Chapter II: Regulation of Carbon Metabolism and Resource Allocation

1. Regulation of the Calvin–Benson Cycle: Light-Dependent Activation (Ferredoxin–Thioredoxin System) and Metabolic Control

The regulation of the Calvin–Benson cycle is essential for coordinating photosynthetic carbon fixation with the availability of light energy and preventing futile cycles at the end of the day or during the night. This regulation is mainly based on two complementary mechanisms: light-dependent activation through the ferredoxin–thioredoxin system and metabolic/allosteric control.

1.1. Light-Dependent Activation: The Ferredoxin–Thioredoxin System

During the light phase, the thylakoids produce ATP and NADPH molecules. At the same time, electron flow enables the selective reduction and activation of key enzymes involved in the Calvin–Benson cycle.

The electron transfer pathway:

- **Light and Photosystem I (PSI):** Photons excite PSI, leading to the reduction of ferredoxin (Fd) on the stromal side of the thylakoid membrane.
- **Ferredoxin–Thioredoxin Reductase (FTR):** Reduced ferredoxin transfers its electrons to FTR, an enzyme containing a [4Fe–4S] iron-sulfur cluster.
- **Thioredoxin (Trx):** FTR reduces the disulfide bond of thioredoxin, converting it from its oxidized form (-S-S-) into its reduced form (-SH, HS-).
- **Target enzymes:** Reduced thioredoxin cleaves the inhibitory disulfide bonds of target enzymes in the Calvin–Benson cycle, converting them from an inactive conformation into an active one.

Light-dependent activation pathway:

Light → PSI → Ferredoxin (Fd red) → FTR → Thioredoxin (Trx red) → Target enzyme (Active -SH, HS-)

Main enzymes regulated by thioredoxin:

- **Fructose-1,6-bisphosphatase (FBPase):** Catalyzes an irreversible step involved in carbohydrate synthesis and regeneration.
- **Sedoheptulose-1,7-bisphosphatase (SBPase):** Catalyzes an essential irreversible step in the regeneration of ribulose-1,5-bisphosphate (RuBP).

- **Phosphoribulokinase (PRK):** Catalyzes the final phosphorylation step required for RuBP regeneration.
- **Glyceraldehyde-3-phosphate dehydrogenase (GAPDH):** Catalyzes a reduction step using NADPH.

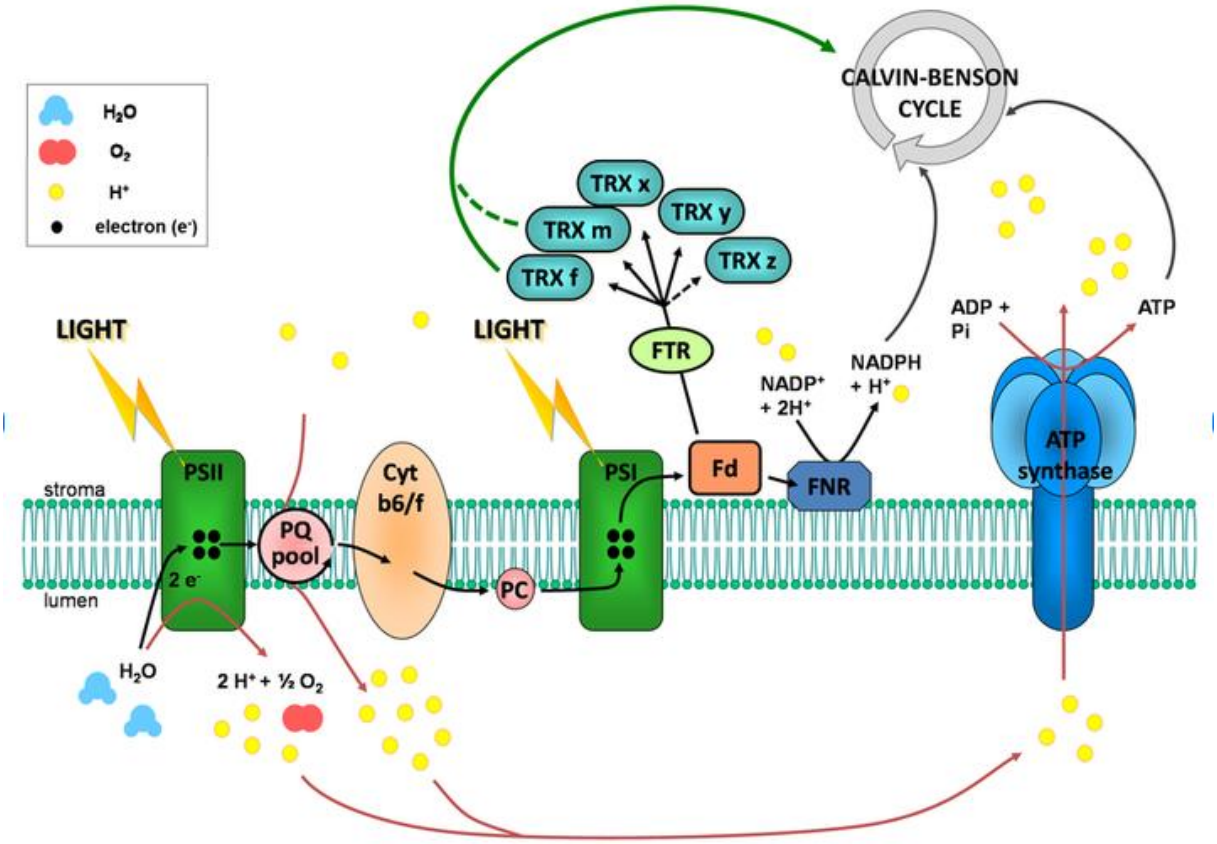

Figure 20. The photosynthetic electron transport chain and the reduction of chloroplastic thioredoxins (TRXs)**.** *In the thylakoid membrane, when Photosystem II (PSII) is excited by the absorption of light energy, its reaction-center chlorophyll molecule loses an electron. This electron is transferred to the plastoquinone pool (PQ), which takes up a proton (H^+) from the stroma. Following electron loss, the oxidized reaction-center chlorophyll becomes a strong oxidizing agent capable of extracting electrons from water molecules, resulting in the release of oxygen (O_2) and protons (H^+) into the thylakoid lumen. The chlorophyll molecule can then be excited again by light absorption.*

Reduced plastoquinone moves through the thylakoid membrane from PSII to the cytochrome b6/f complex (Cyt b6/f), where it is oxidized and releases its protons into the lumen, contributing to proton transport from the stroma to the lumen. Its electrons are subsequently transferred to Photosystem I (PSI) through cytochrome b6/f and plastocyanin (PC).

This electron transfer enables the reduction of the excited reaction-center chlorophyll of PSI. Upon excitation, this chlorophyll transfers its electron to stromal ferredoxin (Fd), which can then transfer electrons to chloroplastic thioredoxins (TRXs) through ferredoxin–thioredoxin reductase (FTR), or reduce $NADP^+$ through ferredoxin–$NADP^+$ reductase (FNR).

Water photolysis and proton transport through plastoquinone contribute to the establishment of a proton gradient across the thylakoid membrane, between the stroma and the lumen. This electrochemical gradient provides the energy required by ATP synthase to produce ATP.

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1.1.1. Additional Stromal Factors Induced by Illumination

In addition to the Fd-Trx system, the activity of the light phase modifies the physicochemical environment of the stroma, thereby optimizing enzymatic activity.

- **Increase in stromal pH:** The pumping of protons (H^+) from the stroma into the thylakoid lumen by the electron transport chain increases the stromal pH from approximately 7.0 in darkness to approximately 8.0 under illumination. The enzymes of the Calvin–Benson cycle have an optimal activity at a pH close to 8.
- **Mg^{2+} efflux:** To compensate for the accumulation of protons in the thylakoid lumen, Mg^{2+} ions are released from the lumen into the stroma. Magnesium acts as an essential cofactor for RuBisCO, FBPase, and SBPase.

1.1.2. Specific Regulation of RuBisCO

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the key enzyme of the Calvin–Benson cycle; however, its regulation differs slightly from the direct Fd-Trx regulatory system.

- **Light-dependent carbamylation:** Activation requires the binding of a non-substrate CO_2 molecule to a lysine residue in the active site (lysine 201), followed by the binding of a Mg^{2+} ion. This process is favored by the alkaline pH and high Mg^{2+} concentration in the stroma under illumination.
- **Role of RuBisCO Activase (RCA):** In darkness, the active site of RuBisCO is often blocked by its substrate (RuBP) or by endogenous inhibitors, such as 2-carboxy-D-arabinitol-1-phosphate (CA1P). RuBisCO activase, which is itself regulated by

thioredoxin or the ATP/ADP ratio, uses energy from ATP hydrolysis to remove these inhibitors and restore access to the active site.

1.2. Metabolic Control and Allosteric Regulation

The Calvin–Benson cycle is also adjusted according to metabolite availability and the metabolic requirements of the cell, particularly the export of photosynthetic products to the cytosol or their storage in the form of starch.

Energy and reducing power ratios (ATP/ADP and NADPH/$NADP^+$)

- High levels of ATP and NADPH stimulate the activity of PRK and GAPDH.
- The accumulation of $NADP^+$ or ADP acts as a negative feedback signal, indicating a shortage of energy or reducing power.

Feedback inhibition and allosteric regulation by triose phosphates

- **FBPase and SBPase:** These enzymes can be inhibited by the accumulation of their products or metabolic intermediates, particularly excess inorganic phosphate (Pi) or certain sugar phosphates.
- **Regulation through triose phosphate export:** The triose phosphate/phosphate translocator (TPT) located in the chloroplast envelope exchanges triose phosphates (photosynthetic products) for cytosolic inorganic phosphate (Pi). When the cytosol becomes saturated with sugars, Pi import into the stroma decreases, thereby slowing ATP synthesis and consequently reducing the activity of the Calvin–Benson cycle.

Comparative Overview of Dark and Light Conditions

- **Dark Conditions (Inactivation Phase):**

In the absence of light photons, the photosynthetic electron transport chain becomes inactive. Consequently, the production of the energy-rich compounds required for carbon assimilation (ATP and NADPH) stops, while the regulatory protein thioredoxin becomes oxidized. This leads to the inactivation of key enzymes of the Calvin–Benson cycle, preventing unnecessary energy consumption and futile metabolic cycles.

- **Light Conditions (Activation Phase):**

Under illumination, light energy activates the light-harvesting complexes of the thylakoid membranes, triggering electron transport and the production of ATP and NADPH. These

changes are accompanied by physicochemical modifications in the stroma, including an increase in pH and Mg^{2+} concentration, as well as the reduction of thioredoxin. Together, these mechanisms promote the activation of the Calvin–Benson cycle, enabling efficient CO_2 assimilation and organic matter production.

Table 1: Comparative Summary of Calvin–Benson Cycle Regulation under Dark and Light Conditions

Parameter / Mechanism	**Darkness**	**Light**
Thioredoxin	Oxidized (-S-S-)	Reduced (-SH, HS-)
Regulated enzymes (FBPase, SBPase, PRK)	Inactive (disulfide form)	Active (reduced thiol form)
Stromal pH	Approximately 7	Approximately 8
Stromal Mg^{2+} concentration	Low	High
RuBisCO	Inhibited by inhibitors / Non-carbamylated	Carbamylated with Mg^{2+} / Active
Calvin–Benson cycle activity	Inactive (prevents ATP wastage)	Maximum activity

2. The Rubisco Dilemma: Mechanisms of Photorespiration and Bypass Strategies (C4 and CAM) from the Perspective of Water-Use Efficiency (WUE)

2.1. Mechanisms of Photorespiration

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is an enzyme responsible for fixing carbon dioxide during photosynthesis. It plays a central role in the Calvin–Benson cycle and is considered one of the most important enzymes in the plant kingdom.

The Rubisco dilemma lies in its dual catalytic activity: it can fix both carbon dioxide (CO_2) through carboxylation and oxygen (O_2) through oxygenation. This lack of absolute substrate specificity leads to photorespiration (the C2 cycle), a metabolically and energetically costly process that reduces the net efficiency of photosynthesis, particularly under hot and dry climatic conditions, when stomata close to limit water loss.

The evolutionary limitation of Rubisco lies in its inability to perfectly distinguish between CO_2 and O_2 molecules at its active site.

Consequently, Rubisco exhibits two competing activities:

A. CO_2 Fixation (Carboxylation)

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is the key enzyme responsible for the initial fixation of inorganic carbon (CO_2) into organic matter during the Calvin–Benson cycle. It is the most abundant enzyme in the biosphere, accounting for up to 50% of the soluble proteins in leaves.

Its primary function is to catalyze the carboxylation reaction:

The resulting 3-phosphoglycerate (3-PGA) then directly enters the Calvin–Benson cycle, where it is used to produce triose phosphates (sugars).

B. O_2 Fixation (Oxygenation)

The Rubisco dilemma also results from its inability to distinguish perfectly between CO_2 and O_2 molecules. Its active site can bind either molecule, leading to a competing oxygenase activity.

- **Oxygenase activity:** The fixation of O_2 leads to the following reaction:

2-phosphoglycolate (2-PG) is a toxic metabolic compound that inhibits several key chloroplastic enzymes, such as triose-phosphate isomerase. Therefore, plants must detoxify and recycle this compound.

To convert 2-phosphoglycolate into 3-PGA, plant cells activate photorespiration, also known as the C2 cycle. This process functions as an inter-organelle recycling pathway that detoxifies 2-PG and recovers approximately 75% of the carbon involved.

This complex metabolic pathway involves three organelles operating in sequence: the chloroplast, peroxisome, and mitochondrion.

1. in the Chloroplast (Initial Stage)

The oxygenation reaction:

- **Formation of products:** When Rubisco fixes O_2 instead of CO_2, it produces one useful molecule of 3-PGA and one molecule of toxic 2-phosphoglycolate (2-PG).

- **Dephosphorylation:** A specific enzyme, 2-phosphoglycolate phosphatase, removes the phosphate group from 2-PG to produce glycolate.
- **Export:** Glycolate leaves the chloroplast through a membrane transporter and enters the peroxisome.

2. In the Peroxisome (Oxidation and Transamination)

- **Oxidation:** Glycolate is oxidized into glyoxylate by glycolate oxidase.

An important point is that this reaction produces hydrogen peroxide (H_2O_2), a highly toxic compound. It is immediately broken down into water (H_2O) and oxygen (O_2) by catalase, an enzyme present in high concentrations in the peroxisome.

- **Transamination:** Glyoxylate receives an amino group (NH_2) transferred from an amino acid, usually glutamate, to form glycine, a two-carbon amino acid.
- **Export:** Glycine is transported from the peroxisome to the mitochondrion.

3. In the Mitochondrion (Decarboxylation and CO_2 Loss)

This is the key stage of photorespiration:

Two molecules of glycine, containing a total of four carbon atoms, are processed by the glycine decarboxylase complex.

The main reactions include:

- **Decarboxylation:** One carbon atom is removed and released as CO_2, representing a net carbon loss associated with photorespiration.
- **Deamination:** An amino group is released in the form of ammonia (NH_3).
- **Condensation:** The remaining three carbon atoms are combined to form one molecule of serine, a three-carbon amino acid.
- **Reduction:** One molecule of NADH and H^+ is produced.

4. Return Pathway (Carbon Recovery)

- **Return to the peroxisome:** Serine (3C) leaves the mitochondrion and returns to the peroxisome, where it undergoes deamination to form hydroxypyruvate, which is subsequently reduced to glycerate.

- **Return to the chloroplast:** Glycerate is transported back into the chloroplast.
- **Phosphorylation:** Using one ATP molecule, glycerate kinase phosphorylates glycerate to regenerate 3-PGA.
- **Reintegration:** The regenerated 3-PGA re-enters the Calvin–Benson cycle and can be used for sugar synthesis.

2.2. Negative Bioenergetic Consequences of Photorespiration

Photorespiration has several negative effects on plant metabolism:

- **Carbon loss:** The recycling of two molecules of 2-PG into one molecule of 3-PGA inevitably releases one molecule of CO_2 in the mitochondrion. This represents a net loss of approximately 25% of the carbon initially involved in the photorespiratory pathway.
- **Energy consumption without net sugar production:** The C2 cycle consumes additional ATP and reducing power to recover part of the carbon involved, without directly producing new sugars.
- **Nitrogen cost:** One molecule of ammonia (NH_3) is released in the mitochondrion and must be reassimilated through the GS-GOGAT system, which requires additional energy.

2.3. Environmental Impact: Hot and Dry Climates

Competition between CO_2 and O_2 at the active site of Rubisco depends strongly on environmental conditions.

- **Stomatal closure:** Under water stress, plants close their stomata to prevent dehydration. This reduces the entry of atmospheric CO_2, while O_2 continues to accumulate in the leaf as a result of photosynthetic activity.
- **Effect of temperature on gas solubility:** As temperature increases, the solubility of CO_2 in the aqueous phase of the cell decreases more rapidly than that of O_2. Furthermore, the relative specificity of Rubisco for CO_2 decreases, favoring oxygenation and increasing photorespiration.

2.4. Bypass Strategies: Spatial (C4) and Temporal (CAM) Separation

To eliminate or significantly reduce Rubisco oxygenation, C4 and CAM plants use phosphoenolpyruvate carboxylase (PEPC) as the primary enzyme for initial CO_2 fixation.

PEPC has a high affinity for bicarbonate ions (HCO_3^-) and does not exhibit oxygenase activity.

A. The C4 Pathway: Spatial Separation

C4 plants separate the initial fixation of CO_2 from its incorporation into the Calvin–Benson cycle by using two different types of specialized cells arranged in a specific anatomical structure.

Kranz Anatomy (Crown-like Arrangement)

At the histological level, bundle sheath cells, which surround the vascular tissues, are concentrically surrounded by mesophyll cells.

In the Mesophyll Cells (CO_2 Capture Stage)

- Atmospheric CO_2 enters the leaf through the stomata.
- PEPC fixes carbon onto phosphoenolpyruvate (PEP, 3C), producing oxaloacetate (OAA, 4C), hence the name C4.
- OAA is rapidly reduced to malate or converted into aspartate.

Transport and Decarboxylation in the Bundle Sheath Cells

- Malate (4C) is transported to the bundle sheath cells through plasmodesmata.
- It undergoes decarboxylation, catalyzed by NADP-dependent or NAD-dependent malic enzyme, or phosphoenolpyruvate carboxykinase (PEPCK).
- This reaction releases pyruvate (3C), which returns to the mesophyll cells to regenerate PEP, while CO_2 is released and concentrated around Rubisco.

Main Benefit:

By increasing the CO_2 concentration around Rubisco (potentially reaching 10–20 times the atmospheric concentration), C4 plants strongly suppress its oxygenase activity and consequently minimize photorespiration.

B. The CAM Pathway: Temporal Separation

CAM (Crassulacean Acid Metabolism) plants perform the entire process within the same type of specialized cell, usually succulent cells with large central vacuoles. However, they separate the different stages between night and day.

Nocturnal Phase (CO_2 Capture and Storage)

- **Environmental conditions:** At night, temperatures decrease and relative humidity increases, resulting in a lower vapor pressure deficit.
- **Mechanism:** Stomata open during the night, allowing CO_2 uptake while minimizing water loss through transpiration. PEPC fixes CO_2 in the form of malic acid.
- **Storage:** Malic acid accumulates in large quantities inside the central vacuole, causing tissue acidification during the night.

Daytime Phase (Decarboxylation and Carbon Assimilation)

- **Environmental conditions:** During the day, temperatures increase.
- **Mechanism:** Stomata close to prevent excessive water loss.
- **CO_2 release:** Malic acid leaves the vacuole and is decarboxylated, releasing CO_2 inside the leaf tissues.
- **Assimilation:** The internally released CO_2 reaches a high concentration, allowing Rubisco and the Calvin–Benson cycle to operate efficiently using ATP and NADPH produced by the light-dependent reactions, while minimizing daytime water loss.

2.5. Comparison of Water-Use Efficiency (WUE)

Water-use efficiency (WUE) measures the amount of carbon assimilated per unit of water lost through transpiration.

$$\mathrm{WUE} = \frac{\text{Assimilation nette de } CO_2\,(A)}{\text{Transpiration } (E)}$$

C4 and CAM metabolisms maximize water-use efficiency (WUE = A/E) through the following mechanisms:

- **Maintaining a high CO_2 gradient with reduced stomatal opening (C4 plants):** Thanks to the high affinity of PEPC for inorganic carbon, C4 plants can maintain high carbon assimilation rates even when stomatal conductance is reduced, thereby limiting water loss through transpiration.

- **Shifting transpiration to nighttime (CAM plants):** By opening their stomata only at night, CAM plants avoid transpiration during the hours when the vapor pressure deficit (VPD) between the leaf and the surrounding air is highest. This minimizes water loss (E) while maintaining carbon assimilation (A).

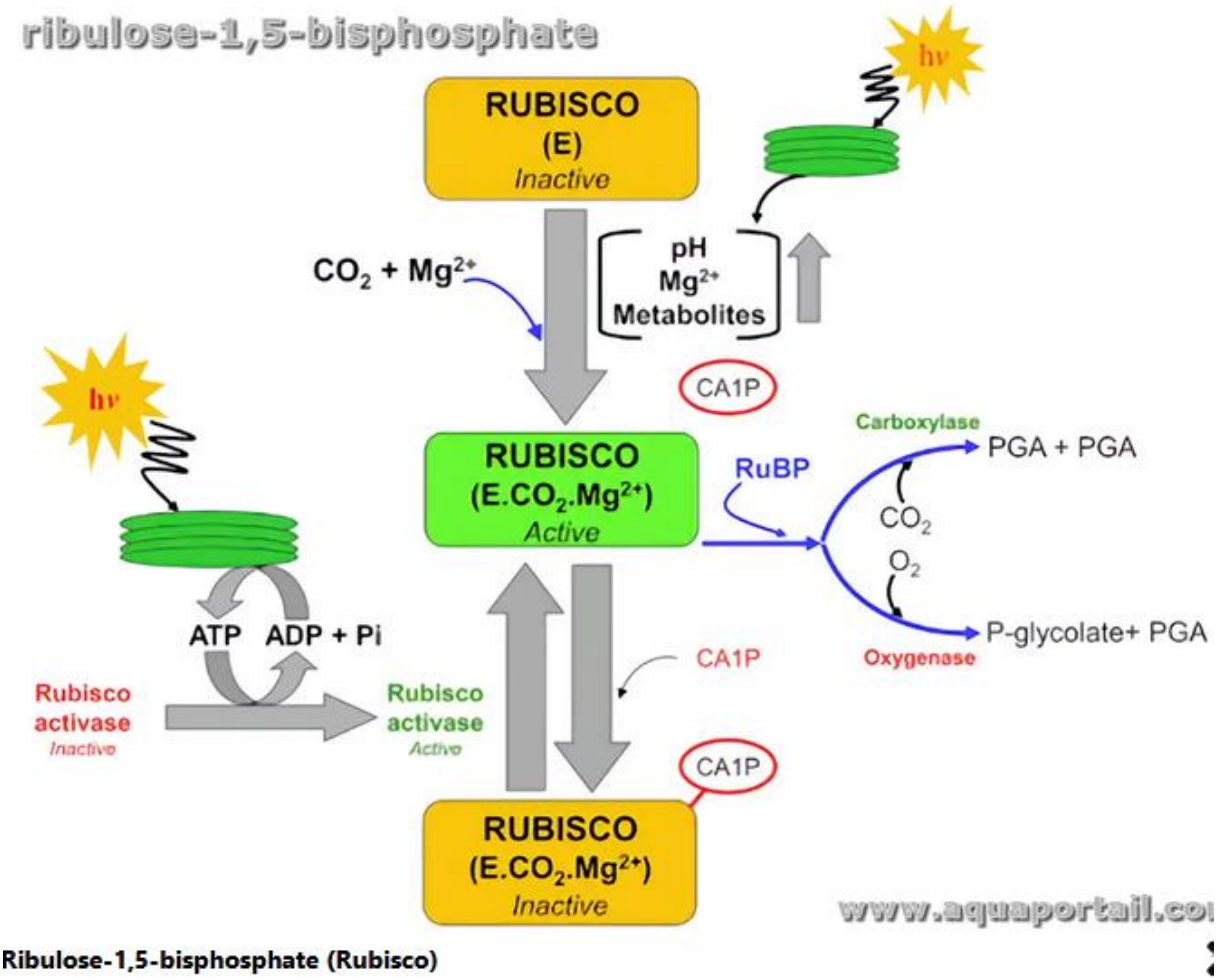

Figure 20. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) *catalyzes the addition of gaseous carbon dioxide (CO_2) to ribulose-1,5-bisphosphate (RuBP), producing two molecules of 3-phosphoglyceric acid (3-PGA). It is therefore the key enzyme responsible for CO_2 assimilation during photosynthesis.*

Inactive Rubisco (E) reacts with CO_2 to form a carbamate, which is stabilized by the binding of a Mg^{2+} ion, resulting in the formation of the active enzyme complex ($E–CO_2–Mg^{2+}$).

3. Source-Sink Relationships: Mechanisms of Phloem Loading and Unloading; Role of Sucrose Transporters (SUT/SWEET)

In plant physiology, source-sink relationships refer to the transport of nutrients (such as sugars) from a producing organ to a consuming or storage organ.

3.1. Mechanisms of Phloem Loading

Phloem loading is the process by which sucrose produced in the mesophyll is transferred into the sieve tube–companion cell complex (CC-ST).

- Sieve tubes: Transport the phloem sap.
- Companion cells: Support the functioning of sieve tubes, particularly in the loading and unloading of phloem sap.
- Sieve tube elements are living cells but lack a nucleus, whereas companion cells contain a nucleus.

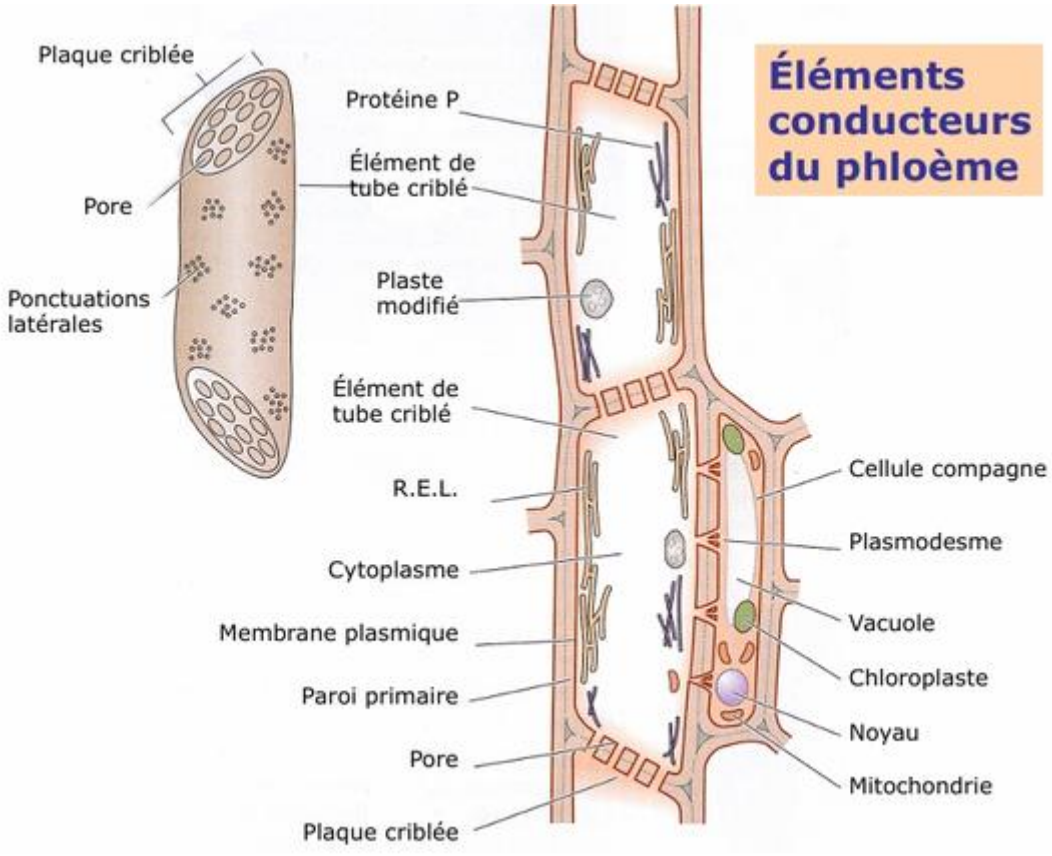

Fig. 20. Conducting elements of the phloem

There are two main pathways of phloem loading:

a. Apoplastic Loading (Active Loading)

This mechanism is mainly found in herbaceous plants (e.g., Arabidopsis, cereals, and potato).

Sucrose (Suc) is the main form of reduced carbon transported over long distances through the plant vascular system. It is synthesized in the cytoplasm, either directly from photosynthetic products or from stored reserves.

A portion of the sucrose is released into the apoplast (the free space formed by cell walls and intercellular spaces), particularly through SWEET family transporters via facilitated diffusion.

Because sucrose is a relatively large, polar molecule, its transport across the plasma membrane against a concentration gradient requires energy-dependent secondary active transport.

Active phloem loading takes place through several successive steps:

a. Establishment of the proton gradient (Driving Force)

- In the plasma membrane of companion cells and sieve elements, membrane pumps called H^+-ATPases consume energy in the form of ATP.
- These pumps actively transport hydrogen ions (H^+) from inside the cell to the outside (into the apoplast).
- The accumulation of protons in the apoplast creates an electrochemical gradient (a difference in H^+ concentration and electrical charge between the outside and inside of the cell).
- Protons naturally tend to move back into the cell down this gradient.

b. Sucrose cotransport (Secondary Active Transport)

Sucrose accumulated in the apoplast cannot freely cross the plasma membrane on its own because it is a relatively large, polar molecule.

Specific transporters called SUT/SUC use the energy released by the inward movement of H^+ to transport sucrose into the cell simultaneously.

This mechanism is known as symport: the proton enters the cell down its electrochemical gradient (a favorable process), providing the energy required to transport sucrose against its own concentration gradient (an unfavorable process).

c. Sucrose Accumulation and Phloem Sap Flow

Through this active transport mechanism, the sucrose concentration becomes very high inside the companion cell/sieve element complex.

This high sucrose concentration draws water osmotically from the xylem, increasing the local hydrostatic pressure. This pressure drives the movement of phloem sap toward the sink organs (roots, fruits, and young leaves).

Notes and Definitions

- **Apoplast:** The entire space outside the plasma membranes, including cell walls and intercellular spaces. It serves as the pathway through which sucrose passes before entering the phloem.

- **Companion Cell/Sieve Element Complex (CC/SE):** The functional unit of the phloem. The sieve element acts as the conducting tube for phloem sap, while the companion cell provides metabolic support and regulates membrane transport.

- **Secondary Active Transport:** A membrane transport mechanism in which the movement of a molecule against its concentration gradient does not directly consume ATP but instead uses the energy stored in an ion gradient (here, the H^+ gradient previously established by the H^+-ATPase pump).

- **SUT/SUC (Sucrose Transporter/Sucrose Carrier):** Specialized membrane proteins that function as sucrose/H^+ symporters.

- **Proton Motive Force (PMF):** The potential energy stored in the form of a pH gradient and an electrical potential difference across the plasma membrane following the outward transport of H^+ ions.

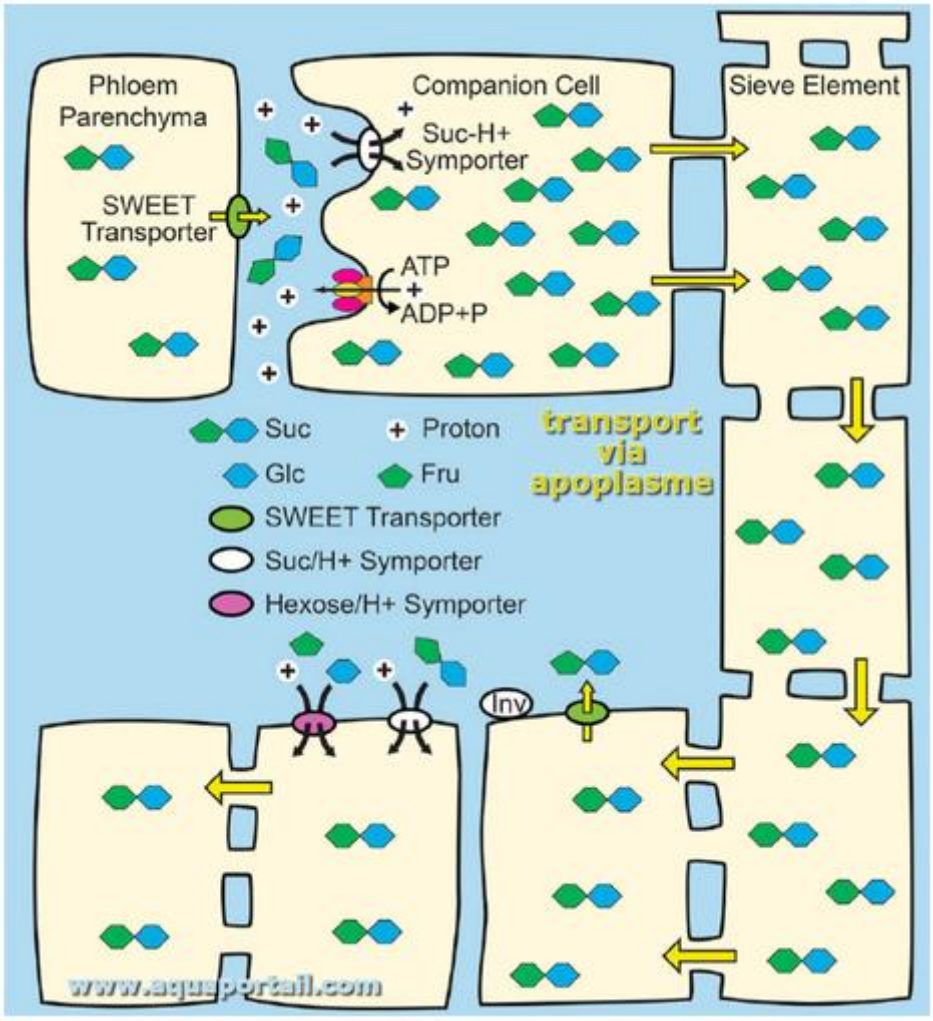

Fig. 21. Transport via the apoplast

b. Symplastic Loading (Passive Loading or Polymer Trapping)

This mechanism is found in many woody species and certain plant families (e.g., Cucurbitaceae).

Symplastic phloem loading takes place entirely within the continuous cytoplasmic network of plant cells, without passing through the cell wall space (the apoplast).

There are two main mechanisms: passive symplastic loading and polymer trapping.

⇨ **Passive Symplastic Loading**

Sucrose produced by photosynthesis in the mesophyll diffuses from cell to cell through numerous plasmodesmata.

This mechanism relies exclusively on a downhill concentration gradient and simple diffusion through regular plasmodesmata connecting the mesophyll cells to the sieve elements.

- Mesophyll cells accumulate very high concentrations of solutes (sucrose).
- Sucrose moves freely from cell to cell, from the mesophyll toward the phloem, following its concentration gradient.
- There is no active concentration step at the mesophyll/phloem interface.
- All cells maintain uniformly high hydrostatic pressures.

⇨ **Polymer Trapping**

This mechanism allows sugars to accumulate in the phloem through a biochemical reaction that prevents their backflow toward the mesophyll.

Sucrose entry into intermediary cells

- Sucrose (Suc) from the mesophyll passively enters specialized companion cells called intermediary cells through highly branched plasmodesmata.

Oligosaccharide synthesis (Polymerization)

Once inside the intermediary cell, sucrose is converted into more complex sugars belonging to the RFO (Raffinose Family Oligosaccharides) group:

- Galactinol (Gol) transfers a galactose unit to sucrose through the action of raffinose synthase, producing raffinose (Raf).
- Raffinose can then be converted into stachyose (Sta) by stachyose synthase.
- The resulting raffinose and stachyose molecules are too large to move backward through the plasmodesmata toward the mesophyll.
- However, the plasmodesmata connecting intermediary cells to sieve elements are wider, allowing RFOs to move into the phloem.

Maintenance of the Gradient and Osmotic Pressure

- The continuous conversion of sucrose into RFOs maintains a low sucrose concentration in the intermediary cells, sustaining the passive influx of sucrose.
- The accumulation of RFOs generates a strong osmotic water influx, creating the hydrostatic pressure required for phloem transport.

Notes and Definitions

- **Symplast:** The continuous network formed by the cytoplasm of all plant cells connected to one another.

- **Plasmodesmata (PD):** Microscopic channels that cross cell walls and establish direct connections between the cytoplasm and endoplasmic reticulum of adjacent cells.

- **Intermediary Cells:** Highly specialized companion cells containing numerous branched plasmodesmata connecting them to surrounding cells.

- **RFO (Raffinose Family Oligosaccharides):** A family of soluble oligosaccharides derived from sucrose, mainly including raffinose and stachyose.

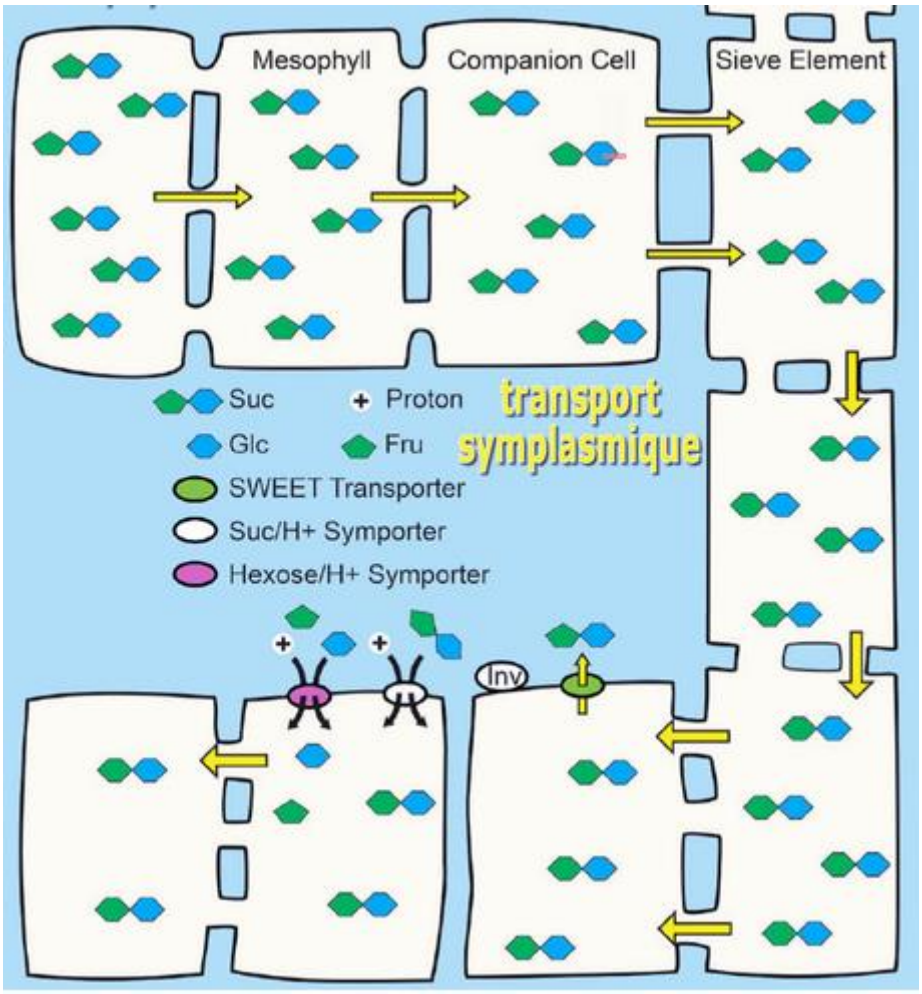

Fig. 23. Symplastic Transport

3.2. Long-Distance Transport (Münch Pressure-Flow Model)

- Sucrose accumulation in the phloem at the source lowers the osmotic potential.

- Water enters by osmosis from the adjacent xylem, generating high hydrostatic pressure (turgor pressure).
- The hydrostatic pressure difference between the source (high pressure) and the sink (low pressure) drives the mass flow of phloem sap along the sieve tubes.

3.3. Phloem Unloading

Phloem unloading occurs throughout the phloem transport pathway. Once nutrients reach the receiving organs (sinks), they leave the sieve tubes through two main pathways, which may operate independently or together:

- **Symplastic pathway:** Solutes move directly from cell to cell through plasmodesmata (PD), entering adjacent cells through their cytoplasmic connections.
- **Apoplastic pathway:** Solutes are released (efflux) across the plasma membrane into the apoplast (the cell wall space), then reabsorbed by neighboring cells.

Post-Phloem Unloading in Sink Organs

After leaving the sieve tubes, short-distance transport within sink tissues (post-phloem transport) depends on tissue connectivity.

A. Vegetative and Growing Organs (Symplastic Unloading)

Symplastic phloem unloading is the preferred mechanism for supplying actively growing vegetative organs, such as developing young leaves and root apices.

These tissues are characterized by intense metabolic activity and a high demand for metabolites to support cell division and elongation.

To ensure a rapid and continuous supply, the cells of the phloem complex (sieve elements and companion cells) are directly connected to neighboring recipient cells through a high density of plasmodesmata, forming an uninterrupted cytoplasmic continuum called the symplast.

Thanks to this structure, sucrose and other nutrients move freely and passively through these intercellular connections, following their concentration gradients without crossing the plasma membrane or entering the external apoplastic space.

This symplastic continuity allows particularly efficient, smooth, and energy-saving transport, ensuring a massive and uninterrupted flow of carbon compounds directly available for the growth and active metabolism of newly developing plant tissues.

Notes:

- **Symplastic Unloading:** An unloading pathway in which nutrients move from one cell to another while remaining within the continuous cytoplasm, passing directly through plasmodesmata without crossing the plasma membrane.
- **Plasmodesmata:** Microscopic channels crossing the cell wall that establish cytoplasmic and membrane continuity between two adjacent plant cells.
- **Symplast:** A continuous compartment formed by all interconnected cellular cytoplasms through plasmodesmata throughout plant tissues.
- **Apoplast:** The extracellular compartment consisting of cell walls and intercellular spaces, as opposed to the symplast.

B. Storage Organs and Seeds (Obligatory Apoplastic Unloading)

Obligatory apoplastic phloem unloading is an essential mechanism for supplying storage organs and reproductive structures, particularly developing seeds (including the embryo and endosperm) and certain ripening fruits.

In these tissues, nutrient transport encounters a major anatomical barrier: a discontinuity in cytoplasmic connections, known as symplastic isolation, which separates the maternal tissue from the filial tissue (the tissues of the new generation).

The process takes place through several coordinated steps:

- Initially, sucrose and other solutes leave the sieve elements of the phloem and move over short distances through the symplastic pathway (via plasmodesmata) within maternal tissues, such as the seed coat (testa).
- However, upon reaching the boundary between maternal and filial tissues, the complete absence of functional plasmodesmata interrupts the symplastic network and forces nutrients to enter the extracellular space, namely the apoplast.
- Once released into this intermediate apoplastic space, sugars are actively or passively reabsorbed across the plasma membranes of the endosperm and embryo through specific membrane transporters.

This obligatory passage through the apoplast not only allows nutrients to cross the anatomical barrier between two genetically distinct generations but also provides the plant with strict regulatory control over the flow of nutrients accumulated in seed or fruit reserves.

Notes:

- Obligatory Apoplastic Unloading: A process in which nutrients leave the phloem through the extracellular space (apoplast) because direct cytoplasmic connections between two tissues are absent.
- **Symplastic Isolation:** The absence or closure of plasmodesmata between adjacent cellular domains, preventing direct exchange of materials through the cytoplasm and creating a transport barrier.
- **Maternal Tissues:** Seed coverings (such as the seed coat or testa) belonging to the mother plant (parental genome).
- **Filial Tissues:** Structures of the new generation, including the embryo and endosperm, resulting from double fertilization (genomes derived from the parental cross).
- **Seed Coat (Testa):** The protective outer covering of the seed, originating from maternal tissue and traversed by phloem strands that initially supply nutrients.
- **Endosperm:** A storage tissue in angiosperm seeds that accumulates nutrients (starch, proteins, and lipids) to nourish the embryo during germination.
- **Membrane Reabsorption:** Active or facilitated uptake of solutes from the apoplast by transport proteins (such as SUTs or hexose transporters) located in the plasma membrane of recipient cells.

Fate of Sucrose in the Apoplast

Once released into the apoplastic space during unloading, sucrose (Suc) can follow two major metabolic pathways:

- **Intact Recovery (via SUT)**

- Sucrose is directly reabsorbed by adjacent cells (or retrieved by the phloem) using membrane sucrose transporters (SUT/SUC).

- **Enzymatic Hydrolysis (via Invertases)**

- Sucrose is broken down in the cell wall by apoplastic invertases (cell wall invertases) into hexoses: glucose and fructose.
- These hexoses are subsequently taken up by recipient cells through specific hexose/H^+ transporters.

4. Starch Metabolism: Transient Synthesis (Leaves) vs. Long-Term Storage (Storage Organs)

Starch metabolism in plants exhibits a marked functional and spatial distinction.

Although the basic biochemical pathways are similar, the dynamics, localization, and regulation differ considerably between source organs (leaves) and sink organs (seeds, tubers, and roots).

Plant starch metabolism is divided into two distinct strategies:

- Transient starch synthesis in photosynthetic tissues (leaves).
- Long-term starch storage in reserve organs (tubers, seeds, and roots).

Although the fundamental chemical reactions involved in glucose polymerization are similar, these two processes differ significantly in their cellular localization, regulation, and physiological purpose.

4.1. Transient Starch (Source Organs / Leaves)

Transient starch metabolism is the mechanism through which leaves manage their energy resources over a 24-hour cycle.

Transient starch acts as a short-term metabolic buffer, preventing excessive accumulation of photosynthetic products in the chloroplast during the day and ensuring a continuous supply of carbon during the night.

a. During the Day (Accumulation)

The plant carries out photosynthesis in leaf chloroplasts and produces excess sugars.

To prevent cellular overload and photosynthetic inhibition, a key enzyme, ADP-glucose pyrophosphorylase (AGPase), converts these sugars into starch, an insoluble storage form.

When photosynthesis is operating at a high rate, the concentration of 3-PGA increases, activating AGPase and promoting starch synthesis. Conversely, an increase in inorganic phosphate (Pi) inhibits this process.

b. During the Night (Mobilization)

In the absence of light, photosynthesis stops. The plant then breaks down the starch accumulated during the day, mainly releasing maltose.

This maltose leaves the chloroplast through the MEX1 transporter, enters the cytosol, and is subsequently converted into sucrose.

c. Export

The resulting sucrose is loaded into the phloem (the plant's transport system) to distribute energy to non-photosynthetic organs, such as roots, stems, and fruits.

Notes:

- **AGPase (ADP-glucose pyrophosphorylase):** A major regulatory enzyme in the starch synthesis pathway. It catalyzes the formation of ADP-glucose from glucose-1-phosphate (G1P) and ATP.
- **3-PGA (3-phosphoglycerate):** The first stable compound formed during CO_2 fixation in the Calvin cycle. Its accumulation indicates active photosynthesis and stimulates starch synthesis.
- **Pi (Inorganic Phosphate):** The free form of phosphate in the cell. An increase in Pi indicates limited energy availability (ATP) and inhibits starch synthesis.
- **Maltose:** A disaccharide composed of two glucose molecules. It is the main product of nighttime starch degradation.
- **MEX1 (Maltose Export 1):** A transport protein located in the chloroplast envelope that allows maltose to move into the cytosol.

4.2. Storage Starch (Sink Organs / Tubers and Seeds)

Storage starch represents the plant's long-term energy reserve. It is essential for survival during dormancy and provides the energy required for germination and vegetative regrowth. It is located in amyloplasts, which are non-photosynthetic plastids found in storage organs (e.g., starch-storing parenchyma cells in tubers and the endosperm of seeds). Storage starch metabolism involves two main phases:

- Filling Phase: Unidirectional accumulation over an extended period (weeks to months) during organ development.

- Mobilization Phase: Massive degradation triggered by developmental or environmental signals (e.g., dormancy release or gibberellin-induced germination).

The Developmental Cycle of Storage Starch

⇨ **Sucrose Transport and Breakdown**

Sugar produced in the leaves travels as sucrose through the phloem toward storage organs (tubers and seeds).

Once it reaches the target cell, sucrose is broken down in the cytosol by two possible enzymes: sucrose synthase or invertase, providing substrates for the formation of glucose-6-phosphate (G6P).

⇨ **Entry and Conversion in the Amyloplast**

G6P enters a specialized organelle called the amyloplast. Inside, amyloplastic AGPase converts the appropriate glucose-derived substrate into ADP-glucose.

⇨ **Starch Granule Formation (The Enzyme Team)**

Three main enzymes work together to assemble the starch granule:

- SS (Starch Synthase): Elongates glucose chains in a linear manner.
- SBE (Starch Branching Enzyme): Introduces branches to produce amylopectin.
- GBSS (Granule-Bound Starch Synthase): Produces long, unbranched chains of amylose.

This highly organized process forms hard, stable, semi-crystalline starch granules.

⇨ **Mobilization (Germination)**

Starch remains stored for months. When vegetative growth resumes or germination begins, plant hormones such as gibberellins trigger extensive starch degradation, supplying energy and carbon to the developing plant.

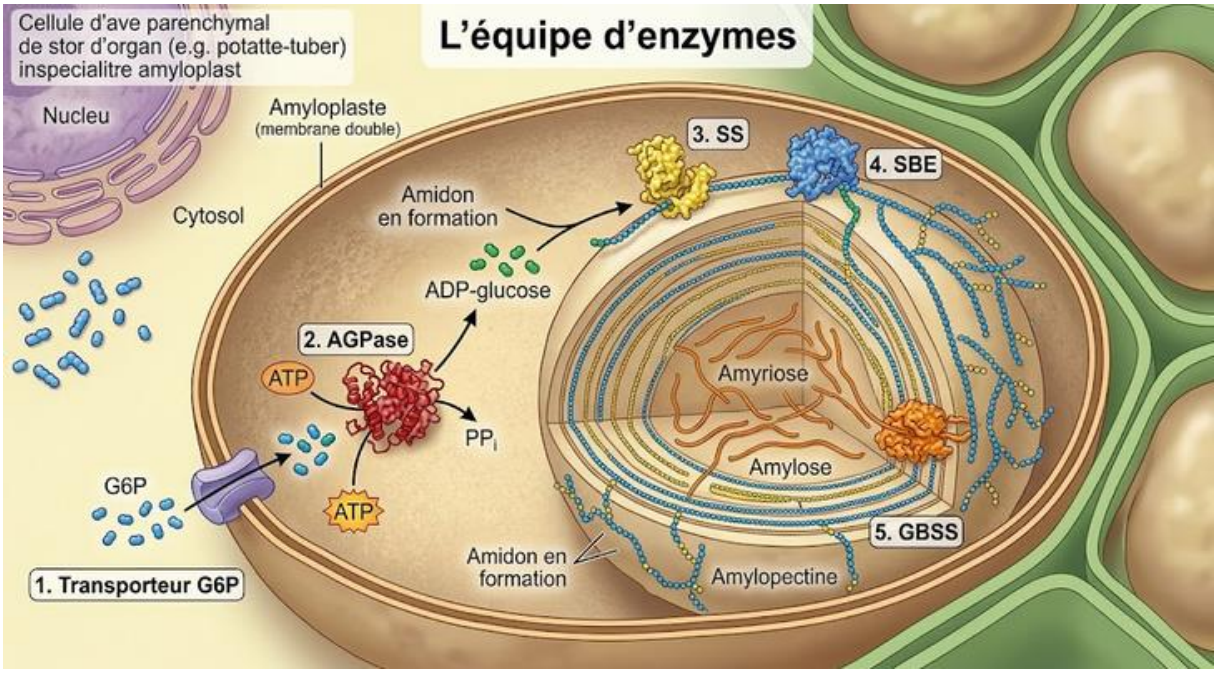

Fig. 25. Storage Starch

Note:

- **Storage starch:** A long-term carbohydrate storage form (weeks to years) accumulated in sink organs. It is stable and insoluble.

- **Amyloplast:** A non-photosynthetic plastid specialized in the synthesis and massive storage of reserve starch.

- **Sink organ:** A non-photosynthetic organ (seed, tuber, or root) that consumes or stores the carbon exported from source leaves.

- **Starch-storing parenchyma:** Plant storage tissue whose cells are filled with amyloplasts (e.g., the flesh of a potato).

- **Albumen (or Endosperm):** A nutritive tissue located inside the seed that stores energy reserves for the future seedling.

- **Sucrose Synthase / Invertase:** Cytosolic enzymes responsible for breaking down imported sucrose to release hexoses (simple sugars).

- **G6P (Glucose-6-phosphate):** The phosphorylated form of glucose that serves as a precursor imported into the amyloplast for starch synthesis.

- **Amyloplastic AGPase:** The form of AGPase specific to amyloplasts, regulated by the plant's developmental program rather than by light.

- **Starch Synthase (SS):** An enzyme responsible for the linear elongation of glucose polymer chains.

- **Starch Branching Enzyme (SBE):** An enzyme that creates branches (α-1,6 linkages) in glucose chains to form amylopectin.

- **GBSS (Granule-Bound Starch Synthase):** An enzyme embedded within the starch granule, specifically responsible for amylose synthesis.

- **Amylose / Amylopectin:** The two main components of starch. Amylose is a relatively simple, mostly linear chain, whereas amylopectin is a large, highly branched structure.