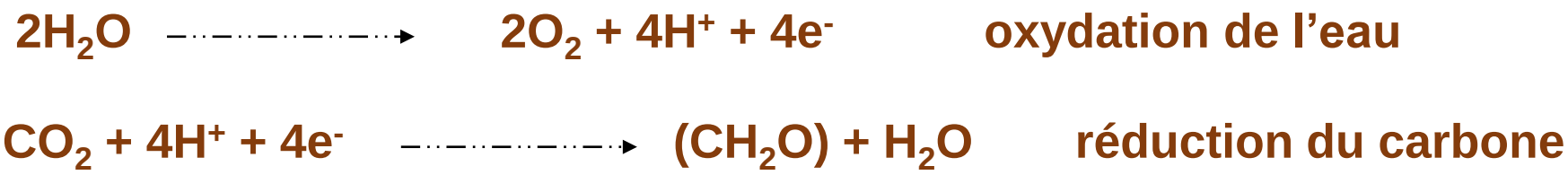
Chap 4-1. The metabolism of plants

A. PHOTOSYNTHESIS

A. Photosynthesis

1. Definition: **Photosynthesis** is a complex biological process that uses light energy to chemically transform carbon dioxide CO2 (in the atmosphere) into hydrocarbon substances (CH2O)n, in particular sugars: in this transformation, water is oxidised.



These 2 formulae represent redox reactions

A redox reaction is a chemical reaction in which electrons are transferred. The chemical species that captures the electrons is called the oxidant and the one that gives them up is called the reductant.

Photosynthesis occurs in 2 main phases :

I. The **light phase**, which is a set of photochemical reactions that depend on light. During this phase, light energy (photons) is converted directly into chemical energy.

II. The **dark phase**, which corresponds to the **Calvin cycle**, is entirely enzymatic and independent of light (does not depend on light).

2. Localisation of photosynthesis

Photosynthesis takes place in chloroplasts, a specific type of plastid. **Chloroplasts** are derived from a non-specialised plant organelle, the proplast. The proplast differentiates into the chloroplast. Chloroplasts are the most common of all plastids and contain chlorophyll, which gives plants their green colour (leaves and young stems).

Chloroplasts are green, lenticular organelles. In spinach, chloroplasts occupy a volume of around 34 μm3 (19 μm3 in lettuce).The number of chloroplasts varies from 20 to 60 per cell,

3. Chloroplast structure

The chloroplast has its own genetic material and a double phospholipid membrane (outer membrane and inner membrane) (Fig. 1) :

Outer membrane: which is a phospholipid double layer (phospholipids and proteins), it is relatively permeable.

Inner membrane: This is not very permeable and has folds called thylakoids. These folds are either stacked to form granules (one granule = granular thylakoid) or isolated (= somatic thylakoid). The inner membrane is the most important for photosynthesis and defines the inner part of the chloroplast, the stroma.

The membrane contains unsaturated fatty acids, responsible for membrane fluidity, and pigments (chlorophyll and carotenoids) often associated with proteins. Transmembrane structures allow the formation of protein complexes associated with chlorophyll, known as photosystems (PSI and PSII).

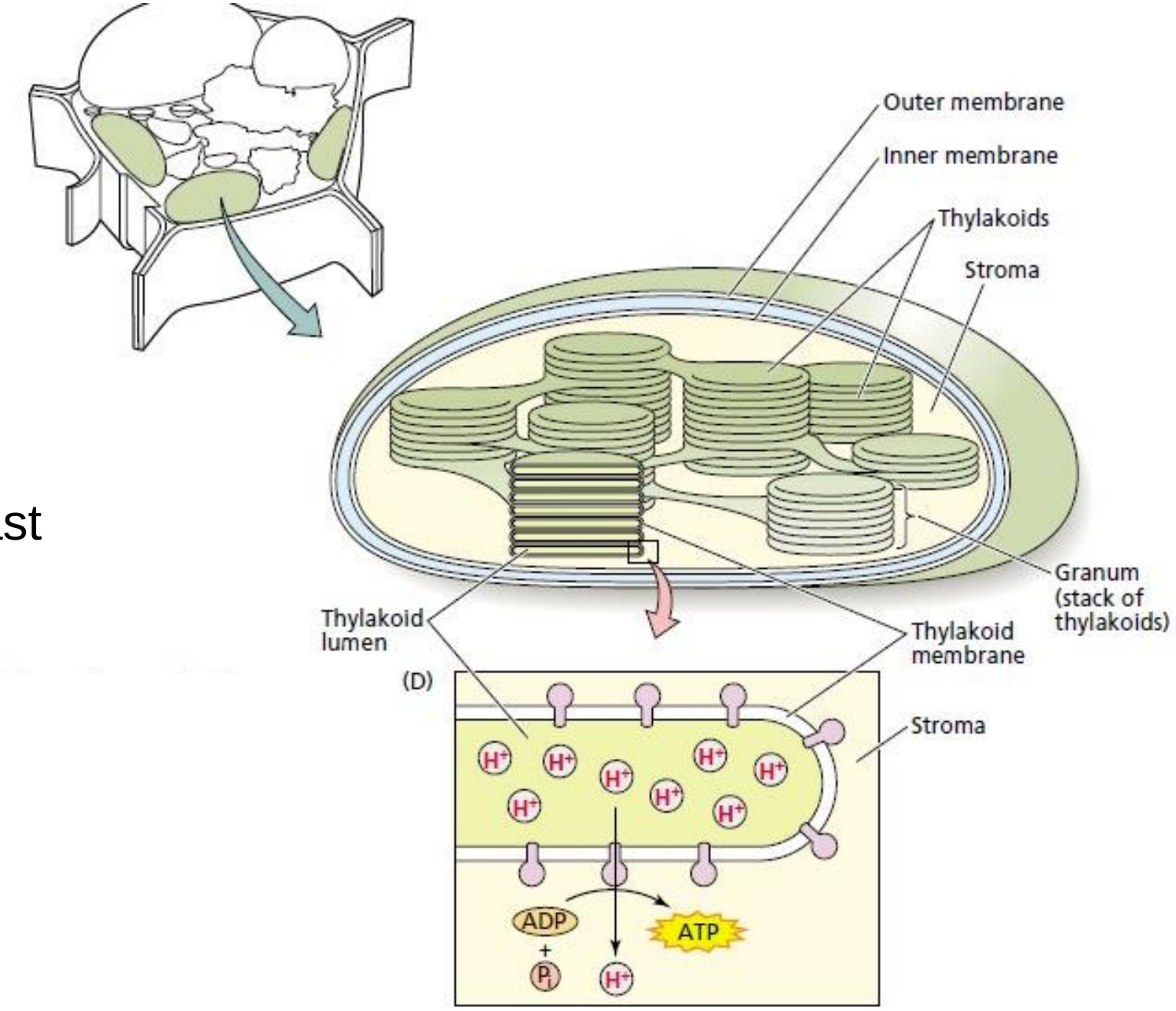


Fig 1. Structure of a chloroplast

Photosystems are the photoreceptor centres of the thylakoid membrane contained in chloroplasts. They consist of a collecting antenna and a reaction centre located at the centre of the antenna. The collecting antenna captures light energy using **pigments** of several types: chlorophyll a, b and carotenoids. The energy captured is transmitted to the reaction centre, which is a specialised site made up of clusters of pigments containing only one pair of chlorophyll a capable of giving up its electrons to the primary acceptor in the chain of electron acceptors.

The primary acceptor in photosystem I (PSI) is chlorophyll A0 (modified chlorophyll a) and in photosystem II (PSII) is pheophytin. The chain of electron acceptors allows electrons to be transported from molecule to molecule in the direction of the increase in potential. The major difference between PSI and PSII is the absorption wavelength. PSII has a molecular complex called P680 and PSI has a molecular complex called P700. During the clear phase, the water supplies the electrons (PSII), and these electrons are then transmitted to the PSI. It is the PSII that starts photosynthesis.

- Mecanisms of the photosystems

Photosystem II (PSII): (Fig. 2)

Light energy is first absorbed by the collecting antenna, which then transmits its energy to the P680 complex, causing ionisation of one of its chlorophyll molecules (i.e. P680). P680 is electronically linked to pheophytin a in D1 (electron transfer to pheophytin a in D2 is never observed).Chlorophyll a (in P680) releases electrons which are captured by the primary acceptor (Q1).

These electrons then pass through the cytochrome complex where they induce the passage of protons from the stroma to the intra-thylakoid space. The accumulated protons form a proton gradient that enables ATP synthetase to produce ATP. The electrons leave the cytochrome complex and are transmitted to photosystem I (PSI). Chlorophyll a (from P680) loses electrons, which it must recover in order to continue to function. These electrons are supplied by the photolysis of water.

Note: The electron remains only transiently in the pheophytin and is transferred to QA and then to QB. For QB to be saturated with electrons (QB^{-2}), two electrons must have successively left P680. These electrons are then injected into the chain of electron transporters, allowing QB to accept new electrons.

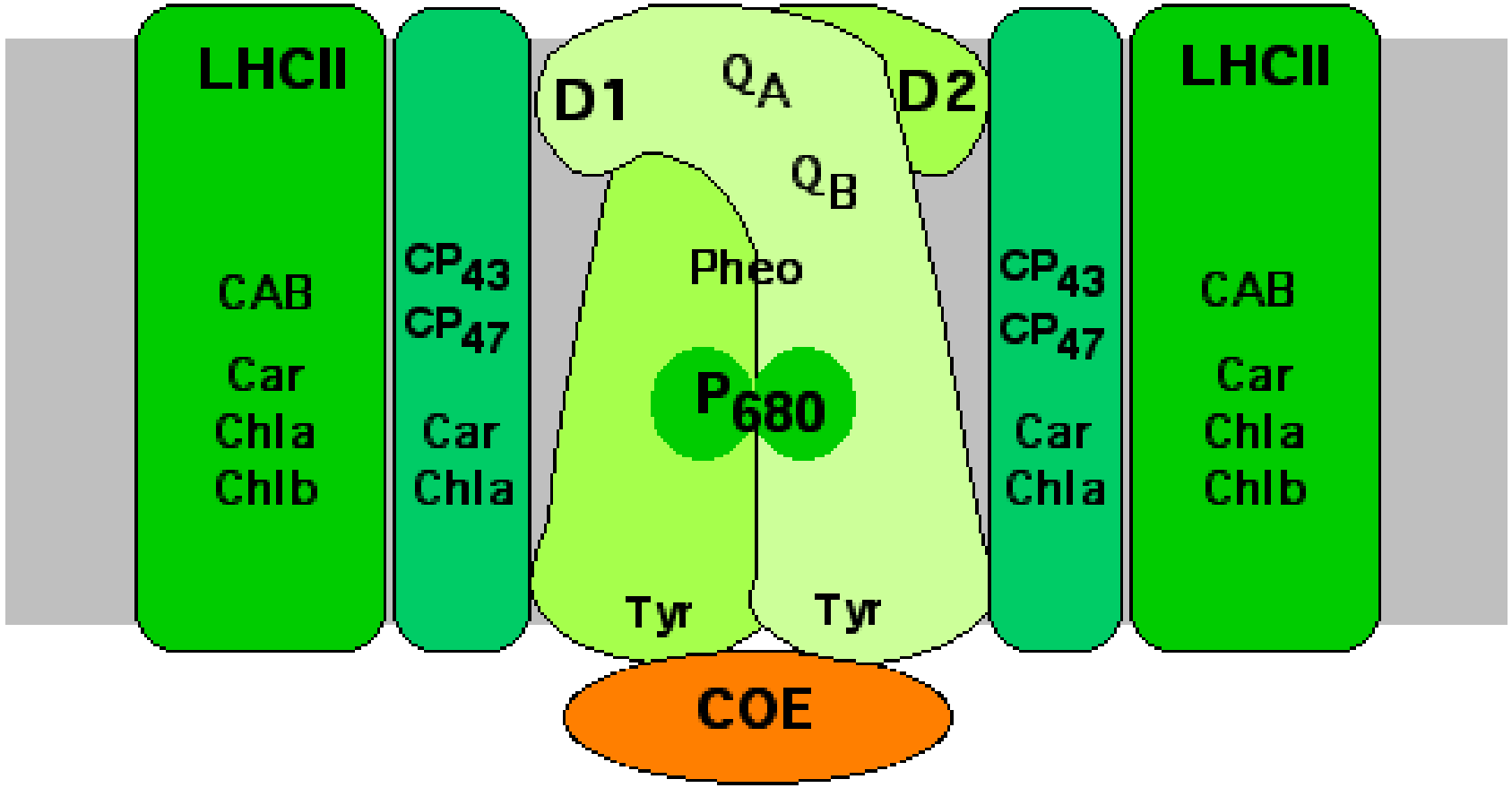
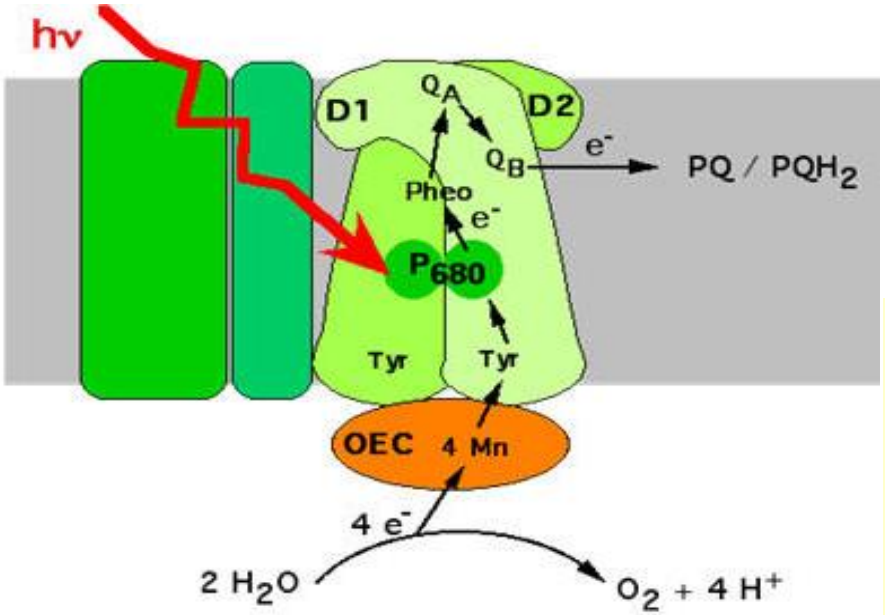


Fig 2. Schematic representation of PSII in the thylakoid membrane. CAB: peripheral (or major) antenna proteins, Car: carotene, Chla: chlorophyll a, Chlb: chlorophyll b, CP: proximal antenna proteins, D1-D2: reaction centre subunits, LHCII: Light Harvesting Complex II (major antenna), OEC: Oxygen Evolving Complex, P680: chlorophyll a dimer (trap molecule of the reaction centre), Pheo: pheophytin, QA-QB: Plastoquinones, Tyr: tyrosine.

The photosystem I (PSI) :

Further photosynthesis still requires light energy, which will be absorbed by the collector antenna and transmitted to the P700 complex (Fig. 3). The role of the P700 is to charge the electrons transmitted by the cytochrome complex with energy. These electrons are captured by the primary acceptor (pheophytin) and transported by the chain of electron acceptors to ferredoxin. Ferredoxin in turn transports the electrons to NADP reductase, which reduces the $NADP^+$ to NADPH + H^+. Chlorophyll a in P700 has therefore lost 2 electrons which it must recover for the system to function. These electrons are supplied by PSII.



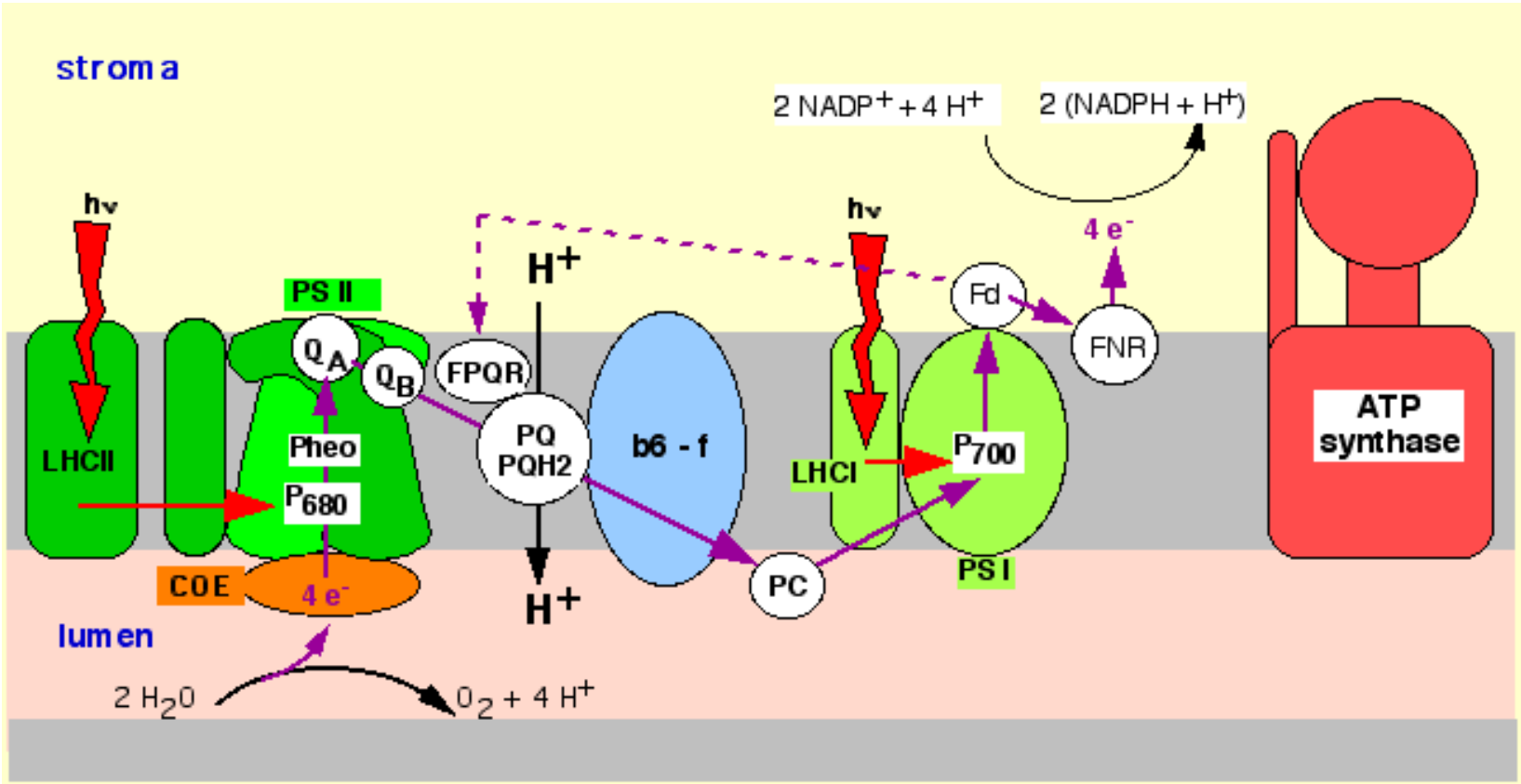


Fig 3. **Schematic representation of PSII and PSI in the thylakoid membrane.**

4. Measuring photosynthetic activity

- Measurement of gas exchange

Counting the number of bubbles released by a fragment of green aquatic plant over a given period of time. All the bubbles are assumed to have the same dimensions, and the bubble is considered to be the unit of volume of gas released.

Air analysis: The composition of the circulating air is analysed at the inlet and then at the outlet of the experimental chamber. The difference corresponds to the quantity of O2 released or CO2 absorbed.

The gases can be measured using chemical substances that absorb CO2 (potash or baryte) or O2 (potassium pyrogallate or phosphorus) or using sensitive magnetic devices (infrared analyser for CO2, paramagnetic analyser for O2).

Manometric methods - warburg apparatus: A CO_3K_2 + CO_3HK buffer keeps the CO_2 level constant. The difference in level observed at a given time between the two sides of the manometer corresponds to the volume of O_2 released.

Example:
Photosynthesis analysis apparatus

5. Metabolic reactions:

5.1. Electron transport in the light phase

Water photolysis and non-cyclic electron transport

At the level of PSII, an important step in photosynthesis takes place, which is the photolysis of water. Each time PSII is photo-oxidised, an electron is supplied by the water to compensate for the loss that PSII has just undergo. Water is therefore the primary electron donor in photosynthesis (Fig 4.). The water molecule must undergo an oxidation reaction under the action of light. This reaction releases electrons, protons and oxygen.

The electrons will be captured by the PSII, the protons produced will accumulate in the intra-thylakoid space to take part in the proton gradient, and the oxygen will be released into the atmosphere. Oxygen is therefore a waste product of photosynthesis. During these various transfers, the electron loses a little energy. This energy is used by certain transporters to carry H+ protons from the stroma (extra-thylakoid space) to the intra-thylakoid space.

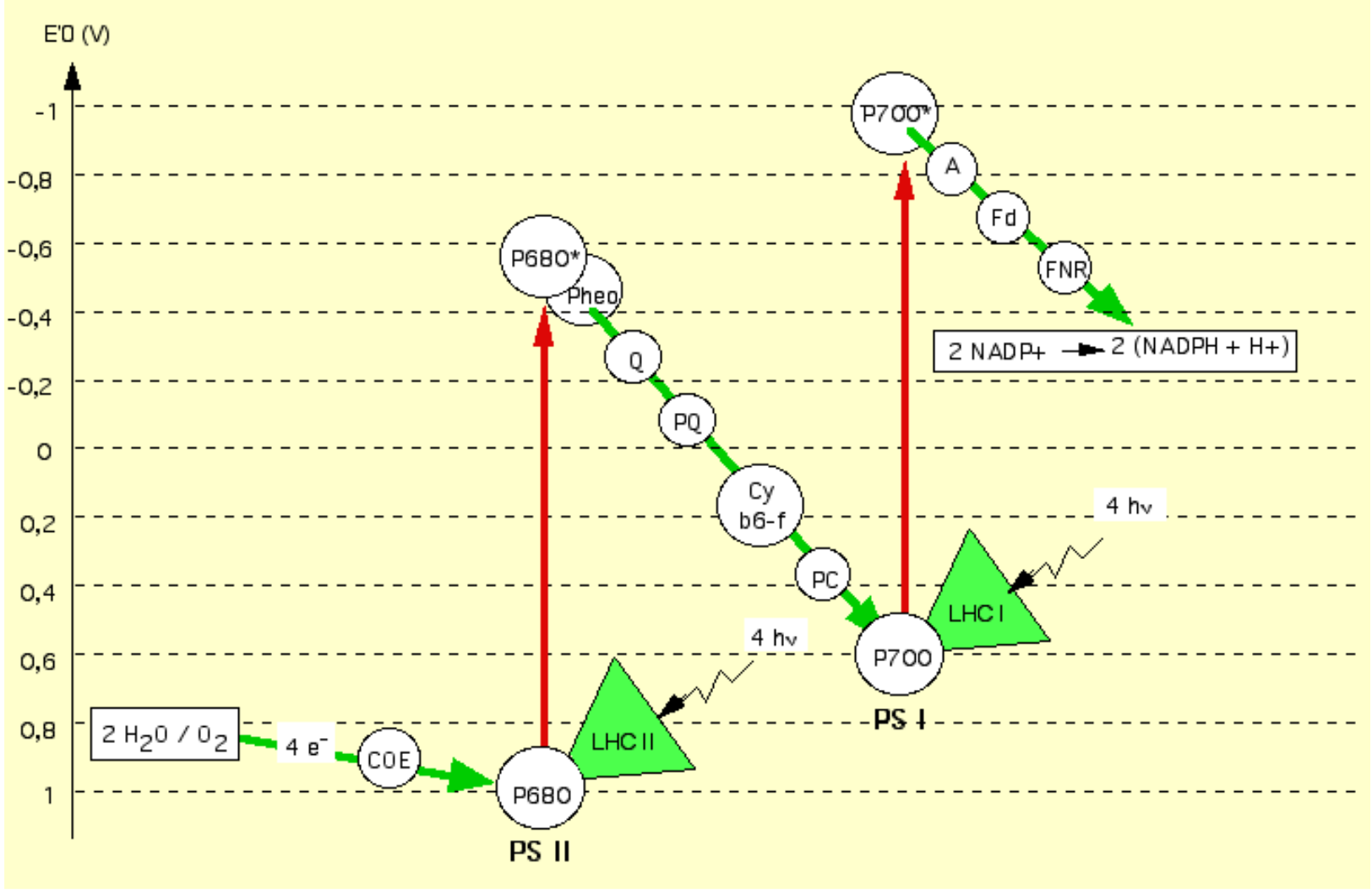


Fig 4. Z" diagram, acyclic electron transfer. Through the integrated action of the two photosystems, electrons are transferred from water to the final acceptor, NADP+. A: PSI acceptor, Cy b6-f: cytochrome protein complex, FD: ferredoxin, FNR: Ferredoxin NADP reductase, LHCI: Light Harvesting ComplexI (PSI branch), LHCII: Light Harvesting ComplexII (major PSII branch), OEC: Oxygen Evolving Complex, P680: PSII chlorophyll trap molecule, P700: PSI chlorophyll trap molecule, PC: plastocyanin, Pheo: pheophytin, PSI: Photosystem I, PSII: Photosystem II, PQ: Plastoquinones, Q: Quinones.

Cyclic electron transport

Electrons can follow a cyclic pathway involving only PSI. Instead of supplying electrons to NADP reductase, ferredoxin transfers them to plastoquinone (PQ) via a cytochrome. The electrons then follow the first chain of transporters back to the PSI, where they fill the gaps they left. This cyclic pathway (Fig. 5) makes it possible to accumulate additional protons in the intra-thylakoid space without reducing NADP+ but by promoting ATP production.

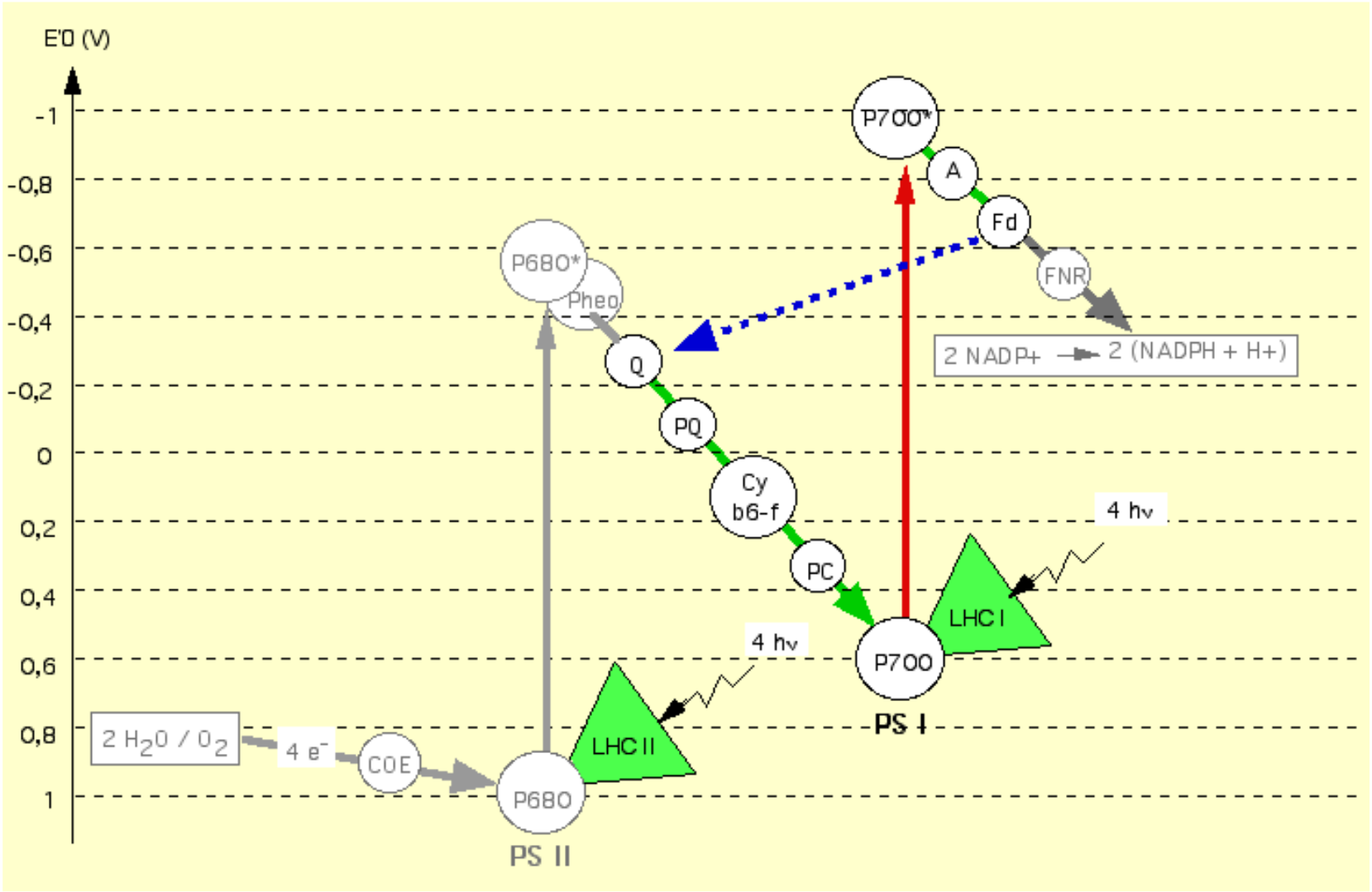


Fig 5. Transfert cyclique des électrons autour du PSI. Le transfert des électrons ne fait pas intervenir le photosystème II. Il n'y a donc pas d'oxydation de l'eau ni de réduction du NADP+.

5.2. The mechanisms of the dark phase:

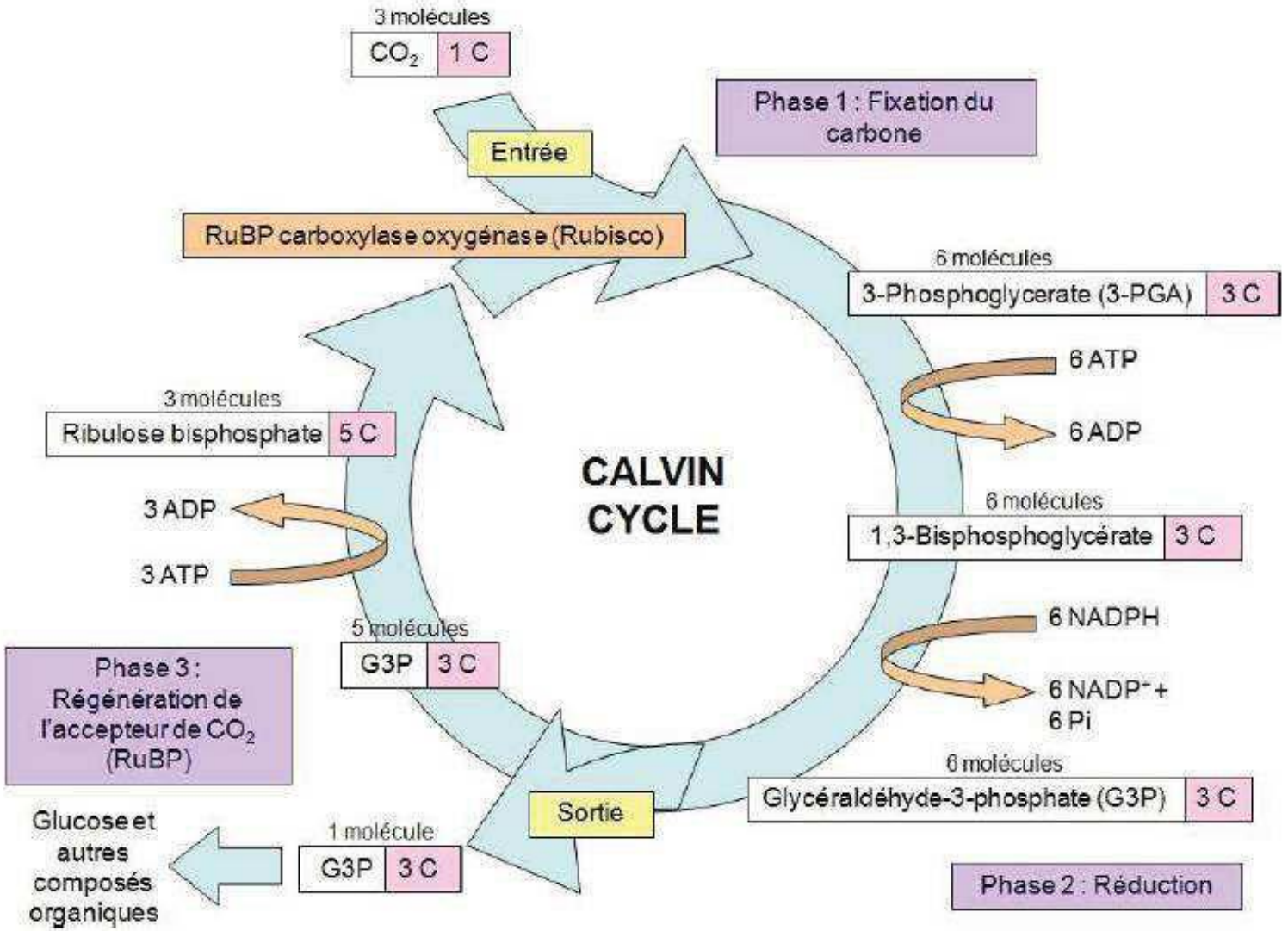
The **dark phase** corresponds to the CO_2 assimilation phase, which uses the energy molecules produced during the light phase and is carried out cyclically. This cycle is called the **Calvin cycle** and takes place in the stroma of the chloroplast. CO2 is assimilated in four main stages, the first three of which take place within the Calvin cycle: CO2 fixation (carboxylation), reduction of the fixed carbon, regeneration of the CO2 acceptor and synthesis of sugars.

- The Calvin cycle
    Sugar synthesis
    Balance sheet
    Photosynthesis yield

The Calvin cycle comprises 3 stages: Carboxylation, reduction and regeneration.

a. The cycle of Calvin

- **CO_2 fixation** : The 1st molecule in the Calvin cycle is ribulose-biphosphate (RuBP), which has 5 carbons. Fixing CO2 on this molecule requires the use of an enzyme (Rubisco) (for Ribulose Biphosphate Carboxylase Oxygenase). This enzyme allows the formation of an unstable 6-carbon molecule which rapidly gives rise to 2 molecules of 3-phosphoglycerate with 3 carbons.



Mode of action of the Rubisco

Rubisco has 2 catalytic activities:

Carboxylase activity: 2 molecules of **phosphoglyceric acid** are formed from RuBP.

Oxygenase activity: One molecule of **phosphoglycolic acid** and one molecule of **phosphoglyceric acid (PGA)** are formed from **RuBP**. This 2nd activity slows down photosynthesis and prevents the Calvin cycle from continuing.

- Reduction of fixed carbon :

This 2nd phase of the Calvin cycle corresponds to the reduction of 3-phosphoglycerate. The latter is phosphorylated by ATP to form biphosphoglyceric acid, which in turn is reduced by NADPH to form **3-phosphoglyceraldehyde (G3P)**, which is the sugar.

- Regeneration of the CO_2 acceptor

The G3P formed can have different uses; 1/6th of it will be used by the cell as a carbohydrate component and the remaining five-sixths will be used to continue the Calvin cycle. The reformation of RuBP, which will be reused to fix CO2, will take place in several stages and will require the use of ATP.

-Synthesis of sugars

As we have already seen, one-sixth of the 3-phosphoglyceraldehyde (G3P) produced in the Calvin cycle will enter the plant's metabolic reactions, in which it will mainly be converted into carbohydrates: - Either in the form of sucrose (α-Glu-Fruct), which is the form transported in the elaborated sap.

- Or in the form of starch, which is the form stored (α-1,4-Glu).