Chapter 3 : Signal Perception and Transduction

1. Molecular Photoreception: Mode of Action of Phytochromes (Pr/Pfr), Cryptochromes and UVR8

Molecular photoreception refers to the process by which plant cells detect environmental light signals through specialized chromoproteins called photoreceptors and convert light energy into internal biochemical signals.

Unlike photosynthesis, which uses light as a source of metabolic energy, photoreception uses light as a source of information (spectral quality, intensity, direction and photoperiod). This signal triggers photomorphogenesis, which includes all the changes in plant growth and development, such as seed germination, stem elongation, leaf expansion, stomatal opening, spatial orientation and flowering.

1.1. Phytochromes (Red Light / Far-Red Light)

Phytochromes perceive red light (R, approximately 660 nm) and far-red light (FR, approximately 730 nm). They are dimers composed of an apoprotein bound to a tetrapyrrole chromophore called phytochromobilin.

Phytochrome is a soluble homodimer composed of two identical subunits, each approximately 120–130 kDa. Each monomer consists of a protein component (the apoprotein) and a non-protein chromophore molecule (phytochromobilin, a linear tetrapyrrole) covalently attached through a thioether bond at the GAF domain.

Each phytochrome subunit is organized into two major functional modules:

- **Photosensory module (N-terminal):** Composed of the PAS, GAF and PHY domains. The GAF domain contains the chromophore, while the PHY domain is essential for stabilizing the active Pfr form after light absorption.

- **Regulatory module (C-terminal):** Contains two PAS-like domains and a histidine kinase-related domain (HKRD). This module regulates protein dimerization, its serine/threonine kinase activity, its transport into the nucleus and its protein–protein interactions.

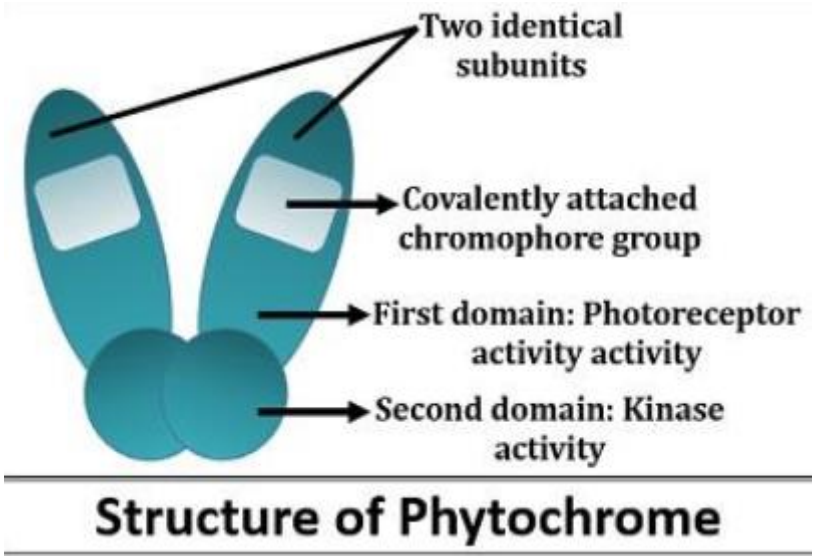


Fig. 27. Structure of phytochrome

- ⇨ **Mechanism of Action (Photoreversibility)**

Photoreversibility is based on a reversible spectral and conformational transition induced by photons:

- **Pr State (Phytochrome Red) – Inactive Form:** Phytochrome is synthesized de novo in the cytoplasm in this conformation under dark conditions. Its absorption spectrum has a maximum peak in the red region (wavelength approximately 660 nm). In this conformation, the nuclear localization signals (NLS) are hidden within the tertiary structure, keeping the protein in the cytosol.

- **Photoconversion (Pr → Pfr):** The absorption of a photon at 660 nm provides the energy required for the photo-induced Z-to-E isomerization of the double bond between rings C and D (C15–C16) of phytochromobilin. This rotation of the chromophore generates mechanical tension that is transmitted to the apoprotein. The apoprotein undergoes a major structural rearrangement: the PHY domain loop changes its conformation, exposing the nuclear localization signal (NLS) as well as the interaction sites for transcription factors.

- **Photoreversion (Pfr → Pr) and Thermal Conversion:** The Pfr form primarily absorbs far-red light (wavelength approximately 730 nm). A photon at this wavelength re-isomerizes the chromophore from E to Z, restoring the Pr conformation. In the absence of light (dark conditions), Pfr can also slowly return to the Pr form through a passive process called thermal reversion or dark reversion.

- **Photostationary Equilibrium (φ):** Under natural light conditions, the absorption spectra of Pr and Pfr partially overlap. Therefore, the proportion of Pfr depends on the red-to-far-red light ratio (R/FR). Under pure red light, approximately 85% of phytochromes are in the Pfr form, whereas under a vegetation canopy (enriched in far-red light), this percentage decreases considerably.

Gene Regulation

In the nucleus, Pfr directly interacts with transcription factors called PIFs (Phytochrome Interacting Factors), which act as repressors of photomorphogenesis under dark conditions.

This interaction induces the phosphorylation and ubiquitination of PIFs, leading to their degradation by the 26S proteasome. The removal of this repression allows the transcription of target genes, resulting in the activation of seed germination, inhibition of hypocotyl elongation and other photomorphogenic responses.

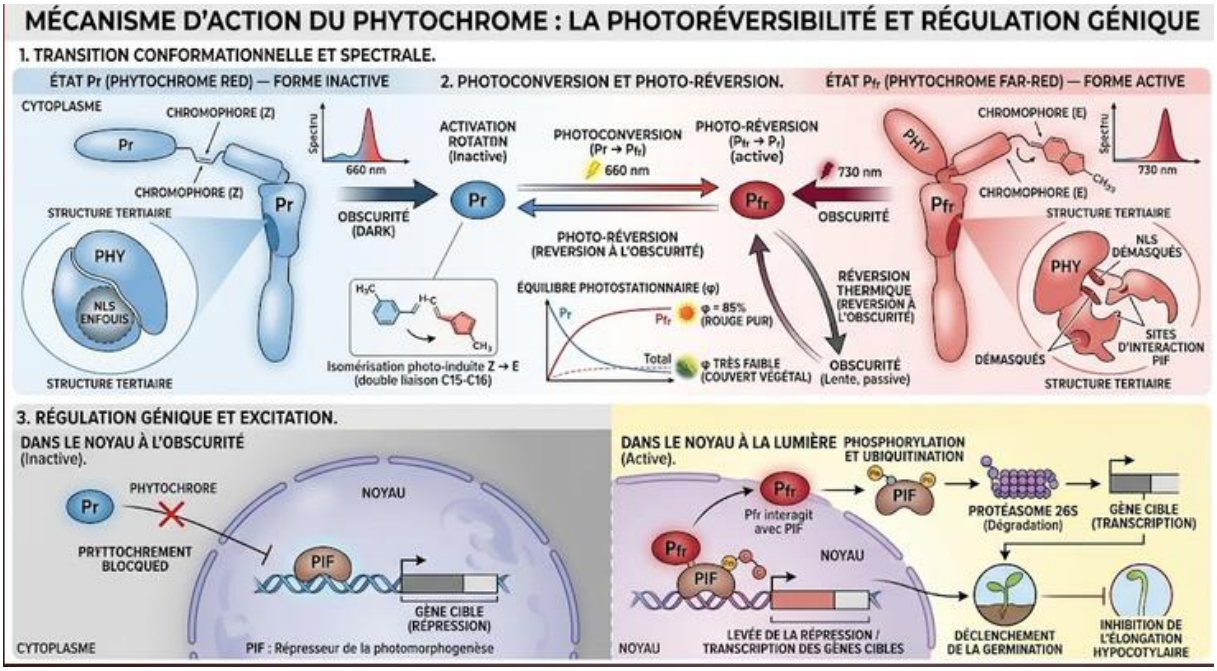


Fig. 28. Mechanism of Action (Photoreversibility)

Cryptochromes (Blue Light / UV-A)

Cryptochromes (CRY1, CRY2) perceive blue light (400–500 nm) and UV-A radiation. They are flavoproteins that bind two chromophores non-covalently: FAD (Flavin Adenine Dinucleotide) and MTHF (a pterin molecule).

Mechanism of Action (Reduction and Conformational Change)

- **Light Absorption:** The MTHF chromophore absorbs light energy and transfers it to FAD. In the inactive state, cryptochrome remains in its non-activated conformation.

- **Redox State Change:** FAD, initially in its oxidized form (FADox), undergoes photoreduction to form a semiquinone radical (FADH•).

- **Conformational Change:** This reduction induces the unfolding of the C-terminal domain of the protein (CCE domain).

- **Signal Transduction:** The activated CCE domain interacts with the COP1/SPA complex. COP1 is an E3 ubiquitin ligase that continuously targets HY5 (a basic leucine zipper transcription factor essential for photomorphogenesis) for degradation under dark conditions.

- **Gene Regulation:** The CRY–COP1 interaction inhibits the ubiquitination activity of COP1. As a result, HY5 is no longer degraded and accumulates in the nucleus, where it activates genes involved in de-etiolation, chloroplast development and circadian clock regulation.

Notes and Definitions

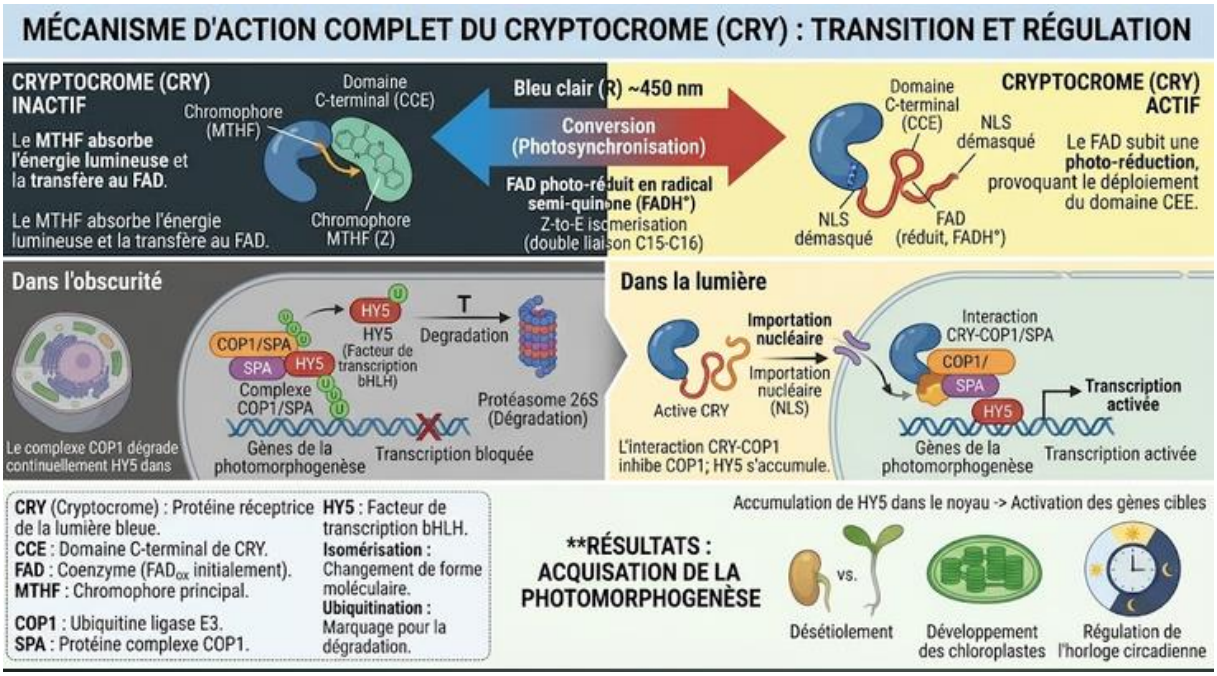
- Cryptochrome (CRY): A photoreceptor protein that detects blue light (400–500 nm) and UV-A radiation.

- **FAD and MTHF:** Two molecules called chromophores, associated with the receptor and responsible for light absorption.

- CCE Domain: The C-terminal region of cryptochrome that undergoes a conformational change upon activation to transmit the signal.

- COP1/SPA: A protein complex containing an E3 ubiquitin ligase that marks other proteins for degradation by the cell.

- HY5: An essential transcription factor that activates key genes involved in light-regulated plant growth and development.



3. UVR8 (UV-B Light)

The UVR8 receptor (UV Resistance Locus 8) is unique because it perceives UV-B radiation (280–315 nm) without any prosthetic chromophore. Instead, it uses aromatic amino acids naturally present in its own peptide sequence, specifically tryptophan residues (particularly Trp285 and Trp233).

Mechanism of Action (Monomerization)

- Inactive state: In the absence of UV-B radiation, UVR8 exists as a homodimer maintained by complex salt bridges between its two subunits.

- Activation: The absorption of UV-B photons by tryptophan residues disrupts these electrostatic interactions, causing the dimer to dissociate into two active monomers.

- Signal transduction: The UVR8 monomers accumulate in the nucleus and interact with the COP1 ubiquitin ligase.

- Gene regulation: UVR8 sequesters COP1, preventing HY5 degradation, just like cryptochromes. The accumulation of HY5 activates the expression of UV-acclimation genes, including those involved in the synthesis of protective flavonoids (which act as a natural sunscreen) and DNA repair mechanisms.

- Feedback regulation (Deactivation): RUP1 and RUP2 proteins bind to active UVR8 monomers and promote their re-dimerization, thereby deactivating the signaling pathway and preventing an excessive, metabolically costly response.

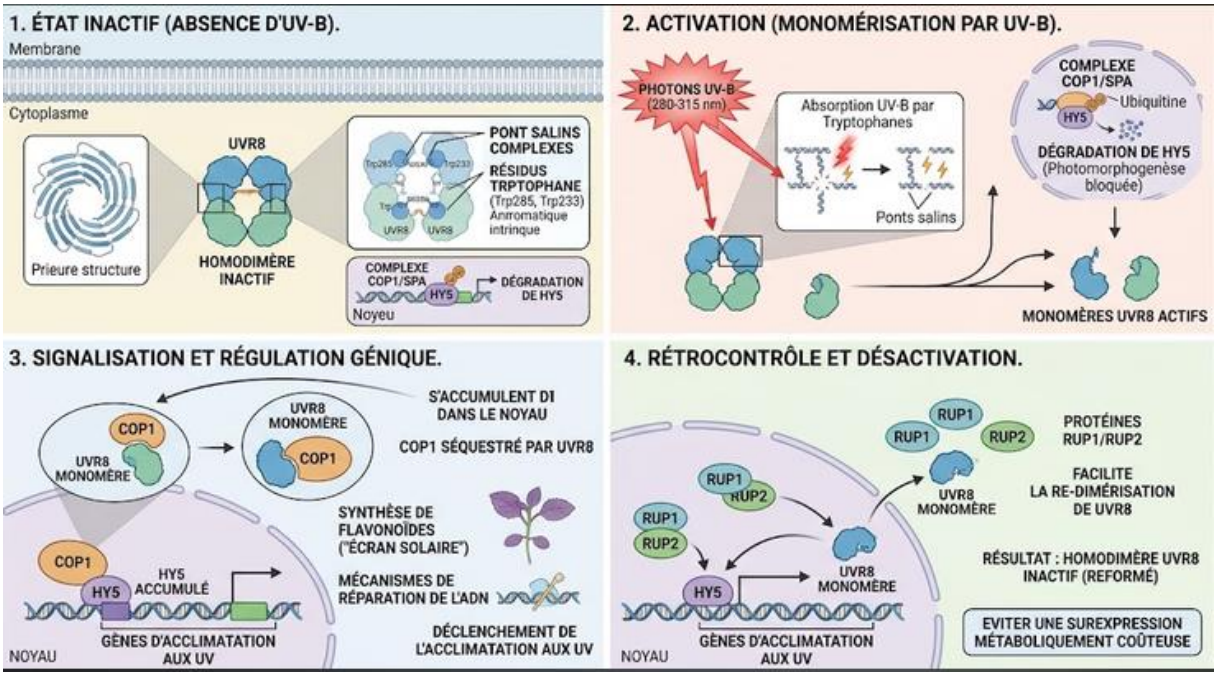


Fig. 29. UVR8 (UV-B Light)

Notes and Definitions

- **UVR8 (UV Resistance Locus 8):** A photoreceptor protein specifically sensitive to UV-B light (280–315 nm). It is unique because it does not use any external non-protein chromophore to absorb light.

- **Prosthetic Chromophore**: A non-protein chemical group (such as FAD or the tetrapyrrole chromophore found in phytochromes) attached to a protein and responsible for light absorption. UVR8 does not contain one.

- **Tryptophan Residues (Trp285, Trp233):** Aromatic amino acids directly incorporated into the UVR8 peptide sequence. They act as intrinsic chromophores by directly absorbing UV-B photons.

- **Homodimer (Inactive State):** A protein complex formed by the association of two identical UVR8 subunits. In the absence of UV-B radiation, the protein remains in this inactive form.

- **Salt Bridges:** Electrostatic interactions (attractive forces between positive and negative charges) that firmly hold the two subunits of the UVR8 homodimer together.

- **Monomerization (Activation):** The process by which the homodimer dissociates into two independent and active monomers, triggered by the disruption of salt bridges under UV-B radiation.

- **COP1 (Constitutive Photomorphogenic 1):** An E3 ubiquitin ligase enzyme. In darkness or in the absence of UV-B radiation, it targets HY5 and marks it for degradation by the 26S proteasome.

- **HY5 (Long Hypocotyl 5):** A key transcription factor. When it escapes degradation by COP1 (through COP1 sequestration by UVR8), it accumulates in the nucleus and activates target genes.

- **Plant Sunscreen (Flavonoids**): Secondary metabolites whose synthesis is stimulated by HY5. They accumulate in the leaf epidermis to absorb and block harmful UV-B radiation, thereby protecting internal plant tissues.

- **RUP1 / RUP2 (Repressor of UV-B Photomorphogenesis 1 and 2):** Negative regulatory proteins involved in feedback regulation. They bind to active UVR8 monomers and promote their re-dimerization into the inactive form, preventing an excessive and energetically costly genetic response.

2. Calcium Signaling: Calcium as a Second Messenger; Role of Protein Kinases (CDPK, MAPK)

Calcium signaling is a central mechanism in plants, in which calcium ions (Ca^{2+}) act as universal second messengers to translate environmental or hormonal signals into appropriate cellular responses.

Cytosolic Ca^{2+} concentrations are normally very low (approximately 100 nM, around 20,000 times lower than in the extracellular environment). An increase in this concentration, caused by the opening of calcium channels in the endoplasmic reticulum or plasma membrane, activates specific proteins and triggers signaling cascades.

Depending on the cellular context, changes in cytosolic calcium concentration may vary in amplitude and duration and may also occur as oscillations. Each stimulus and cellular context produces a specific calcium signature.

There are two major sources of Ca^{2+} ions that increase cytosolic calcium concentration:

- Internal calcium stores, mainly in the endoplasmic reticulum (ER) and, to a lesser extent, in the mitochondria and vacuoles.
- The extracellular environment.

The movement of Ca^{2+} between these compartments depends on the activity of calcium-permeable transmembrane channels and pumps. Transmembrane channels facilitate calcium movement along its electrochemical gradient, whereas pumps transport calcium against this gradient, requiring energy.

2.1. Calcium (Ca^{2+}) as a Second Messenger

a. Maintenance of the Gradient and Homeostasis

- Resting state: The concentration of free Ca^{2+} in the cytosol is maintained at a very low level, approximately 100 nM (around 20,000 times lower than in the extracellular environment).

- Storage compartments (Reservoirs): Calcium ions are stored at much higher concentrations (µM–mM) in:

- The extracellular environment (or the cell wall/apoplast in plants).
- Intracellular organelles: the endoplasmic reticulum (ER), mitochondria, and vacuoles (specific to plant cells).

- Calcium signature: Upon stimulation, transmembrane channels open, causing a rapid influx of Ca^{2+} into the cytosol. Variations in calcium concentration in terms of amplitude, duration, frequency, and spatial localization constitute the specific calcium signature associated with each stimulus.

b. Pathways of Calcium Entry and Removal from the Cytosol

- Mobilization of internal calcium (PLC/IP3 pathway): The activation of membrane receptors (e.g., Gq/11-type G-protein-coupled receptors) activates phospholipase C (PLC). PLC cleaves PIP2 into two second messengers:

- DAG (Diacylglycerol): Remains anchored to the membrane and activates Protein Kinase C (PKC).
- IP3 (Inositol-1,4,5-trisphosphate): Diffuses through the cytosol and binds to its receptors (IP3R) on the endoplasmic reticulum, triggering the release of Ca^{2+} into the cytosol.

- Refilling of calcium stores (SOCE pathway – Store-Operated Calcium Entry):

When the Ca^{2+} concentration inside the ER decreases, the luminal protein STIM1 undergoes a conformational rearrangement and physically interacts with the Orai1 membrane channel, triggering the entry of extracellular Ca^{2+} into the cell.

- Calcium extrusion mechanisms: The return to the resting state is actively ensured by Ca^{2+}-ATPase pumps (ACA/ECA) and ion exchangers (such as H^+/Ca^{2+} or Na^+/Ca^{2+} exchangers).

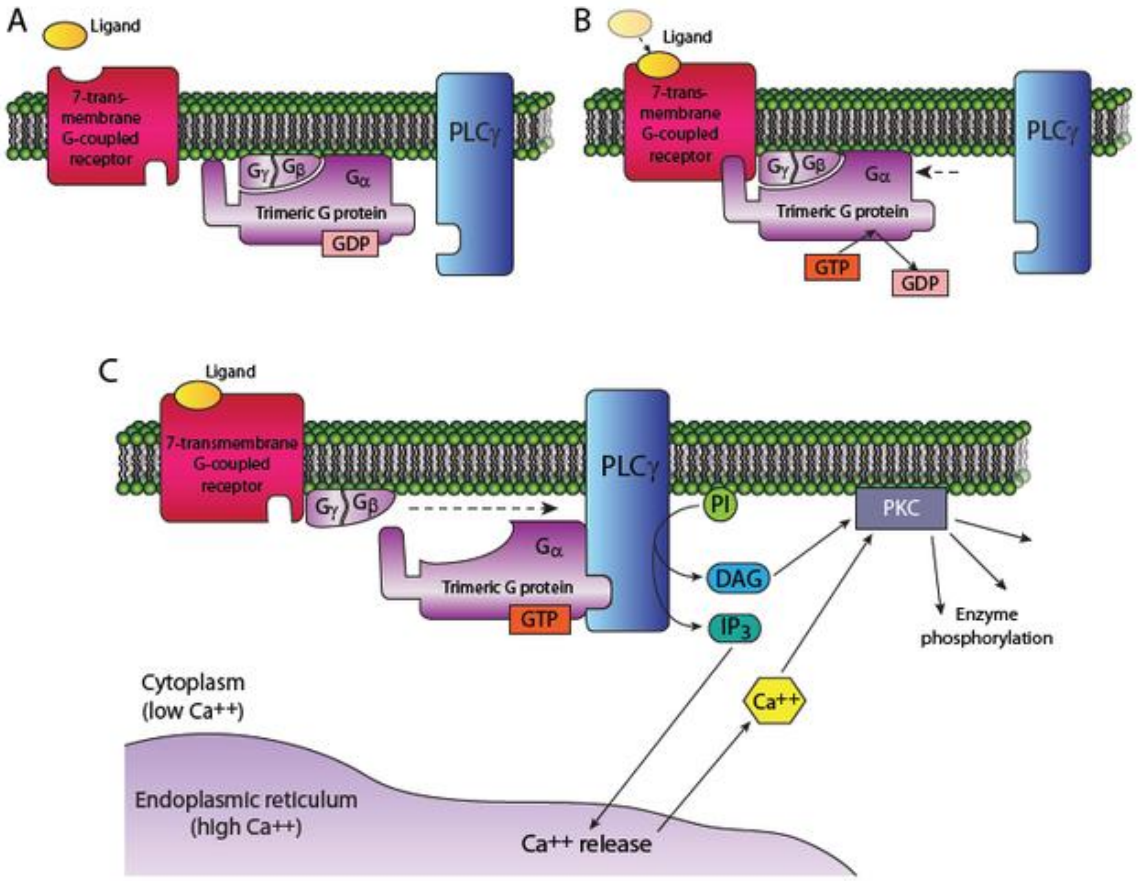


Fig. 30. Activation of Phospholipase C by a G Protein. *(A) The G protein is inactive when GDP is bound to it. (B) When a ligand binds to a receptor containing seven transmembrane domains, the G protein interacts with the receptor and exchanges GDP for GTP, causing the Gα subunit to dissociate. (C) The Gα-GTP subunit activates PLCγ, which produces two second messengers: DAG and IP3. IP3 induces the release of Ca^{2+} into the cytoplasm from the endoplasmic reticulum. Together with DAG, it also contributes to the activation of Protein Kinase C (PKC). An annotated version of this figure is available in video format.* Source: https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/Book%3A_Cells_-_Molecules_and_Mechanisms_(Wong)

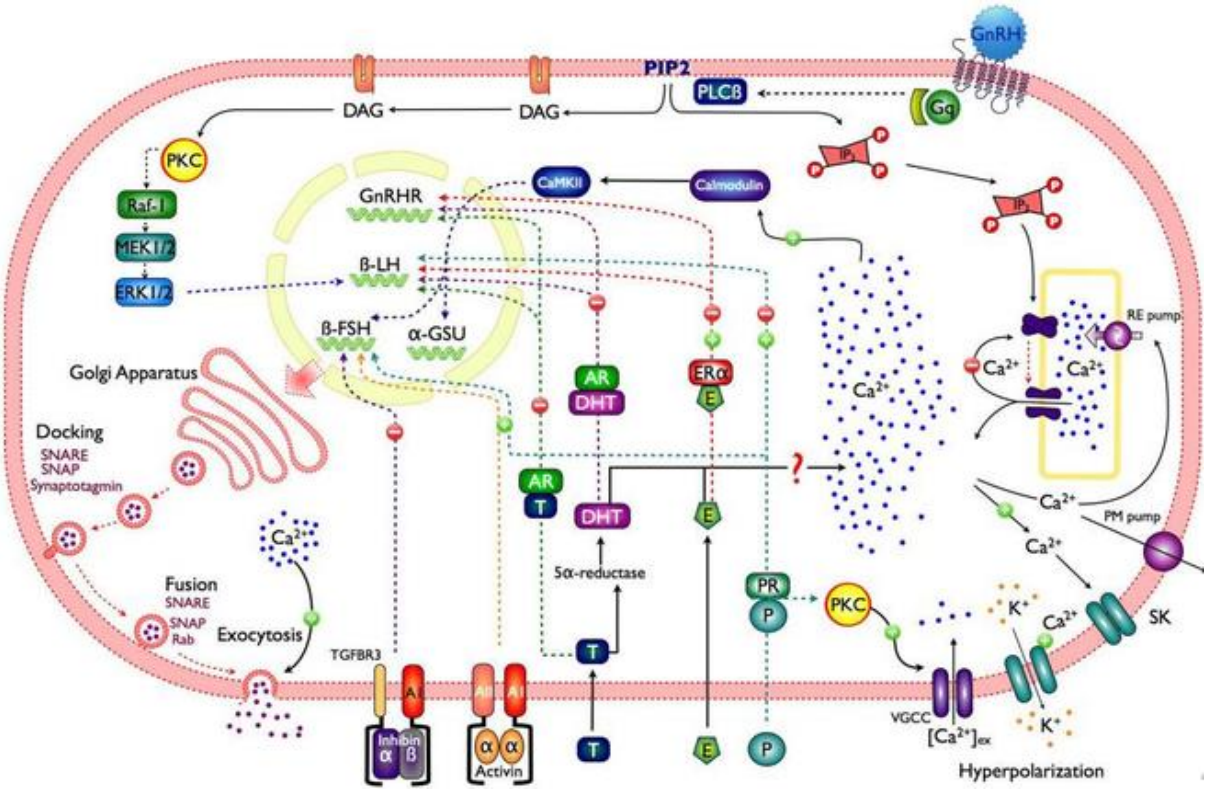


Fig. 31. Example of a Signaling Pathway Activated by a Neurohormone (GnRH), Involving Calcium at Multiple Levels. *GnRH (Gonadotropin-Releasing Hormone) acts on cells of the anterior pituitary gland (adenohypophysis), stimulating the synthesis and release of FSH and LH. This signaling pathway depends on calcium at several different levels. Abbreviations: GnRH: Gonadotropin-Releasing Hormone. Gq: Gq/11 G protein. PLCβ: Phospholipase C beta. PIP2: Phosphatidylinositol 4,5-bisphosphate. DAG: Diacylglycerol. IP3: Inositol 1,4,5-trisphosphate. PKC: Protein Kinase C. VGCC: Voltage-Gated Calcium Channels. CaMKII: Calcium/Calmodulin-Dependent Protein Kinase II. ER pump: Endoplasmic Reticulum Calcium Pump. PM pump: Plasma Membrane Calcium Pump. SK: Small-Conductance Calcium-Activated Potassium Channels. P: Progesterone. PR: Progesterone Receptor. E: Estradiol. ERα: Estrogen Receptor Alpha. T: Testosterone. AR: Androgen Receptor. Raf: Serine/Threonine Protein Kinase. MEK: Mitogen-Activated Protein Kinase Kinase. ERK: Extracellular Signal-Regulated Kinase.* Source: https://www.frontiersin.org/journals/endocrinology/articles/10.3389/fendo.2013.00127/full

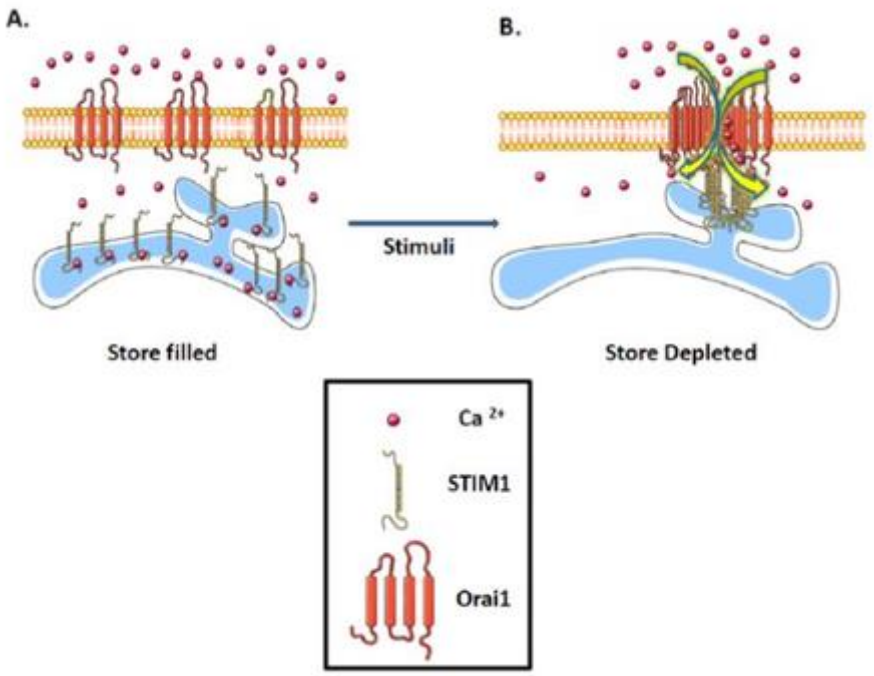


Fig. 32. Activation of Calcium Entry Controlled by Store Depletion (SOCE Pathway) *A: Under resting conditions, the Ca^{2+} stores in the endoplasmic reticulum (ER) are full, and Ca^{2+} is bound to STIM1. The Orai1 tetrameric channels are closed and distributed throughout the plasma membrane. B: When the Ca^{2+} stores become depleted, Ca^{2+} dissociates from STIM1, causing STIM1 proteins to aggregate in nearby regions of contact between the ER and the plasma membrane (called puncta). Following aggregation, STIM1 physically interacts with Orai1 channels, opening them and allowing Ca^{2+} to flow into the cell.*

2.2. Decoding the Calcium Signature: The Role of Protein Kinases

The increase in free Ca^{2+} is detected by calcium-sensing proteins (such as Calmodulin – CaM) or directly by calcium-dependent protein kinases. These proteins transmit the signal through phosphorylation cascades.

a. Calcium-Dependent Protein Kinases (CDPK / CPK)

CDPKs (Calcium-Dependent Protein Kinases) are major components involved in decoding calcium signals, particularly well-developed and extensively studied in plants and certain protists.

Unique structure:

Unlike most animal kinases (such as CaMKII, which requires a separate calmodulin protein), CDPKs are unique chimeric molecules. They contain three functional domains within a single polypeptide chain:

1. Catalytic kinase domain: A serine/threonine kinase responsible for protein phosphorylation.
2. Autoinhibitory domain: Blocks the catalytic activity in the absence of Ca^{2+}.
3. Calcium-binding domain: Contains EF-hand motifs, similar to those found in calmodulin.

Activation mechanism:

In the absence of Ca^{2+}, the autoinhibitory domain blocks the catalytic site. The direct binding of Ca^{2+} ions to the EF-hand motifs induces a conformational change that removes autoinhibition and activates the kinase.

Biological roles:

- Response to abiotic stress: Regulation of stomatal closure (through the SLAC1 anion channel during water stress).
- Immunity and defense: Phosphorylation of enzymes that generate reactive oxygen species (ROS) and activation of transcription factors during pathogen attacks.

b. Mitogen-Activated Protein Kinase Cascades (MAPK)

MAPKs represent a universal signal transduction module consisting of three kinases acting sequentially in a cascade:

⇨ MAP3K
⇨ MAP2K
⇨ MAPK

Activation of target proteins and gene expression

Relationship between Ca^{2+} and the MAPK pathway:

The increase in cytosolic Ca^{2+} generally does not activate MAPKs directly. Instead, it acts upstream through intermediate signaling proteins:

- Ca^{2+} activates calmodulin-dependent kinases (CaMK) or CDPKs.

- These kinases (or upstream proteins such as PKC) phosphorylate and activate MAP3K.
- MAP3K activates MAP2K, which subsequently phosphorylates MAPK.
- Activated MAPK translocates into the nucleus, where it phosphorylates key transcription factors (e.g., CREB, MYB and WRKY).

Biological roles:

- Control of the cell cycle and development (cell division and differentiation).
- Responses to complex environmental signals through the integration of mechanical, thermal and hormonal stress signals.

c. Summary and Overall Mechanism

Calcium signaling is based on the conversion of a physical ionic flow into a biochemical code.

1. Signal generation: A stimulus triggers the opening of Ca^{2+}-permeable channels, causing a rapid increase in cytosolic Ca^{2+} concentration.

2. Signal decoding by kinases:

- CDPKs directly bind Ca^{2+} and respond rapidly at the local cellular level.
- MAPKs receive calcium signals relayed through other intermediate proteins (PKC/CaMK) and amplify the signal toward long-term gene regulation.

3. Cellular response: The cell adapts to the stimulus through changes in metabolism, gene transcription or cell viability.

Notes and Definitions

- Calcium Signature: A specific spatial and temporal pattern of changes in cytosolic Ca^{2+} concentration (amplitude, duration, oscillation frequency and localization), characteristic of each stimulus and cell type.

- Apoplast: The extracellular compartment in plants, consisting of cell walls and intercellular spaces. It acts as an external reservoir of calcium.

- Phospholipase C (PLC): A membrane-associated enzyme activated by cellular receptors. It cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into two second messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3).

- CDPK (Calcium-Dependent Protein Kinase): A calcium-dependent protein kinase, widely found in plants, containing both a catalytic domain and a calcium-binding domain (EF-hand motifs) within a single polypeptide chain, allowing direct activation by Ca^{2+}.

- MAPK Cascade (Mitogen-Activated Protein Kinase): A universal three-level signaling module (MAP3K → MAP2K → MAPK) that transmits signals from the cell membrane to the nucleus to regulate gene expression.

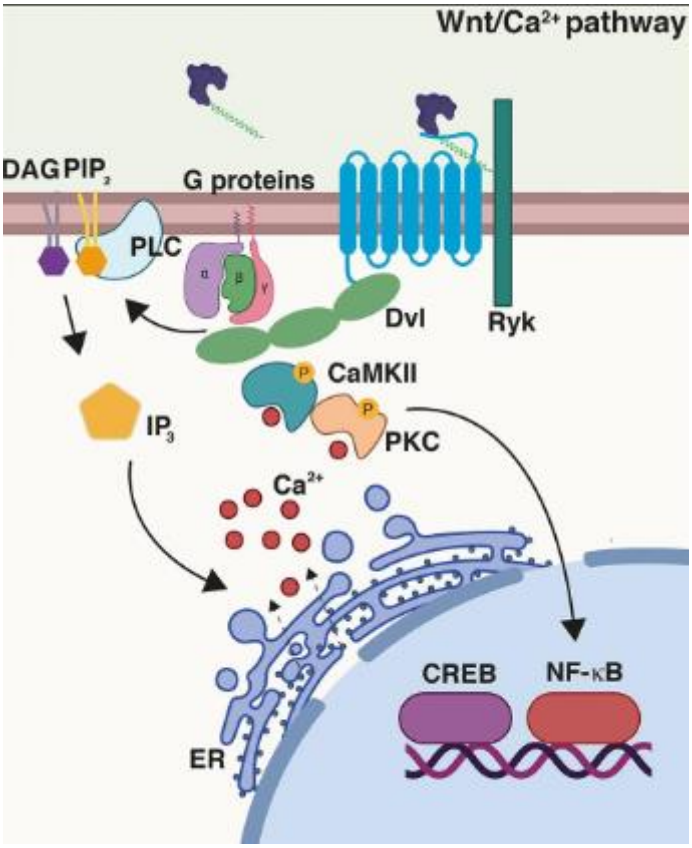
- Calmodulin (CaM): A ubiquitous calcium-binding protein that undergoes a conformational change upon binding Ca^{2+} ions, allowing it to interact with and activate target proteins.

- IP3 Receptor (IP3R): A ligand-gated calcium channel located on the membrane of the endoplasmic reticulum. It opens when IP3 binds to it, allowing Ca^{2+} to be released into the cytosol.

- SOCE Pathway (Store-Operated Calcium Entry): A mechanism of extracellular Ca^{2+} influx triggered by the depletion of internal calcium stores in the endoplasmic reticulum, involving the interaction between STIM1 and Orai1 proteins.

- ACA / ECA Pumps (Ca^{2+}-ATPases): Primary active transporters that consume ATP to move Ca^{2+} out of the cytosol into the extracellular environment or intracellular storage compartments, against its electrochemical gradient.

- CAX Exchangers (H^+/Ca^{2+}): Secondary active transporters that use the proton (H^+) gradient to sequester Ca^{2+} inside the vacuole or remove it from the cell.



Écriture

Fig. 33. Wnt/Ca^{2+} Signaling Pathway. *Activation of the Wnt/Ca^{2+} pathway, a β-catenin-independent signaling pathway, induces the release of Ca^{2+} from the endoplasmic reticulum (ER), which subsequently activates the protein kinases CaMKII and PKC. This pathway triggers downstream signaling events, including the activation of the transcription factors NF-κB and CREB, leading to the transcription of their target genes.* Source: https://www.frontiersin.org/articles/10.3389/fcell.2021.692888/full

3. Hormonal Signaling Models: Focus on Auxin Perception (TIR1/AFB Proteins) and Abscisic Acid (ABA) Perception (PYR/PYL Receptors)

In plants, development, growth, and responses to environmental stresses are orchestrated by phytohormones. At the cellular level, hormonal signal transduction relies on receptor-mediated perception, followed by cascades of gene repression or derepression and metabolic regulation. Two major models illustrate the diversity of these mechanisms:

- **Auxin-induced protein degradation pathway:** A transcriptional derepression pathway mediated by the SCF TIR1/AFB E3 ubiquitin ligase complex.

- **Phosphatase inhibition pathway:** A signaling pathway regulated by abscisic acid (ABA) through the PYR/PYL/RCAR receptor module.

3.1. Auxin Perception Pathway (TIR1/AFB Proteins)

Auxin, particularly indole-3-acetic acid (IAA), is a key hormone involved in cell division, elongation, and differentiation. Nuclear auxin signaling operates through a transcriptional derepression mechanism mediated by the ubiquitin–proteasome system.

a. Key Components of the Pathway

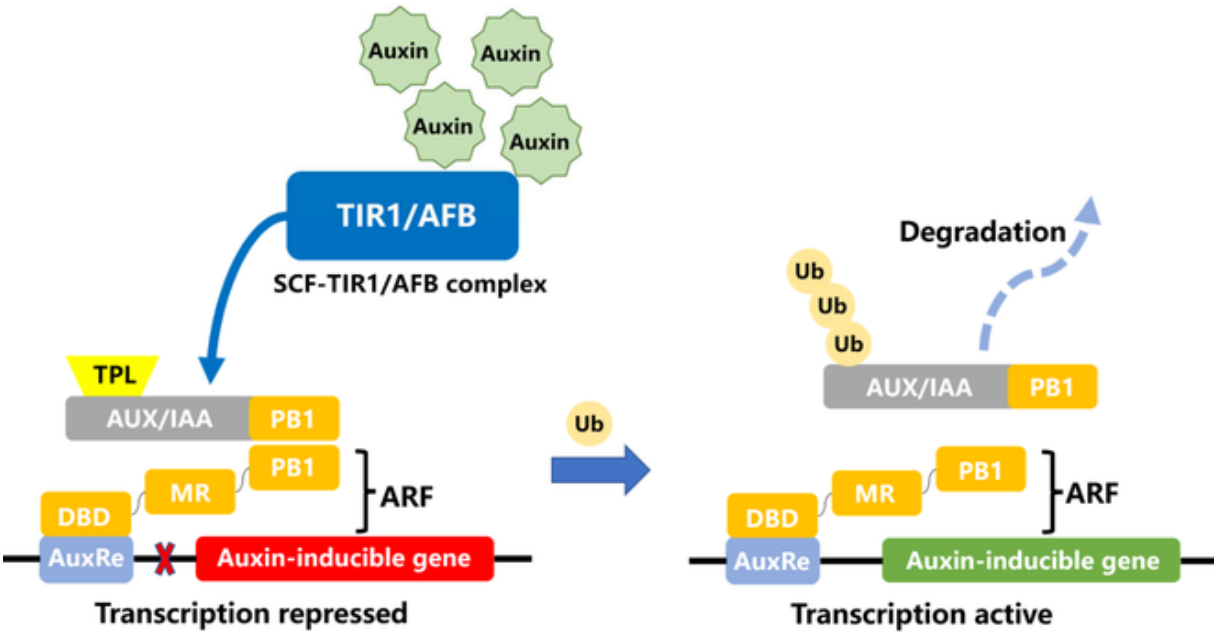
- **TIR1/AFB (TRANSPORT INHIBITOR RESPONSE 1 / AUXIN SIGNALING F-BOX):** These are soluble nuclear receptors that constitute the F-box subunits of the SCF TIR1/AFB E3 ubiquitin ligase complex.
- **Aux/IAA (Repressor Proteins):** These are short-lived nuclear proteins that act as transcriptional repressors. They bind to ARF transcription factors and recruit corepressors, such as TOPLESS (TPL), to promote chromatin condensation and inhibit gene expression.
- **ARF (Auxin Response Factors):** These are transcription factors that bind to auxin response elements (AuxREs) in DNA to regulate the transcription of target genes.

b. Mechanism of Perception and Degradation (The Molecular Glue Model)

- **Absence of auxin:** Aux/IAA repressors interact with ARF transcription factors, preventing them from activating the transcription of auxin-responsive genes.
- **Auxin entry:** Auxin enters the nucleus and binds to the ligand-binding pocket of the TIR1/AFB receptor.
- **Complex formation:** Auxin acts as a molecular glue by stabilizing the interaction between TIR1/AFB and the Aux/IAA protein.
- **Ubiquitination and degradation:** Aux/IAA binding promotes its ubiquitination by the SCF TIR1/AFB complex, targeting it for degradation by the 26S proteasome.
- **Transcriptional activation:** Once the Aux/IAA repressors have been degraded, ARF transcription factors are released from repression and activate the transcription of auxin-responsive genes.

Remarks

- **The 26S proteasome:** A large multiprotein complex located in the cytosol and nucleus of eukaryotic cells. It is responsible for the selective and controlled degradation of cellular proteins.
- **Ubiquitination (or ubiquitylation):** A biochemical process in which a small, highly conserved protein consisting of 76 amino acids, called ubiquitin, is covalently attached to a target protein, usually through a lysine residue. This modification acts as a cellular targeting signal or molecular tag.
    - **Monoubiquitination:** The attachment of a single ubiquitin molecule to a target protein. It is often involved in endocytosis and chromatin regulation.
    - **Polyubiquitination:** The attachment of a chain of ubiquitin molecules linked together, particularly through lysine 48 (K48) of ubiquitin. This modification serves as a specific signal for degradation by the 26S proteasome.



Écriture

Fig. 33. Auxin-Mediated ARF Transcriptional Activation.

Intracellular auxin signal transduction is coordinated by the TIR1/AFB receptor, together with AUX/IAA proteins and ARF transcription factors. Auxin acts as a "molecular glue", facilitating the interaction between the SCF-TIR1/AFB complex and Aux/IAA proteins.

At low auxin concentrations, the AUX/IAA repressor binds to the ARF transcription factor and forms a complex that recruits the TPL (TOPLESS) co-repressor, thereby inhibiting ARF activity and the expression of auxin-responsive target genes.

When auxin concentrations increase, AUX/IAA binds to the SCF-TIR1/AFB complex and is ubiquitinated, then degraded by the 26S proteasome. ARF transcription factors are subsequently released from repression and activate the transcription of downstream target genes.

DBD = DNA-Binding Domain; MR = Middle Region; PB1 = Phox and Bem1 Domain.

Source: https://www.mdpi.com/2076-3417/12/3/1360

3.2. Abscisic Acid (ABA) Perception Pathway (PYR/PYL/RCAR Receptors)

Abscisic acid (ABA) is the main plant hormone involved in responses to water stress (drought and salinity) and seed dormancy. Unlike auxin, ABA perception operates through a mechanism known as the inhibition of inhibitors (double inhibition of a phosphatase).

a. Key Components of the Pathway

- **PYR/PYL/RCAR (Pyrabactin Resistance / PYR1-Like / Regulatory Components of ABA Receptors):** Soluble ABA receptors located in the cytosol and nucleus.
- **PP2C (Type 2C Protein Phosphatases):** Negative regulators of the ABA signaling pathway (e.g., ABI1 and ABI2). They maintain the pathway in an inactive state in the absence of the hormone.
- **SnRK2 (Sucrose Non-Fermenting 1-Related Protein Kinase 2):** Positive regulatory protein kinases that activate downstream cellular targets when the pathway is stimulated.
- **ABF/ABI (ABA-Responsive Element-Binding Factors):** Nuclear transcription factors that regulate the expression of stress-responsive genes.

b. Mechanism of Perception and Signal Transduction

- **Absence of ABA:** PP2C phosphatases remain active and continuously dephosphorylate SnRK2 protein kinases, keeping them in an inactive state.
- **Presence of ABA:** ABA binds to the hydrophobic pocket of the PYR/PYL/RCAR receptor.

- **Conformational change:** ABA binding induces the closure of a receptor loop known as the "gate", creating an interaction surface for the PP2C phosphatase.
- **PP2C inhibition:** The ABA-PYR/PYL complex binds to and sequesters the PP2C phosphatase, thereby inhibiting its activity.
- **SnRK2 kinase activation:** Once released from PP2C-mediated inhibition, SnRK2 kinases become activated through autophosphorylation.
- **Cellular responses:**
    - **In the nucleus:** SnRK2 phosphorylates transcription factors (ABF/ABI), inducing the expression of drought-responsive genes.
    - **At the plasma membrane (stomatal closure):** SnRK2 phosphorylates ion channels (e.g., SLAC1), triggering ion efflux and a loss of turgor pressure in guard cells.

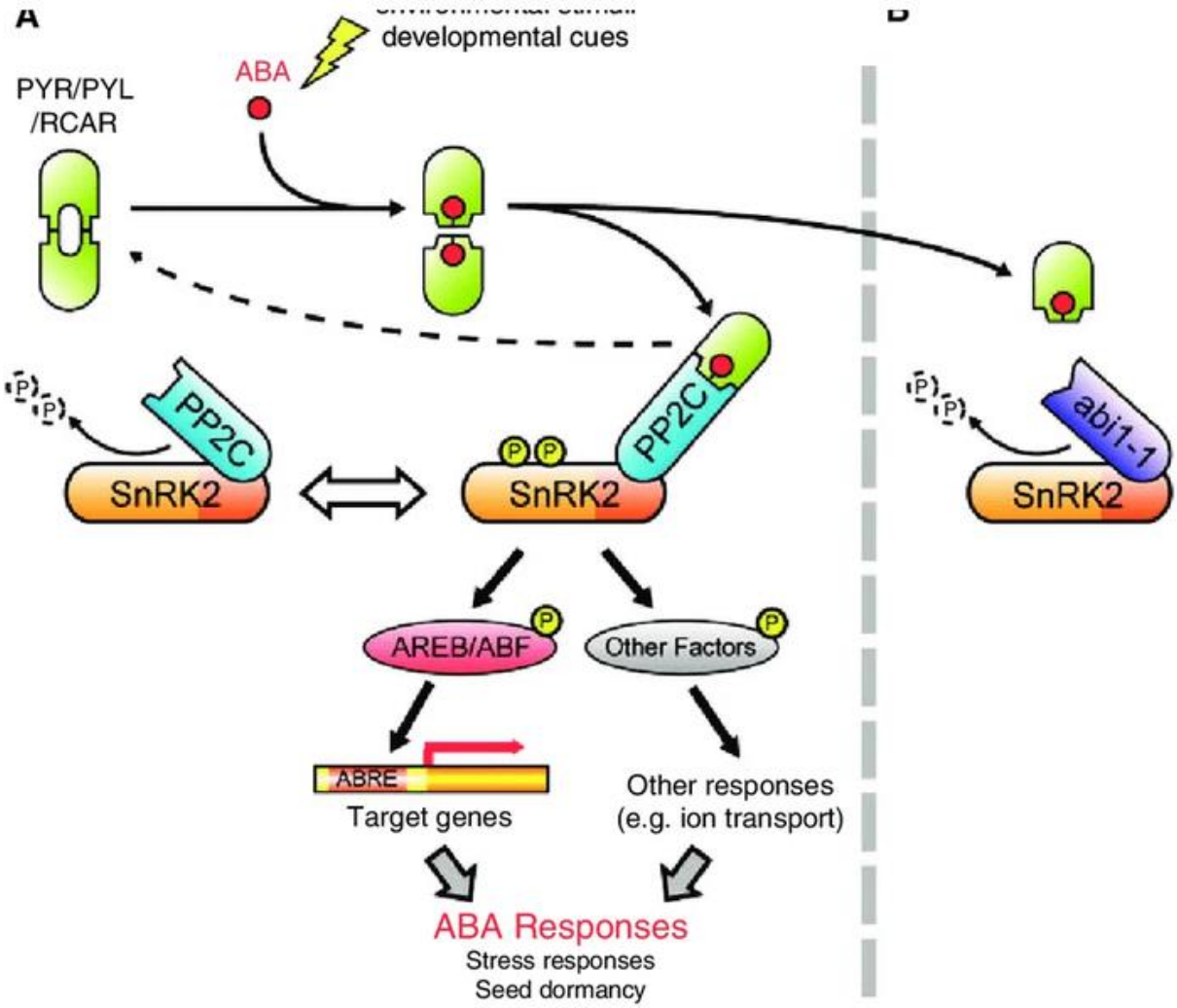
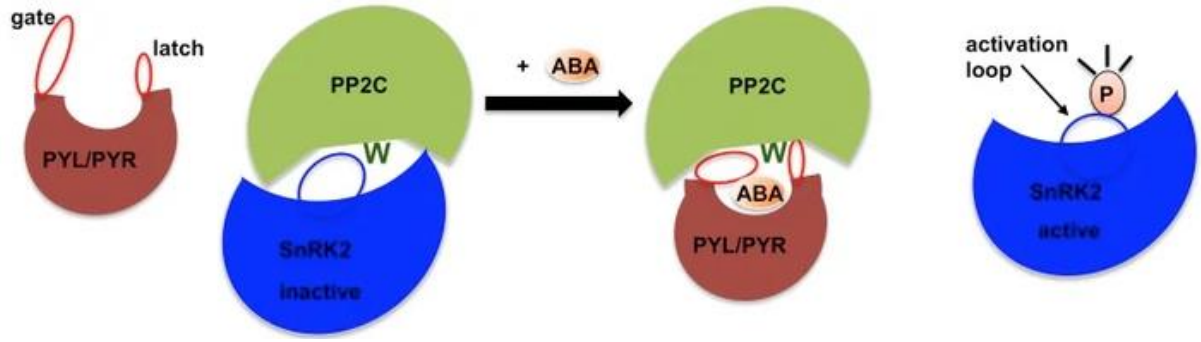


Fig. 34. Proposed Model of the Major ABA Signaling Pathway. *PYR/PYL/RCAR, PP2C, and SnRK2 form a signaling complex known as the "ABA signalosome". **(A)** Under normal conditions, PP2C negatively regulates SnRK2 through direct interactions and the dephosphorylation of multiple SnRK2 residues. When abiotic stresses or developmental signals increase endogenous ABA levels, PYR/PYL/RCAR receptors bind to ABA and interact with PP2C, thereby inhibiting its phosphatase activity. Consequently, SnRK2 is released from PP2C-mediated inhibition and becomes activated, allowing it to phosphorylate downstream targets, such as AREB/ABF bZIP-type transcription factors and membrane proteins, including ion channels. **(B)** In contrast, the abi1-1 mutant protein is unable to bind PYR/PYL/RCAR receptors. This results in the constitutive inhibition of SnRK2, even in the presence of ABA, leading to strong ABA insensitivity in the abi1-1 mutant.*



Écriture

Fig. 35. ABA-Dependent Regulation of PYR/PYL Receptors, PP2Cs, and SnRK2 Kinases. *In the absence of ABA, PYR/PYL receptors adopt an "open" conformation, with the "gate" loop oriented away from the ligand-binding pocket and the "latch" loop. PP2C phosphatases remain active and bind to SnRK2 kinases, inhibiting their activity through dephosphorylation of the activation loop as well as allosteric inhibition. ABA binding induces a conformational change in the receptors, causing the "gate" loop to swing into a closed position. A conserved tryptophan residue (represented by the dark green W) on the PP2C surface acts as a molecular lock, stabilizing the ligand within the binding pocket and forming a catalytically inactive receptor–ligand–phosphatase complex. Inhibition of PP2C allows SnRK2 kinases to become activated through phosphorylation of their activation loops. Activated SnRK2 kinases subsequently transduce the ABA signal by phosphorylating downstream effector proteins.*

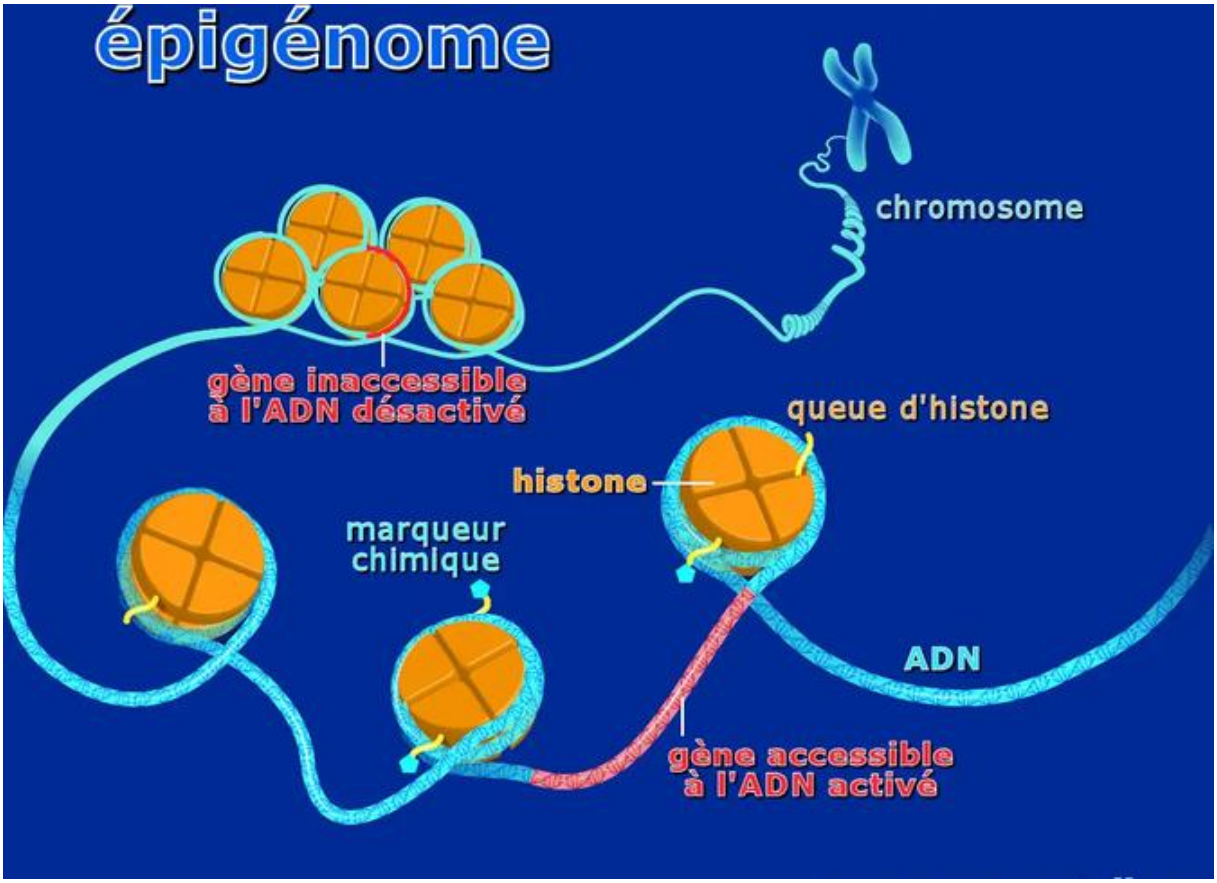
4. Epigenetics and Memory: Concepts of Priming and Chromatin Modifications in Response to Stimuli

Epigenetics is the study of reversible and heritable changes in gene expression that do not involve alterations in the underlying DNA sequence. In both plants and animals, epigenetic mechanisms play a central role in cellular memory and adaptation to environmental stresses, such as drought, pathogen attacks, and temperature fluctuations.

Epigenetics (epigenesis + genetics) refers to the study of heritable changes in gene expression that occur without modifications to the underlying DNA sequence. These changes can lead to alterations in phenotype without changing the genotype, thereby influencing how cells read and express their genes.

Epigenetic priming is an important mechanism of rapid adaptation. Dynamic modifications of chromatin leave a "molecular imprint" following an initial stimulus, transforming a previous

environmental experience into a functional memory that enables plants to respond more efficiently to subsequent stress exposure.



Écriture

Fig. 36. The Epigenome.

The epigenome consists of a set of chemical compounds that interact with the genome and influence its activity.

4.1. Chromatin: The Structural Basis of Epigenetic Signaling

Chromatin (DNA wrapped around histone proteins) alternates between two dynamic states:

- **Euchromatin (open chromatin):** A loosely packed structure that is accessible to RNA polymerase and transcription factors, allowing gene expression. Genes are transcriptionally active.
- **Heterochromatin (condensed chromatin):** A tightly packed structure that is inaccessible to transcription factors, resulting in gene silencing.

The main chromatin remodeling mechanisms include:

1. DNA Methylation: The "OFF" Switch

Small chemical groups called methyl groups are directly attached to DNA, mainly to cytosine bases (C).

These methyl groups act as molecular locks, preventing the cellular machinery from accessing and reading certain genes.

Result: Gene repression (the gene is switched off or transcriptionally silenced).

2. Covalent Histone Modifications

DNA is an extremely long molecule. To fit inside the cell nucleus, it is wrapped around proteins called histones, much like a thread wrapped around spools. Together, DNA and histones form chromatin.

- **Histone acetylation (Examples: H3K9ac, H3K27ac):** An acetyl group is added to a histone protein. This weakens the interaction between histones and DNA, allowing chromatin to become less condensed and more accessible.

Result: Gene activation (the gene becomes accessible to the transcriptional machinery and can be expressed).

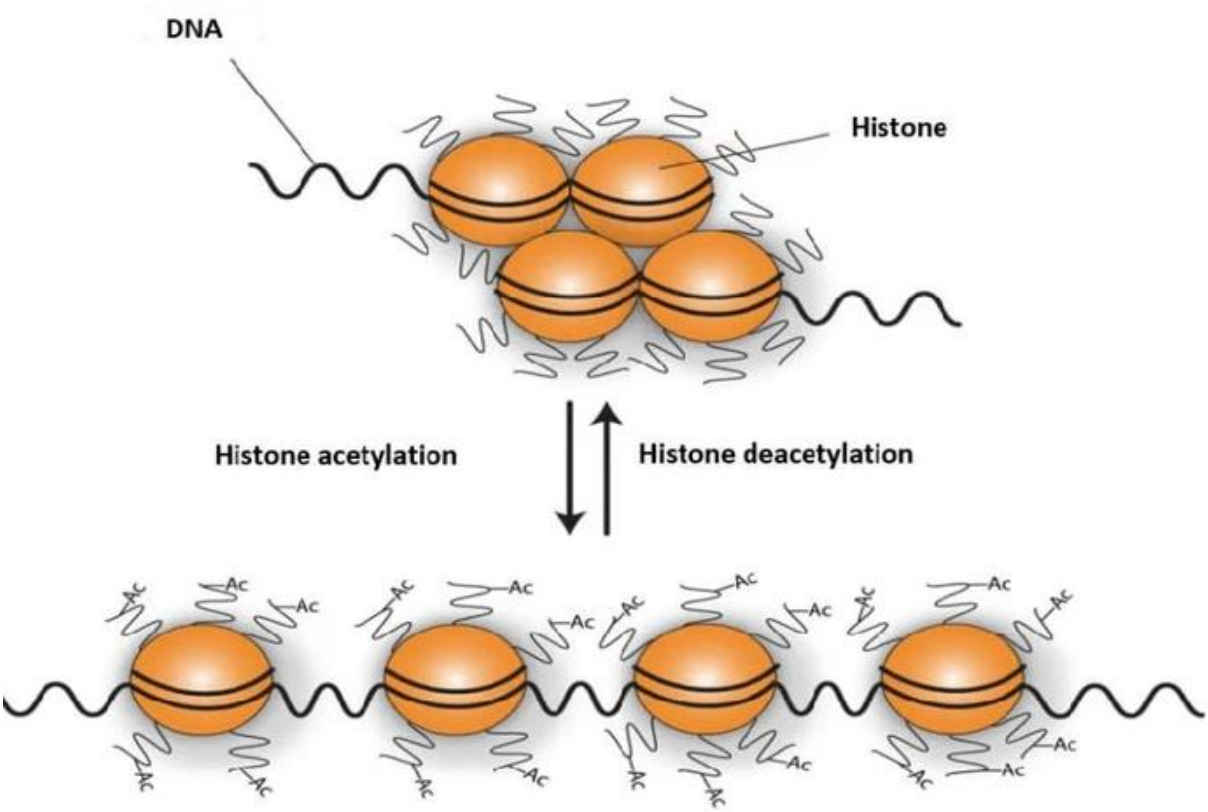


Fig. 36. Schematic Representation of Histone Acetylation Leading to Transcriptional Activation.

- **Histone methylation (Examples: H3K4me3 vs. H3K27me3):** A methyl group is added to a histone protein. Depending on the exact position where the modification occurs, its effect on gene expression can differ:
    - **H3K4me3:** An activating mark that promotes chromatin accessibility and gene transcription.
    - **H3K27me3:** A repressive mark that promotes chromatin compaction and gene silencing.

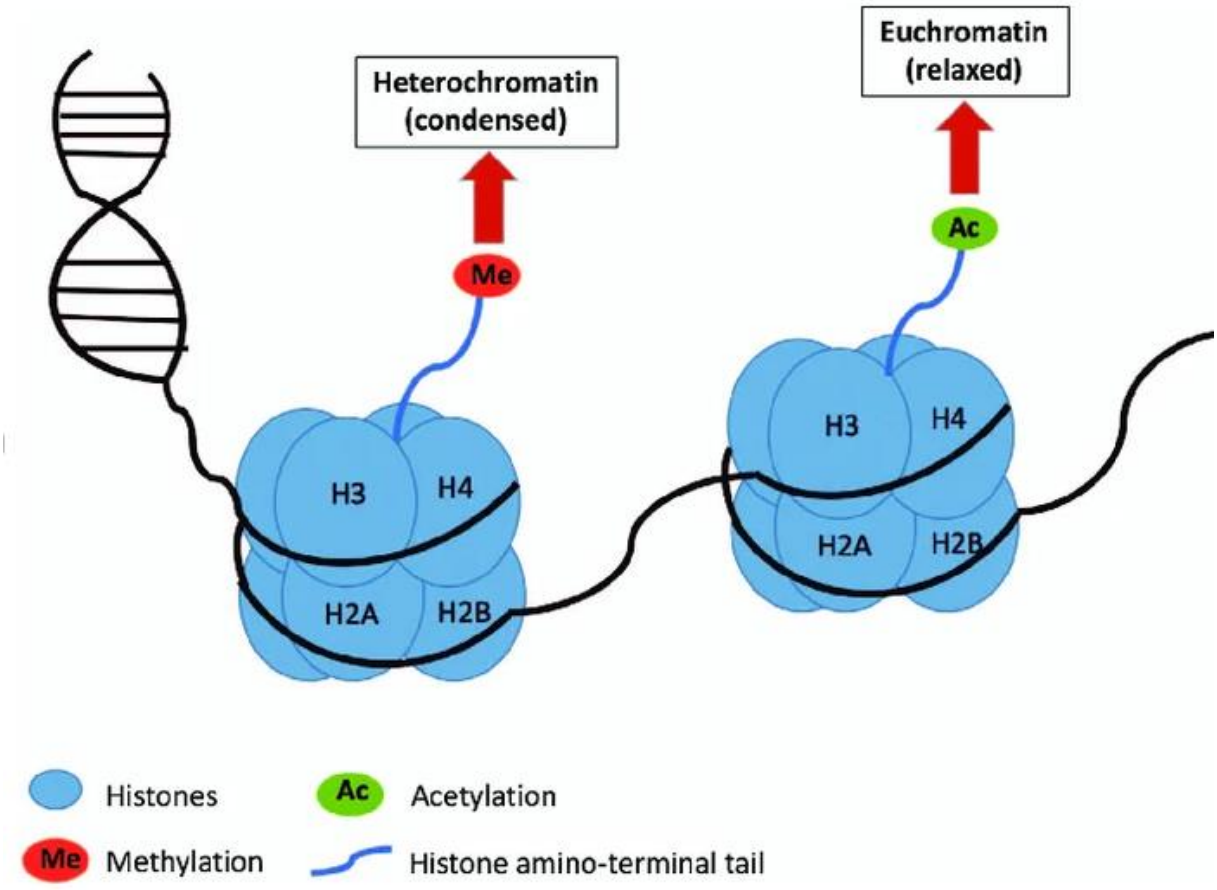


Fig. 38. Schematic Representation of Histone Methylation and Acetylation in Chromatin Remodeling. *The addition of methyl groups to the tails of histone core proteins results in histone methylation, which can lead to the formation of a condensed chromatin state known as* ***heterochromatin****. Heterochromatin prevents the transcriptional machinery from accessing DNA, resulting in transcriptional repression. In contrast, the addition of acetyl groups to lysine residues on the N-terminal tails of histones leads to histone acetylation, promoting a relaxed chromatin state known as* ***euchromatin****. In this state, transcription factors and other regulatory proteins can access their DNA-binding sites, allowing active transcription to take place.*

4.2. Epigenetic Priming

Priming is a physiological state of alertness induced by an initial stimulus (moderate stress, a signaling molecule, or pathogen attack). It does not necessarily trigger an immediate, massive response but prepares the cell to respond more rapidly and strongly to subsequent stress.

a. Molecular Mechanism of Priming

- **During the initial stress:** Activating epigenetic marks (e.g., H3K4me3 or H3K9ac) accumulate at the promoters of stress-responsive genes.

- **After the return to normal conditions:** Transcription levels decrease, but activating histone marks or transcriptional complexes maintained in a poised state (poised RNA Polymerase II) remain associated with DNA.
- **Upon re-exposure (second stress):** The presence of these residual molecular marks allows the transcriptional machinery to be recruited almost immediately, resulting in a faster and stronger response.

4.3. Establishment of Epigenetic Memory

Environmental memory can be maintained at two different levels:

- **Somatic Memory (Intragenerational Memory):** Maintained through mitotic cell divisions throughout the organism's lifetime. Maintenance enzymes (e.g., DNMT1 for DNA methylation) reproduce epigenetic patterns on newly synthesized DNA strands.
- **Transgenerational Memory:** Transmitted to offspring through gametes when epigenetic marks escape reprogramming and erasure during meiosis and fertilization.