Chapter I: Membrane Transport and Bioenergetics

Introduction

Membrane transport refers to all the mechanisms by which substances (ions, molecules, and solutes) cross the cell membrane. These mechanisms are essential for homeostasis, which is the ability of a living organism to maintain a relatively stable and dynamic internal environment despite external changes and disturbances.

⇨ **Role of Regulatory Mechanisms**

- Ensure the proper functioning of physiological systems.
- Preserve the essential conditions required to sustain life.
- Facilitate cellular communication and adaptation to environmental changes.

⇨ **Role of Solute Transport**

(Diffusion of solutes and water across the membrane)

- Regulate cell volume.
- Adjust intracellular pH.
- Maintain ionic balance within the cell.

⇨ **Functions of Homeostasis**

- Maintain a stable and optimal internal environment for the organism.
- Regulate fundamental vital parameters, such as temperature, pH, ion concentrations, and blood pressure.

⇨ **Mechanisms of Homeostasis**

- Mainly rely on negative feedback loops.
- Detect changes and disturbances in vital parameters.
- Trigger corrective responses to restore these parameters to their normal (reference) values.

- **Structure of the Cell Membrane**

The cell membrane is a dynamic lipid bilayer in which proteins, lipids, and carbohydrates are dispersed, forming a fluid mosaic. It acts as a semi-permeable barrier separating the intracellular and extracellular environments.

Key Components and Their Functions:

- **Phospholipids:** Amphipathic molecules consisting of a hydrophilic head (oriented outward toward the aqueous environment) and hydrophobic tails (oriented inward), forming the core of the lipid bilayer.
- **Membrane Proteins:**

- **Integral proteins:** Embedded in or spanning the membrane.
- **Peripheral proteins:** Associated with the membrane surface.
- **Include transport proteins** (channels, carriers, and pumps), which are essential for the movement of solutes across the membrane.
  - **Cholesterol:** Regulates membrane fluidity and stabilizes its structure.
  - **Glycoproteins** and Glycolipids: Play important roles in cell recognition and cellular signaling.

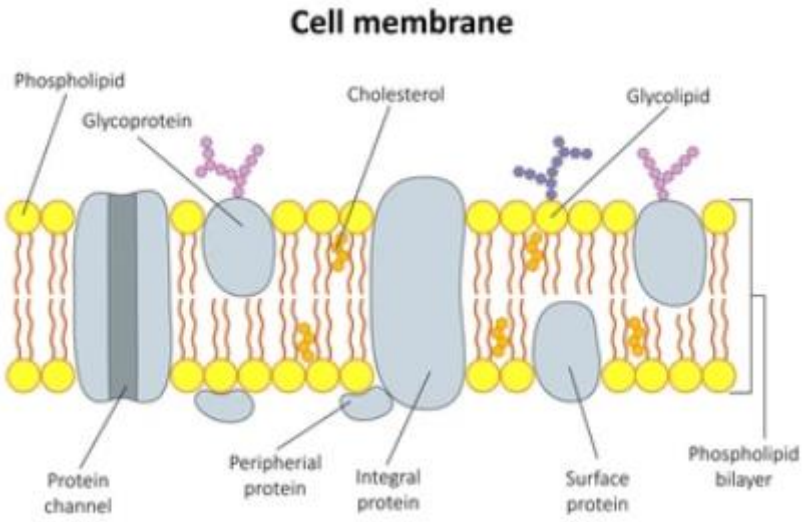

Fig. 1. Structure of the Cell Membrane

I. Thermodynamics of Transport

The thermodynamics of membrane transport is a branch of biophysics and physiology that studies the forces and energy governing the movement of molecules, ions, and water across biological membranes (such as the plasma membrane and the tonoplast).

The thermodynamics of transport is based on the application of the laws of thermodynamics to determine:

- **Direction of spontaneous transport:** Determines whether a substance will move spontaneously across the membrane.
- Type of transport in terms of energy requirements:
  - **Passive transport:** Occurs spontaneously without the consumption of biological energy (ATP), when a substance moves down (along) its electrochemical gradient.
  - **Active transport:** Requires cellular energy (such as ATP hydrolysis), because a substance moves against its electrochemical gradient.
- The main thermodynamic variables used are:
  - Chemical potential.
  - Electrochemical potential.
  - Nernst equation.

1.1. Chemical Potential

In physics, the chemical potential of a substance i is a measure of the free energy, or the energy available for the movement and diffusion, of one mole of that substance within a solution or cellular tissue.

The fundamental rule of transport is that substances spontaneously move from a region of higher chemical potential to a region of lower chemical potential.

From a thermodynamic point of view, adding a certain amount of substance i to a solution contributes energy to the system.

Under plant physiological conditions, where temperature (T) and pressure (p) are approximately constant, the chemical potential is defined as the partial molar Gibbs free energy, and its final equation is expressed as follows:

$$\mu i = \left(\frac{\partial G}{\partial ni}\right) p, T, nj = Gi$$

Explanation of the Components of the Equation

- **μ_i (Chemical potential):** The chemical potential of species i, expressed in joules per mole (J/mol).

- **G (Gibbs free energy):** The total free energy available in the cellular system.

- **n_i (Amount of substance):** The quantity of substance i, expressed in moles (mol).

- **($\partial G/\partial n_i$):** Represents the rate of change in the system's Gibbs free energy when the amount of substance i changes by an infinitesimal quantity.

- **Subscripts below the parenthesis (T, p, n_j):** Indicate that the measurement is performed under the following conditions:

  - Constant temperature (T).
  - Constant pressure (p).
  - Constant amounts of all other substances present in the solution (n_j).

- **G_i:** An abbreviated symbol representing the partial molar Gibbs free energy of substance i.

A. Importance of Chemical Potential in Plant Physiology

The importance of this concept lies in its ability to explain many biological processes within plants, including:

- **Water Absorption and Transport (Water Potential Ψw):**

  - The water potential of a plant is an expression of the chemical potential of water divided by its molar volume.
  - It explains how water moves spontaneously from the soil (higher water potential) toward the roots, then to the stem and leaves (lower water potential).

- **Ion Absorption Across Membranes:**

  - It helps determine whether the transport of ions (K^+, NO_3^-, etc.) across the cell membrane occurs through passive diffusion (along the chemical potential gradient) or

active transport (against the chemical potential gradient, requiring energy in the form of ATP).

- **Movement of Leaf Stomata:**

- The movement of ions and water into and out of the stomatal guard cells depends on changes in the chemical potential of ions inside these cells.

1.2. Electrochemical Potential

When the chemical species being studied carry an electrical charge (such as K^+, Na^+, Cl^-, or NO_3^- ions) and are located in an environment subjected to an electric field (for example, on either side of a biological membrane), the chemical potential alone (μ_i) is no longer sufficient to describe their behavior. In this case, it is necessary to use the electrochemical potential ($\bar{\mu_i}$).

The electrochemical potential ($\bar{\mu_i}$) of an ionic species i takes into account two types of energy:

- Chemical energy: Energy related to the concentration of the ion (chemical work).
- Electrical energy: Energy related to the electrical charge of the ion within the electrical potential of its environment (electrical work).

Its mathematical expression is written as follows:

$$\bar{\mu}i = \mu i + ziF\Phi$$

Explanation of the Components

- **$\bar{\mu_i}$ (Electrochemical potential):** The electrochemical potential of species i, expressed in joules per mole (J/mol).

- **μ_i (Chemical potential):** The classical chemical potential of species i, expressed in joules per mole (J/mol).

- **z_i (Valence):** The electrical charge (valence) of the ion.

- +1 for K^+.
- +2 for Ca^{2+}.
- −1 for NO_3^-.

- F (Faraday constant): The Faraday constant (F ≈ 96,485 C/mol).

- Φ (Electrical potential): The electrical potential of the environment or phase under consideration, expressed in volts (V).

- **Complete Electrochemical Potential Equation**

By expanding the chemical potential using its thermodynamic expression, the complete equation becomes:

$$\bar{\mu i} = \mu i \circ + RT\ lnai + ziF\Phi$$

Explanation of the additional components:

- $\mu_i°$ (Standard chemical potential): The standard chemical potential of species i.

- R (Universal gas constant): 8.314 $J \cdot mol^{-1} \cdot K^{-1}$.

- T (Absolute temperature): The absolute temperature expressed in Kelvin (K).

- a_i (Activity): The activity of species i in the solution, which can be approximated by its concentration in dilute solutions.

- **Application in Plant Physiology**

In plant cells, the plasma membrane maintains an electrical potential difference between the intracellular and extracellular environments, known as the membrane potential (ΔΦ).

- Electrochemical Gradient: The spontaneous movement of an ion across the membrane depends on the difference in electrochemical potential ($\Delta\bar{\mu_i}$).

- Passive Transport: If an ion moves down its electrochemical gradient ($\Delta\bar{\mu_i} < 0$), its entry into or exit from the cell occurs spontaneously without energy consumption. This takes place through ion channels or diffusion.

- Active Transport: If an ion must be accumulated against its electrochemical gradient ($\Delta\bar{\mu_i} > 0$), the cell must provide chemical energy in the form of ATP. This occurs through pumps such as the H^+-ATPase proton pump.

1.3. The Nernst Equation Applied to Plant Ions

The Nernst equation makes it possible to determine the electrochemical equilibrium potential (Eeq) of a given ion across the plasma membrane of a plant cell.

It helps determine whether an ion enters or leaves the cell passively (by diffusion under the influence of the electrochemical gradient) or whether active transport is required (energy consumption through a pump or cotransporter).

General Formula

For an ion X with valence z, the Nernst equation is written as:

$$Eeq = \frac{R.T}{Z.F}\ Ln\left(\frac{(X)EXT}{(X)INT}\right)$$

By converting to the decimal logarithm ($\log_{10}$) at the standard physiological temperature of 25°C (298 K):

$$Eeq = \frac{59.2}{Z.F}\ Log10\left(\frac{(X)EXT}{(X)INT}\right)$$

Explanation of the Components

- **Eeq:** Equilibrium potential of the ion (mV).
- **R:** Universal gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$).
- **T:** Absolute temperature (K).
- **z:** Electrical charge (valence) of the ion:
  - +1 for K^+.
  - +2 for Ca^{2+}.
  - −1 for NO_3^- or Cl^-.
- **F:** Faraday constant (96,485 $C \cdot mol^{-1}$).
- **[X]ext:** Ion concentration (or activity) in the external environment (apoplast/soil).
- **[X]int:** Ion concentration (or activity) inside the cell (cytosol).

- **Specific Characteristics of the Membrane Potential in Plants**

Unlike animal cells, whose resting membrane potential (Em) is maintained mainly by the Na^+/K^+-ATPase pump at approximately −70 mV, plant cells possess a highly active plasma membrane H^+-ATPase pump.

- The actual membrane potential (Em) of plant cells is strongly negative, generally ranging between −120 mV and −200 mV.

- Thermodynamic interpretation is based on comparing the measured membrane potential (Em) with the theoretical equilibrium potential (Eeq) calculated using the Nernst equation:

$$\Delta E = E_m - E_{eq}$$

Interpretation of the Results

- **If Em = Eeq:** The ion is at electrochemical equilibrium (no net ion flux).

- **If the electrochemical driving force favors ion movement:** Transport is passive through ion channels or passive carriers.

- **If the ion accumulates against the electrochemical driving force:** Active transport is required through pumps or H^+/ion cotransporters.

- **Application to the Main Ions in Plants**

A. Potassium (K^+)

- Valence: z = +1.

- Behavior:

- At a high external concentration, the equilibrium potential (Eeq K^+) is close to the membrane potential (Em). Potassium absorption then occurs passively through inward-rectifying potassium channels (e.g., KAT1).
- At a low external concentration in the soil, Eeq K^+ becomes significantly more negative than Em. Potassium uptake then requires secondary active transport through H^+/K^+ symporters or HAK-type transporters.

B. Anions (NO_3^-, Cl^-, $H_2PO_4^-$)

- Valence: z = −1 (or −2, depending on the ion).

- Behavior:

- Since the cytoplasm is highly electronegative (e.g., −160 mV), the electrical force strongly repels anions toward the outside.
- To allow anions such as NO_3^- or Cl^- to enter against their concentration gradient and the electrical potential, the Nernst equilibrium potential (Eeq) is much higher than Em (often around +30 to +50 mV).
- Conclusion: The uptake of major anions generally requires active transport, mainly through H^+/NO_3^- or $H^+/H_2PO_4^-$ symporters coupled to the proton-motive force.

C. Calcium (Ca^{2+})

- Valence: z = +2.

- Behavior:

- The electrochemical gradient strongly favors Ca^{2+} entry into the cytosol, where its concentration is extremely low (approximately 100 nM).
- Passive Ca^{2+} entry through ion channels acts as an important biological signal.
- In contrast, Ca^{2+} extrusion toward the apoplast or its sequestration into the vacuole requires active transport through Ca^{2+}-ATPases or H^+/Ca^{2+} antiporters.

2. P-Type and V-Type ATPases: The Central Role of the Proton Pump (H^+-ATPase) as the Primary Driving Force for Transport

ATPases (adenosine triphosphatases) are enzymes found in all living organisms. They catalyze the hydrolysis of the terminal phosphoanhydride bond of adenosine triphosphate (ATP), producing adenosine diphosphate (ADP) and inorganic phosphate (Pi).

This hydrolysis releases a significant amount of free energy, which is used by the cell to drive many essential biological processes. Depending on the cell type, this energy is used for:

- Transporting ions and solutes across membranes.

- Movement of fibers, particularly during muscle contraction.
- Various energy metabolism reactions and cellular signaling processes.

Although some ATPases are soluble enzymes, many are membrane proteins. Based on their structure and function, these membrane transporters are classified into several major families:

- **P-type ATPases (Phosphorylated ATPases):** Mainly involved in the active transport of ions across biological membranes.

- **V-type ATPases (Vacuolar ATPases):** Responsible for acidifying intracellular compartments.

- **F-type ATPases (or ATP synthases, F_0F_1-ATPases):** Mainly located in mitochondria, chloroplasts, and bacteria, where they function primarily in the synthesis of ATP using an electrochemical proton gradient.

- **A-type ATPases (Archaeal ATPases):** Found mainly in archaea and involved in ion transport and ATP synthesis.

- **E-type ATPases (Ectonucleotidases):** Located on the extracellular surface of cell membranes and involved in the hydrolysis of extracellular nucleotides.

2.1. V-ATPase

The V-ATPase (Vacuolar ATPase) is a large, multimeric proton-pumping protein (composed of several subunits). It is found in the membranes of cellular organelles (vacuoles, lysosomes, endosomes, and secretory vesicles), as well as in the plasma membrane of certain specialized cells.

It functions as a rotary motor that pumps protons (H^+) across membranes against their concentration gradient, using energy derived from ATP hydrolysis.

2.1.1. Structure of the V-ATPase

The pump consists of two main functional domains:

1. The Cytosolic V_1 Domain

- Located in the cytosol (peripheral region of the membrane).
- Composed of eight different subunits (A–H).
- Contains the catalytic site responsible for ATP hydrolysis (ATP → ADP + Pi), mainly carried out by subunits A and B.

2. **The Membrane V_0 Domain**

- Embedded in the phospholipid bilayer of the membrane.
- Composed of several subunits (a, c, c′, c″, d, and e).
- Forms the proton (H^+) translocation pathway, allowing protons to cross the membrane.

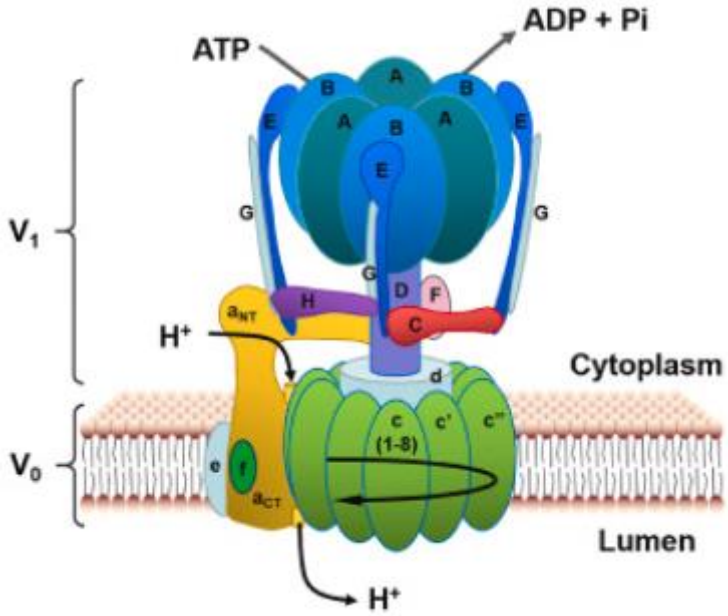

Fig. 1. Structure of the V-ATPase.

2.1.2. Mechanism of Action

The functioning of the V-ATPase is based on a rotary motor mechanism:

- ATP Hydrolysis: The reaction takes place in the V_1 catalytic complex through the A/B subunits, generating mechanical energy.

- Rotation of the Central Stalk: The energy released by ATP hydrolysis drives the rotation of the central stalk (the rotor, composed of subunits D, F, d, and the c-ring).

- Proton Pumping: This rotational movement induces conformational changes in the c-ring of the V_0 domain. The interaction between acidic residues (e.g., glutamate) in the c-ring and arginine residues in subunit a allows H^+ protons to be transferred from the cytosol into the lumen of the organelle.

2.1.3. Functional Roles of the V-ATPase

Beyond its basic role in proton pumping, the V-ATPase performs several essential functions:

- Acidification of Cellular Organelles: It lowers the intraluminal pH of lysosomes and secretory vesicles (approximately pH 4–5), which is essential for activating hydrolytic enzymes and supporting secondary active transport.

- pH Sensor: It acts as a sensitive detector of changes in proton concentration, thereby regulating membrane fusion processes.

- Dynamic Regulation Through Reversible Dissociation: Under certain cellular conditions (such as energy depletion or pH variations), the V_1 domain can temporarily dissociate from the V_0 domain, stopping proton pumping to conserve ATP.

- Membrane Fusion and Exocytosis: The subunits of the V_0 membrane domain play a direct role, independently of proton pumping, in the formation of fusion pores and the release of neurotransmitters and hormones.

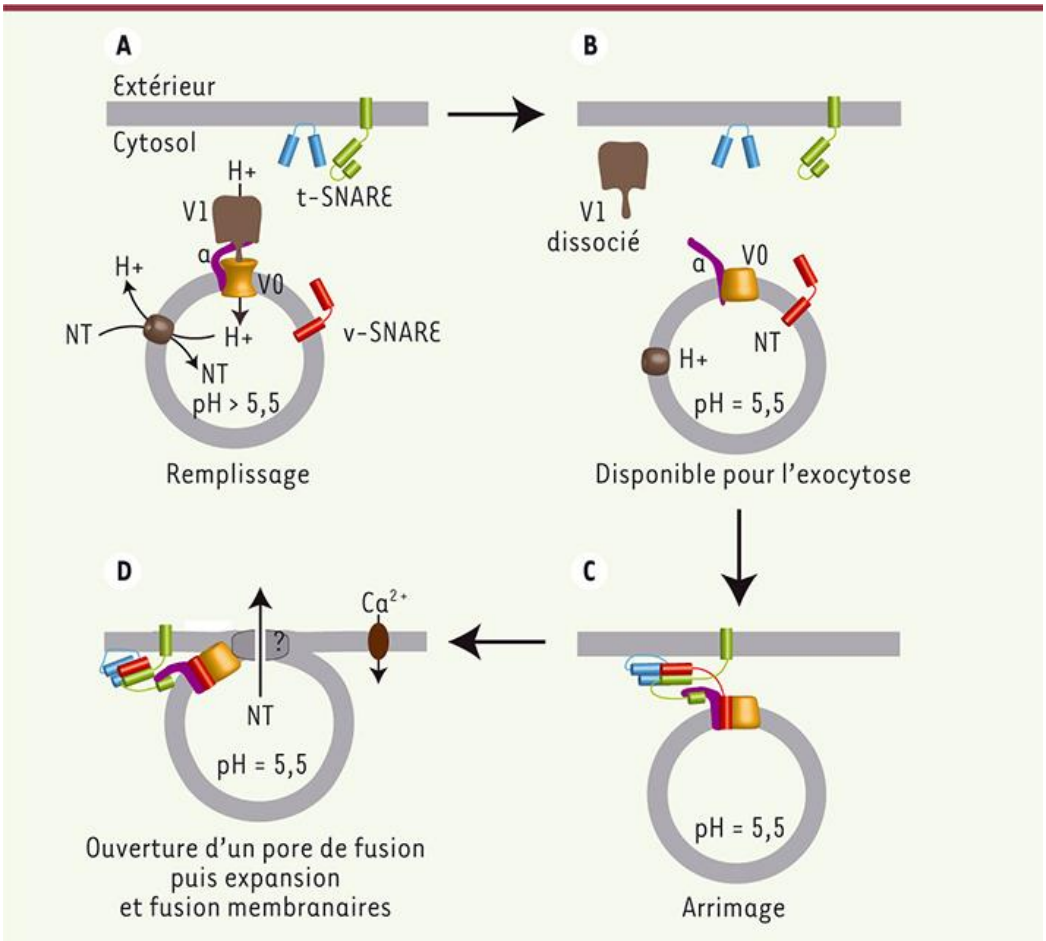

Fig. 2. Model illustrating the mechanism by which V-ATPase regulates neurosecretion. *At pH > 5.5, the V_1–V_0 complex enables neurotransmitter (NT) loading into the vesicle by generating a proton gradient (A). Dissociation of the V_1–V_0 complex at acidic pH (B) makes V_0 available to interact with the v-SNARE protein VAMP-2 (red) and the t-SNARE protein syntaxin-1 (green). This interaction promotes the formation of complexes involving VAMP-2, syntaxin-1, and SNAP-25 (blue), thereby allowing the synaptic vesicle to dock at the plasma membrane (C). Upon stimulation, V_0 regulates the formation and/or opening of a fusion pore (D), whose expansion ultimately leads to membrane fusion.*

2.2. P-Type ATPases

P-type ATPases are primary active transport membrane proteins that are essential for the functioning of plant cells. They use the energy released from ATP hydrolysis to transport cations or lipids across membranes (plasma membrane, tonoplast, and endoplasmic reticulum) against their concentration gradients.

They are called "P-type" because a transient phosphorylated intermediate is formed during the catalytic cycle, in which a phosphate group from ATP is transferred to a conserved aspartate residue of the enzyme.

2.2.1. Mechanism of Action (The Post-Albers Cycle)

The transport mechanism is based on a transition between two major structural conformations (E1 and E2).

A. Architecture of the Protein Complex

- **N Domain (Nucleotide-binding):** Binds the ATP molecule and possesses kinase activity.

- **P Domain (Phosphorylation):** Contains the conserved aspartate residue that undergoes autophosphorylation.

- **A Domain (Actuator):** Acts as a phosphatase to release the phosphate group and reset the pump.

- **Transmembrane Domain (T/M):** Forms the transport pathway and contains specific ion-binding sites.

B. Steps of the Transport Cycle

a. **E1 State:** The pump is open toward the cytosolic side and has a high affinity for the ions to be transported.
b. **Phosphorylation (E1 → E1P):** ATP binds to the N domain and transfers its gamma phosphate group (γ-phosphate) to the P domain.
c. **Conformational Change (E1P → E2P):** Phosphorylation induces a rearrangement of the transmembrane helices. The pathway opens toward the outside of the cell (or the organelle lumen), reducing the affinity for the ions, which are subsequently released.
d. **Dephosphorylation (E2P → E2 → E1):** The A domain catalyzes phosphate hydrolysis. The protein returns to its initial E1 conformation, ready to begin another transport cycle.

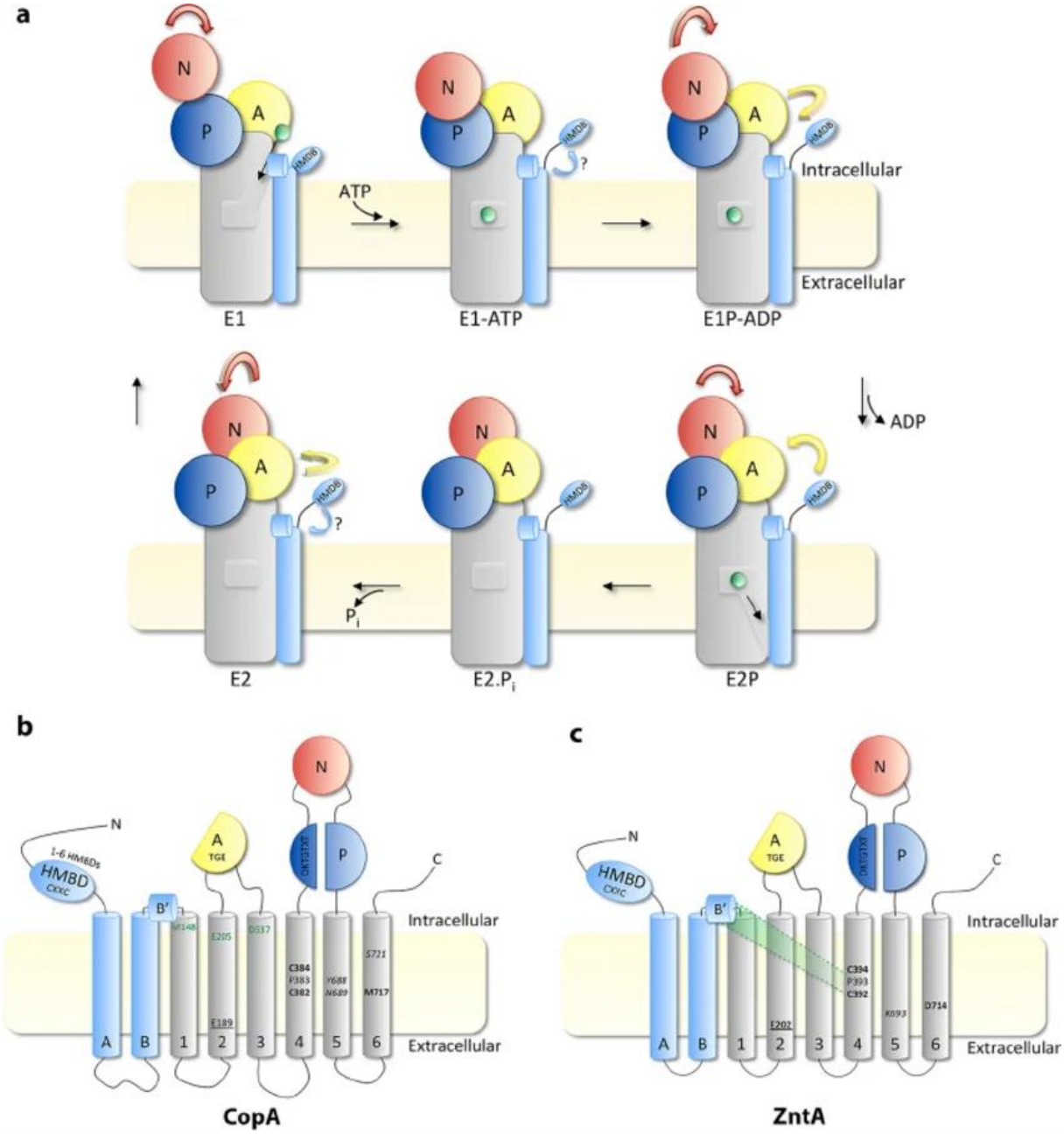

Fig. 3. Schematic representation of the reaction cycle and topology of P1B-type ATPases. *These proteins consist of an M domain (transmembrane, wheat and cyan), an A domain (actuator, yellow), a P domain (phosphorylation, blue), an N domain (nucleotide-binding, red), and one or several heavy metal-binding domains (HMBDs, cyan). (a) Post-Albers reaction cycle of P-type ATPases: P-type ATPases cycle through four main conformational states: E1, E1P, E2P, and E2.*

- *In the E1 state, the metal ion (green) binds to a high-affinity binding site within the membrane and triggers conformational changes. These changes lead to ion occlusion*

and phosphorylation of a conserved aspartate residue in the P domain, forming the E1P-ADP state.

- *The protein then releases ADP and undergoes a conformational rearrangement into the outward-open E2P state, allowing the metal ion to be released. (Note that countertransport has not been demonstrated for any P1B-type ATPase.)*
- *Dephosphorylation is associated with ion occlusion and the release of inorganic phosphate (Pi) through the E2·Pi transition state, resulting in the fully dephosphorylated E2 state.*
- *Finally, the protein returns to the E1 state, initiating a new reaction cycle.*

(b) Topology of P1B-type ATPases:

The topology highlights key residues within the different protein domains. Structural features involved in metal uptake are shown in green, including:

- *Ligands involved in metal entry in CopA.*
- *A negatively charged entry funnel in ZntA.*

Highly conserved residues proposed to participate in high-affinity metal binding within the membrane, metal release, and other transport processes are highlighted in bold, underlined, and/or italicized text.

2.2.2. Roles and Functions in Plants (Functional Classification)

In plant physiology, P-type ATPases are divided into several key subfamilies:

A. Plasma Membrane H^+-ATPases (P3 Subfamily)

These are the primary driving forces of plant cells. By pumping protons (H^+) out of the cytosol into the apoplast (extracellular environment), they generate a proton-motive force consisting of a pH gradient and an electrically negative membrane potential.

- **Physiological Functions:**

- **Mineral Nutrition:** This gradient enables the secondary active uptake of ions (K^+, NO_3^-, PO_4^{3-}) and sugars through symporters and antiporters.

- **Cell Growth (Acid Growth Theory):** Acidification of the cell wall activates expansins, which loosen the cell wall, allowing cell elongation under the effect of turgor pressure.

- **Stomatal Opening:** Activation of H^+-ATPases in guard cells induces massive K^+ and water uptake, resulting in stomatal opening.

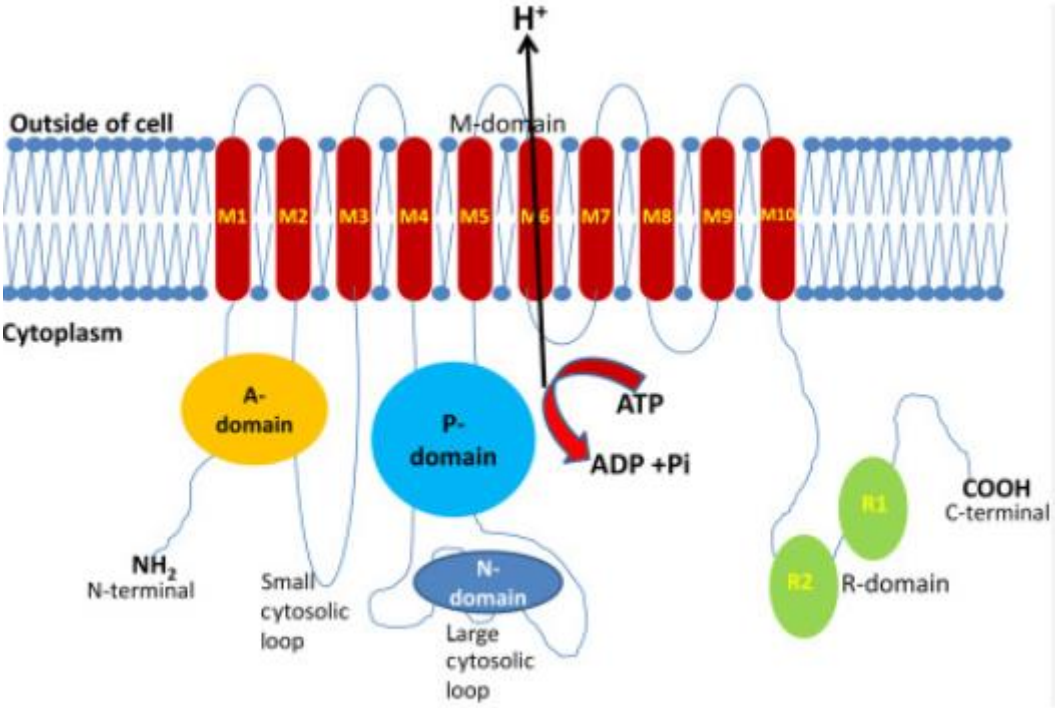

Fig.3. Plasma membrane H+-ATPase in plants

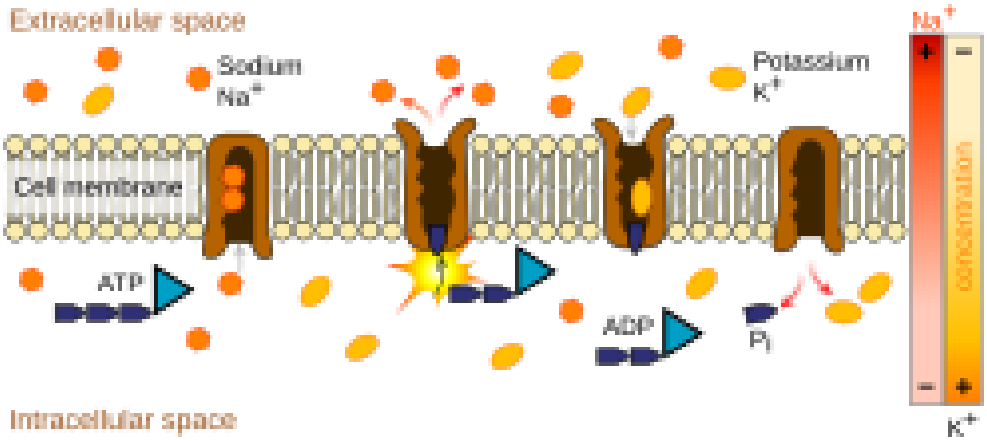

Fig. 4. Sodium-Potassium Pump (Na^+/K^+-ATPase).

B. Ca^{2+}-ATPases / ACA and ECA (P2 Subfamily)

-Maintaining a very low cytosolic calcium (Ca^{2+}) concentration.

- Physiological Functions:

- Pumping excess Ca^{2+} into the vacuole, endoplasmic reticulum, or apoplast.
- Resetting calcium signaling after environmental stress, such as drought, salinity, or pathogen attack.

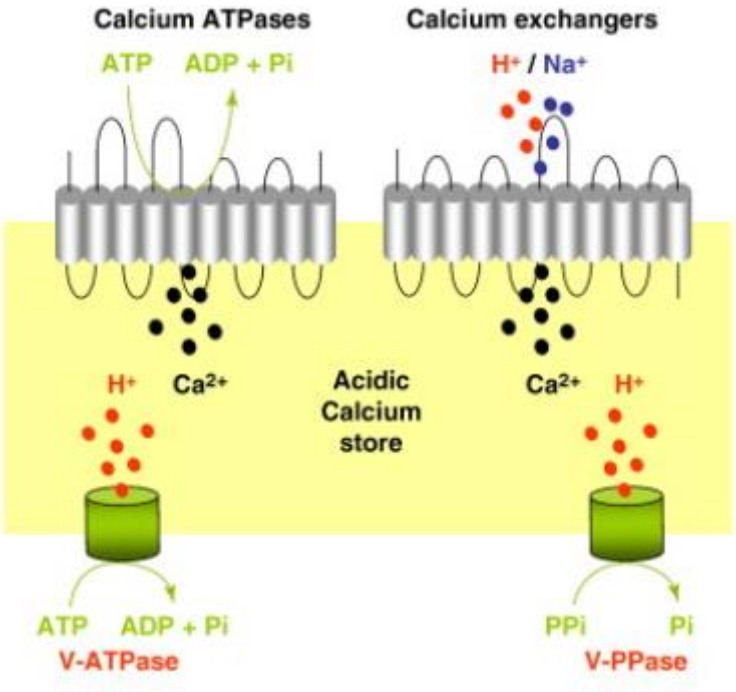

Fig. 5. Calcium ATPase (Ca^{2+}-ATPase).

C. P1B-ATPases (Heavy Metal Transporters – HMA)

- Major Role: Transport of micronutrients and maintenance of metal homeostasis.

- Physiological Functions:

- Transport of essential ions such as copper (Cu^{2+}), zinc (Zn^{2+}), and iron (Fe^{2+}) to plastids and mitochondria.
- Tolerance to Metal Stress: Sequestration of toxic metals (cadmium Cd^{2+} and lead Pb^{2+}) into the vacuole or their export out of the roots.

D. P4-ATPases (Phospholipid Flippases)

- Major Role: Transport of lipids across the membrane bilayer.

- Physiological Functions:

- Maintaining membrane asymmetry.
- Regulating vesicular trafficking dynamics.
- Contributing to plant responses to water and heat stress.

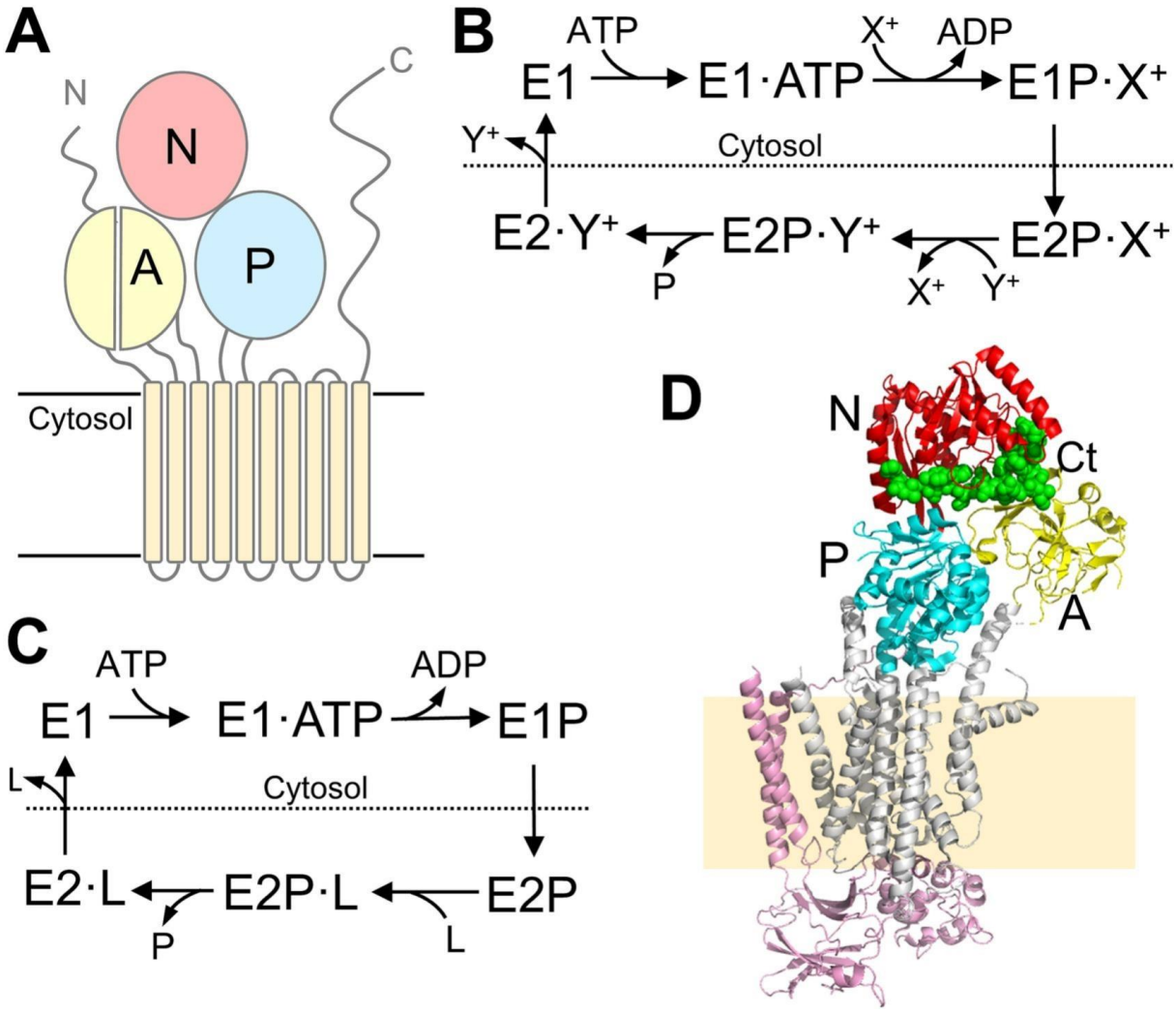

Fig. 6. General features of P4-type ATPases.

(*A) Schematic representation of the typical P-type ATPase structure, showing the 10 transmembrane segments characteristic of P4-type ATPases. N: nucleotide-binding domain; P: phosphorylation domain; A: actuator domain.*

(B) Post-Albers model of the catalytic cycle of a P-type ATPase, illustrated using a hypothetical protein transporting a cation (X^+) from the cytosol to the extracellular or luminal side of the membrane, and a counterion (Y^+) in the opposite direction.

Starting from the E1 conformation, the protein binds ATP and a cation on the cytosolic side, forming the phosphorylated substrate-bound E1P intermediate. Following the transition to E2P, the protein releases the cation on the extracellular side and binds a counterion. Dephosphorylation and spontaneous conversion back to the E1 state result in the release of the counterion into the cytosol.

(C) Modified Post-Albers cycle for a P4-type ATPase transporting a generic lipid molecule (L).

(D) Position of the C-terminal end of human ATP8A1 during autoinhibition (PDB accession: 6K7L).

- *Red: N domain.*
- *Cyan: P domain.*
- *Yellow: A domain.*
- *Green spheres: C-terminal region.*
- *Pink: CDC50A β-subunit.*
- *The approximate position of the membrane is represented by an ochre-colored box.*

- **Agronomic and Biotechnological Importance**

4. Agronomic and Biotechnological Importance

- **Adaptation to acidic or saline soils:** The overactivation of H^+-ATPases enables plants to better tolerate soil acidity and excrete excess sodium (Na^+) through the Na^+/H^+ transporter (SOS1).

- **Phytoremediation:** The overexpression of P1B-type ATPases (HMA) makes it possible to select or develop plants capable of accumulating heavy metals and cleaning up contaminated soils.

- **Nutrient Use Efficiency (NUE):** Optimizing proton pump activity improves the absorption of nitrogen- and phosphate-based fertilizers by plant roots.

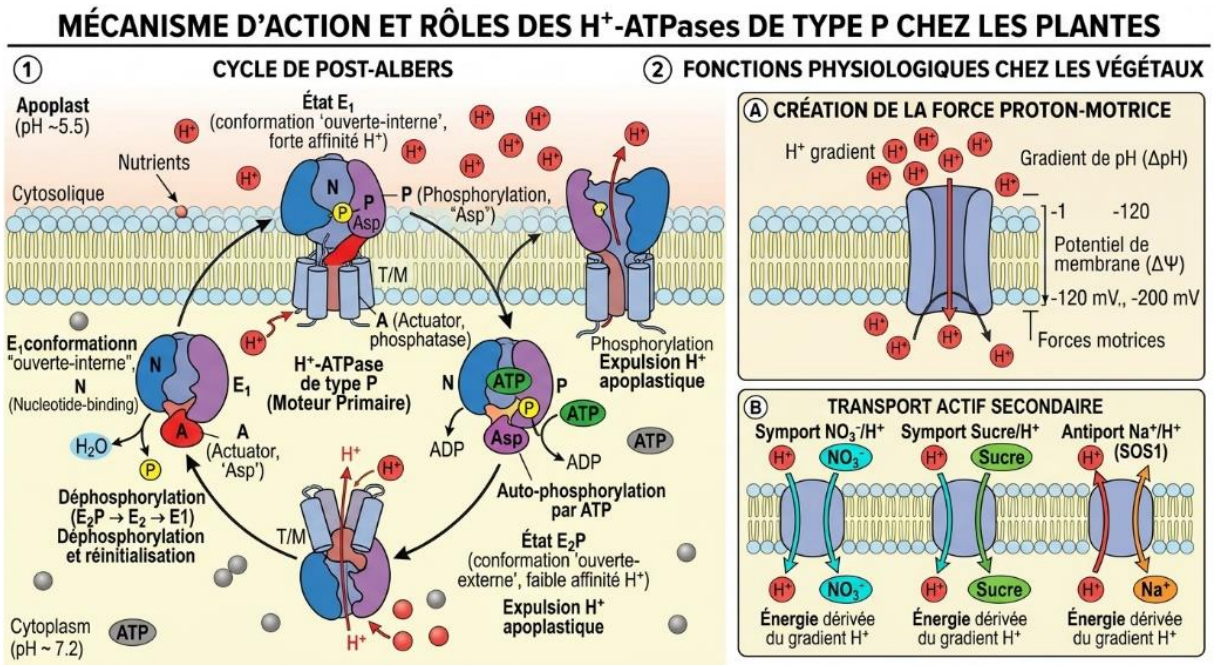

Fig. 4. Mechanism of action and roles of P-type H^+-ATPases in plants.

3. Channels and Transporters

Channels and transporters are intrinsic membrane proteins that are essential for cellular functioning. They enable the selective movement of water, ions, and solutes across the lipid bilayer of the plasma membrane and endomembranes.

3.1. Membrane Channels

An ion channel is a transmembrane protein that facilitates the transport of ions down their electrochemical or concentration gradients. Two main types of channels can be distinguished: passive channels, which are constitutively open, and gated channels, which remain closed unless stimulated. The latter include voltage-gated and ligand-gated channels.

- Mechanism: They form a continuous hydrophilic pore across the membrane. When open, they allow extremely rapid transport (up to 10710^7–10810^8 molecules or ions per second).

- **Selectivity:** A selectivity filter within the pore allows ions or molecules to be selected according to their size and electrical charge.

- **Gating (Regulation of channel opening):** Channels are not continuously open; their opening depends on specific stimuli:

- **Voltage-gated channels:** Open in response to changes in membrane potential (e.g., K^+, Na^+, or Ca^{2+} channels).

- **Ligand-gated channels:** Open following the binding of a specific molecule (e.g., neurotransmitters, hormones, or metabolites).

- Mechanosensitive channels: Respond to membrane stretching and mechanical stress.

- **Aquaporins (AQPs):** Specialized channels that facilitate the rapid movement of water across membranes (hydraulic conductivity).

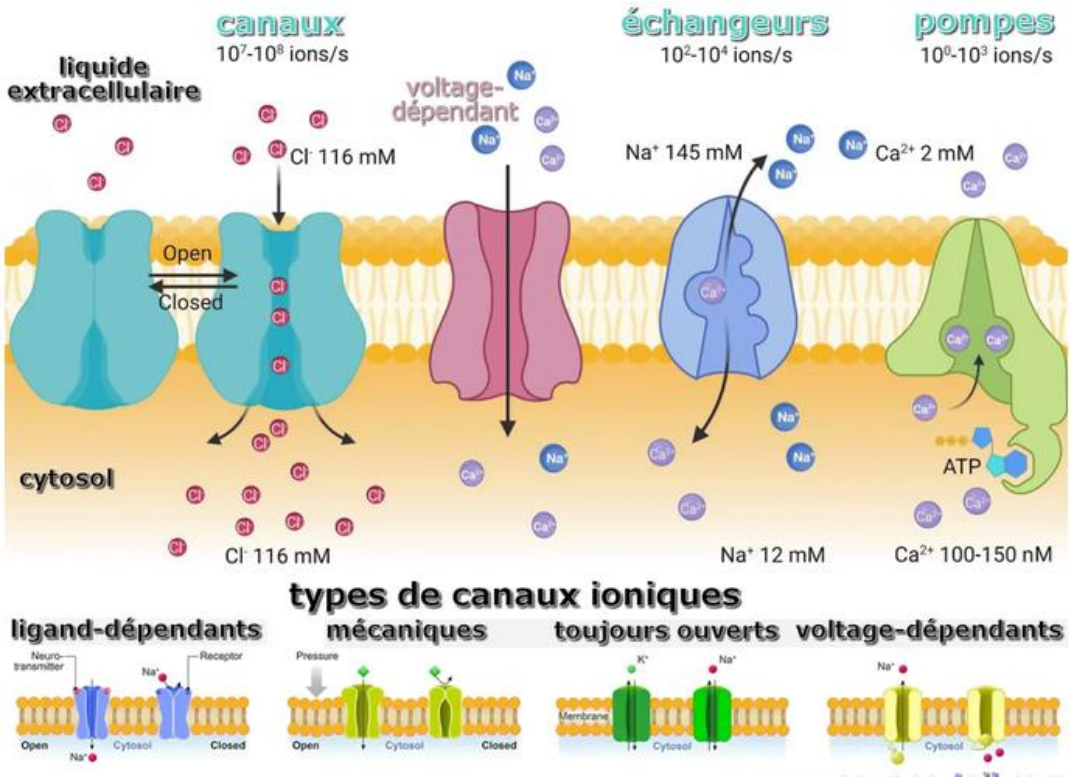

Fig. 7. Ion channels.

3.2. Membrane Transporters

A membrane transporter is a transport protein integrated into or associated with the lipid bilayer of cellular membranes. These proteins are involved in regulating the movement of molecules and ions across the membrane, either into or out of the cell, or between intracellular compartments.

Unlike channels, transporters undergo sequential conformational changes to move substrates from one side of the membrane to the other. Consequently, their transport rate is slower (10^2 to

10^4 molecules per second) and subject to saturation (Michaelis–Menten kinetics). These proteins facilitate the transport of substances through either facilitated diffusion or active transport.

3.2.1. Passive Transport (Facilitated Diffusion)

Passive transport, unlike active transport, is the net movement of a substance across a membrane down its concentration gradient. It does not require energy from the cell.

It involves the transfer of molecules (biochemical, atomic, or molecular substances) across a lipid membrane in the direction of their electrochemical gradient, without requiring an external energy source. The passive transport mechanism is called uniport when a single type of substance is transported by a transport protein.

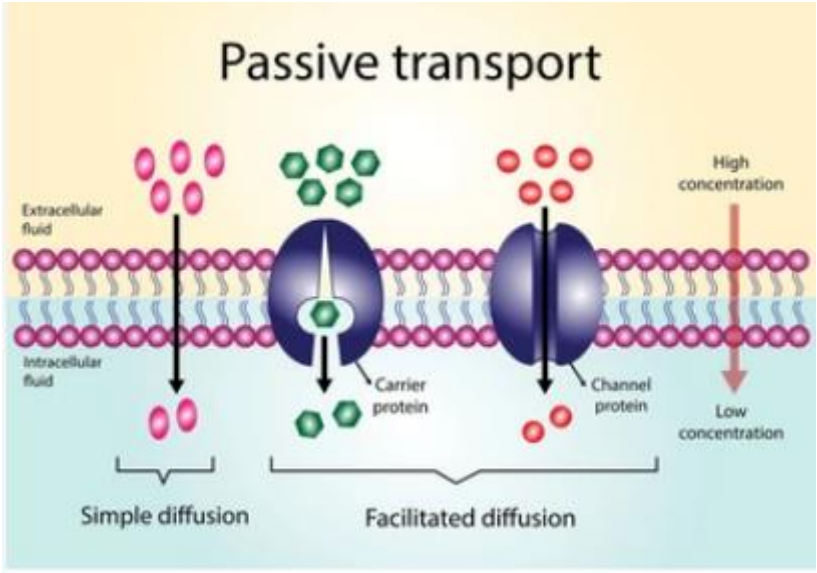

Fig. 8. Passive transport: simple diffusion across the membrane.

3.2.2. Active Transport

Active transport refers to the net movement of an ion or molecule across a membrane against its concentration gradient. When this process uses chemical energy produced, for example, by the hydrolysis of a nucleoside triphosphate such as adenosine triphosphate (ATP), it is referred to as primary active transport. Secondary active transport, on the other hand, involves the use of an electrochemical gradient to drive the transport of another substance across the membrane.

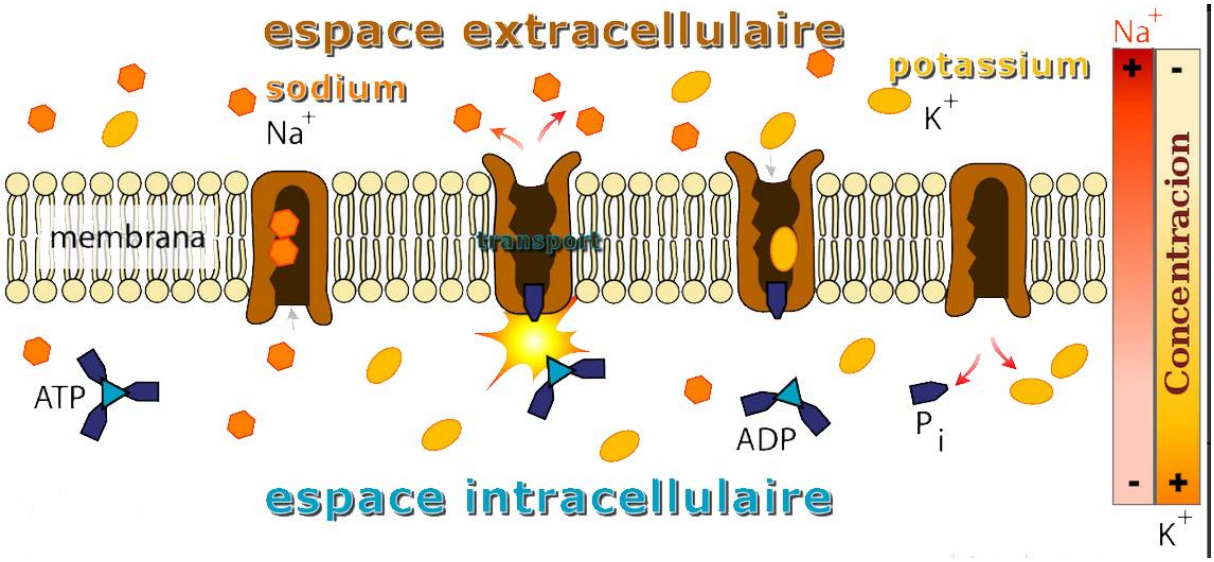

Fig. 9. Primary active transport of Na^+ and K^+.

The Two Main Types of Active Transport

a. Primary Active Transport (Direct)

Primary active transport directly uses a source of metabolic energy to pump substances against their concentration or electrochemical gradients.

The main energy sources used include:

- ATP hydrolysis: Through transmembrane ATPases.
- Oxidation–reduction reactions: For example, the mitochondrial electron transport chain using NADH.
- Photon energy (light): For example, during photosynthesis at the thylakoid membrane.

Main types of ATP-driven transporters:

- **P-type ATPases:** Na^+/K^+ pumps, Ca^{2+} pumps, and proton (H^+) pumps.
- **F-type ATPases:** ATP synthases found in mitochondria and chloroplasts.
- **V-type ATPases:** Vacuolar ATPases.
- **ABC transporters (ATP-Binding Cassette):** Include MDR and CFTR transporters.

b. Secondary Active Transport (Coupled Transport or Cotransport)

Secondary active transport does not use ATP directly. Instead, it exploits the potential energy stored in an electrochemical gradient previously established by a primary active transporter.

- **Mechanism:** The passive movement of an ion (e.g., Na^+ or H^+) down its electrochemical gradient releases energy that drives another molecule against its own gradient.

Types of cotransporters:

- **Symport:** Both substances are transported in the same direction across the membrane (e.g., the sodium–glucose cotransporter).

- **Antiport:** Two substances are transported in opposite directions across the membrane.

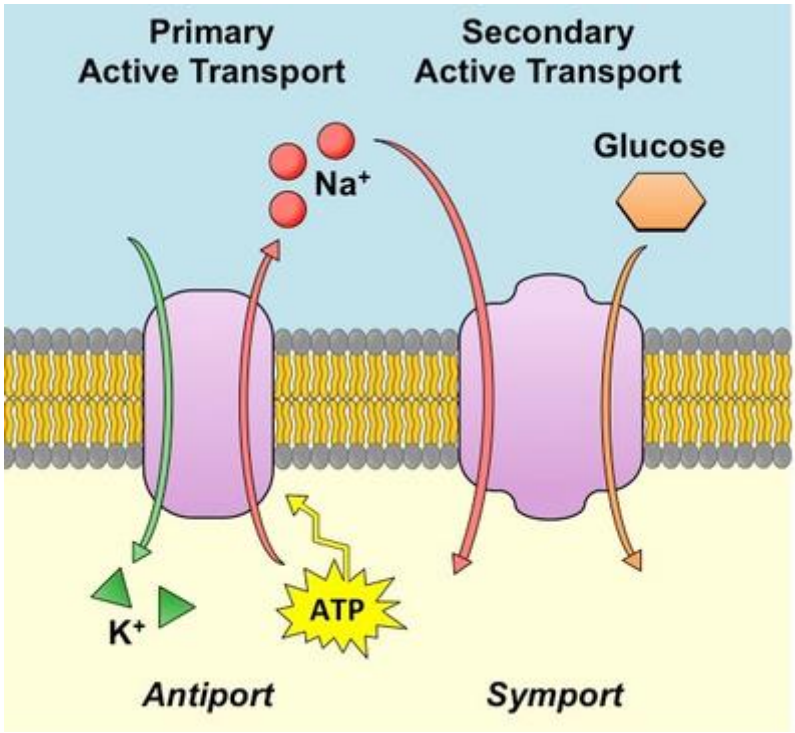

Fig. 10. Types of cotransporters.

3.2.3. Vesicular Transport (Large Molecules / Particles)

When substances are too large to pass through membrane transport proteins, the cell uses vesicular transport, which also requires energy.

- Endocytosis (Uptake): The cell membrane invaginates and forms a vesicle around the external material.

  - Pinocytosis: Uptake of liquid droplets (e.g., absorption of fats in the intestine).
  - Phagocytosis: Uptake of solid particles.

- Exocytosis (Secretion): The reverse process, in which secretory vesicles fuse with the plasma membrane and release their contents outside the cell.

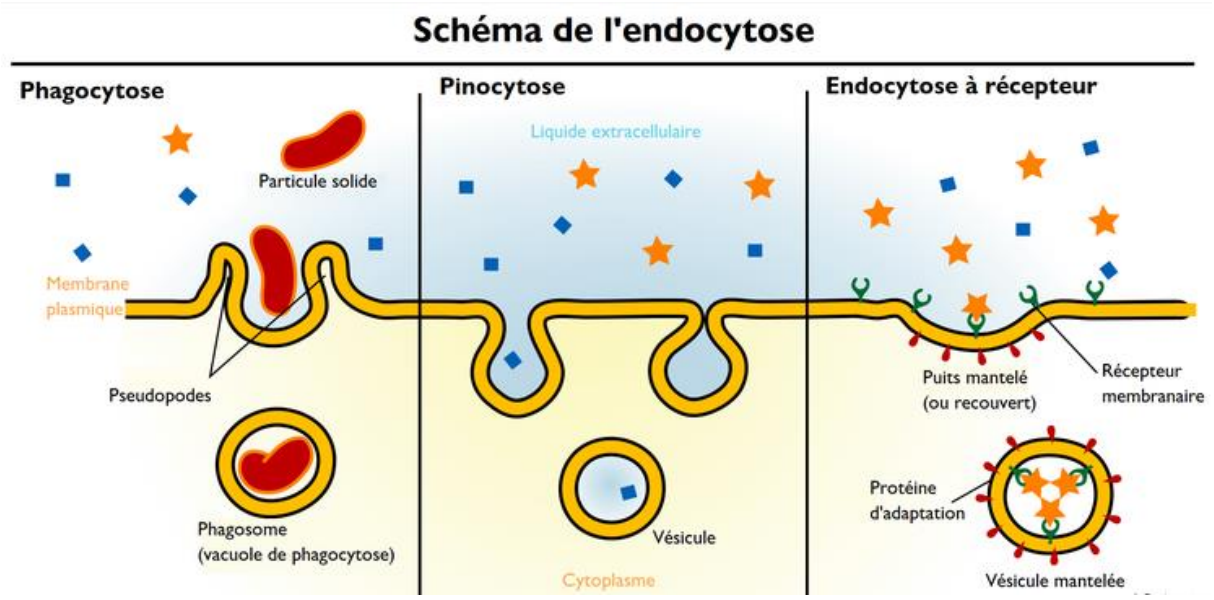

Fig. 11. Endocytosis.

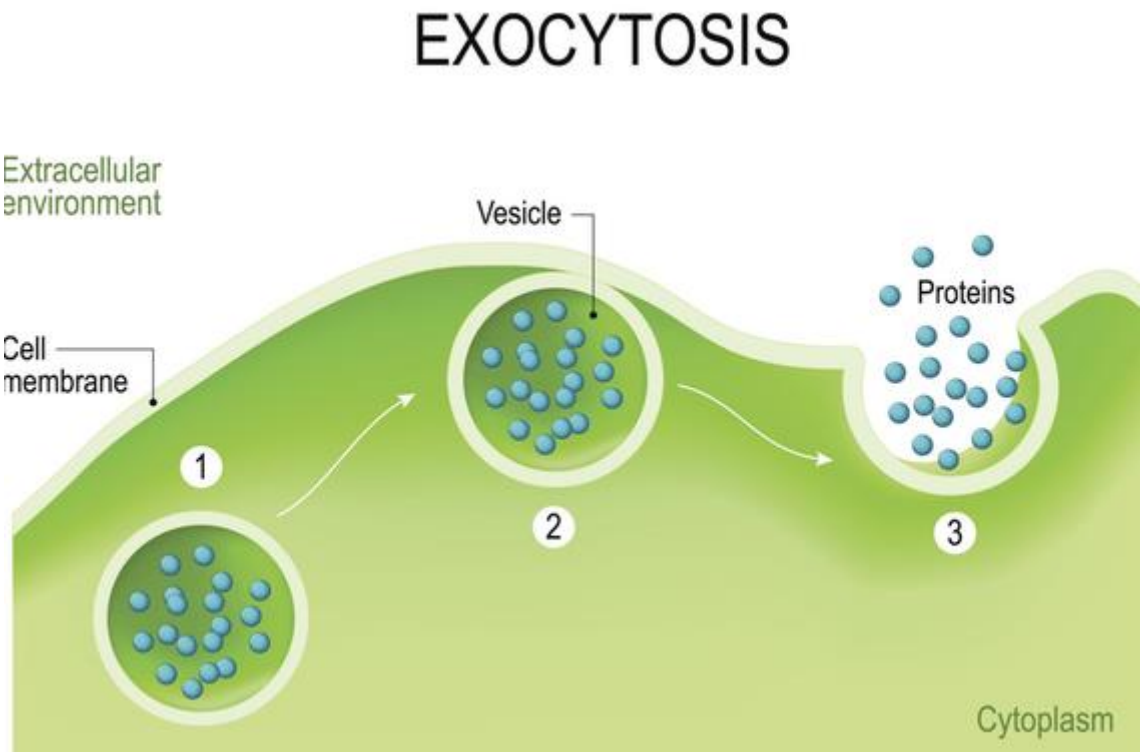

Fig. 12. Exocytosis.

3.3. Study of Aquaporins (Water Regulation) and High- and Low-Affinity Nitrate Transporters (NRT1, NRT2)

Aquaporins and nitrate transporters (NRT) are two major components of plant physiology, responsible for regulating hydraulic conductivity and the uptake of mineral nitrogen, respectively.

3.3.1. Aquaporins

Aquaporins (AQPs) are tetrameric membrane proteins belonging to the Major Intrinsic Proteins (MIP) family. Each monomer forms an individual pore that allows the rapid and selective passage of water along a water potential gradient (ΔΨ).

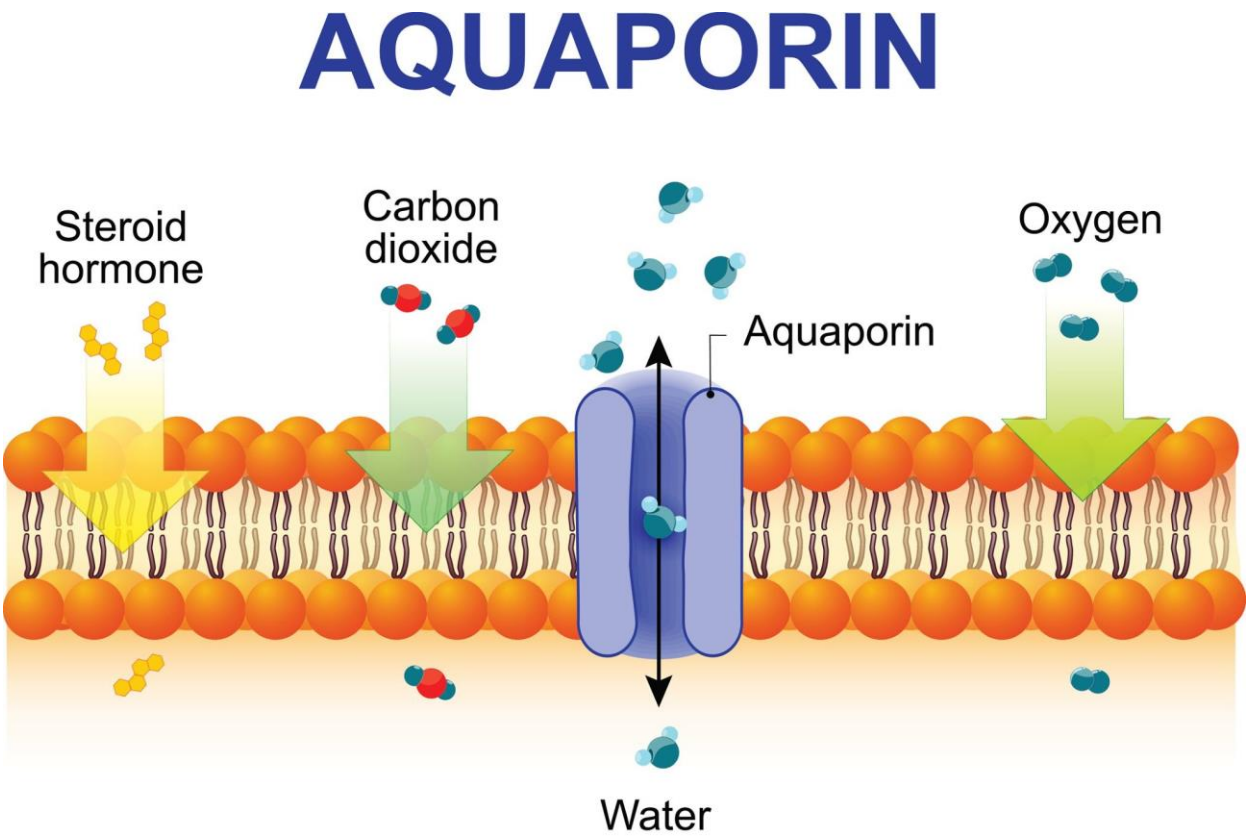

Fig. 13. Structure of an aquaporin in the membrane. Source: ttsz / Getty Images.

Subcellular Classification in Plants

- PIPs (Plasma Membrane Intrinsic Proteins): Located in the plasma membrane and divided into PIP1 and PIP2 subfamilies. They regulate water entry into the cell and the symplastic pathway in roots.

- TIPs (Tonoplast Intrinsic Proteins): Located in the tonoplast. They regulate water flow between the cytosol and the vacuole, thereby maintaining turgor pressure.

- NIPs (Nodulin 26-like Intrinsic Proteins) and SIPs (Small basic Intrinsic Proteins): They also facilitate the transport of other small, uncharged molecules, such as glycerol, boron, and silicon.

Modes of Regulation of Hydraulic Activity

- Gating (Opening and Closing):

- Phosphorylation: The phosphorylation of specific serine residues (through CDPKs or PKA) induces pore opening.

Phosphorylation is a biochemical process in which specific enzymes, such as CDPKs (Calcium-Dependent Protein Kinases, which are particularly common in plants) or PKA (Protein Kinase A), add a negatively charged phosphate group (PO_4^{3-}) to the hydroxyl group (-OH) of a serine residue, generally located in the cytoplasmic loops or terminal regions of the protein.

The introduction of this negative charge generates electrostatic interactions (attractions or repulsions) with surrounding amino acids. This induces a conformational change in the protein structure, which shifts the internal gate of the channel, thereby opening the pore and facilitating the passage of water or ions across the membrane.

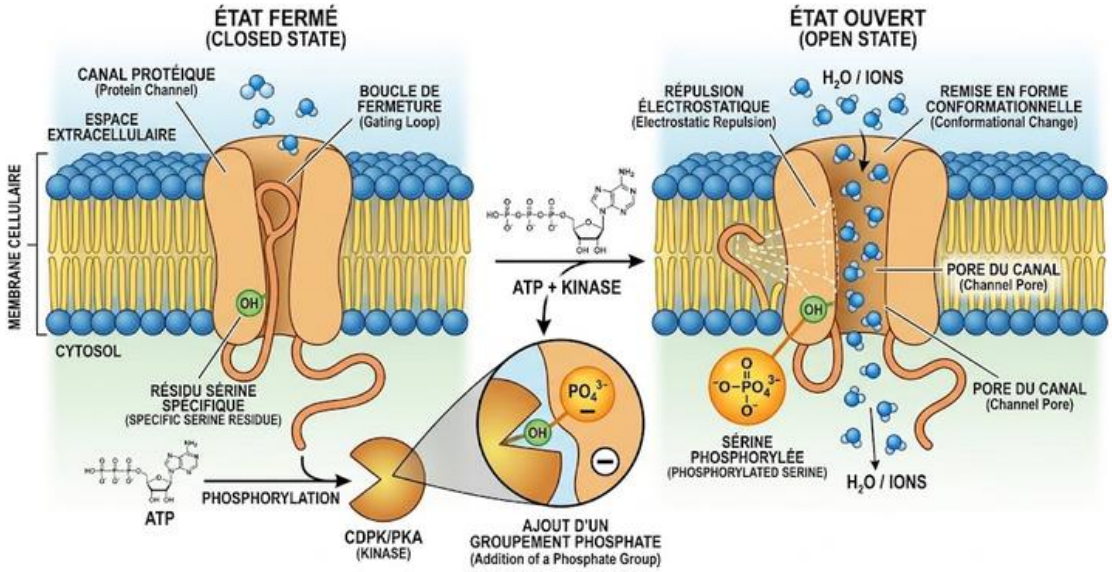

Figure: *Régulation de l'ouverture d'un pore par phosphorylation*

Fig. 13. Regulation of pore opening by phosphorylation.

- Protonation / Cytosolic pH: A decrease in cytosolic pH (e.g., during root anoxia) leads to the protonation of a conserved histidine residue, resulting in pore closure. Under environmental stress, such as root anoxia caused by waterlogging, cellular metabolism shifts towards fermentation, leading to a decrease in cytosolic pH (cytosolic acidosis).

The accumulation of H^+ ions causes the protonation of a conserved histidine residue located in the cytoplasmic loops of the channel protein. The acquisition of a positive charge by histidine enables it to form stable ionic interactions and hydrogen bonds with nearby negatively charged amino acids, such as aspartate.

This structural rearrangement blocks the channel's closing loop, resulting in pore closure and thereby preventing water loss and cellular osmotic imbalance.

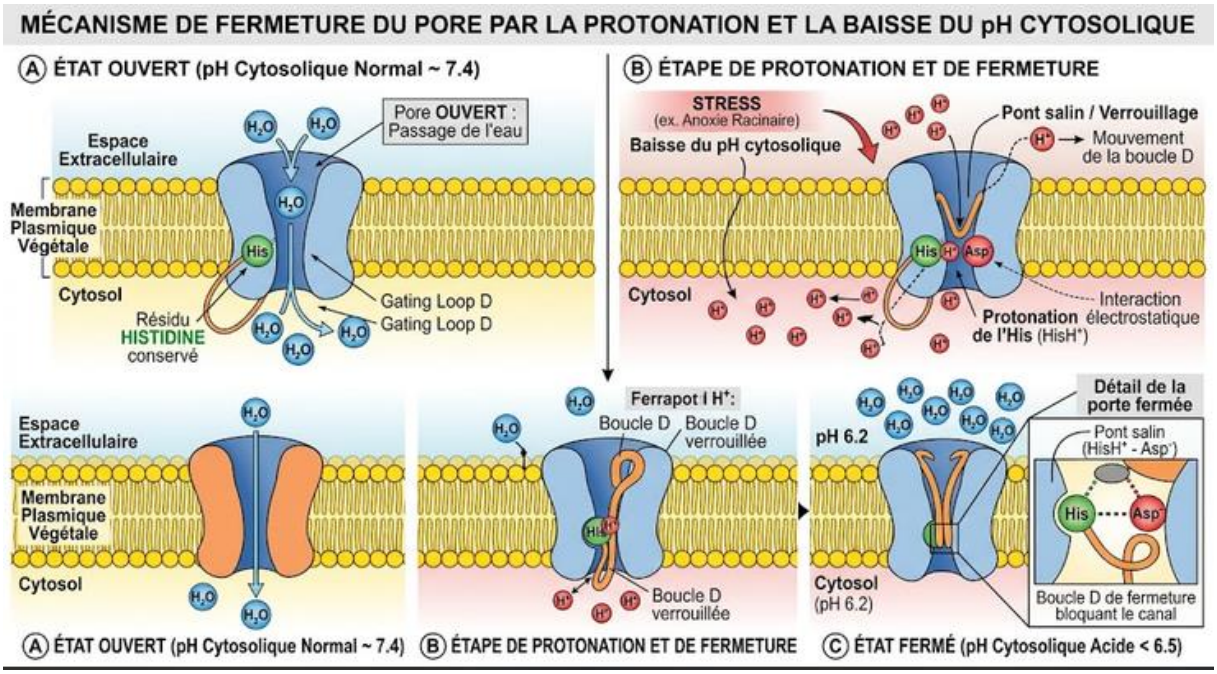

Fig. 15. Mechanism of pore closure through protonation and cytosolic pH regulation.

- Divalent Cations: Ca^{2+} binding stabilizes the closed conformation of the pore.

During environmental stress (such as drought or salinity), the cytosolic calcium concentration (Ca^{2+}) increases considerably. Ca^{2+} ions bind specifically to negatively charged amino acid residues, such as aspartate and glutamate, located on the cytoplasmic loop (D-loop) of the channel.

Due to its double positive charge, Ca^{2+} acts as an ionic bridge (calcium bridge), connecting the negative charges of the loop to those of other regions of the protein.

This interaction firmly stabilizes the loop in a locked position above the channel entrance, maintaining the pore in a closed state and thereby blocking the flow of water or ions.

- **Trafficking (Membrane Trafficking):** In response to osmotic or salt stress, aquaporins are internalized from the plasma membrane into endosomes, thereby reducing root hydraulic conductivity (Lpr).

In response to osmotic or salt stress, plant cells adjust their water permeability by modifying the density of aquaporins (PIPs) present in the plasma membrane.

This process, known as membrane trafficking, begins with the endocytosis of aquaporins. These channel proteins are internalized through vesicles (either clathrin-dependent or clathrin-independent) from the plasma membrane into endosomes, particularly the trans-Golgi network and early endosomes.

This intracellular sequestration significantly reduces the number of functional water channels at the cell surface, resulting in a rapid decrease in root hydraulic conductivity (Lpr).

Depending on the intensity and duration of the stress, internalized aquaporins may either be recycled back to the plasma membrane when favorable conditions are restored or transported to the vacuole for degradation.

3.3.2. Nitrate Transporters: High- and Low-Affinity Systems

Nitrate (NO_3^-) is the main source of mineral nitrogen for most higher plants. Due to fluctuations in its concentration in the soil solution (from 10 μM to more than 50 mM), plants have developed two complementary transport systems.

The high-affinity nitrate uptake system (HATS) operates through two distinct modes: a constitutive mode (cHATS) and an inducible mode (iHATS).

- High-Affinity Transport System (HATS): The HATS, characterized by a low Km value (10–100 μM), operates at very low nitrate concentrations (less than 0.5 mM). It includes a constitutive component (cHATS), which ensures basal nitrate uptake, and an inducible component (iHATS), which is activated by the presence of nitrate in the soil.

- Low-Affinity Transport System (LATS): The LATS operates when nitrate is abundant (greater than 0.5 mM) and is characterized by a high Km value (greater than 1 mM). It enables high-capacity and high-rate nitrate uptake to meet the plant's increased nutritional requirements.

The uptake of nitrate (NO_3^-) by plant roots relies on two major families of membrane transporters adapted to nitrogen availability in the soil.

3.3.2.1. NRT2 Family (High Affinity)

This family is responsible for the efficient uptake of nitrate at very low concentrations (less than 0.5 mM).

It also operates through an NO_3^-/H^+ symport mechanism, allowing nitrate to be transported against its concentration gradient through the inward movement of protons.

The main transporter, NRT2.1, cannot function independently in the membrane. It requires direct physical interaction with the non-transporting partner protein NAR2.1 (NRT3.1).

The formation of this heteromeric complex [NRT2.1–NAR2.1] is essential for stabilizing NRT2.1, ensuring its targeting to the plasma membrane, and activating high-affinity nitrate transport under nitrogen-deficient conditions.

3.3.2.2. NRT1 / NPF Family (Low and Dual Affinity)

This family operates mainly when nitrate is abundant in the soil (greater than 0.5 mM).

It involves secondary active transport through an H^+/NO_3^- symport mechanism, coupling the uptake of one NO_3^- ion with two H^+ ions and using the electrochemical gradient generated by the plasma membrane H^+-ATPase.

The key member, AtNRT1.1 (NPF6.3), is a dual-affinity transceptor (transporter and receptor).

Under nitrogen-deficient conditions, its phosphorylation at Threonine 101 (T101) by the CIPK23 kinase induces a conformational change that switches the transporter to a high-affinity state.

At high nitrate concentrations, its non-phosphorylated form functions in a low-affinity state and triggers extensive gene signaling known as the Primary Nitrate Response (PNR).

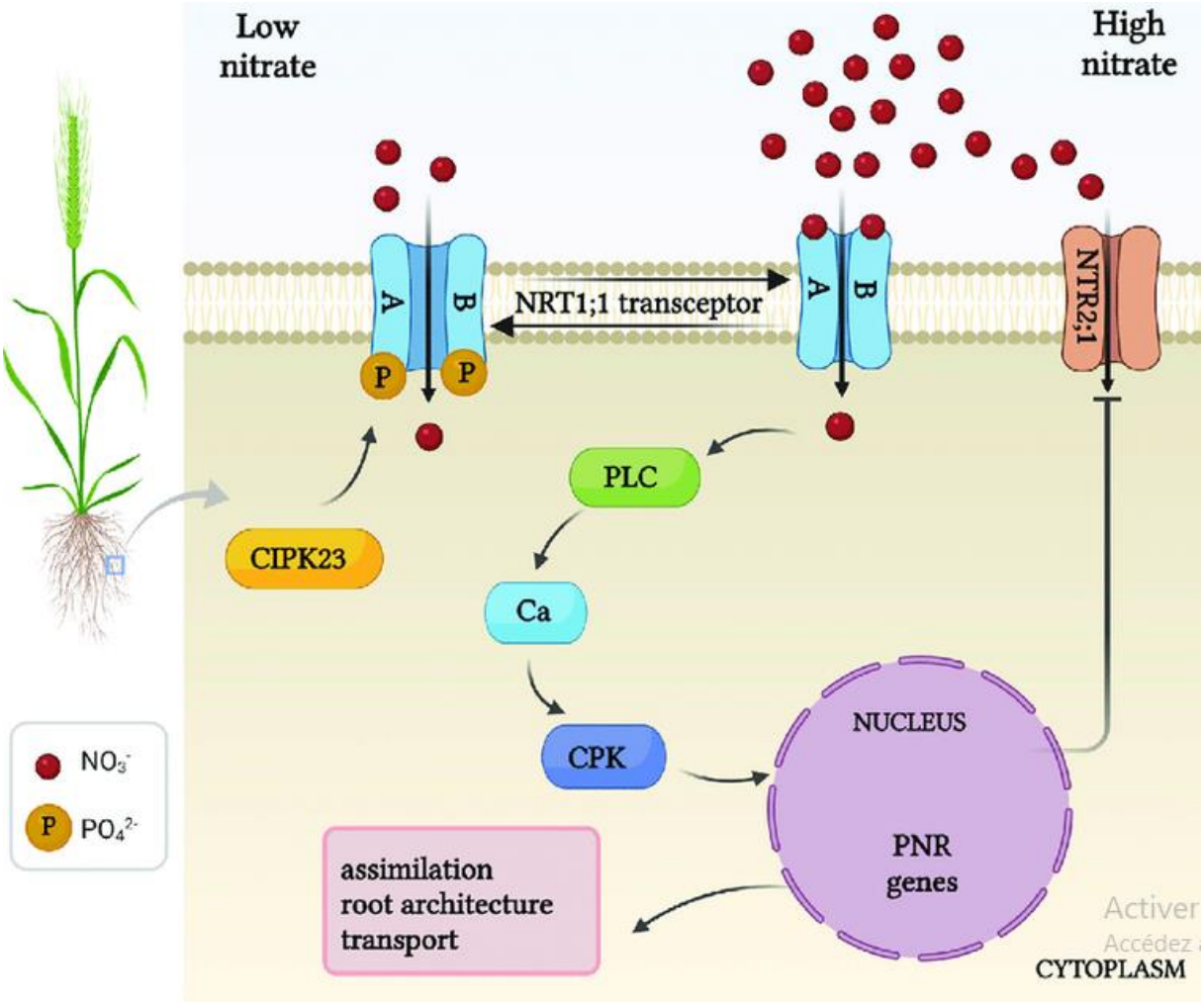

Fig. 16. Transceptor-mediated nitrate signaling. *Under conditions of low external NO_3^- concentration, only one of the NRT1.1 monomers binds NO_3^- with high affinity. This triggers conformational changes in the protein, switching NRT1.1 to its high-affinity transport mode and allowing the CIPK23 kinase to access and phosphorylate NRT1.1.*

When the external NO_3^- concentration increases, NRT1.1 becomes dephosphorylated, and both NRT1.1 monomers bind NO_3^-. The resulting conformational change causes the monomers to interact, switching NRT1.1 to a low-affinity, high-capacity transport mode.

Simultaneously, a PLC-mediated Ca^{2+} signal is generated and transmitted to the nucleus through CPKs, activating the transcription of Primary Nitrate Response (PNR) genes.

The PNR includes the downregulation of other high-affinity NO_3^- uptake transporters, such as NRT2.1, as well as physiological responses, including increased nitrogen assimilation and modifications to root architecture.

Abbreviations: NRT, nitrate transporter; CIPK, CBL-interacting protein kinase; PLC, phospholipase C; CPK, Ca^{2+}-dependent protein kinase; PNR, Primary Nitrate Response.

Figure created with BioRender.com.

3.3.2.3. Interactions and Energy Coordination

Water and nitrate uptake are closely coupled at the root level.

1. Generation of the Proton-Motive Force ($\Delta\mu H^+$)

- Plant cells rely on the proton pump H^+-ATPase, located in the plasma membrane.
- This pump actively exports hydrogen ions (H^+) from the cytosol into the apoplast (the extracellular space and cell wall) by consuming energy in the form of ATP.
- This pumping generates an electrochemical proton gradient ($\Delta\mu H^+$), making the apoplast more acidic and positively charged relative to the cytosol.
- This gradient provides the driving force required for nitrate transporters (NRT1 and NRT2) through an H^+/NO_3^- symport mechanism, in which nitrate ions enter the cell by utilizing the spontaneous inward movement of protons towards the cytosol.

2. Water and Mineral Coupling

- The continuous activity of NRT1 and NRT2 transporters promotes the uptake and accumulation of nitrate ions (NO_3^-) in the symplast, followed by their loading into the xylem vessels.
- The accumulation of these solutes (nitrates) inside the xylem causes a decrease in osmotic potential (Ψs), making the total water potential (Ψw) more negative.
- This difference in osmotic potential creates an osmotic driving force that causes water molecules to move passively through the roots, from the soil towards the xylem vessels.
- PIP-family aquaporins facilitate and accelerate the movement of water molecules through the symplastic pathway to meet this osmotically driven water demand.

4. Mitochondrial Bioenergetics: Alternative Respiration (AOX) and Its Role in Excess Energy Dissipation

In plants, respiration does not serve exclusively to produce energy in the form of ATP. It also plays a crucial role in protecting cells against environmental stress through a specific metabolic

pathway known as the alternative respiratory pathway, which involves the activity of Alternative Oxidase (AOX).

AOX is a component of plant mitochondria that provides an alternative route for electron transport.

4.1. The Classical Mitochondrial Respiratory Chain

The respiratory chain consists of four enzymatic complexes, ubiquinone, and cytochrome c, located in the inner mitochondrial membrane. These components act as electron carriers and, in some cases, proton pumps, representing the final stage of energy production during the oxidation of energy-rich molecules.

Under normal conditions, mitochondria break down carbon compounds (derived from glycolysis) to synthesize ATP:

- The reduced coenzymes (NADH and $FADH_2$) transfer their electrons to the electron transport chain located in the inner mitochondrial membrane.
- Electrons pass through a series of protein complexes: Complex I → Complex II → Ubiquinone (UQ) → Complex III → Cytochrome c → Complex IV (Cytochrome c oxidase).
- This electron transfer is accompanied by the pumping of protons (H^+) from the mitochondrial matrix into the intermembrane space, generating an electrochemical gradient.
- Protons flow back into the matrix through ATP synthase, producing a large amount of ATP through oxidative phosphorylation.

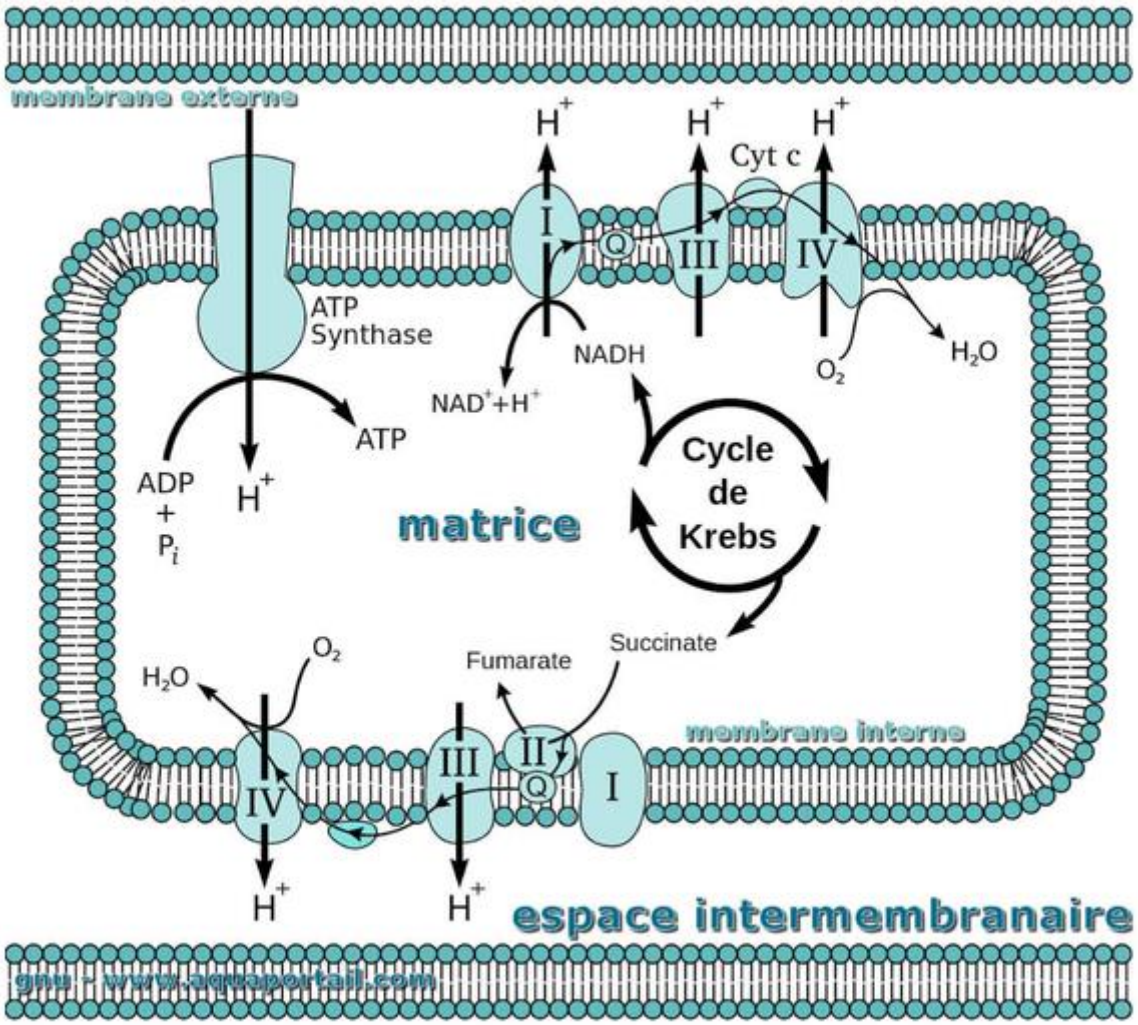

Fig. 17. Schematic representation of the mitochondrial respiratory chain. *The respiratory chain, consisting of Complexes I, II, III, and IV, as well as ATP synthase (Complex V), is located in the inner mitochondrial membrane.*

Upper pathway: Electrons enter the respiratory chain through Complex I following the oxidation of NADH to NAD^+. They are then transferred via coenzyme Q (ubiquinone) to Complex III, followed by cytochrome c to Complex IV, where molecular oxygen (O_2) is reduced to water (H_2O).

Lower pathway: Electrons enter the respiratory chain through Complex II following the oxidation of succinate to fumarate. They are subsequently transferred via coenzyme Q to Complex III, then through cytochrome c to Complex IV, where molecular oxygen (O_2) is reduced to water (H_2O).

4.2. Alternative Oxidase (AOX)

Alternative Oxidase (AOX) is an enzyme specific to plants (as well as certain fungi and protists), located in the inner mitochondrial membrane.

Mechanism of the Alternative Respiratory Pathway:

- **Bypassing the classical respiratory chain:** AOX accepts electrons directly from the ubiquinone (UQ) pool and transfers them directly to molecular oxygen (O_2), reducing it to water (H_2O).

- **Avoidance of Complexes III and IV:** By bypassing Complexes III and IV, this pathway does not involve additional proton pumping across the inner mitochondrial membrane by these complexes.

- **Result:** The energy released during electron transfer is not conserved in the form of ATP but is instead dissipated as heat (thermal energy).

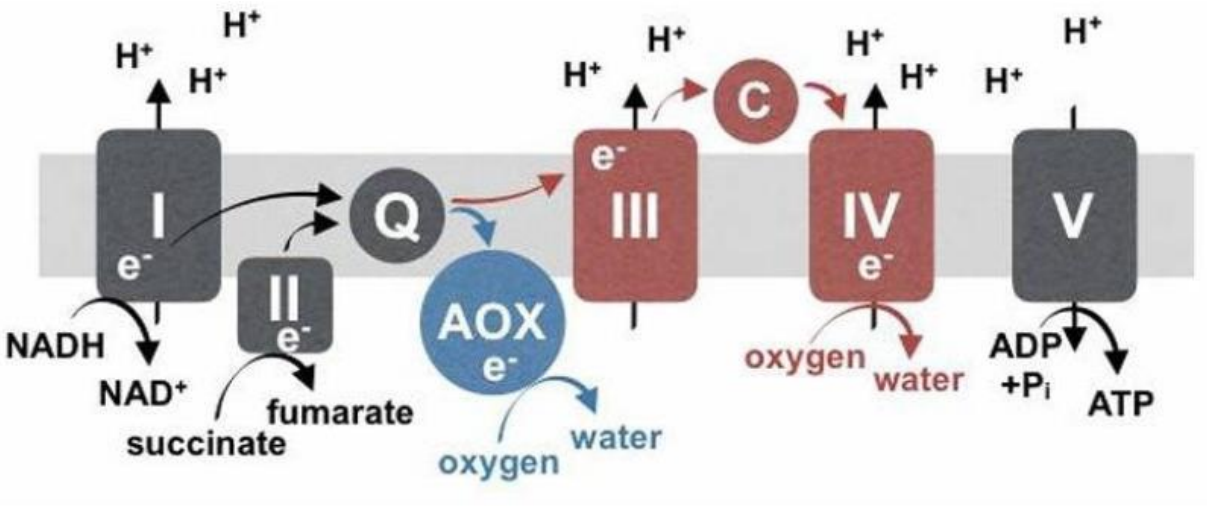

Fig. 18. Alternative Oxidase (AOX).

4.3. The Role of Alternative Respiration in Excess Energy Dissipation

Why would a plant use a pathway that "wastes" energy instead of producing ATP? This is an essential protective mechanism that operates under environmental stress conditions.

A. Prevention of Electron Overload (Electron Bottleneck)

When plants experience stress (cold, drought, high light intensity, or salinity), cellular ATP demand decreases, or the activity of the cytochrome pathway (Complex IV) becomes restricted.

- In the absence of AOX, the classical respiratory chain would rapidly become saturated with electrons.

- This electron saturation can promote the direct transfer of electrons to oxygen, leading to the formation of Reactive Oxygen Species (ROS), which are toxic molecules that damage cellular membranes and proteins.
- AOX acts as a safety valve by draining excess electrons from the ubiquinone pool, preventing excessive reduction of the respiratory chain and limiting ROS production.

B. Maintenance of Intermediate Metabolism

Even when the cell does not require additional ATP production, it still needs carbon skeletons (organic acids) for the synthesis of amino acids, pigments, and secondary defense metabolites.

- AOX allows the Krebs cycle to continue operating without being inhibited by excess ATP or NADH, thereby ensuring a continuous supply of essential metabolic precursors under stress conditions.

C. Thermogenesis (Heat Production)

In certain plant species, such as some members of the Araceae family and the leopard lily, energy dissipation as heat through AOX can increase the local temperature of flowers.

This process helps volatilize aromatic compounds to attract pollinators and can protect reproductive organs against freezing temperatures.

Note

- **Heat Dissipation:** AOX bypasses part of the electron transport chain, dissipating excess energy as heat rather than producing ATP.

- **Cellular Protection:** It protects plants against oxidative stress by preventing the excessive accumulation of Reactive Oxygen Species (ROS) under unfavorable environmental conditions.

- **Metabolic Flexibility:** It allows mitochondria to maintain the activity of the Krebs cycle and produce defense-related molecules, even when the cell is already saturated with ATP.