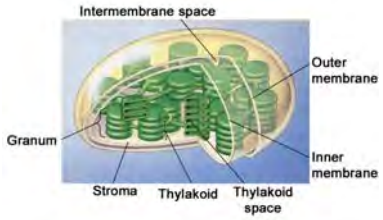

## Chloroplasts most complex




**Outer membrane:** Outer envelope proteins, translocons
**Inner membrane:** Transporters e.g. triose phosphate translocator
**Inter membrane space:** Links between translocons
**Stroma:** Rubisco, starch, lipids, DNA, ribosomes
**Thylakoid membranes:** Reaction centres, electron carriers
**Thylakoid lumen:** Plastocyanin

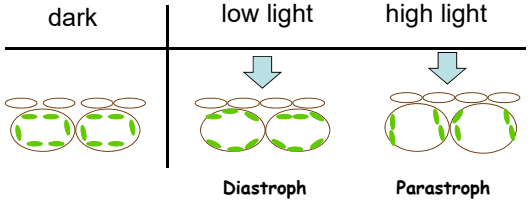
**Crete:** تاج
**Grana: pl. Granum**
**Loculus= Lumen=** روزن
**Saccule= Intergrana=** کیسه چه
**Thylakoid= Lamellae=** تیغه

منشا کلروپلاست و میتوکندری:
باکتری های درون همزیست

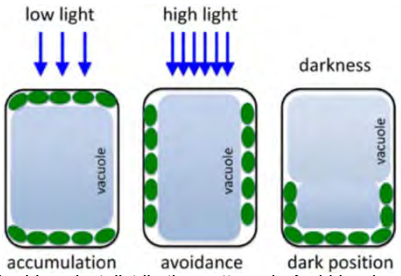
شرایط استخراج:
1. pH=7
2. فشار اسمزی حدود 0.4 M با ساکارز
3. دمای نزدیک صفر

سانیدن در هاون و صاف کردن از میان پارچه تنظیف
سانتریفوژ کردن
بقایای دیواره و درشت
محلول
سانتریفوژ کردن 1000g*12min
رسوب
محلول
انتقال به لوله سانتریفوژ با شیب غلظت ساکارز (46 و 50%)
کلروپلاستهای کلاس I در بخش بین دو غلظت ساکارز

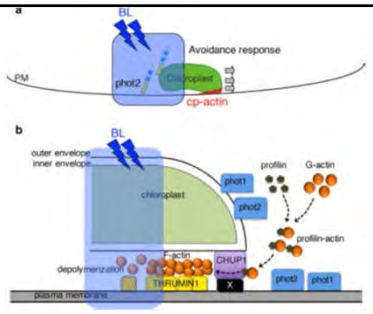
## Chloroplast movement




*Lemna, Adiantum, Oxalis, Acer*




Schematic chloroplast distribution patterns in Arabidopsis mesophyll cells. Chloroplasts are embedded in a thin layer of cytoplasm between the plasma membrane and the tonoplast of a large vacuole.
**Left**. Accumulation response. Chloroplasts re-distribute to the upper and lower sides of palisade cell under low light conditions, achieving maximal light absorption.
**Center**. Avoidance response. Chloroplasts re-distribute on the side walls of palisade cell, minimizing the absorption of strong light.
**Right**. Dark positioning. Chloroplasts distribute on the bottom of the cell in the dark, although the physiological function of this distribution is not yet known.



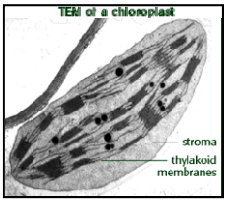

Cp-actin-based chloroplast photorelocation movement.

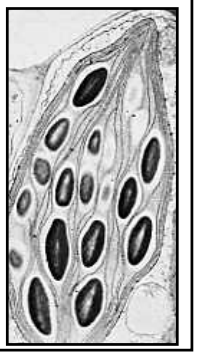
**a** Avoidance response induced by high intensity of ***blue light*** **(BL)**. The avoidance response is mediated by phot2 localized not only in the plasma membrane but also in the chloroplast envelope: phot2 probably in chloroplast envelope mediates rapid severing and random movements of cp-actin filaments to induce the disappearance of cp-actin filaments in the irradiated region but active actin polymerization in the non-irradiated region. The resulting highly asymmetric distribution of cp-actin filaments induces rapid directional movement.

**b** Cp-actin-based motive force generation for chloroplast photorelocation movement. CHUP1 is localized to the chloroplast envelope via its N-terminal hydrophobic region and mediates chloroplast anchoring to the plasma membrane via its coiled-coil region. In addition, CHUP1 might be involved in the polymerization of cp-actin filaments by inserting G-actin. THRUMIN1 bundles cp-actin filaments at the plasma membrane side. Cp-actin filaments are dominantly depolymerized in the light-irradiated region of the moving chloroplast. However, cp-actin filaments are actively polymerized in the front non-irradiated region of the moving chloroplast. During the process, phot2 acts as the main photoreceptor.

## Photosynthetic capacity

$CO_2$

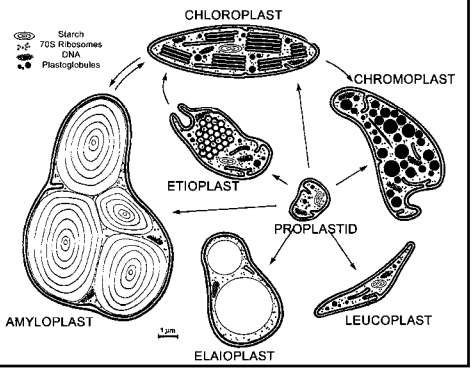


A) Accumulation of starch

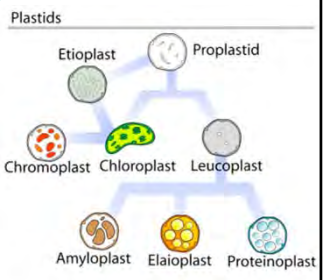
## Chloroplasts belong to a family

- Plastids are found in all cells of all higher plants



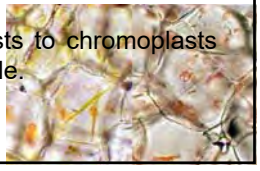

## Plastids

- Chloroplast and Etioplast
- Chromoplast
- Leucoplast
  - Amyloplasts,
  - Elaioplasts
  - Proteinoplasts



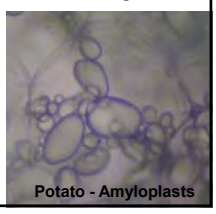

## Chromoplasts

- Chromoplasts are plastids responsible for pigment synthesis and storage.
- Chromoplasts → are found in coloured organs of plants such as fruit and floral petals.
- This is always associated with a massive increase in the accumulation of carotenoid pigments.
- The conversion of chloroplasts to chromoplasts in ripening is a classic example.



## Leucoplasts

- **Leucoplasts** are non-pigmented plastid.
- leucoplasts have been observed in epidermal cells of roots, hypocotyls and petals, and in callus and suspension culture cells of tobacco
- They may become specialized for bulk storage of
  - starch → amyloplasts
  - lipid → elaioplasts
  - Protein → proteinoplasts


Potato - Amyloplasts

## Proteinoplast

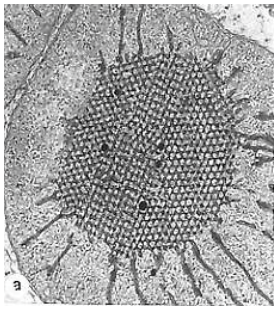
*= Proteoplasts, Aleuroplasts, Aleuronaplasts*

- They contain crystalline bodies of protein and can be the sites of enzyme activity involving those proteins.
- Proteinoplasts are found in many seeds, such as brazil nuts and peanuts.

## Elaioplasts

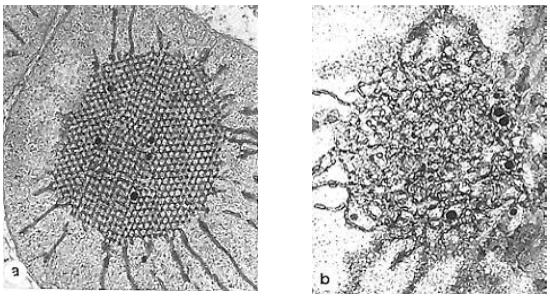
- Colourless plastid (leucoplast) storing oil;
- Common in liverworts and monocotyledons.
- Term is also applied to chloroplasts of many brown algae in which reserve storage product is **oil**.
-

## Internal membrane development



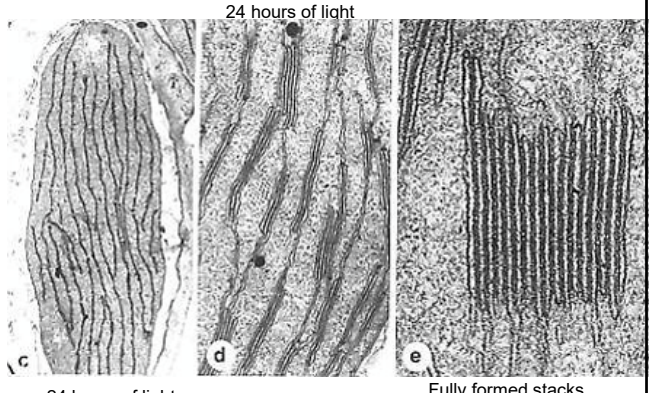
Without exposure to light inner membrane forms lattice-like (توری یا مشبک مانند) prolamellar body (جسم پیش تیغه ای)

## Internal membrane development



Within 1 minute the prolamellar body starts to disperse

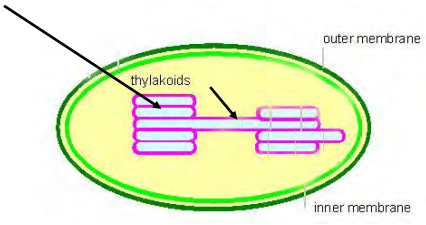
## Internal membrane development




24 hours of light

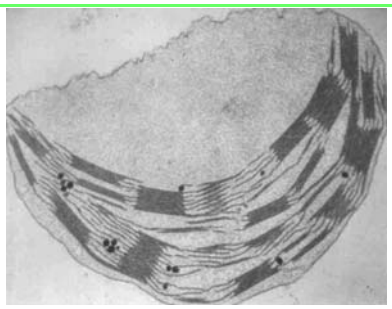
Fully formed stacks

## Internal membrane development




Freeze fracture of chloroplasts can split lipid bilayer down middle

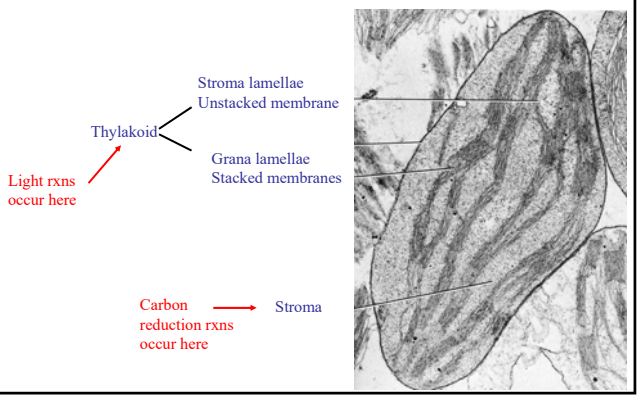
## Internal membrane development



Chloroplasts are not static, inflexible, oval structures

## Photosynthesis - light

P/S takes place in chloroplasts (cp)




# Photosynthesis Photochemistry

## Ps General Equation

$$6\,CO_2 + 6\,H_2O \xrightarrow[\text{Light}]{\text{Chlorophyll}} C_6H_{12}O_6 + 6\,O_2$$

**1. Photo**chemistry Reactions

**Chl.** + **Light** + ***NADP⁺ + ADP + Pi*** → ***ATP + NADPH + H⁺*** + **Chl.**

**2. Bio**chemistry Reactions

$CO_2 + H_2O$ + ***NADPH + H⁺ + ATP*** → $CH_2O$ + ***NADP⁺ + ADP + Pi***

## Two Photochemical Law

1. Grotius- Draper Law (قانون گروتوث-دراپر)

کوانتوم یا فوتون باید جذب شود تا واکنش شیمیایی انجام گردد.

دلیل: طیف کنش و جذب یکسان

1. The Photochemical Equivalence Einstin's

(قانون برابری فتوشیمیایی انشتین)

هر واکنش شیمیایی نیاز به یک فوتون مجرد دارد (انرژی کافی).

پتانسیل احیا (Redox Potential= $E_0$)

$E_0\ H_2$= -0.42 eV
$E_0\ O_2$= +0.82 eV
} اختلاف ولتاژ ≈ 1.2 eV (الکترون ولت)

1 eV = 96 KJ energy → 1.2 eV = 115.5 KJ

یک مول فوتون نور قرمز (1 En, λ=700 nm)= 167 KJ

کارایی فتوسنتز حداقل 70% است

- Blue- Red Transition (انتقال آبی - قرمز)
- در فتوسنتز نور آبی (ایجاد سطح هیجان دوم) مشابه نور قرمز (ایجاد سطح هیجان اول) عمل میکند. زیرا سطح هیجان دوم به اول تبدیل شده و در فتوسنتز وارد میشود و مابقی به صورت گرما هدر میرود.
- ← لذا در مباحث فتوشیمی فتوسنتز فقط نور قرمز و قرمز دور مورد استفاده قرار میگیرد.

## Emerson Enhancement Effect
## (اثر ترغیبی یا افزایشی امرسون)

- نور قرمز (640 nm ) + نور قرمز دور (720) ← افزایش 25% فتوسنتز (اثر سینرژیتیک)
- (در مقایسه با نورهای تک رنگ از قرمز یا قرمز دور)
- لذا ← وجود دو نوع سیستم نوری

## Two Photosystems (PS)
## دو سیستم نوری

1. PSI ➔ P700, P for pigment. In 1961 so I = PSI reaction centre

قله جذبی در 703 nm

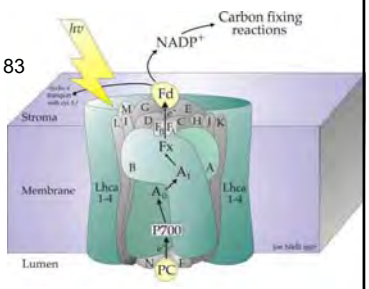
1. PSII ➔ P680, P for pigment. In 1969 so II = PSII reaction centre

قله جذبی در 682 nm

At least 22 polypeptides and 200-300 chlorophyll molecules associated with PSII


- Major distal *L*ight *h*arvesting *c*hlorophyll binding proteins (*Lhcb*1-3)
- Minor distal *Lhc*'s (*Lhcb*4-6)
- 47 and 43kDa *c*hlorophyll binding *p*roteins (CP47 & CP43)
- **PSII core**
- $O_2$ evolving complex

- PSI contains 11 sub-units
- **Core** contains:
  a heterodimer of related 83 kDa polypeptides,
  ~100 chlorophylls
  2 phylloquinones
  3 Fe-S centres
- P700 is a chlorophyll dimer




- **Ferrodoxin is used to reduce NADP to $NADPH_2$ in stroma via ferrodoxin NADP+ reductase**
- **Thus the trans thylakoid $H^+$ gradient has been produced, and $NADPH_2$ generated**

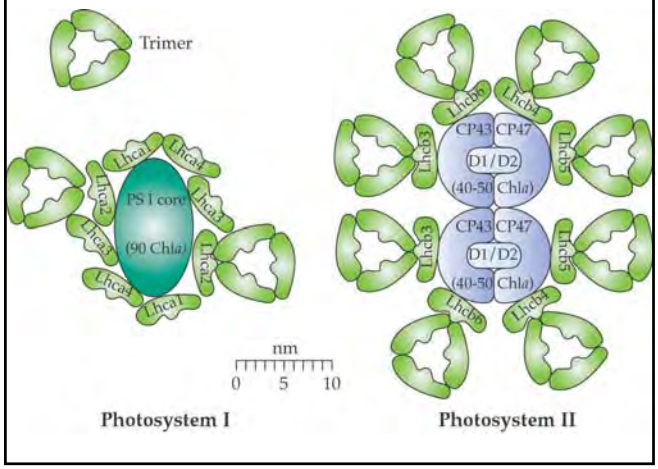
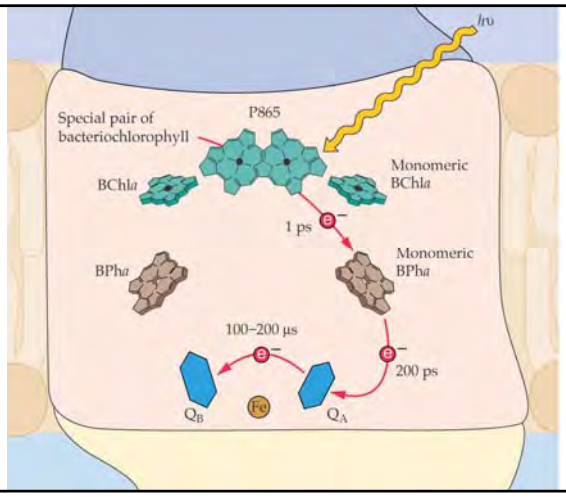
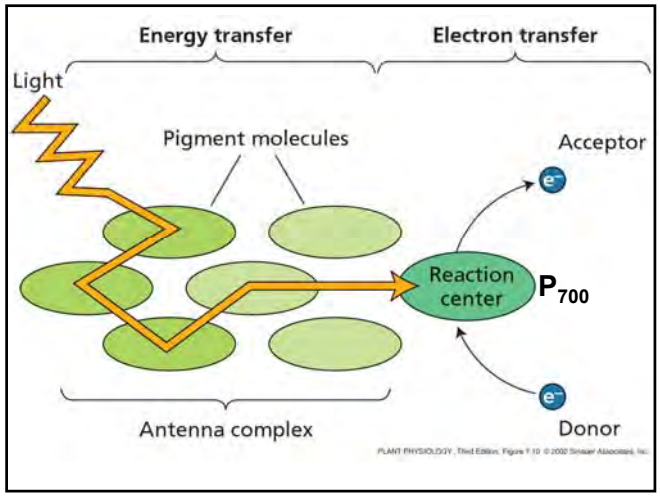


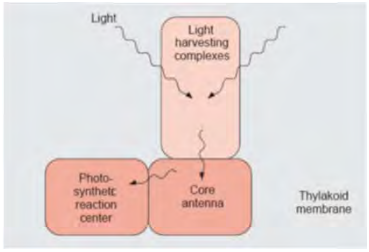

TABLE 12.3 *Properties of light-harvesting chlorophyll protein complexes.*

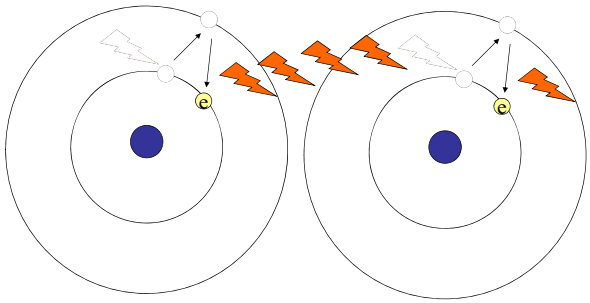
| Complex | Number of Chls | Gene* | Mol. mass (kDa) |
|---|---|---|---|
| **Light-harvesting complexes (LHC) associated with PSI** | | | |
| LHCI 680 | 14 | *lhca3* | 24.9 |
| | 14 | *lhca2* | 23.2 |
| LHCI 730 | 14 | *lhca1* | 21.5 |
| | 14 | *lhca4* | 22.3 |
| **Light-harvesting complexes associated with PSII** | | | |
| CP29 | 13 | *lhcb4* | 27.3–28.2 |
| LHCII | 14 | *lhcb1* | 24.7–24.9 |
| | 14 | *lhcb2* | 24.9 |
| | 14 | *lhcb3* | 24.3 |
| CP26 | 9 | *lhcb5* | 26.1 |
| CP24 | 10 | *lhcb6* | 23.2 |

*Genes are nuclear-encoded

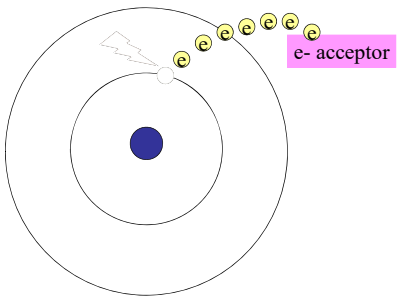


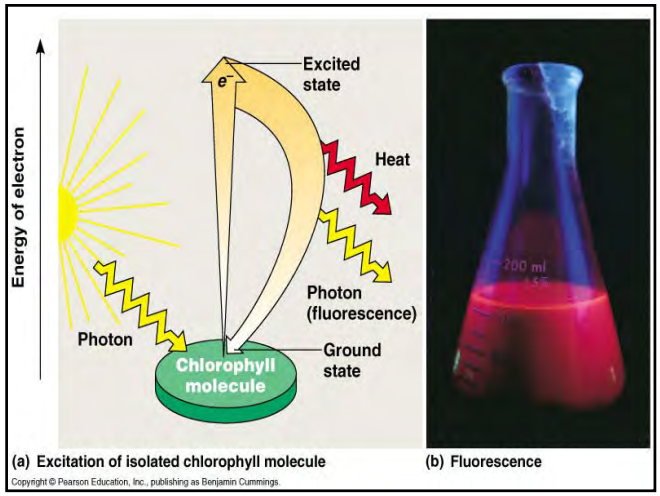



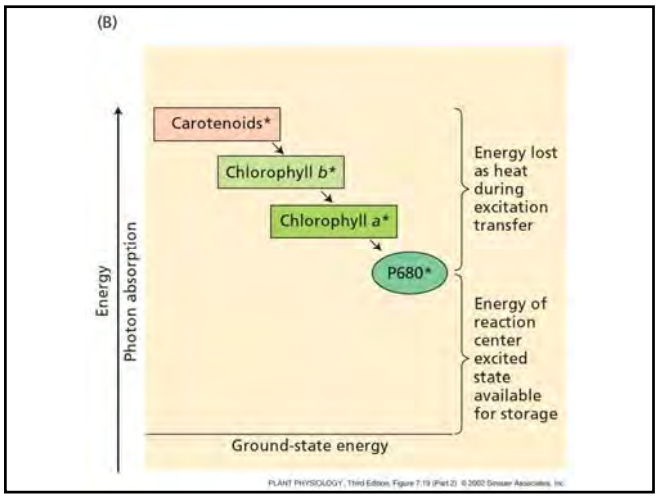



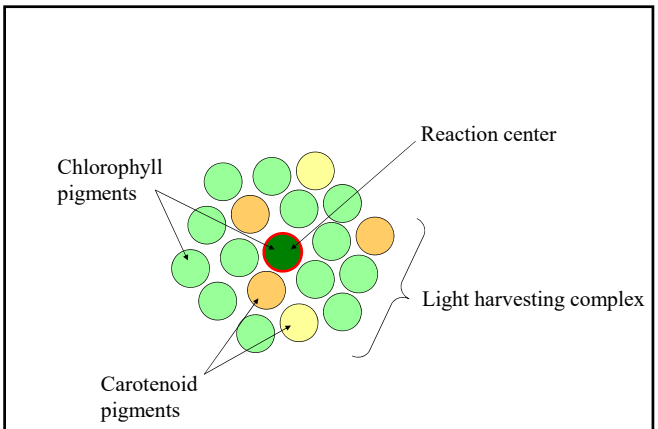



Resonance energy transfer

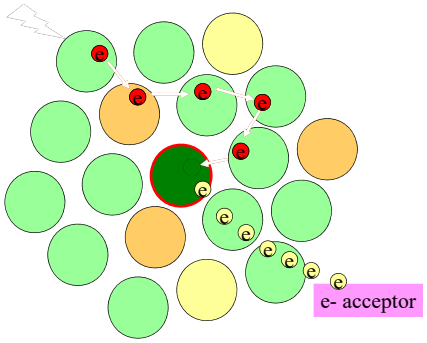


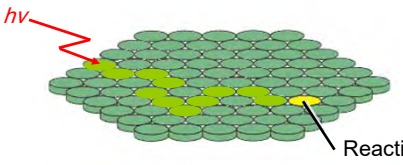






**Generalized photosystem**

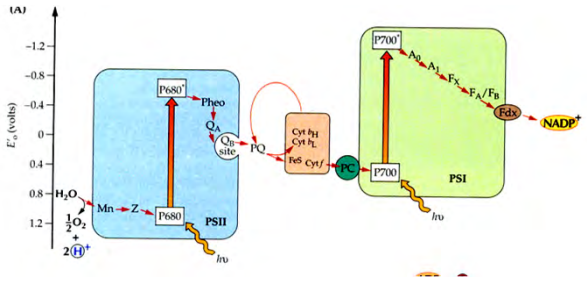


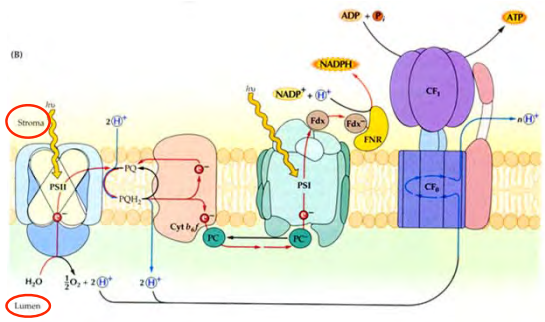

FOUR sequential processes:

1. **LIGHT HARVESTING** (OR ANTENNAE) COMPLEXES. Pigment protein complexes which collect light and pass energy to reaction centres

2. **REACTION CENTRES**. Pigment-protein complexes containing a "special pair" of chlorophyll molecules where light energy is used to initiate electron transfer




3. **ELECTRON TRANSFER**. From reaction centre to acceptor, and from donor to reaction centre




4. **FORMATION OF PROTON GRADIENT**. Across membrane, used for ATP synthesis

## Hill's Reaction
## واکنش هیل

$CO_2 + 2\ H_2O \longrightarrow CH_2O + O_2 + H_2O$

$CO_2 + 2\ H_2S \longrightarrow CH_2O + 2S + H_2O$

- $4Fe^{3+} + 2H_2O \xrightarrow{\text{احیا شدن}} 4Fe^{2+} + 4H^+ + O_2$

فرّیک فرّو

## فرایند انتقال الکترون در فتوسنتز

$H_2O + X + Y \xrightarrow[\text{کلروپلاست}]{\text{نور}} XH + YOH$

$XH \longrightarrow X + (H)$

$YOH \longrightarrow Y + {}^1/_2\,H_2O + {}^1/_4\,O_2$

${}^1/_2\,H_2O \xrightarrow[\text{کلروپلاست}]{\text{نور}} (H) + {}^1/_4\,O_2$

## Hill's Reaction
## واکنش هیل

- $2H_2O + 2NADP \longrightarrow 2NADPH_2 + O_2$

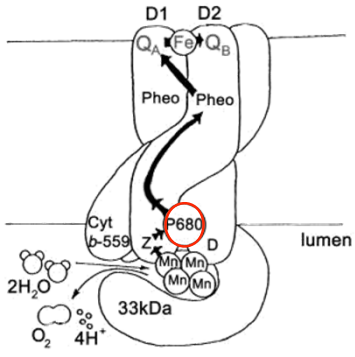
بجای فری سیانید
$[Fe(CN)_6]^{3-}$

# Photophosphorilation

1. **Non-Cyclic**
2. **Cyclic**

**Reaction centre core contains:**

- P680
- D1 and D2 (for diffuse on gels), heterodimer at core of RC
- Cytochrome-*b*559



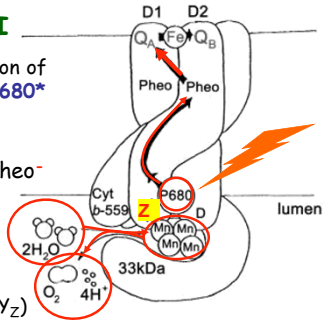

2H2O $\xrightarrow{\text{نور}}$ **2 (H)** + 2 (OH)

**2 (H)** + NADP + ADP + Pi $\longrightarrow$ $NADPH_2$ + ATP

2 (OH) $\longrightarrow$ $H_2O + {}^1/_2O_2$

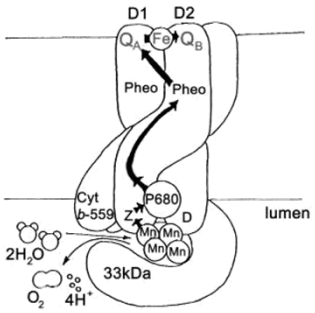
**Photochemistry at PSII**

- Light induced charge separation of chlorophyll converts P680 to P680*
- **e**⁻ acceptor = pheophytin
  P680* $\longrightarrow$ $P680^+$ and $Pheo^-$
- $e^-$ passed to $Q_A$ = form of plastoquinone
- $P680^+$ oxidises tyrosine 161 ($Y_Z$)
- Mn cluster provides $e^-$ to $Y_Z$



## The OEC

- Oxygen Evolving Complex - 4x Mn
- Mn cluster bounded by polypeptides, (33 kDa largest plus one)
- After 4 oxidations, molecular oxygen and $H^+$s liberated into lumen




## Photochemical Reaction

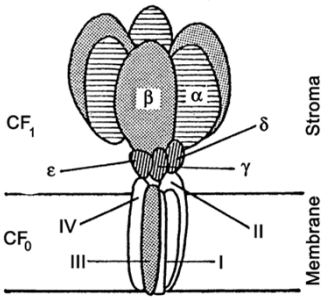
- **Oxidation/Reduction:**
  - Pigment is photo-oxidized.
  - Acceptor molecule is reduced.
  - Photo-oxidation of chlorophyll is the primary photochemical act in photosynthesis.

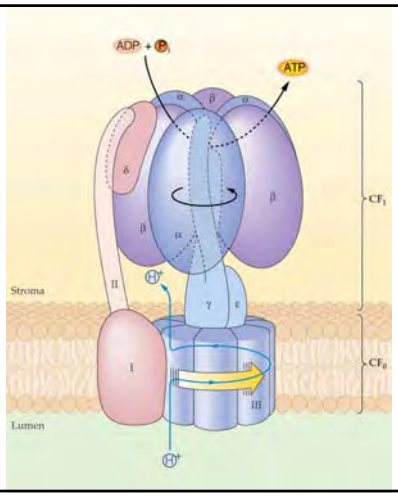
## Photophosphorylation

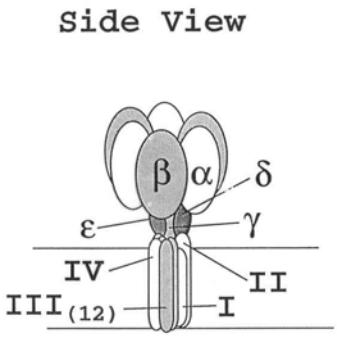
**Proton gradient generated by:**

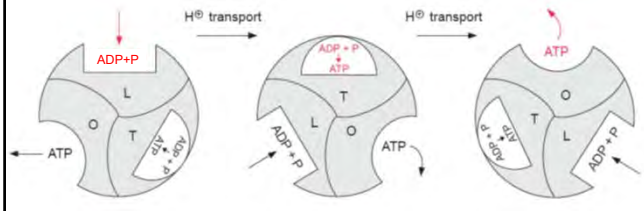
- Water splitting
- Transport by $PQH_2$

→ Trans membrane electrochemical potential → drives ATP synthesis



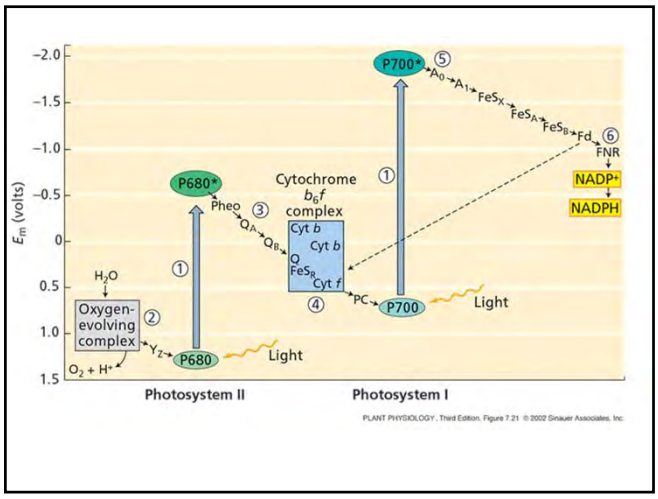

ATP synthesis by the binding changeme chanism as proposed by Boyer.

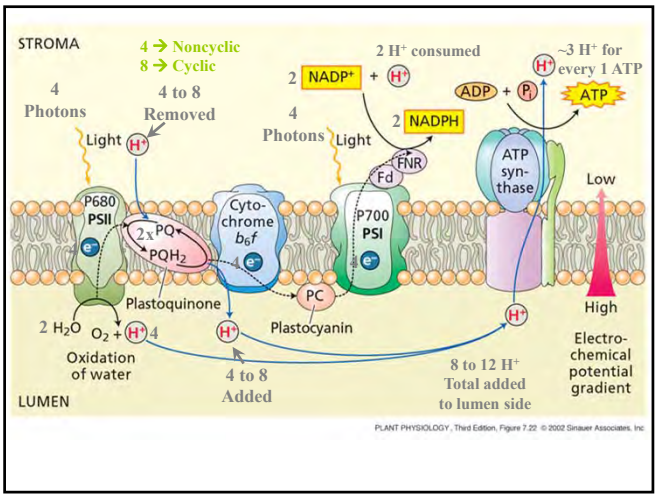
The central feature of this postulated mechanism is that synthesis of ATP proceeds in the F1 complex by three nucleotide binding sites, which occur in three different conformations:

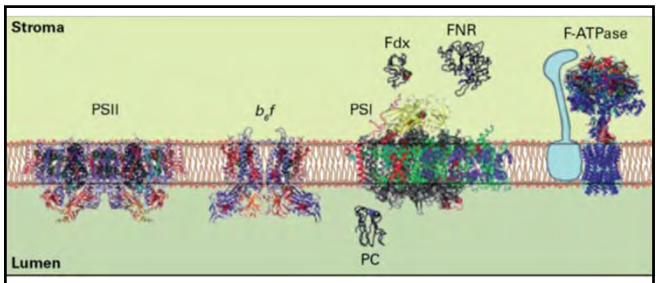
conformation L binds ADP and P loosely, T binds ADP and P tightly and catalyzes the ATP formation; the ATP thus formed is tightly bound.

The open form, O, releases the newly formed ATP.

The flux of protons through the F-ATP synthase, as driven by the proton motive force, results in a concerted conformation change of the three binding sites, probably as a rotation.

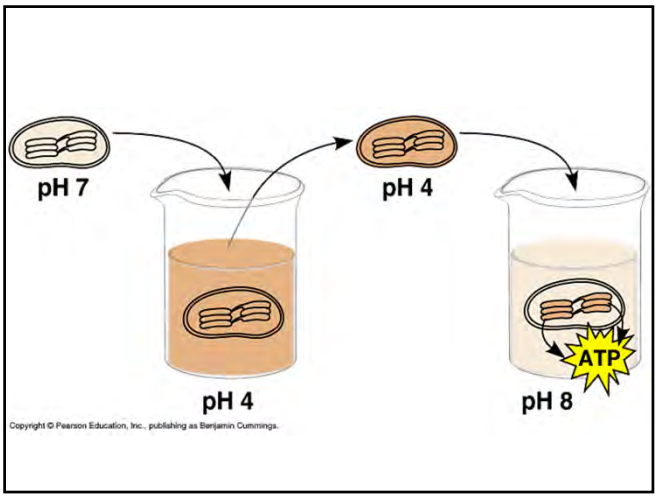


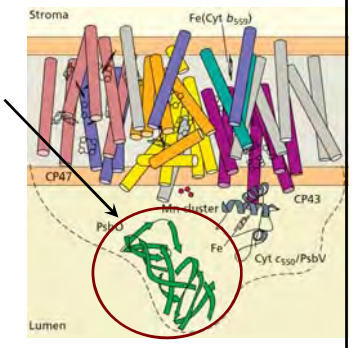

A structural view of the Z-scheme showing structures of the major thylakoid membrane complexes (PSII, cytochrome b6 f complex, PSI, and ATP synthase) involved in the light reactions of oxygenic photosynthesis.

Also shown are the structures of the soluble proteins Fdx and FNR on the stromal side of PSI, as well as PC in the lumen."



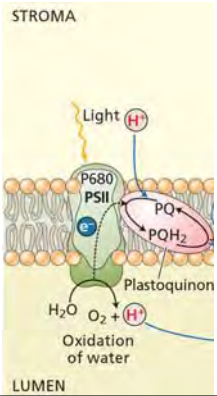

# The Light Reactions

- Oxygen evolving complex provides $e^-$ for Photosystem II.
  - Located on the lumen side of the thylakoid membrane.
  - Where water is split (photolysis) releasing $O_2$ , 4 $e^-$, and 4 $H^+$ for every 2 $H_2O$.

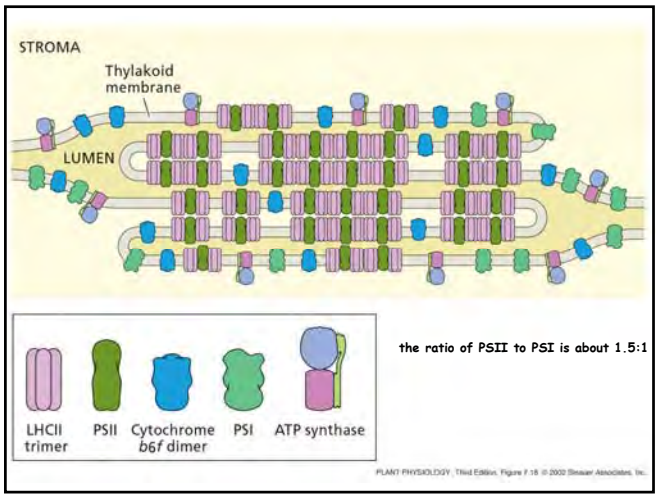


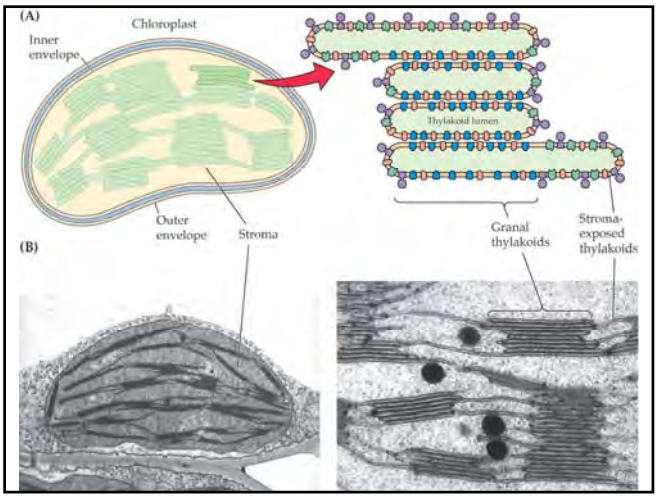

# Photosystem II

- Optimum absorption peak for chlorophyll a 680 nm ($P_{680}$).
- Primarily on grana lamellae (stacks).

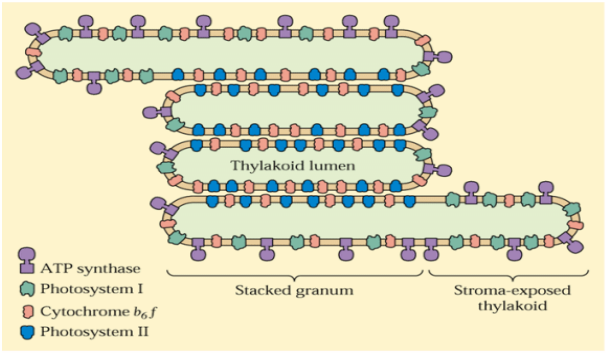



Taiz & Zeiger, 2002,



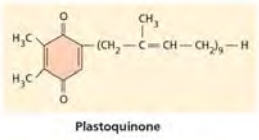



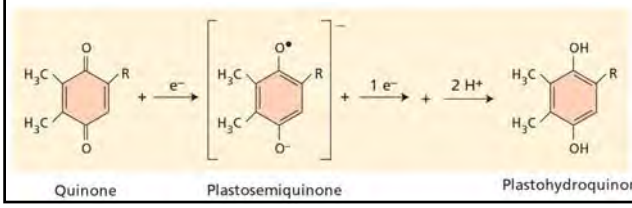

**Protein complexes in thylakoid membrane exhibit lateral heterogeneity:**




# Electron Transport

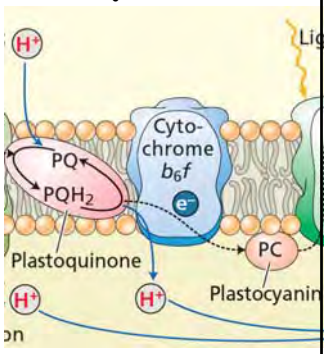
- Plastoquinone (PQ).
  - Carries an H with the e-.



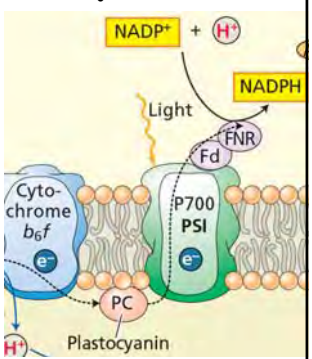

# Electron Transport

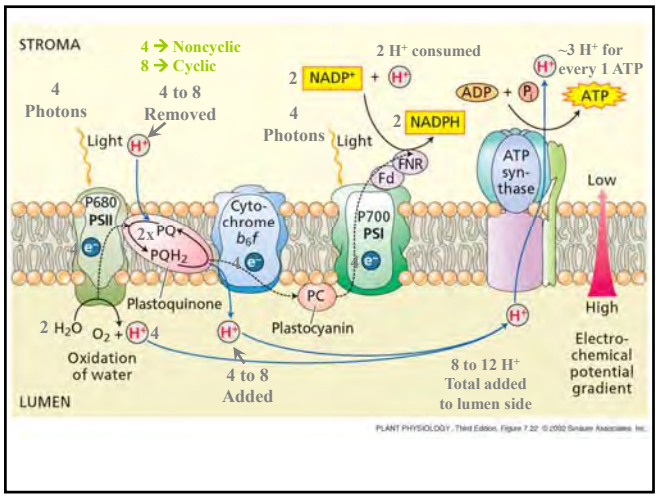
- Cytochrome $b_6$/f complex.
  - Multiprotein complex.
  - Evenly distributed on the membranes.
  - Does not carry and H with the e-.
  - $H^+$ from PQ accumulate in the lumen.

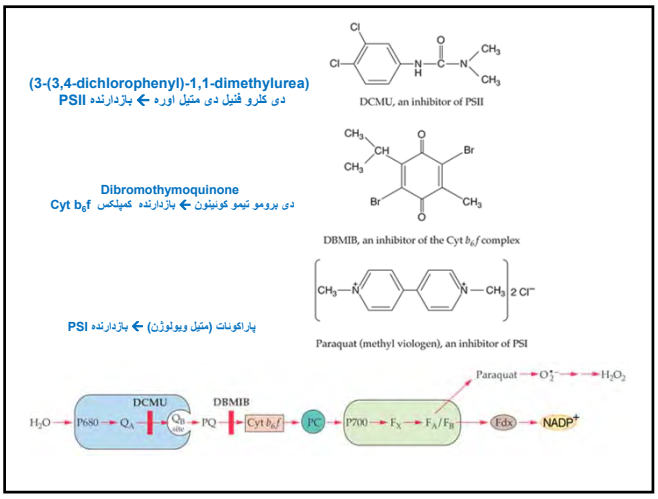



# Electron Transport

- Plastocyanin (PC).
  - Peripheral protein on the lumen side.
  - Shuttles e- between cytochrome $b_6$/f complex and PS I.

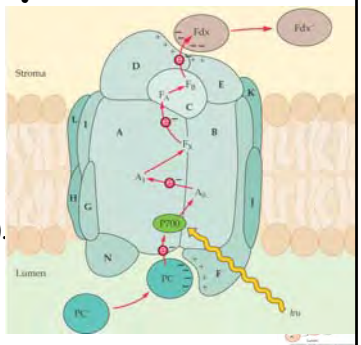





# Photosystem I

- First photosystem discovered.
- Optimum absorption peak for chlorophyll a is 700 nm ($P_{700}$).
- Primarily on stroma lamellae (non-stacked).

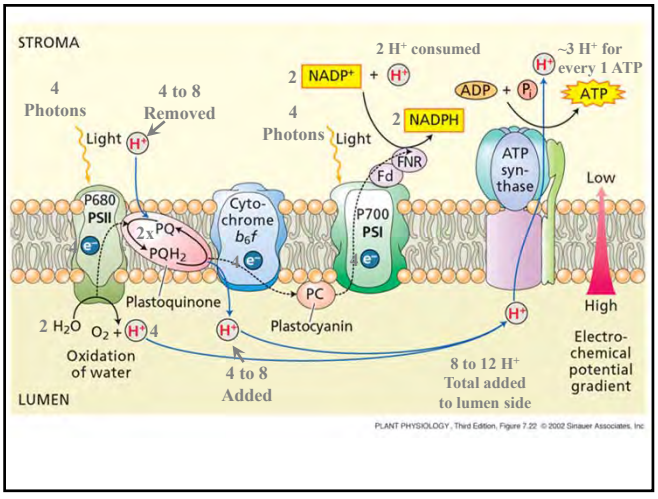



# NADP Production

- PS I passes **e**$^-$ to Ferredoxin
  - Small, water soluble Fe-S protein.
  - Located on the stroma side of thylakoid.
- Then to a NADP reductase.
  - Forms NADPH from $NADP^+$ and $H^+$ on the stroma side of thylakoid.

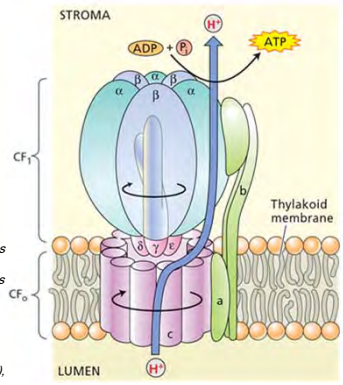
# ATP Synthesis

- ATP synthase couples the transport of $H^+$ with ATP synthesis (photophosphorylation).
  - $H^+$ gradient created through:
    - Photolysis of water in lumen.
    - Electron transport chain ($H^+$ into lumen).
    - Use of $H^+$ to make NADPH in stroma.
  - Thylakoids - quite impermeable to $H^+$ except through ATP synthase.



# ATP Synthesis

- As $H^+$ flow down their concentration and electrochemical gradients, ATP synthase uses the energy to create ATP = the chemiosmotic mechanism.
- ATP formed on the stroma side of the membrane.




*Model for the chloroplast ATP synthase complex. The subunit structure of the ATP synthase indicates two major regions in the protein: an integral membrane protein portion ($CF_o$), which functions as a channel for protons passing through the membrane, and an extrinsic portion ($CF_1$), which contains the catalytic sites involved in ATP synthesis. $CF_1$ consists of five different subunits (α, β, γ, δ, and ε), whereas $CF_o$ contains four different subunits (I, II, III, and IV, of which three are shown), including 14 copies of subunit III in the membrane.*

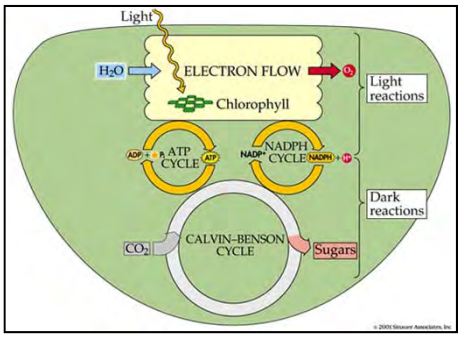
**What is the first biochemical consequence of light absorption by chlorophyll?**

$$Chl^* + A \longrightarrow Chl^+ + A^-$$

- **chlorophyll becomes a reducing agent**
- **Chl* (not Chl) participates in a redox reaction**
- **this reaction only occurs in the light**

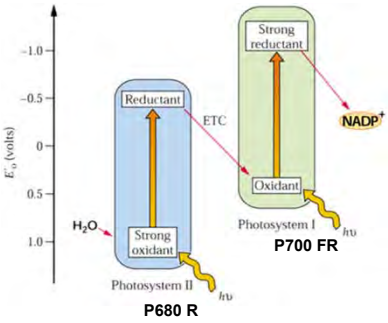
## Light reactions:

**electron flow, photophosphorylation and reductions**




**noncyclic electron flow & cyclic electron flow**

**Oxygenic photosynthetic organisms contain two photochemical reaction centers, PSI and PSII:**

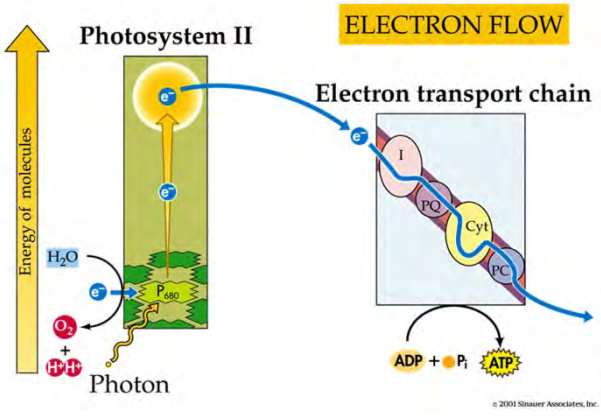



**Noncyclic electron** transport chain produces:

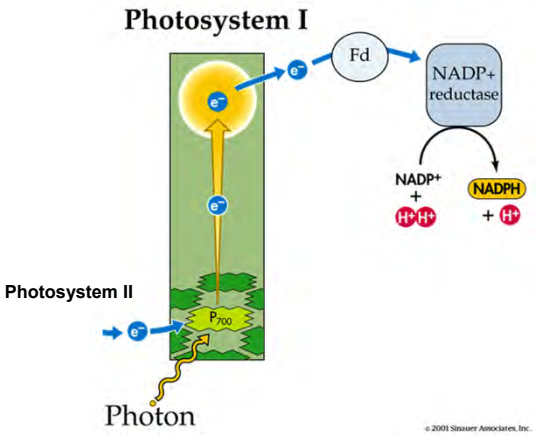
**$O_2$, NADPH, ATP,**

and involves cooperation of **PSI** and **PSII**.

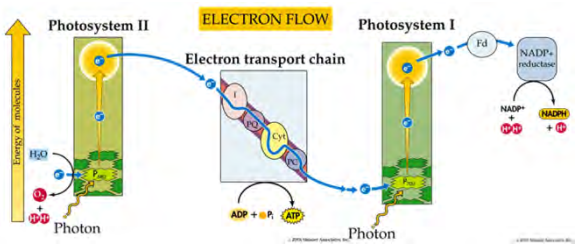
**noncyclic electron flow**




**photophosphorylation: chemiosmotic synthesis of ATP**



## Net reaction for non-cyclic electron flow:




| reactants | products |
|---|---|
| 1 $H_2O$ | 1/2 $O_2$ |
| | 1 $H^+$ |
| 1 $NADP^+$ | 1 NADPH |
| 1 ADP | 1 ATP |
| 1 $P_i$ | |

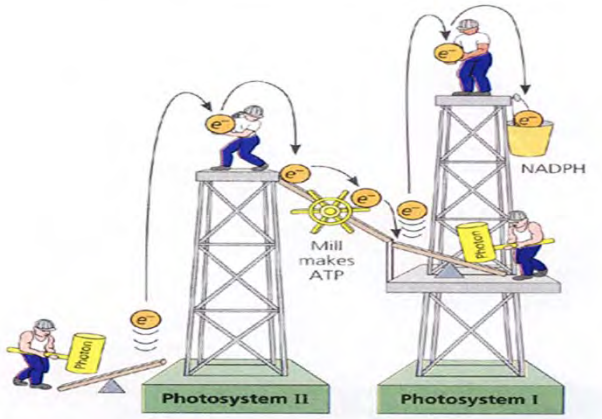
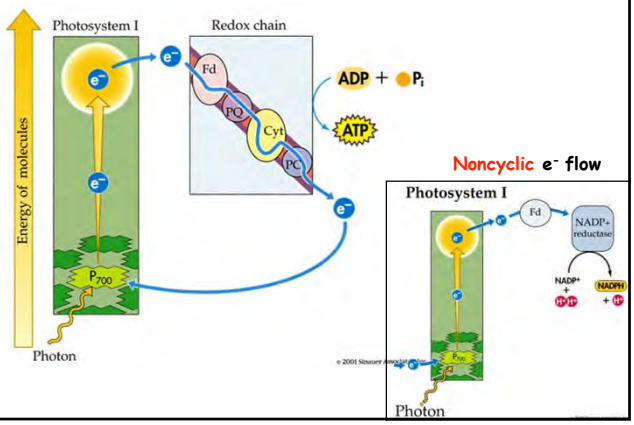
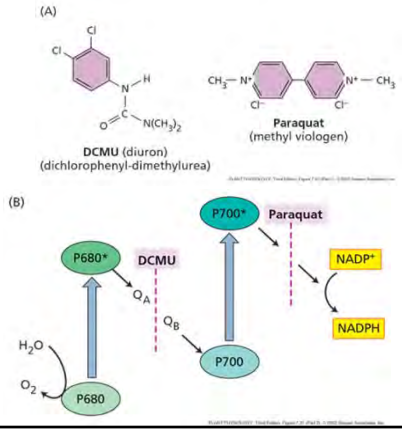


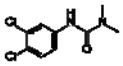

FIGURE 10.13 A mechanical analogy for the light reactions.

## Cyclic electron flow traps light energy as ATP:




## Some herbicides block e⁻ flow



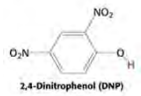



**مواد بازدارنده فتوسیستم ها:**

- DCMU (دی کلروفنیل دی متیل اوره)
- هیدروکسیل آمین
- اورتوفنانترولین
- اوره تانها

**موادی که باعث فتوفسفریلاسیون چرخه ای می شوند:**

- DCPIP (دی کلروفنل ایندوفنل)
- FMN (فلاوین منو نوکلئوتید)
- PMS (فنازین متوسولفات)
- Vit K3

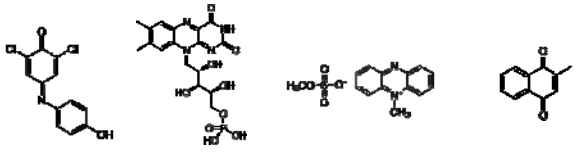



**موادی که از فتوفسفریلاسیون جلوگیری میکنند:**

- DNP (دی نیترو فنل)
- $NH_4^+$

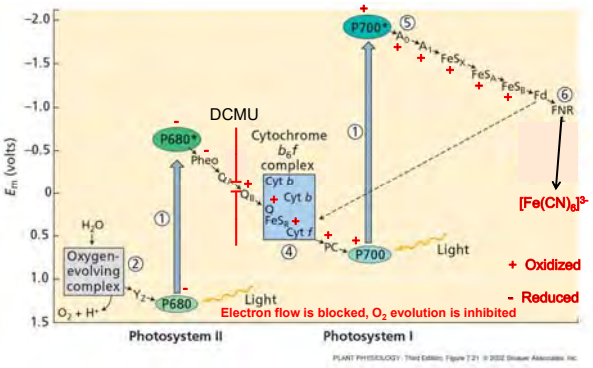
**زمانی که فتوفسفریلاسیون چرخه ای انجام می شود:**

- تجمع فراورده های فتوسنتزی (تجمع NADPH)
- کمبود ATP و زیادی ADP و Pi
- وجود برخی مواد در محیط از جمله: DCPIP ،FMN، PMS، Vit K3

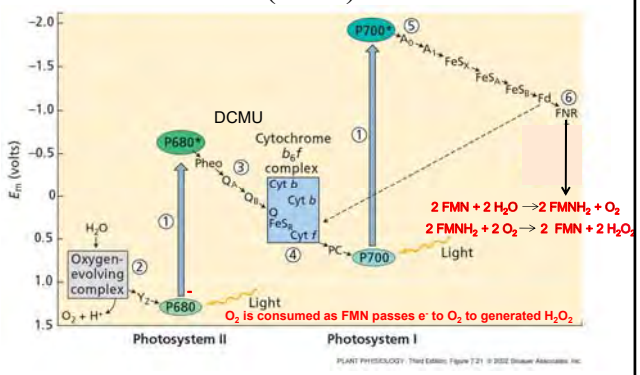


DCPIP FMN PMS Vit K3

## Photosynthesis – blocking e⁻ transport




## Photosynthesis – flavin mononucleotide (FMN)




## $O_2$ can accept e⁻ from PSI

- **Mehler reaction** (light dependent consumption of $O_2$):
  واکنش مهلر: مصرف اکسیژن در نور

$$O_2 + Fd_{red} \longrightarrow O_2^- + Fd_{ox}$$

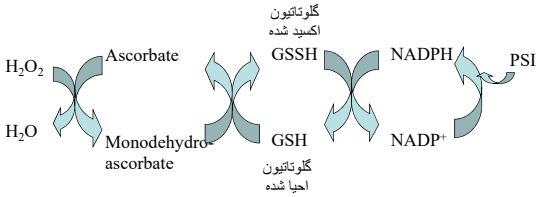
- 5 - 10% of e⁻ generated by PSI react with $O_2$
- Plants have built in systems to "scavange" ROS
  - SOD (superoxide dismutase):

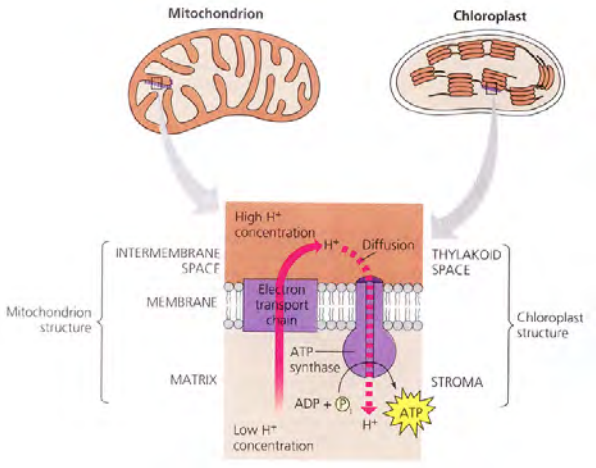
$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

  - $H_2O_2$ must be reduced to prevent reaction with $O_2^-$ to form the highly toxic hydroxyl radical ($OH^\bullet$)

## $O_2$ can accept e⁻ from PSI

- **Asada-Halliwell pathway** reduces $H_2O_2$:




## Photosynthesis reactants and products:

$$6CO_2 + 12H_2O + light \longrightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

$$6RuBP + 6CO_2 + 6H_2O$$

18ATP

12NADPH + 12H⁺

18ADP + 18$P_i$

12NADP⁺

$$6RuBP + C_6H_{12}O_6 + 6H_2O$$

**NET RXN**

- Subsequently, the proportion of carbon, hydrogen and oxygen atoms in sugars and starches was found:

  1 atom of C per molecule of water : ($CH_2O$)
  as the word "carbohydrate" indicates

- Thus, the overall photosynthesis reaction was assumed:

  $CO_2 + H_2O$ + light energy $\rightarrow$ $(CH_2O) + O_2$

reasonable hypothesis… but it turned out to be **wrong**