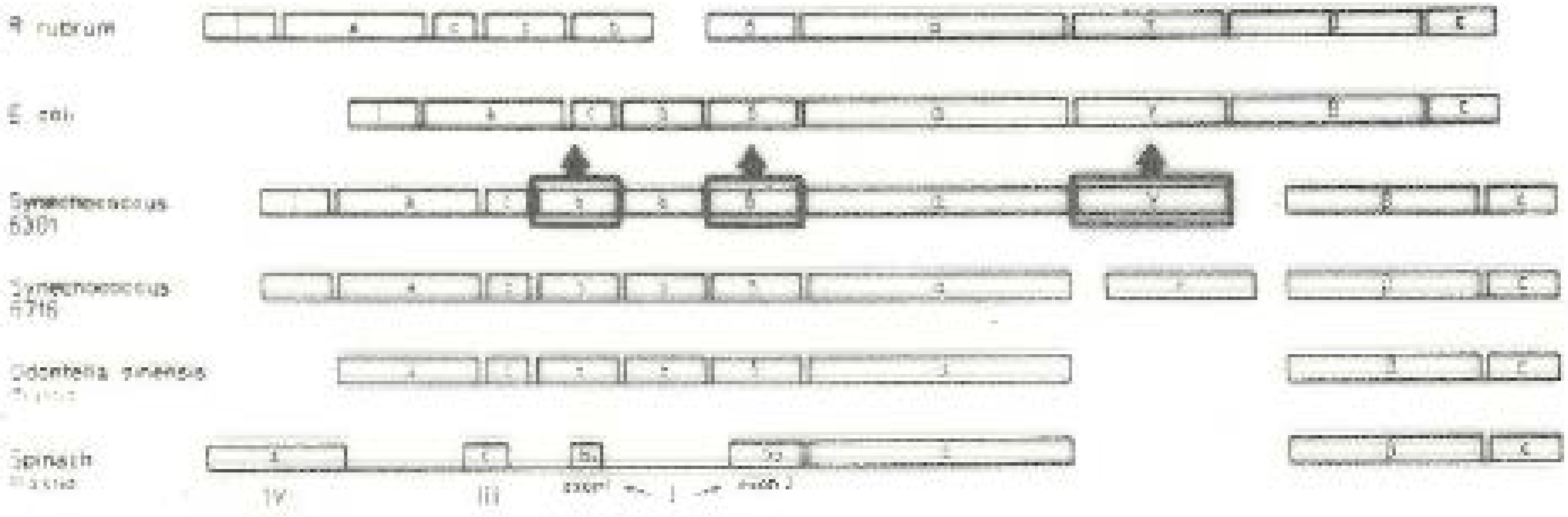
β_L binds ADP and inorganic phosphate (P_i).

The γ subunit has rotated. β_T becomes open and ATP is released. β_L becomes tight and β_O becomes loose.

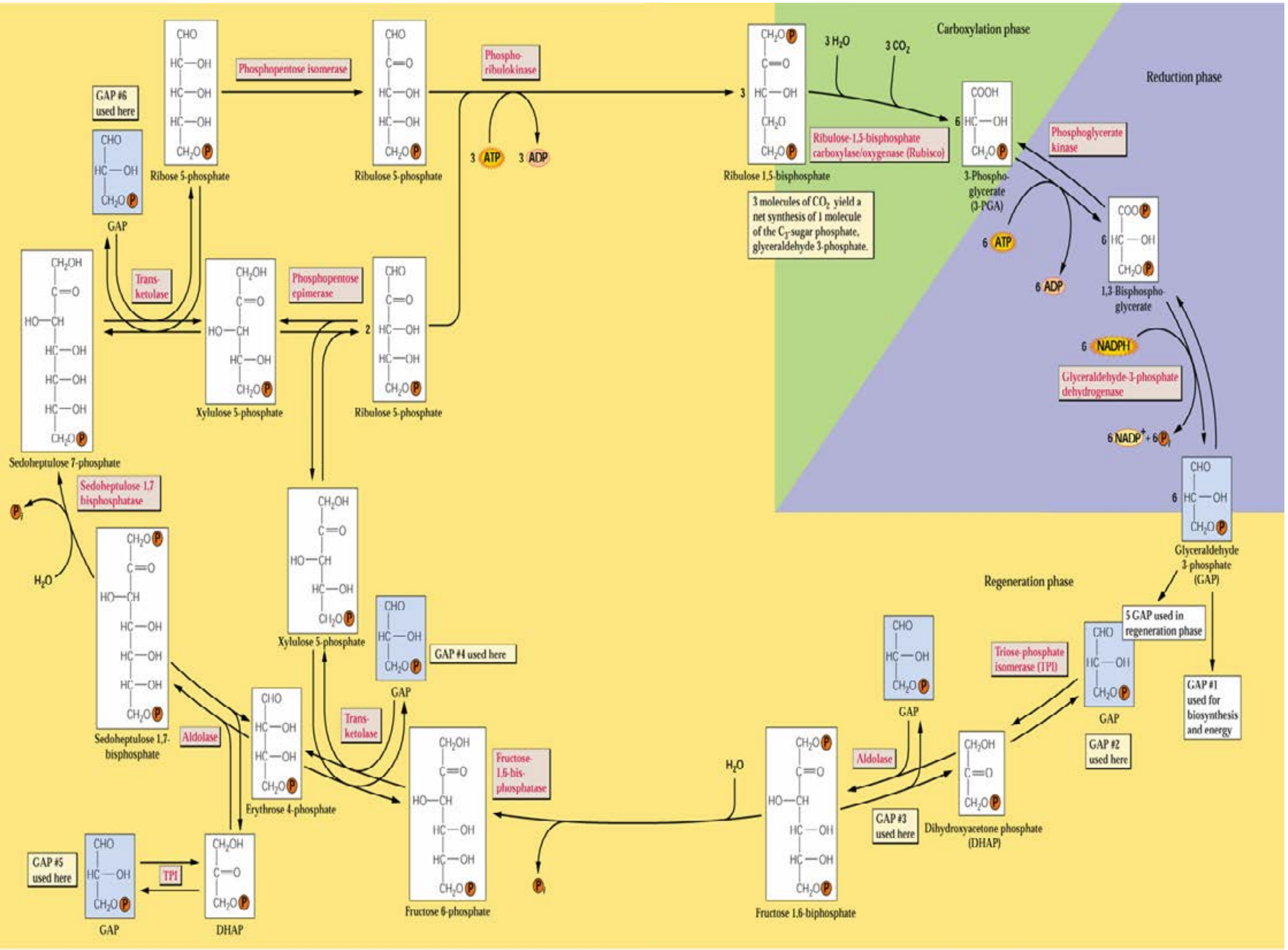
The phosphate ion reacts with the ADP molecule so that a new ATP molecule is formed. We are back to the first stage.


ADP
ADP*
ATP
Substrate binding
Proton transfer with rotation
H_o^+
H_i^+
ATP
ADP*
ATP
ADP*
Binding change
H_o^+
H_i^+
Proton transfer with rotation
ATP formed *in situ*
ATP
ATP**
H_2O
P_i^*
fast
ATP
ADP*
Proton transfer with rotation
H_o^+
H_i^+
ATP**
ATP
ATP**
ATP
Binding change
ATP
Product release



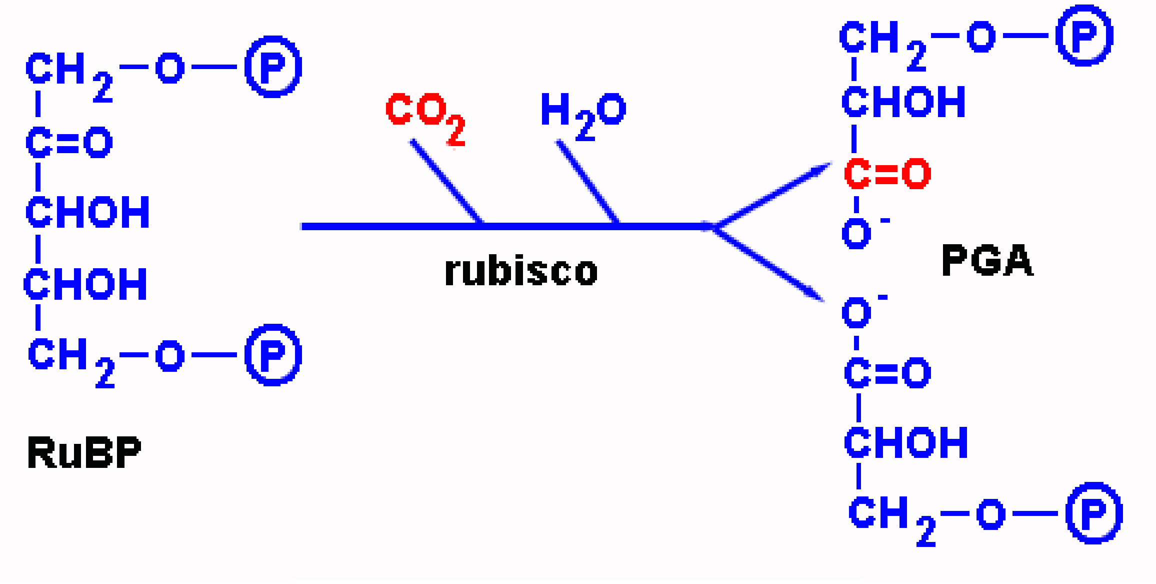

R. rubrum
E. coli
Synechococcus 6301
Synechococcus 6716
Odontella sinensis Plastid
Spinach Plastid
A
C
ε
IV
III

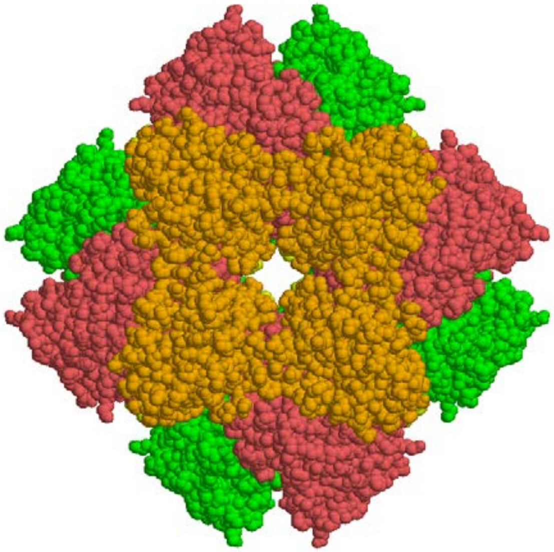
3. Dark reaction: C3 photosynthesis and photorespiration



3 molecules of CO_2 yield a net synthesis of 1 molecule of the C_3-sugar phosphate, glyceraldehyde 3-phosphate.

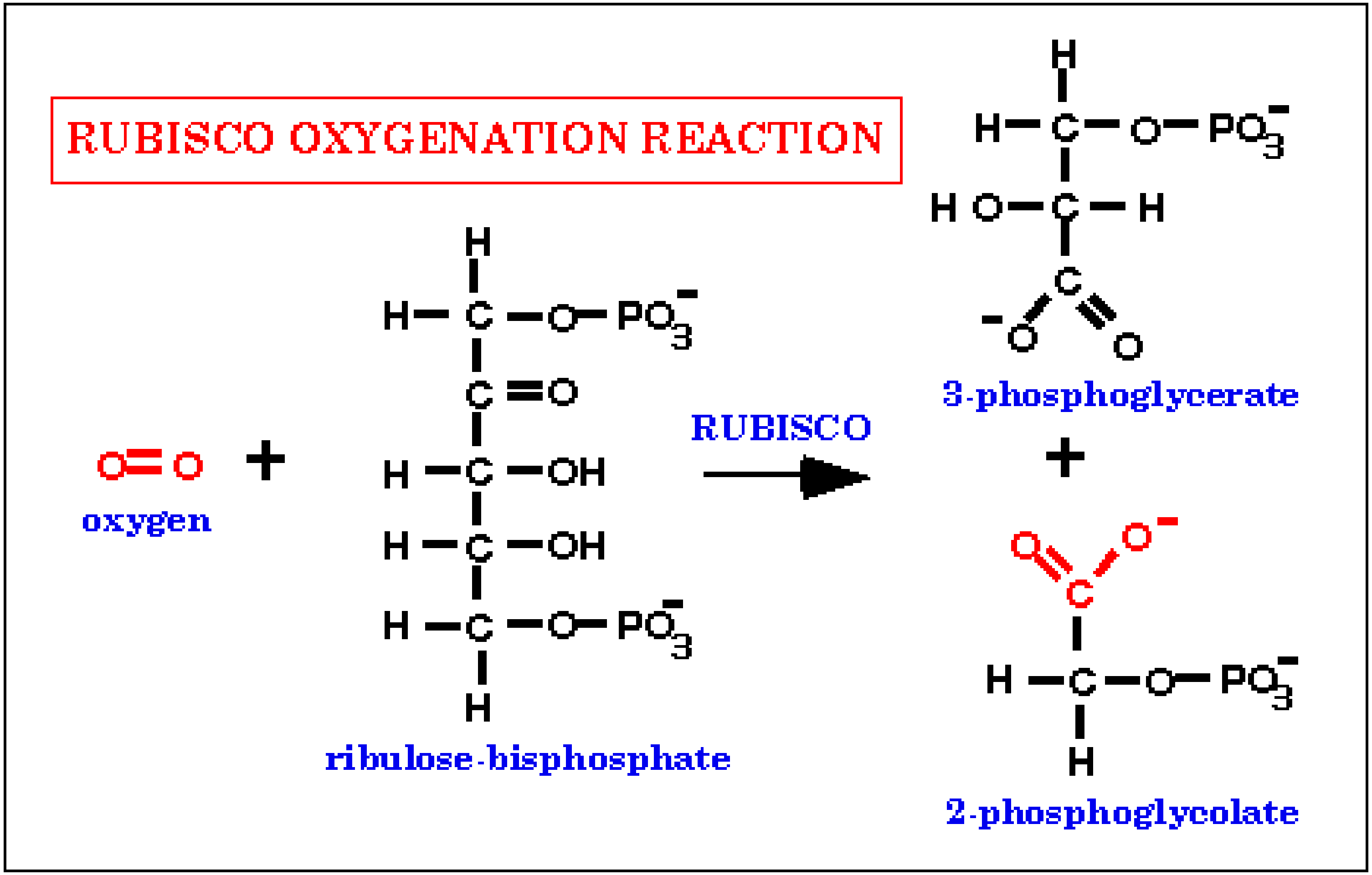
Ribulose-1,5-bisphosphate-carboxylase fixes CO_2





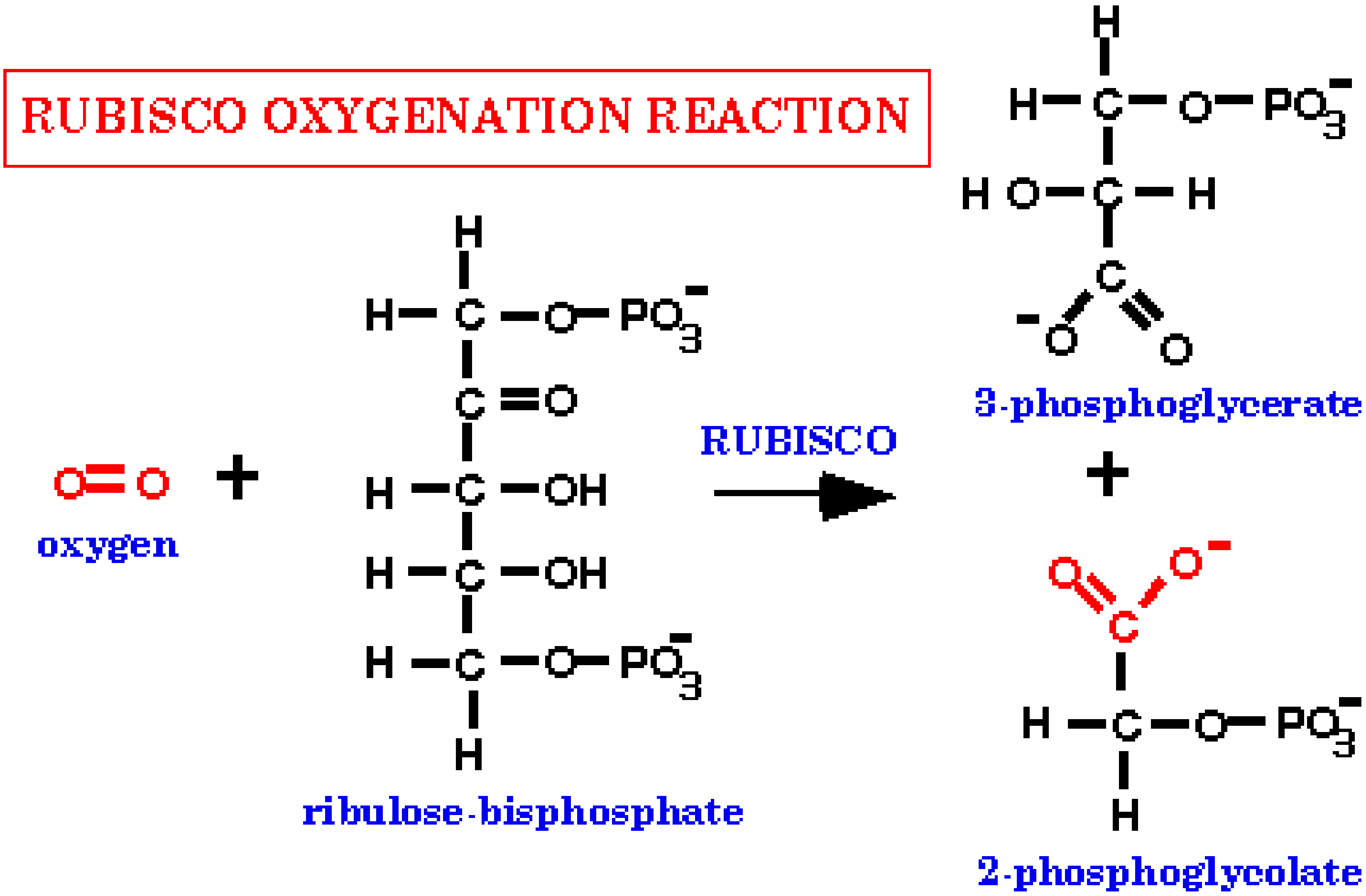
Bifunctionality of Rubisco

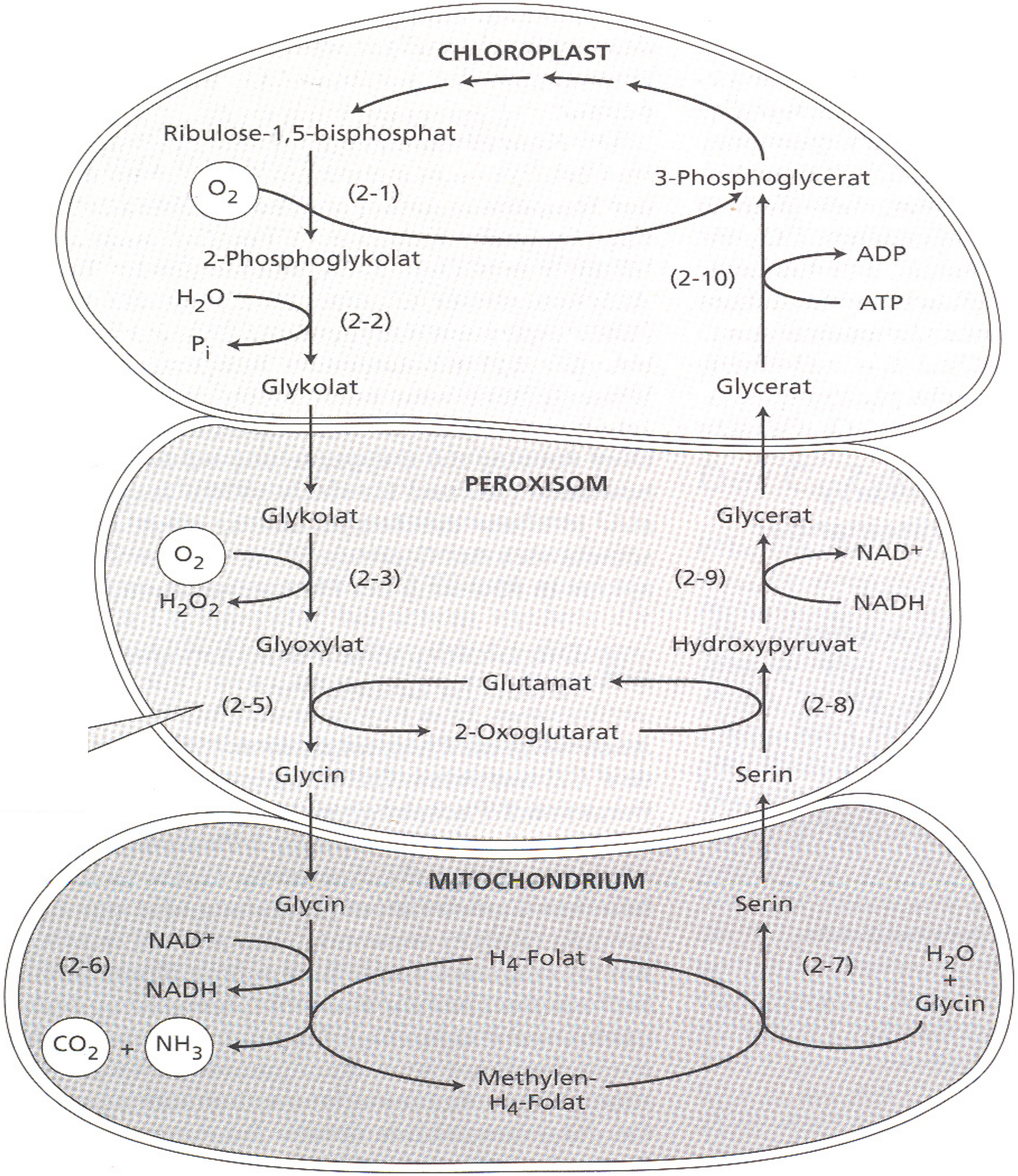
- binding O_2 instead of CO_2
- photorespiration



Photorespiration

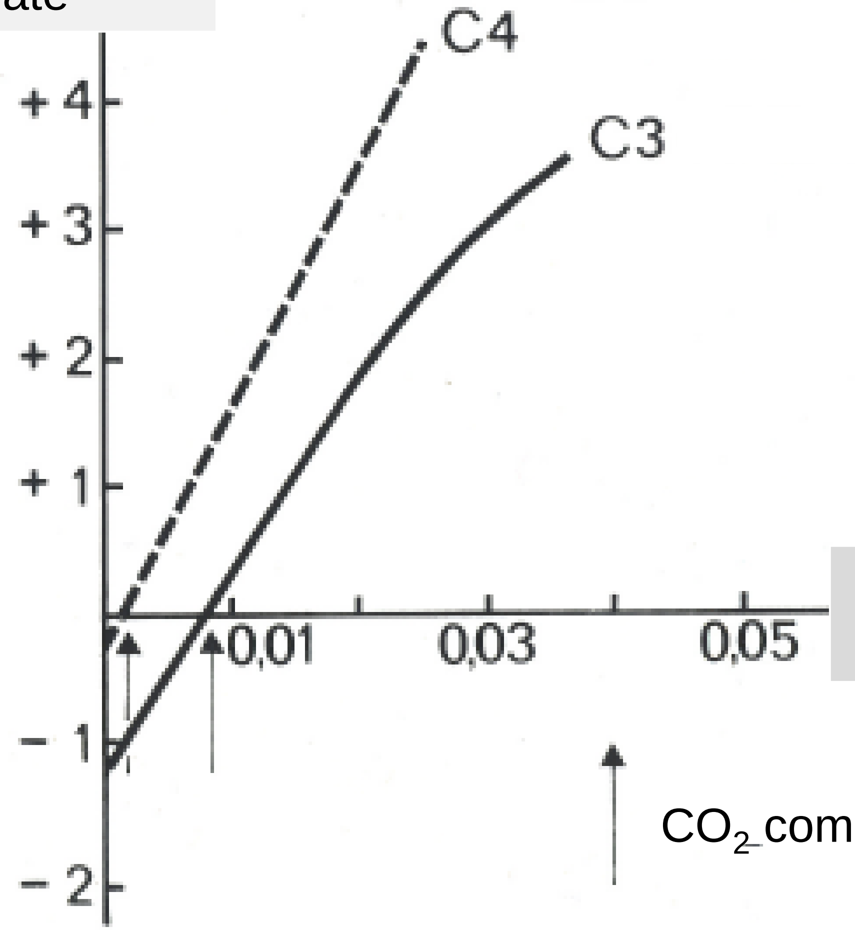
RUBISCO OXYGENATION REACTION




CHLOROPLAST
Ribulose-1,5-bisphosphat
O_2
(2-1)
3-Phosphoglycerat
2-Phosphoglykolat
H_2O
(2-2)
P_i
(2-10)
ADP
ATP
Glykolat
Glycerat
PEROXISOM
Glykolat
Glycerat
O_2
(2-3)
H_2O_2
(2-9)
NAD^+
NADH
Glyoxylat
Hydroxypyruvat
Glutamat
(2-5)
(2-8)
2-Oxoglutarat
Glycin
Serin
MITOCHONDRIUM
Glycin
Serin
NAD^+
(2-6)
NADH
H_4-Folat
(2-7)
H_2O + Glycin
CO_2 + NH_3
Methylen-H_4-Folat

4. C4 photosynthesis & CAM plants

photosynthesis rate



CO$_2$ concentration [%, v/v]

CO$_2$ compensation point

- High light intensity ’ high photorespiration, stomata closure

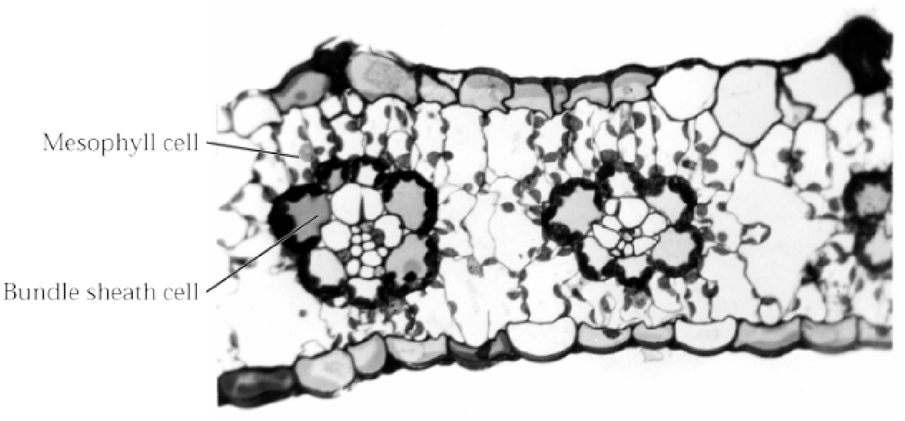
- C4 plants:
  more efficient CO_2 fixation by phosphoenolpyruvate (PEP) carboxylase

- Polyphyletic origin, convergent development

- C4 *vs*.Crassulaceae acid metabolism (CAM) plants:
  samle biochemistry, different morphology and CO_2 fixation

- Examples for C4 plants: maize, sugar cane, *Sorghum*, Chenopodiaceae, Euphorbiacecae

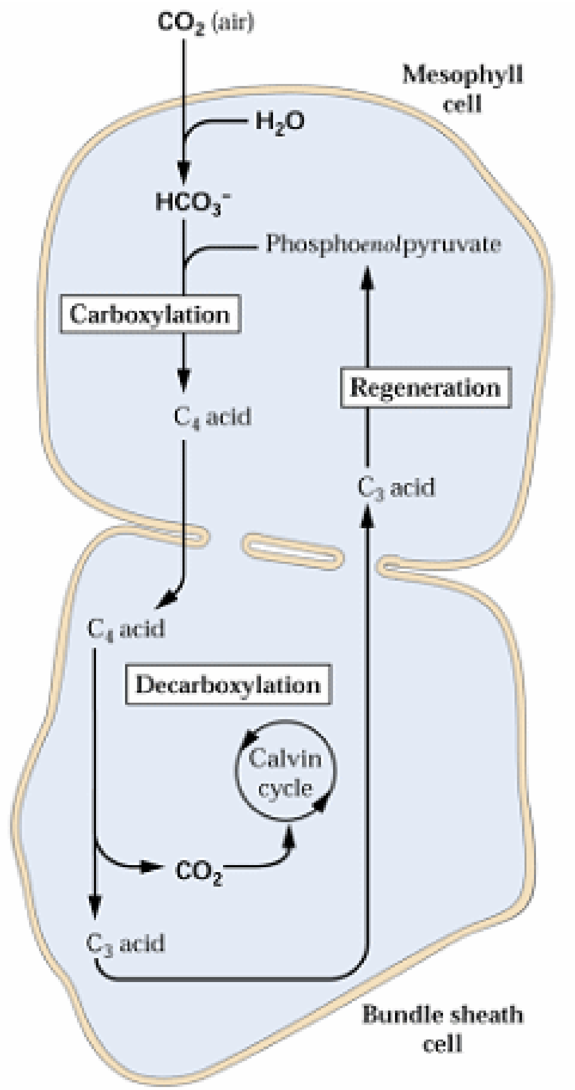
- Examples for CAM plants: succulent plants of Crassulacecae, Cactaceae,
  Compositae, Euphorbiaceae



Mesophyll cell:
Normal chloroplasts with grana

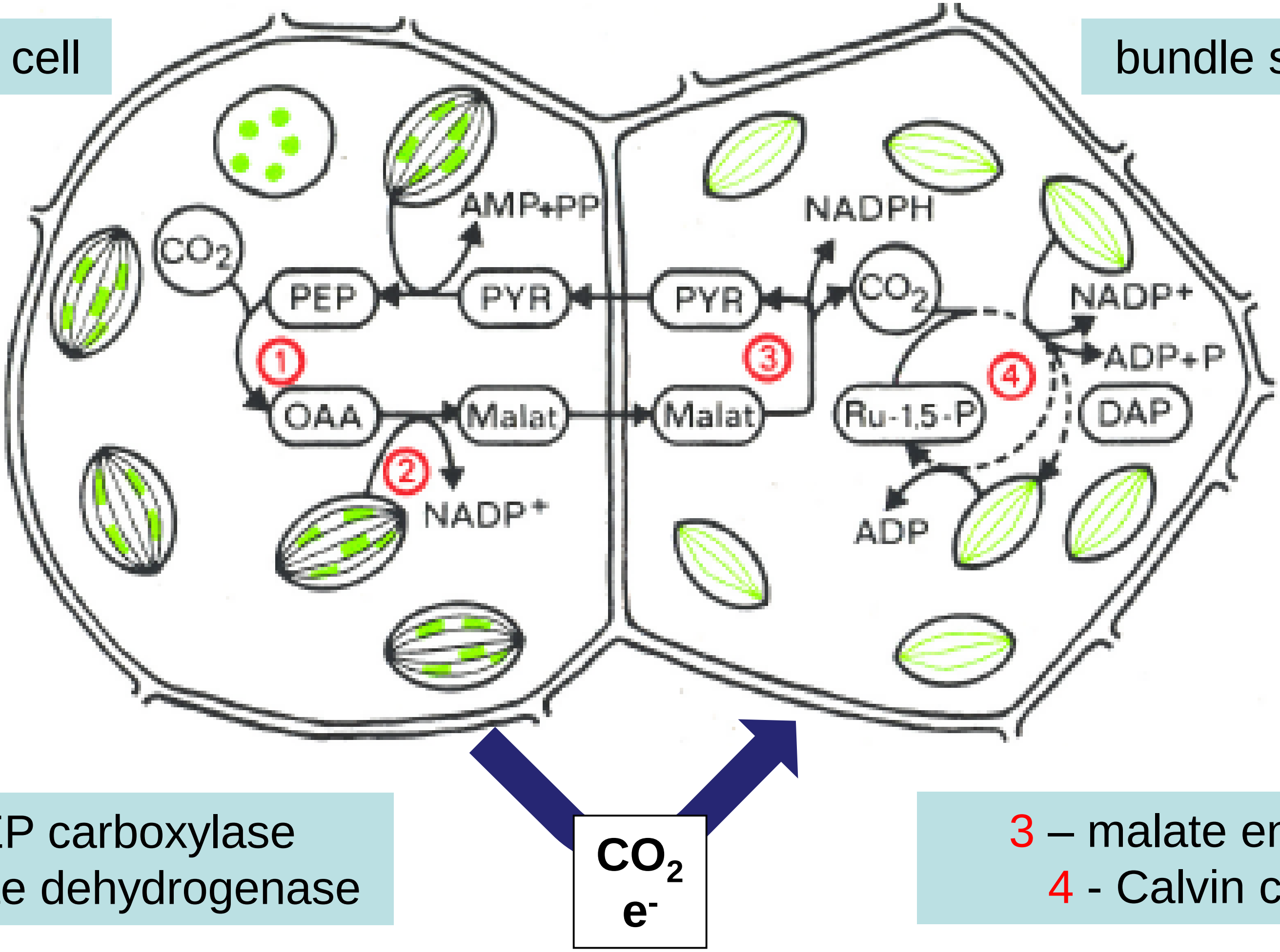
Chloroplast dimorphism

Bundle sheath cell:
Starch-rich plastids without grana (no PS II)


CO2 (air)
Mesophyll cell
H2O
HCO3-
Phosphoenolpyruvate
Carboxylation
Regeneration
C4 acid
C3 acid
C4 acid
Decarboxylation
Calvin cycle
CO2
C3 acid
Bundle sheath cell

mesophyll cell

bundle sheath cell

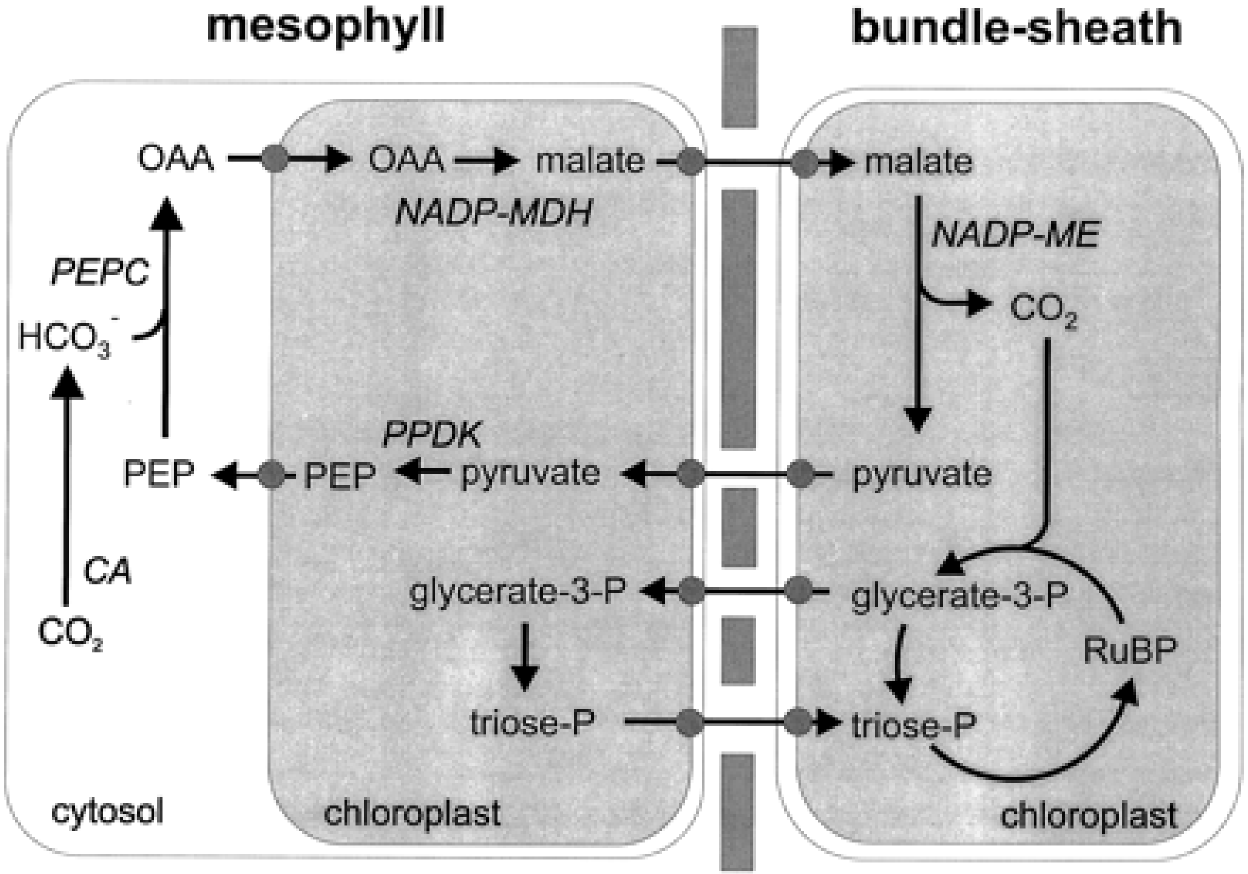


CO_2
e^-

1 - PEP carboxylase
2 – malate dehydrogenase

3 – malate enzyme
4 - Calvin cycle

Biochemistry in different cellular compartments



Summary C4 photosynthesis

- Spacial separation of CO_2 fixation and carbohydrate biosynthesis

Mesophyll cells:

- PEP carboxylase high affinity to CO_2, no O_2 fixation
- More efficient CO_2 fixation in spite of closed stomata than C3 plants
- First C fixation product: C4 compound oxalacetate
- Reduction of oxalacetate to malate in plastids of mesophyll cells requires NADPH from photosynthesis
- Malate is transported to bundle sheath cells via plasmodesmata

Bundle sheath cells:

- Oxidation of malate to pyruvate and CO_2 generates NAPDH
- ’ chloroplast dimorphism

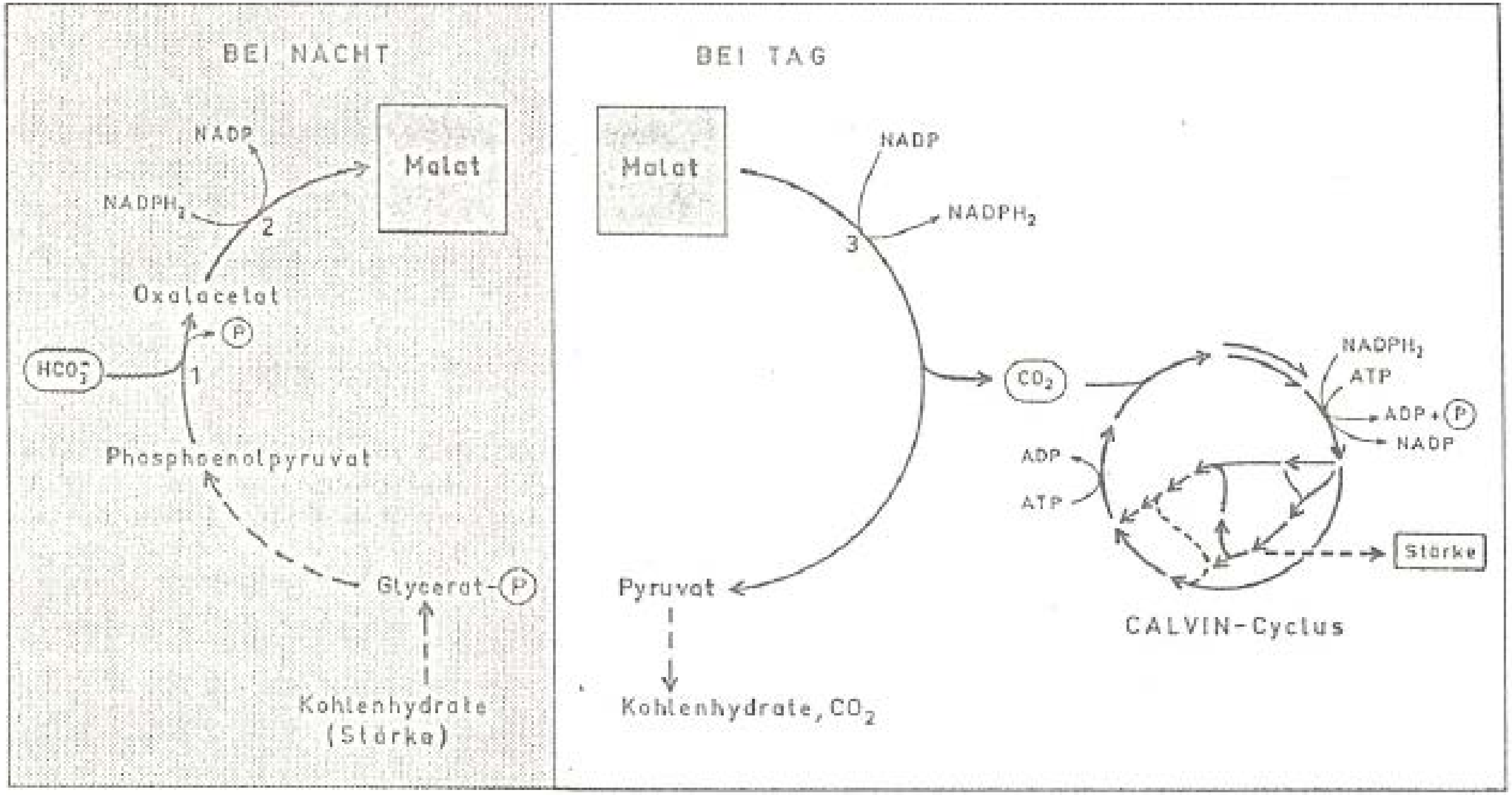
CAM plants

Temporal separation of CO_2 fixation and carbohydrate biosynthesis

Night: stomata open: CO_2 fixation

Day: stomata closed: carbohydrate biosynthesis and photosynthetic light reactions

Storage of CO_2 fixation product: malate in vacuole (acidification during night)


BEI NACHT
NADP
NADPH$_2$
Malat
2
Oxalacetat
Ⓟ
HCO_3^-
1
Phosphoenolpyruvat
Glycerat-Ⓟ
Kohlenhydrate
(Stärke)
BEI TAG
Malat
NADP
NADPH$_2$
3
CO_2
NADPH$_2$
ATP
ADP + Ⓟ
NADP
ADP
ATP
Stärke
Pyruvat
Kohlenhydrate, CO_2
CALVIN-Cyclus

Evolution of PEP carboxylase

A PEP carboxylase gene (*PEPC*) is present in many eukaryotic cells, but the gene product does not play an important role in the metabolism.

PEPC gene

1- Stronger promoter: higher expression level
2- Tissue- specific cis-elements in promoter: expression in mesophyll cells

PEPC enzyme

3- Optimization of CO_2 binding side: higher affinity to CO_2

5. N and S metabolism

NADPH and reduced ferredoxin from light reaction is also used to reduce NO_2^- and SO_4^{2-}.

Nitrate reduction

$$NO_3^- \xrightarrow{NR} NO_2^- \xrightarrow{NiR} NH_4^+$$

Sulfate reduction

$$SO_4^{2-} \xrightarrow{SR} SO_3^{2-} \xrightarrow{SiR} SH^+$$

5. N and S metabolism

NADPH and reduced ferredoxin from light reaction is also used to reduce NO_2^- and SO_4^{2-}.

Nitrate reduction

$$NO_3^- \xrightarrow{NR} NO_2^- \xrightarrow{NiR} NH_4^+$$

cytosol

Sulfate reduction

$$SO_4^{2-} \xrightarrow{SR} SO_3^{2-} \xrightarrow{SiR} SH^+$$

5. N and S metabolism

NADPH and reduced ferredoxin from light reaction is also used to reduce NO_2^- and SO_4^{2-}.

Nitrate reduction

$$NO_3^- \xrightarrow{NR} \boxed{NO_2^- \xrightarrow{NiR} NH_4^+}$$

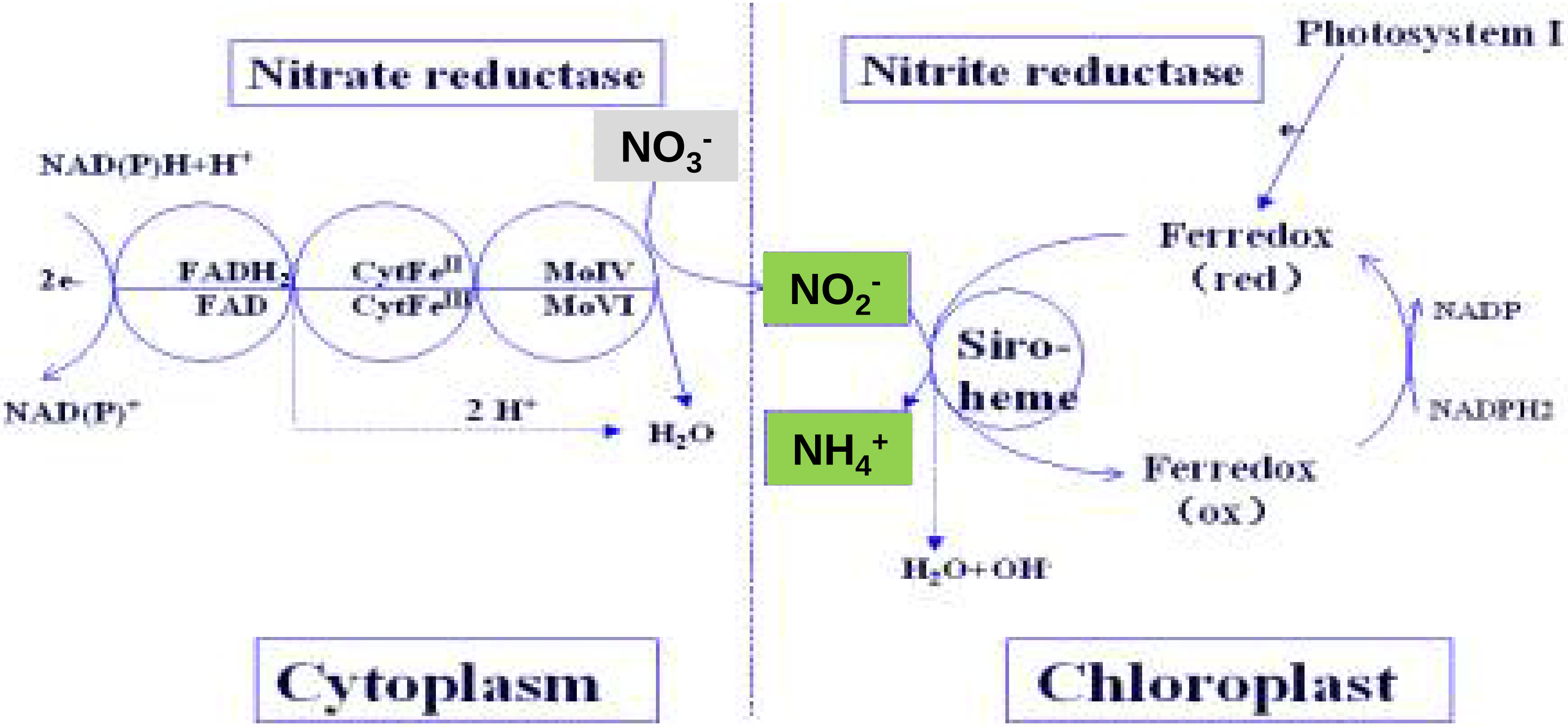
plastid

Sulfate reduction

$$\boxed{SO_4^{2-} \xrightarrow{SR} SO_3^{2-} \xrightarrow{SiR} SH^+}$$

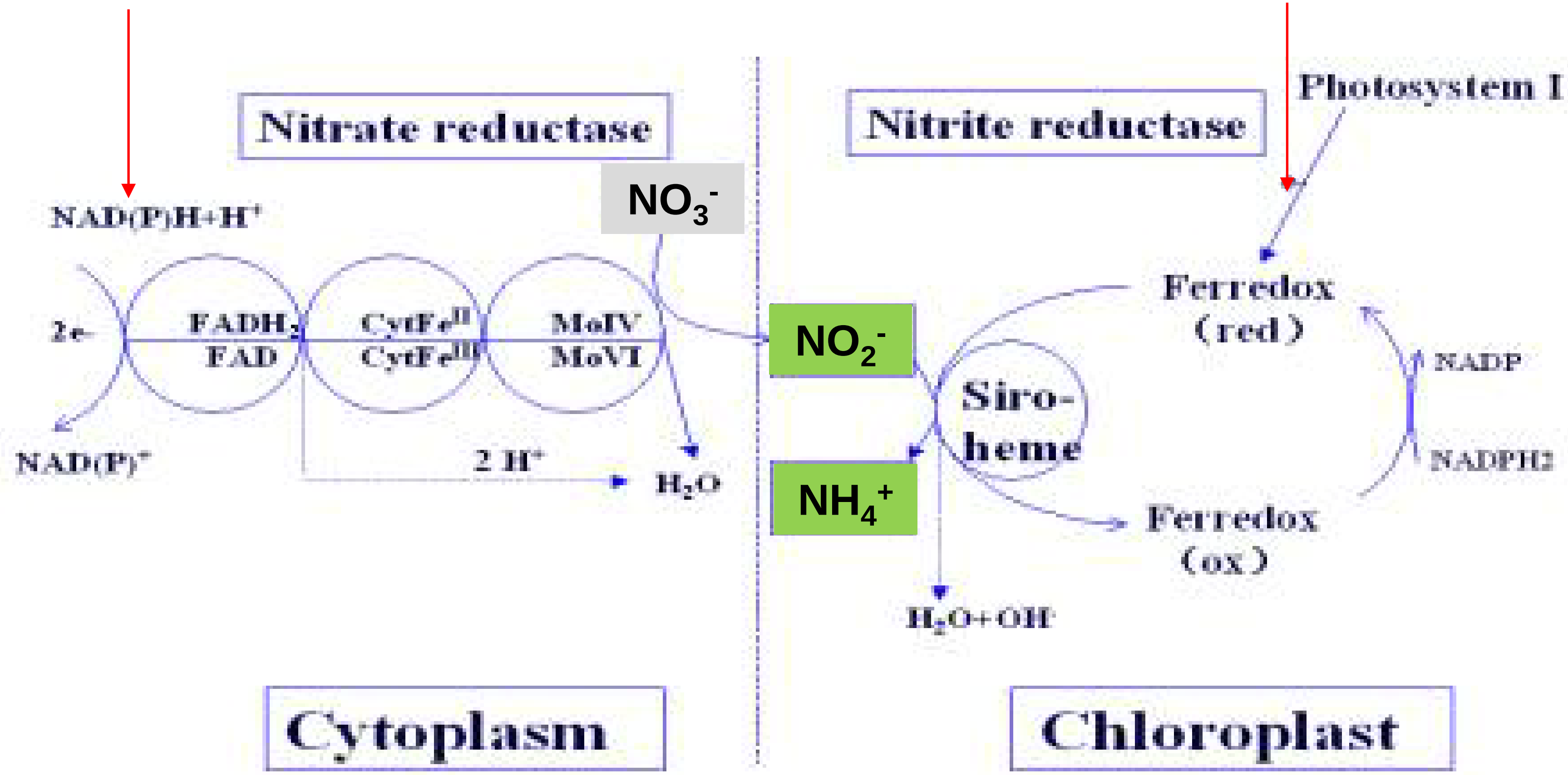
plastid

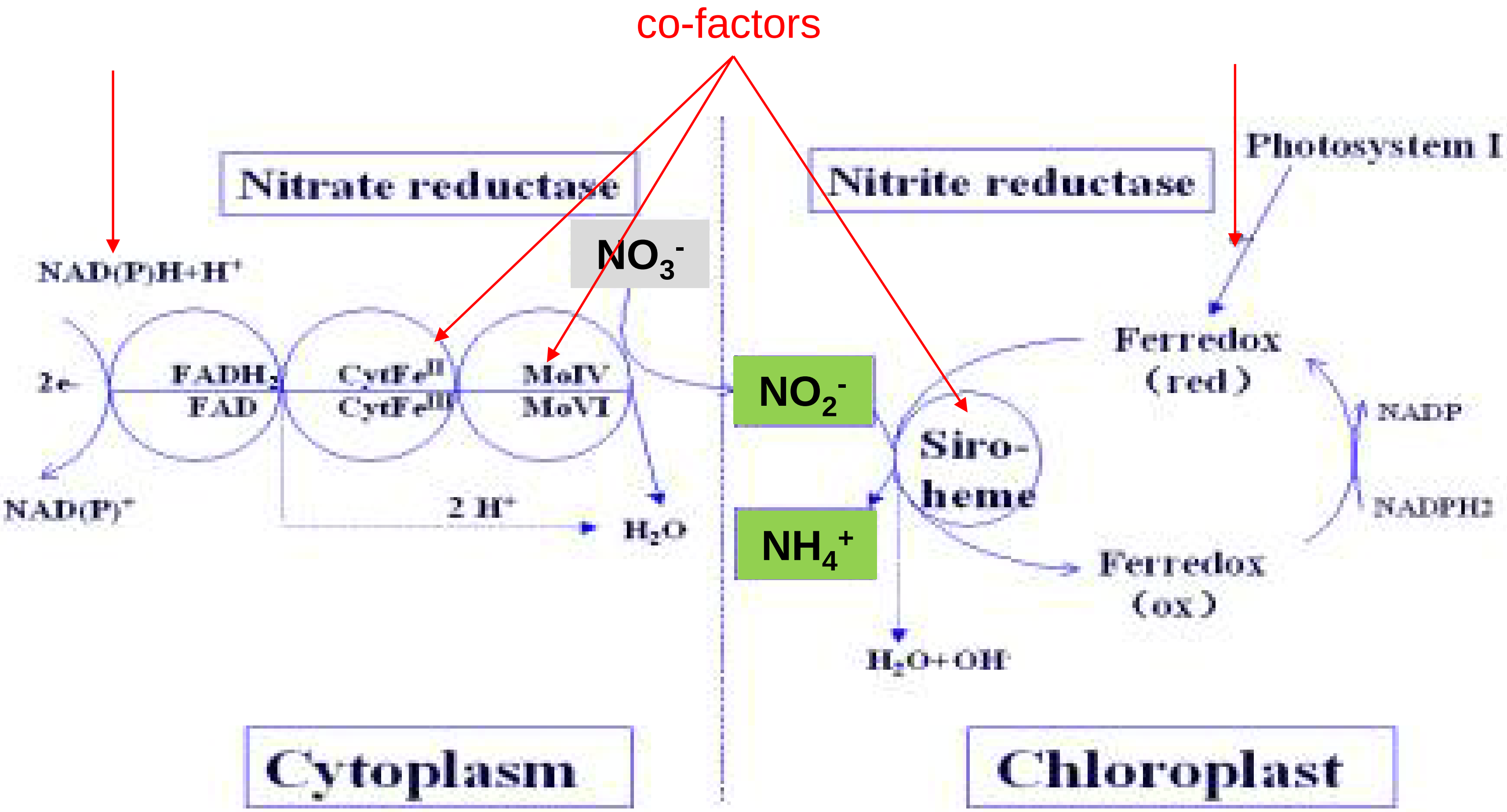
Nitrate assimilation



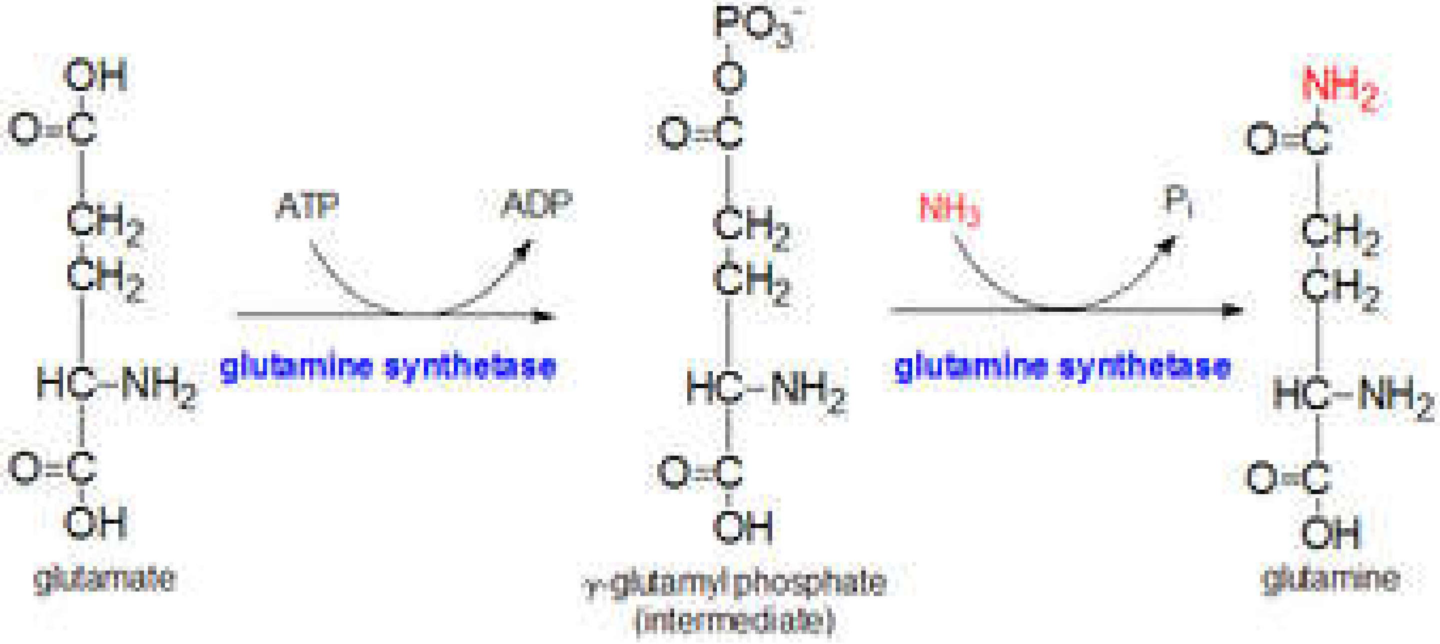
cytoplasmic energy source

photosynthesis

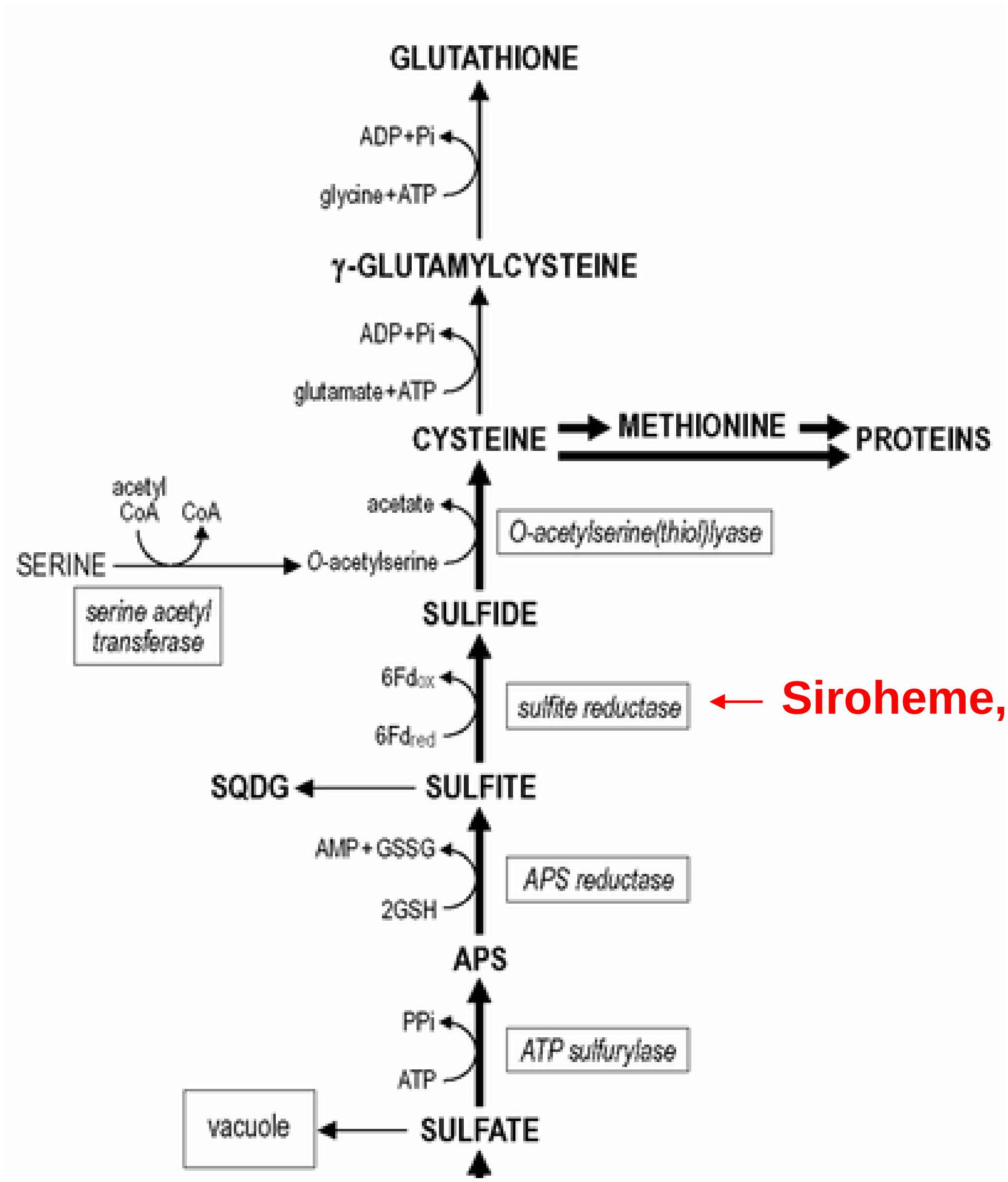



co-factors
Nitrate reductase
Nitrite reductase
Photosystem I
NO_3^-
$NAD(P)H+H^+$
2e-
$FADH_2$
FAD
$CytFe^{II}$
$CytFe^{III}$
MoIV
MoVI
$NAD(P)^+$
$2 H^+$
H_2O
NO_2^-
NH_4^+
Siro-
heme
Ferredox (red)
Ferredox (ox)
NADP
NADPH2
H_2O+OH^-
Cytoplasm
Chloroplast

Ammonium is toxic and rapidly metabolized

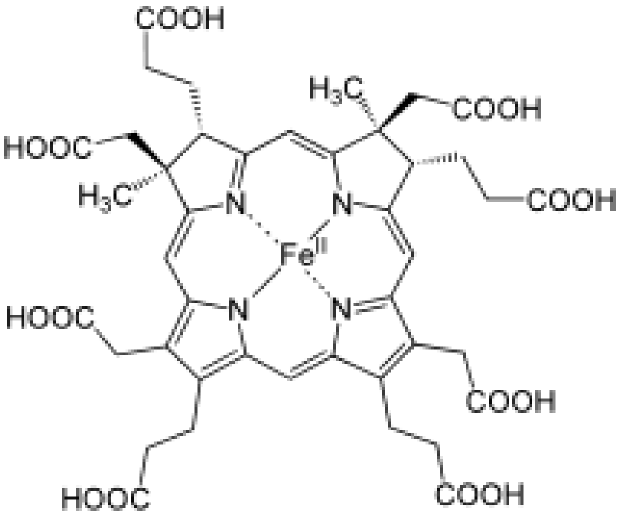


Sulfate assimilation



← **Siroheme, e^- from ferredoxin**

Siroheme



Life on earth is dependent on sulphur (S) and nitrogen (N). In plants, the second step in the reduction of sulphate and nitrate are mediated by the enzymes sulphite and nitrite reductases, which contain the iron (Fe)-containing siroheme as a cofactor. It is synthesized from the tetrapyrrole primogenitor uroporphyrinogen III in the plastids via three enzymatic reactions, methylation, oxidation and ferrochelatation. **Without siroheme biosynthesis, there would be no life on earth.**

Baishnab C Tripathy, Irena Sherameti, and Ralf Oelmüller (2010) Siroheme. An essential component for life on earth
Plant Signal Behav. 2010 Jan; 5(1): 14–20.

6. Plastid gene expression

Plastids contain DNA – plastome

- **Maternal inheritance**

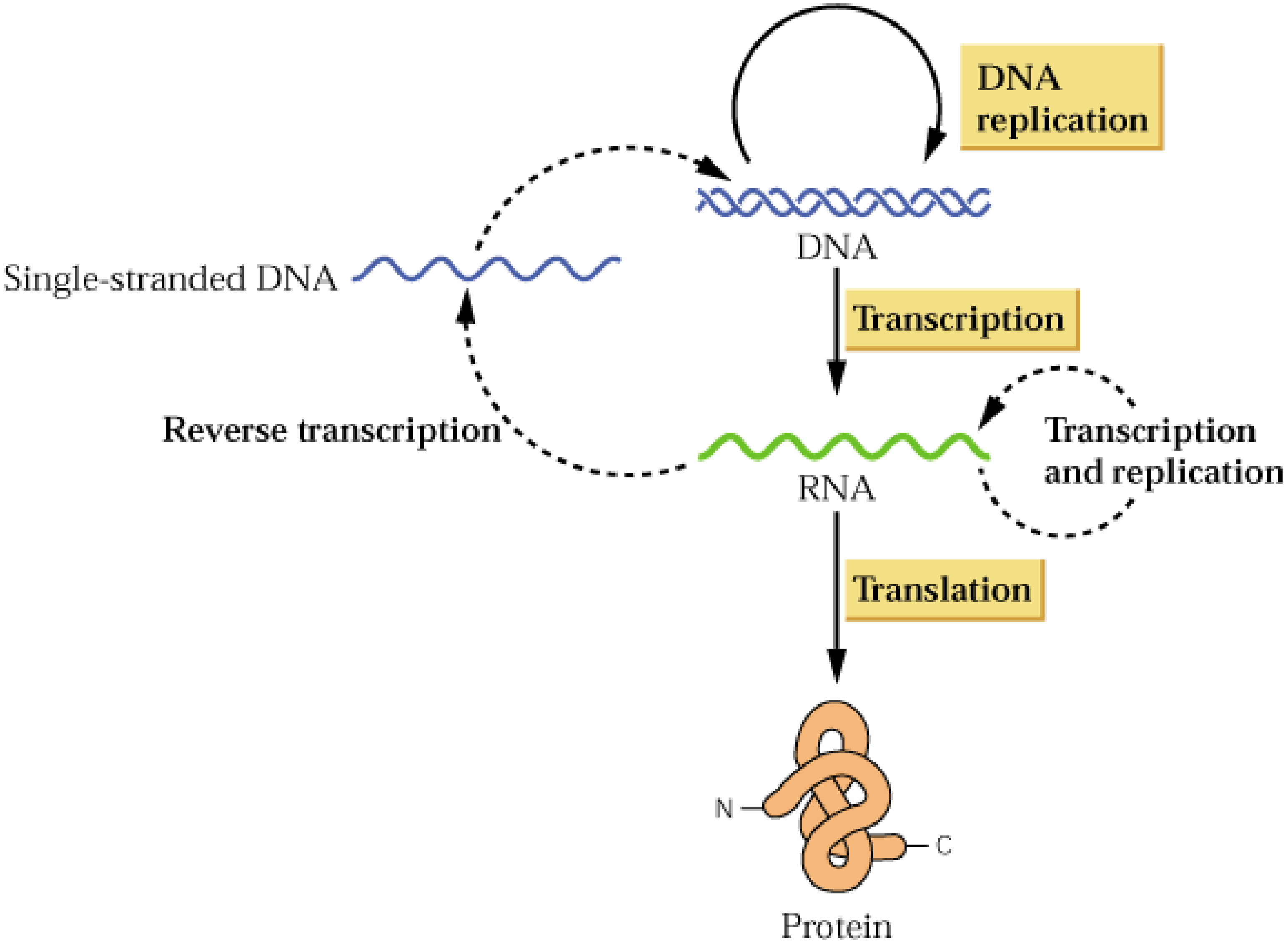
  (advantage for biotechnological application)
  (*Mirabilis japonica*, Correns 1909)

- **100 x 100 plastoms/cell**

- **prokaryotic origin, procaryotic genes and expression**

- **gene transfer to the nucleus**

Gene expression in plastids is procaryotic



Inheritance in plastids

- *Pelargonium*:
  - biparental

- maternal (most of the angisperms)

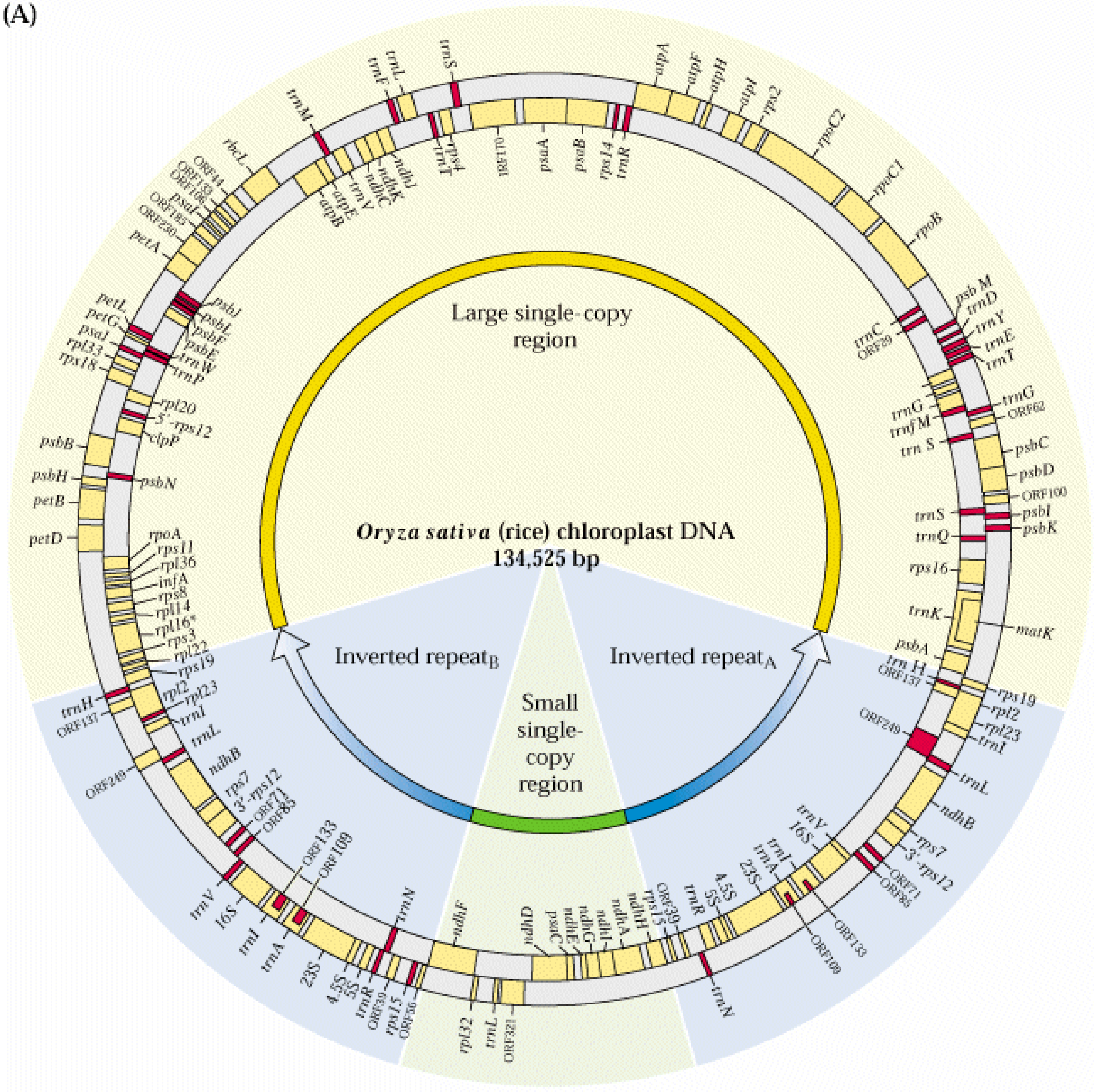
- paternal (gymnosperms, *Sequoia*, *Pinus*)

plastome

- DNA is attached to thylakoid membrane (nucleoid)
- 15 nucleoids/plastid, 10 DNA molecules/nucleotid (polyploid)
- circular DNA
- 130 bis 160 kb
- inverse duplication
- small and large single copy region
- loss of inverse duplication e.g. conifers, *Papilionaceae*

  Epiphagus

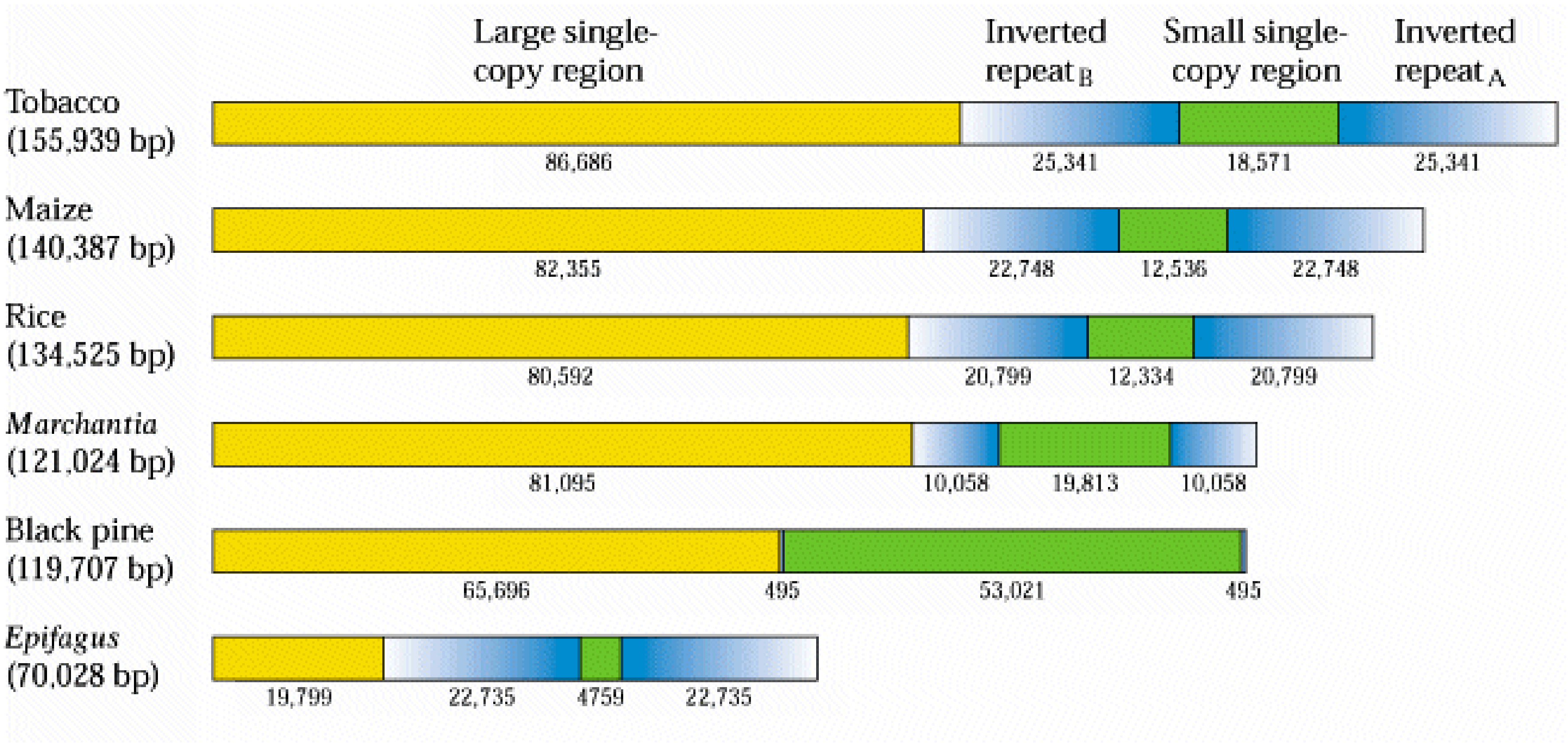
(A)


Large single-copy region
Oryza sativa (rice) chloroplast DNA
134,525 bp
Inverted repeat$_B$
Inverted repeat$_A$
Small single-copy region
trnS
trnL
trnF
trnM
rbcL
atpA
atpF
atpH
atpI
rps2
rpoC2
rpoC1
rpoB
psaA
psaB
rps14
trnR
trnT
ndhJ
ndhK
ndhC
trnV
trnE
psbM
trnD
trnY
trnE
trnT
trnC
ORF29
trnG
ORF62
trnG
trnfM
trn S
psbC
psbD
ORF100
trnS
psbI
psbK
trnQ
rps16
trnK
matK
psbA
trn H
ORF137
rps19
rpl2
rpl23
trnI
ORF249
trnL
ndhB
rps7
3'-rps12
ORF71
ORF85
trnV
16S
trnI
trnA
23S
4.5S
5S
trnR
ORF39
rps15
ndhH
ndhA
ndhI
ndhG
ndhE
psaC
ndhD
ORF133
ORF108
trnN
ndhF
rpl32
trnL
ORF321
ORF36
petA
psaI
ORF44
ORF133
ORF106
ORF185
ORF230
petL
petG
psaJ
rpl33
rps18
psbJ
psbL
psbF
psbE
trnW
trnP
rpl20
5'-rps12
clpP
psbB
psbH
psbN
petB
petD
rpoA
rps11
rpl36
infA
rps8
rpl14
rpl16
rps3
rpl22
rps19
rpl2
rpl23
trnI
trnH
ORF137
trnL
ORF249
ndhB
rps7
3'-rps12
ORF71
ORF85
ORF133
ORF108
trnV
16S
trnI
trnA
23S
4.5S
5S
trnR
ORF39
rps15
ORF36
trnN

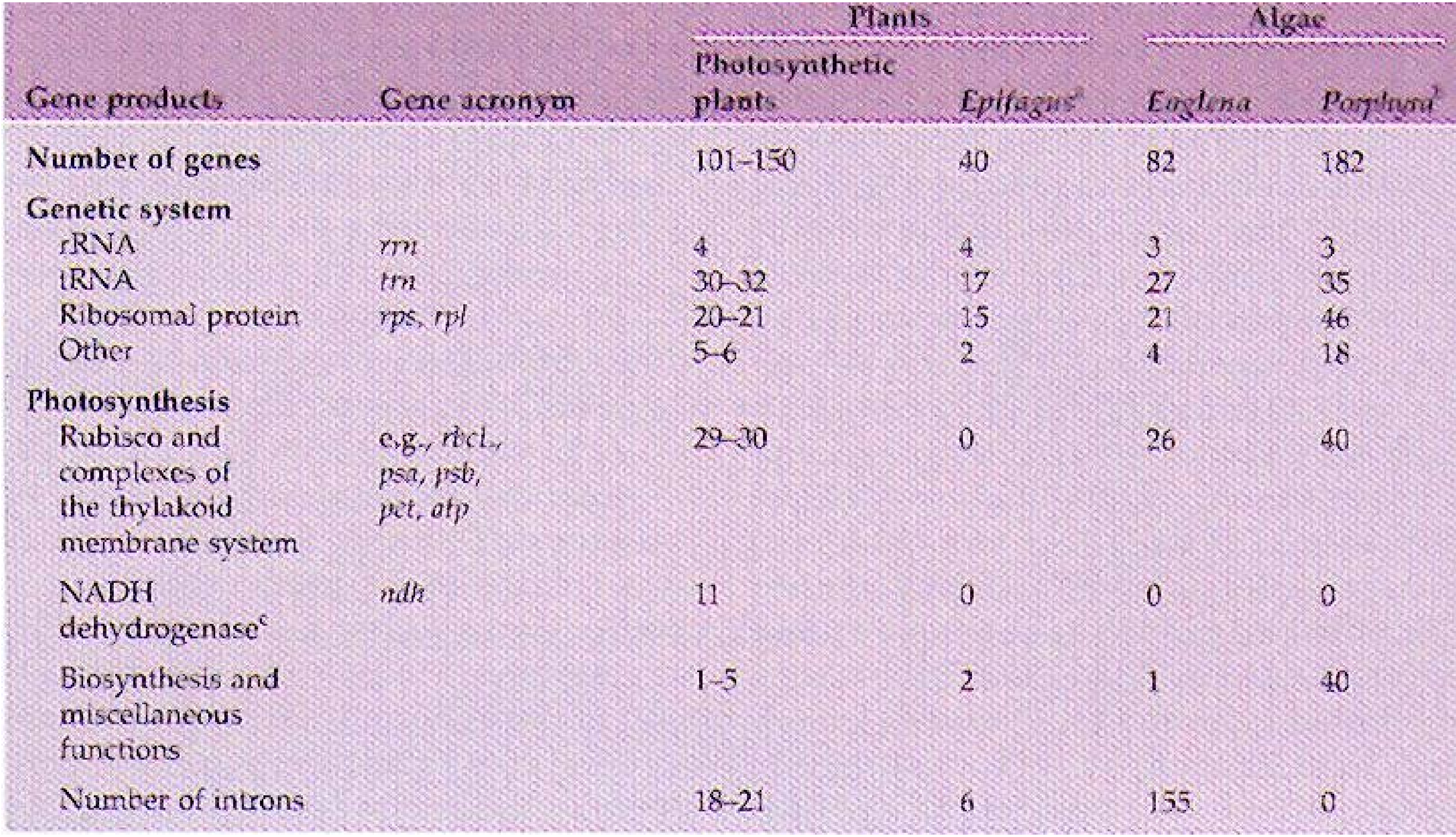
The plastome of the holoparasite *Epifagus virginiana* is substantially reduced

Model system for plastid genetics

Plastomes of land plants

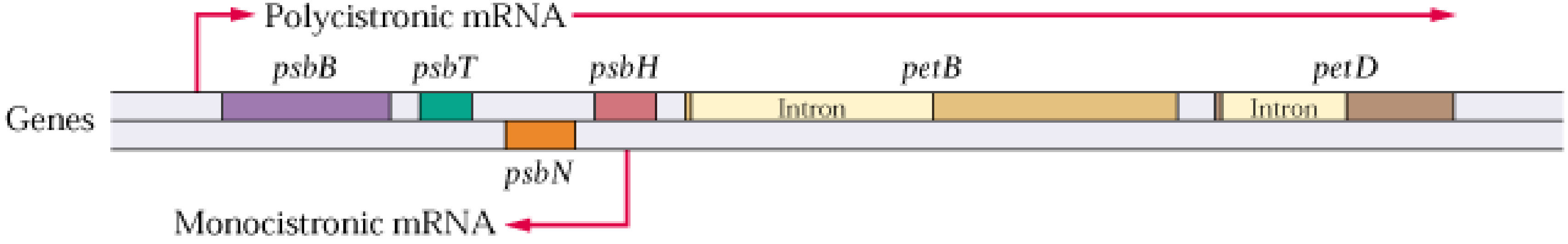


Genes of the plastome



Gene products	Gene acronym	Plants		Algae	
		Photosynthetic plants	*Epifagus*[a]	*Euglena*	*Porphyra*[b]
Number of genes		101–150	40	82	182
Genetic system					
rRNA	*rrn*	4	4	3	3
tRNA	*trn*	30–32	17	27	35
Ribosomal protein	*rps, rpl*	20–21	15	21	46
Other		5–6	2	4	18
Photosynthesis					
Rubisco and complexes of the thylakoid membrane system	e.g., *rbcL*, *psa, psb, pet, atp*	29–30	0	26	40
NADH dehydrogenase[c]	*ndh*	11	0	0	0
Biosynthesis and miscellaneous functions		1–5	2	1	40
Number of introns		18–21	6	155	0

Gene expression in plastids requires pro- and eukaryotic elements

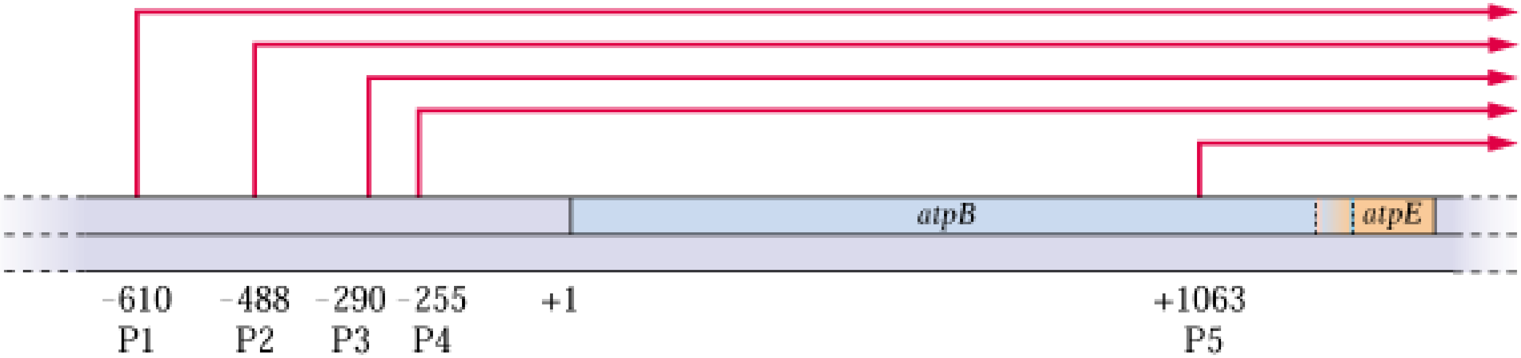


Operons und Introns

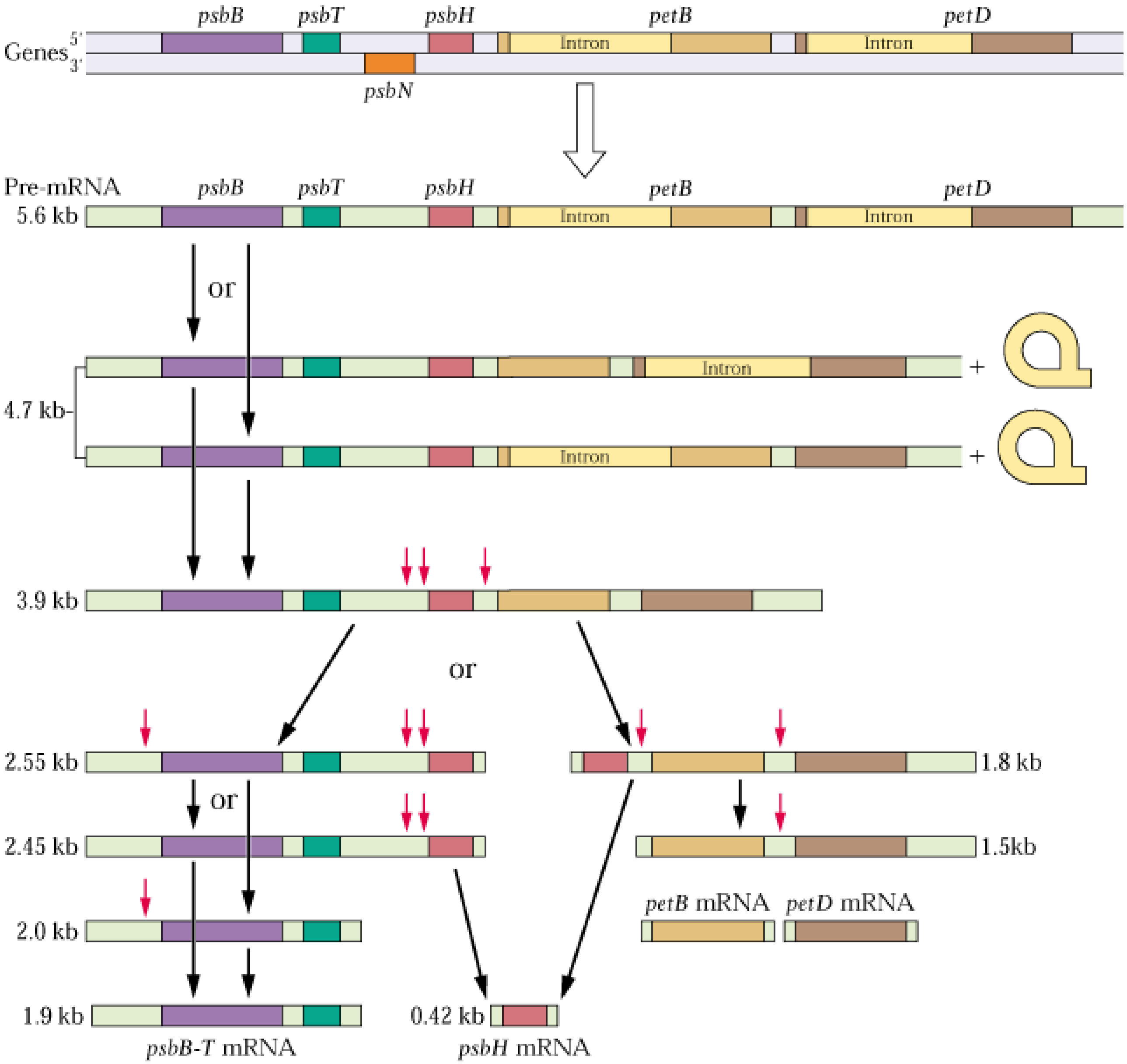
Most of the promoters are procaryotic – but not all of them

		„–35"		„–10"	
rbcL	TACGGTTGGG	TTGCGC	CATATATATGAAAGAGTA	TACAAT	AATGATGT
atpB	AAATTTACTC	TTGACA	GTGGTATATGTTGTATATG	TATATC	CTAGATGT
psbA	TAGATATTGG	TTGAGA	CGGGCATATAAGGCATGT	TATACT	GTTGAATA
psbB	TCAACTCCCA	TTGCGT	ATTGCTACTTATCGAGTA	TAGAAT	AGATTTGT
E. coli	......	TTGACA	...... 17 bp	TATAAT	......

psbD/C GAAAGAAGCATAAAGTAAGTAGACCTGACTCCTTGAATGATGCCTC TATCCGCTATTTCTGATATATAAA



psbB operon: complex processing steps



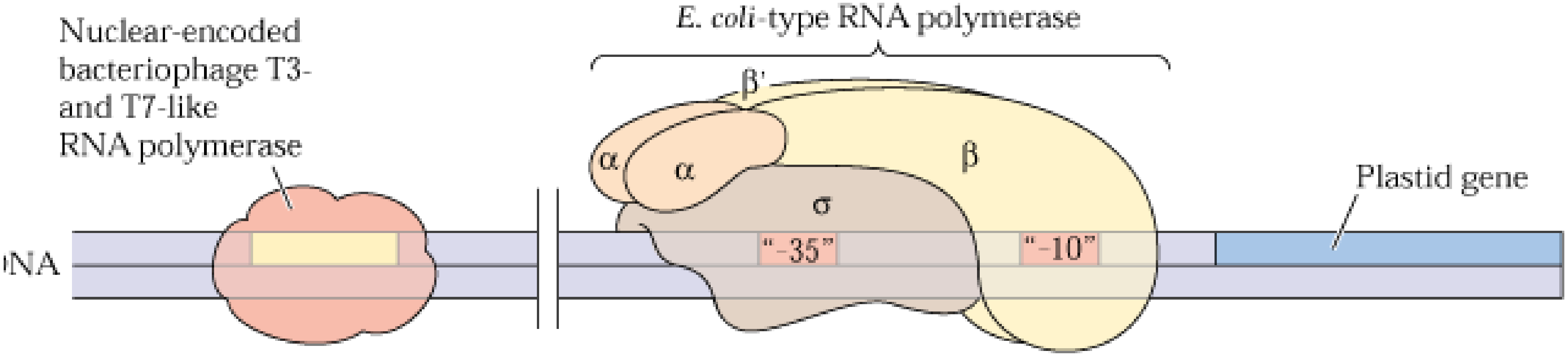
psbB operon

- multiple promotors, multiple transcription start sites
- both strands encode genes
- polycistronic transcripts
- primary transcript is large and unstable
- RNA codes for independent proteins
- transcript ripening, oligocistronic transcripts
- monocistronic transcripts
- specific endonucleases
- Exonucleases: processing of 3´-ends
    - hair pin loops stabilizes RNA
    - secondary structures prevent degradation

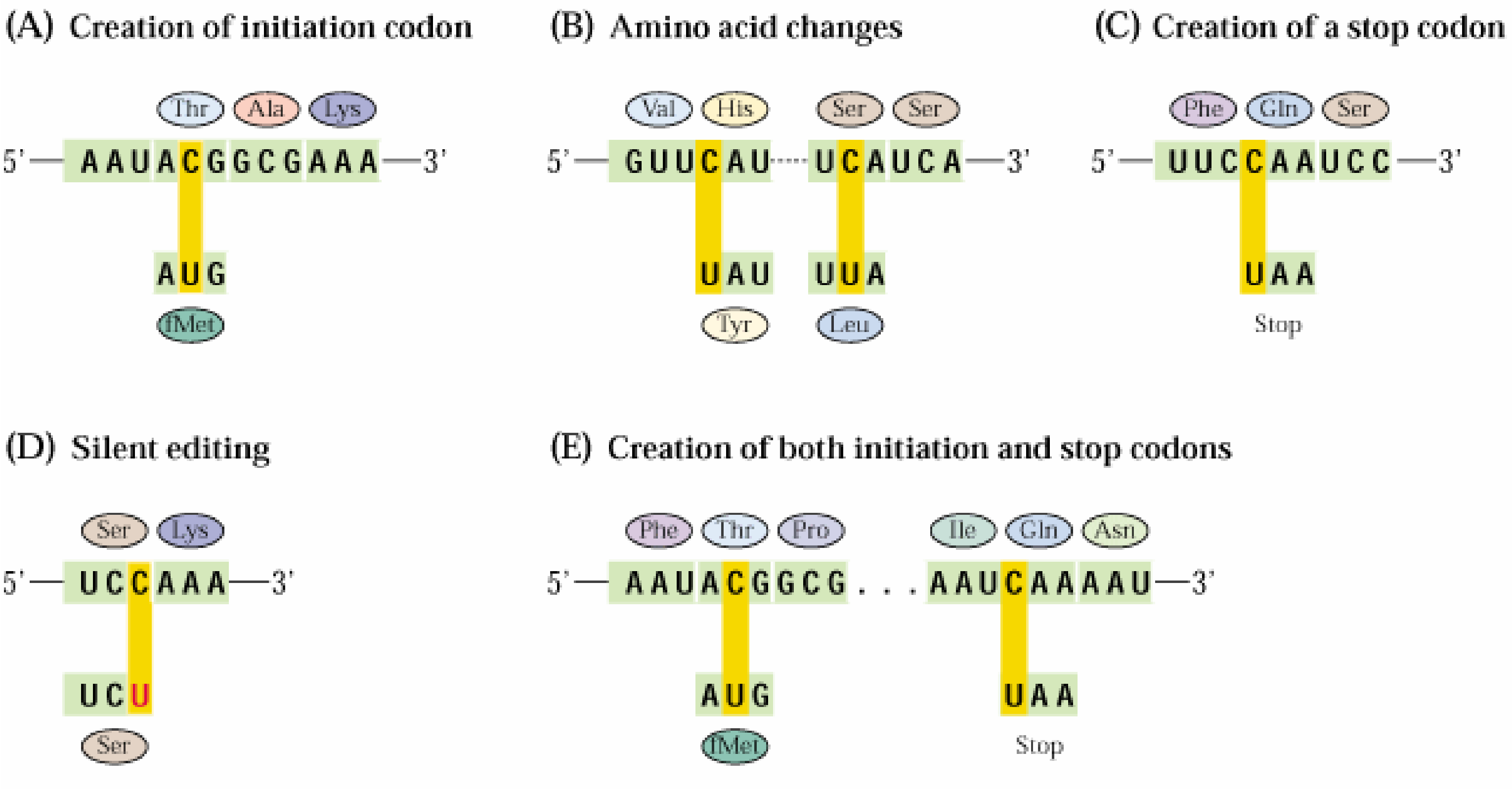
Plastids contain two RNA-polymerases

- *Epiphagus*: lost the genes for RNA-polymerases, but still contain white plastids

- nuclear-encoded RNA-polymerase	- plastid-encoded RNA-polymerase
- phage type	- bacterial type
- one subunit	- ~13 subunits, nuclear- and plastid-encoded
	- sigma factors (bacteria-like)
- Expression of early genes	- Expression of late genes



Editing change plastid transcripts – Hydrolytic deamination of cytidine to uridine


(A) Creation of initiation codon

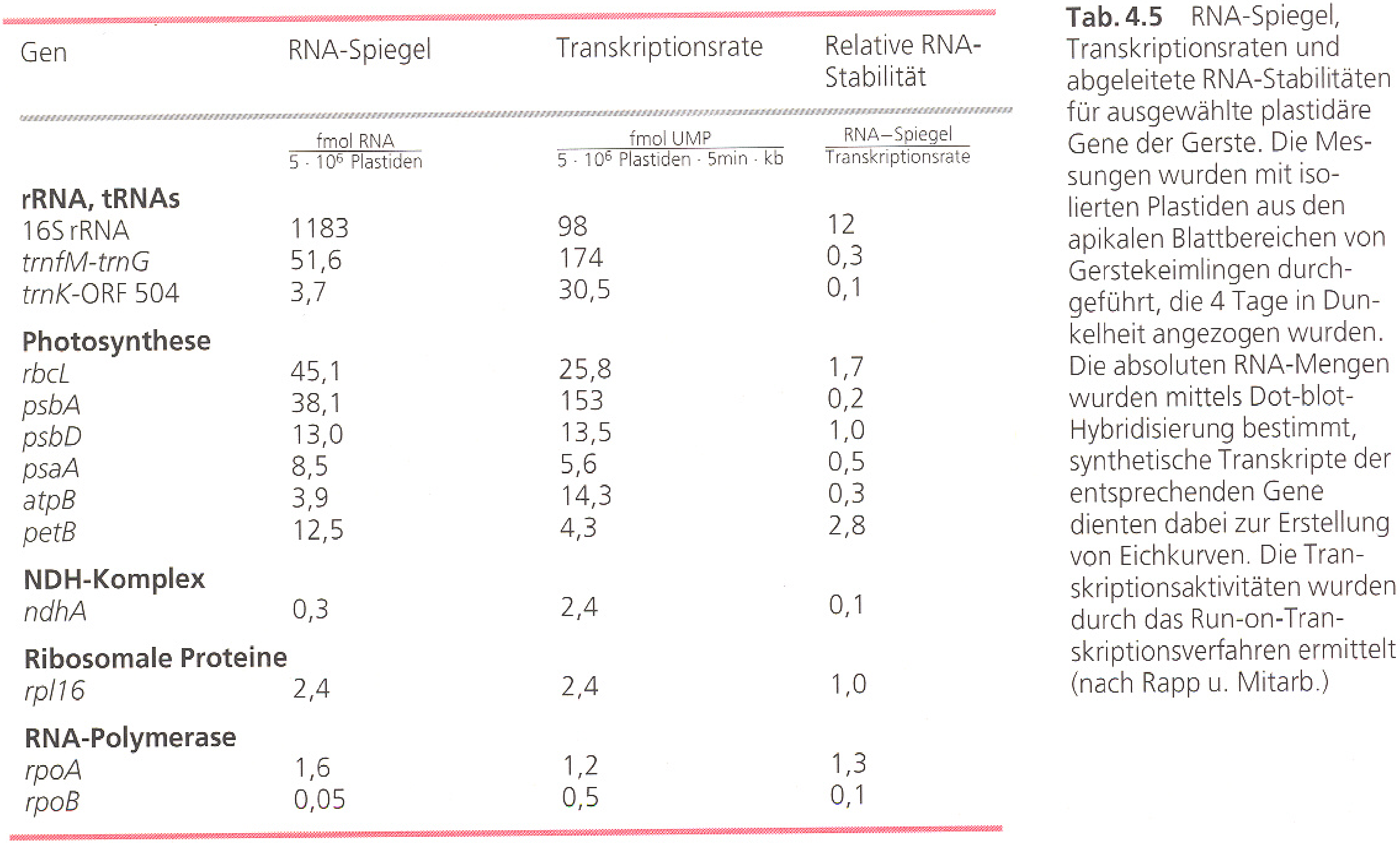
(B) Amino acid changes

(C) Creation of a stop codon

(D) Silent editing

(E) Creation of both initiation and stop codons

Most of the transcripts are stable



Gen	RNA-Spiegel	Transkriptionsrate	Relative RNA-Stabilität
	$\frac{\text{fmol RNA}}{5 \cdot 10^6\ \text{Plastiden}}$	$\frac{\text{fmol UMP}}{5 \cdot 10^6\ \text{Plastiden} \cdot 5\text{min} \cdot \text{kb}}$	$\frac{\text{RNA–Spiegel}}{\text{Transkriptionsrate}}$
rRNA, tRNAs			
16S rRNA	1183	98	12
trnfM-trnG	51,6	174	0,3
trnK-ORF 504	3,7	30,5	0,1
Photosynthese			
rbcL	45,1	25,8	1,7
psbA	38,1	153	0,2
psbD	13,0	13,5	1,0
psaA	8,5	5,6	0,5
atpB	3,9	14,3	0,3
petB	12,5	4,3	2,8
NDH-Komplex			
ndhA	0,3	2,4	0,1
Ribosomale Proteine			
rpl16	2,4	2,4	1,0
RNA-Polymerase			
rpoA	1,6	1,2	1,3
rpoB	0,05	0,5	0,1

Tab. 4.5 RNA-Spiegel, Transkriptionsraten und abgeleitete RNA-Stabilitäten für ausgewählte plastidäre Gene der Gerste. Die Messungen wurden mit isolierten Plastiden aus den apikalen Blattbereichen von Gerstekeimlingen durchgeführt, die 4 Tage in Dunkelheit angezogen wurden. Die absoluten RNA-Mengen wurden mittels Dot-blot-Hybridisierung bestimmt, synthetische Transkripte der entsprechenden Gene dienten dabei zur Erstellung von Eichkurven. Die Transkriptionsaktivitäten wurden durch das Run-on-Transkriptionsverfahren ermittelt (nach Rapp u. Mitarb.)

Many genes from plastids were transferred to the nucleus

- DNA fragment
- as RNA after reverse transcription (e.g. as edited transcripts)