Lipid-Protein Interplay: How the Plant Plasma Membrane H-ATPase Dynamically Interacts with Its Lipid Environment: A Review

Ram Kumar

ABSTRACT

The plasma membrane (PM) H^+-ATPase is the primary electrogenic proton pump in plants, energising secondary transport systems by generating a proton motive force essential for nutrient uptake, cell expansion, and stress responses. This review synthesizes current knowledge on the dynamic interplay between the PM H^+-ATPase and its lipid microenvironment, focusing on three critical regulatory dimensions: (i) the structural and mechanistic basis of proton transport, (ii) the modulation of pump activity by membrane lipids (phospholipids, sphingolipids, and sterols), and (iii) the potential role of membrane lateral heterogeneity in spatially organizing H^+-ATPases with their regulatory partners. We highlight how specific lipid-protein interactions governed by anionic phospholipids, sterol-dependent membrane properties, and sphingolipid-mediated trafficking fine-tune H^+-ATPase function. Emerging evidence suggests that lipids act not only as modulators of pump activity but also as facilitators of nanodomain formation, enabling efficient coupling with phosphorylation-dependent signaling cascades (e.g., auxin, blue light, and abscisic acid pathways). Critically, we evaluate methodological challenges in studying lipid-protein interactions and propose future directions, including high-resolution structural analyses and in vivo imaging, to resolve controversies surrounding detergent-resistant membranes and functional microdomains. By integrating structural, biochemical, and biophysical insights, this review underscores the PM H^+-ATPase as a central hub where lipid dynamics and cellular signaling converge to regulate plant growth and adaptation.

Keywords: H^+-ATPase regulation, Lipid-protein interactions, Plant plasma membrane, Sterol influence, Sphingolipid role

Department of Plant Protection, Agritec Plant Research, Sumperk, Czech Republic

Correspondence to:
Ram Kumar,
kumar@agritec.cz

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Introduction

The plasma membrane (PM) H^+-ATPase is the primary electrogenic proton pump in plants. It actively uses ATP hydrolysis to extrude protons through the PM, generating a proton motive force ($\Delta\mu H^+$).[1] This electrochemical gradient is the fundamental energy currency that drives secondary active transport systems through proton-coupled symport and antiport mechanisms.[1] As the principal energizer of PM transport, PM H^+-ATPase activity is physiologically indispensable, directly mediating critical processes including but not limited to: nutrient acquisition through proton-coupled transporters, cellular expansion via acid growth-dependent cell wall loosening, stomatal regulation for transpiration control, and stress-responsive signaling during pathogen defence.[2–4]

The pleiotropic nature of PM H^+-ATPase function in plant physiology[5,6] reflects its central role as the dominant source of free energy for PM transport systems. Current evidence suggests that approximately 70–80% of PM transporters depend on $\Delta\mu H^+$ as their primary energy source,[7] necessitating precise regulation of PM H^+-ATPase activity through multiple coordinated mechanisms. These include post-translational modifications such as phosphorylation/dephosphorylation cycles, modulation of the lipid microenvironment, and dynamic interactions with 14-3-3 regulatory proteins. Such comprehensive regulatory networks enable rapid metabolic adjustments in response to environmental fluctuations while maintaining essential cellular homeostasis under optimal and stress conditions.

Like all integral membrane transporters, the PM H^+-ATPase interacts with its lipid environment through specific and general mechanisms. Specific interactions occur via direct lipid binding to the protein, while general interactions depend on the bulk physicochemical properties of the lipid bilayer.[8] Structural studies of P-type ATPases have identified well-defined lipid-binding sites crucial in stabilizing the enzyme and regulating its catalytic activity.[9,10] Fundamental bilayer characteristics govern general lipid-protein interactions, including membrane thickness, fluidity, and dipole potential. Notably, electrostatic interactions between cytoplasmic domains of membrane proteins and anionic phospholipid head groups significantly modulate enzymatic activity.[8] Furthermore, the catalytic cycle induces conformational changes in the protein's hydrophobic regions, necessitating dynamic adaptation of the surrounding lipid matrix to prevent hydrophobic mismatch. Sterols emerge as important regulators in this context, constituting up to 30% of total PM lipids.[11] These molecules profoundly influence membrane properties by modulating thickness, fluidity, and permeability.[12,13] Sterols preferentially interact with saturated fatty acid chains of phospholipids and sphingolipids, and can affect membrane protein activity through both specific binding sites and indirect modulation of the local lipid environment at the protein-bilayer interface.[14,15]

Notably, the original hypothesis of PM horizontal heterogeneity emerged from studies on detergent-resistant membrane fractions (DRMs), initially referred to as "lipid rafts." Though contemporary high-resolution fluorescence microscopy techniques have demonstrated that none of the classical DRM markers, including enrichment in sterols and sphingolipids, presence of acylated integral membrane proteins, glycosylphosphatidylinositol-anchored proteins, and other characteristic components, are sufficient to establish protein segregation into specific membrane domains in vivo, definitively. While resistance to cold non-ionic

detergent extraction remains a useful experimental tool for studying specific membrane properties.[16–20] It is now well-established that these biochemical fractions do not accurately reflect functional membrane domains in living cells.[21,22]

Lipid-mediated regulation of H^+-ATPases is increasingly recognized as a crucial mechanism linking crop stress adaptation (such as drought and salinity) with promising biotechnological applications. Multiple studies have shown that the composition and dynamics of membrane lipids, including anionic phospholipids and sterols, directly modulate the activity of plant PM H^+-ATPases, promoting efficient proton pumping by forming specific contact sites within the transmembrane domain and enhancing pump activation in response to environmental stimuli.[23–26] Recent advances have revealed that the PATROL1 protein governs the translocation and activation of H^+-ATPase in both roots and shoots—overexpression of PATROL1 leads to significant increases in root length, lateral root number, shoot dry weight, and shoot nitrogen content under drought conditions, identifying H^+-ATPase regulation as a promising target for improving crop drought resilience and nutrient uptake.[27] Similarly, under salt stress, exogenous melatonin enhances H^+-ATPase activity and synergistically operates with endogenous hydrogen sulfide to maintain K^+/Na^+ homeostasis, enabling better plant stress tolerance by supporting secondary active transport energized by proton gradients.[28] Genetic manipulation studies further show that specific transcription factors (e.g., MYB308) induce H^+-ATPase gene expression, thereby facilitating root iron uptake in iron-limited soils.[29] Together, these findings highlight a mechanistic bridge from lipid environment modulation and lipid-microdomain engineering to advanced crop biotechnology. Proteomic assessment of detergent-resistant fractions of plant PMs showed the presence of P-type H^+-ATPases.[18,21,30,31] Over the past few decades, research has been conducted to understand the molecular mechanisms underlying the regulation of plant cell PM H^+-ATPase by phosphorylation. Some individual protein kinases/phosphatases involved in this process have also been identified.[32,33] Interestingly, many of these proteins belong to the family of receptor-like kinases, which have also been detected in detergent-resistant fractions of plant PM. Whether this indicates a tendency for H^+-ATPases to colocalize with their regulatory kinases is a matter of debate.

This review aims to synthesize current knowledge on three critical aspects of plant PM H^+-ATPase regulation: (i) the structural organization of the pump, (ii) the functional modulation by diverse membrane lipids, and (iii) the potential regulatory influence of PM lateral heterogeneity. While P-type ATPases are ubiquitously distributed across cellular membranes with distinct lipid compositions, a direct comparison of experimental data remains challenging due to system-specific variations in lipid-protein interactions affecting their hydrolytic and transport activities.[10,34] By critically evaluating structural, biochemical, and biophysical evidence, this work seeks to establish unifying principles of lipid-mediated regulation while highlighting plant-specific regulatory mechanisms of this essential proton pump.

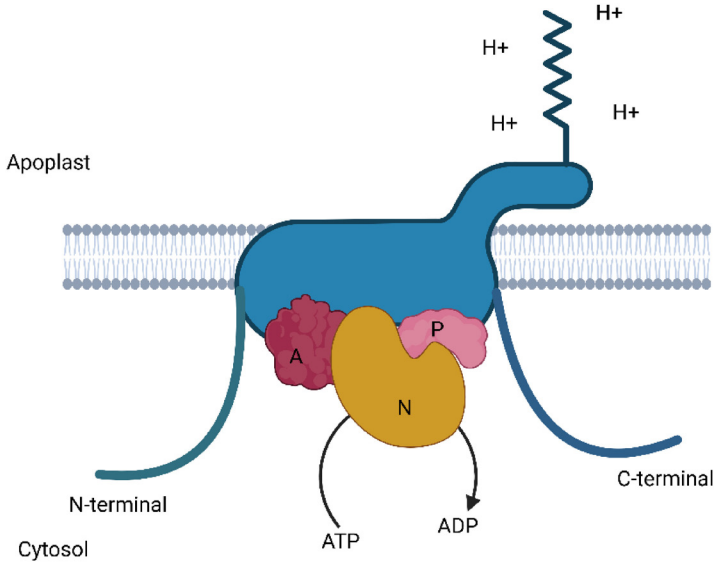

Fig 1 | Structure and functional domains of the PM H-ATPase. The enzyme contains 10 transmembrane helices, with an extracellular N-terminal regulatory domain and a cytosolic C-terminal autoinhibitory domain. Cytosolic catalytic domains include the nucleotide-binding (N), phosphorylation (P), and actuator (A) domains

Catalytic Cycle, Assembly, and Regulation of Plant PM H^+-ATPase

Plant genomes encode multiple isoforms of PM H^+-ATPase, forming a functionally diverse gene family. In Arabidopsis thaliana, the most abundant and well-characterized isoforms are AHA1 and AHA2 (Autoinhibited H^+-ATPase 1 and 2), which have been extensively studied as model proton pumps in this plant.[35,36]

As members of the P3A-type ATPase superfamily, plant PM H^+-ATPases share a conserved structural architecture with other P-type ATPases.[37,38] The enzyme's tertiary structure consists of a single polypeptide chain organized into 10 transmembrane α-helices (TM1-TM10) (Figure 1). Structural and functional studies reveal distinct roles for these domains. Catalytic Core (TM1-TM6 helices form the cytoplasmic region responsible for ATP hydrolysis and energy transduction). Structural Stabilization (TM7-TM10 helices maintain functional integrity, analogous to their role in sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)-type Ca^{2+}-ATPases).[39] Conserved Mechanism critical aspartate residue undergoes phosphorylation during the catalytic cycle, inducing conformational changes that facilitate ion binding site accessibility and proton translocation across the membrane.[7,40] The cytosolic N- and C-terminal domains serve as regulatory modules, containing autoinhibitory regions that control pump activity through intramolecular interactions and post-translational modifications.

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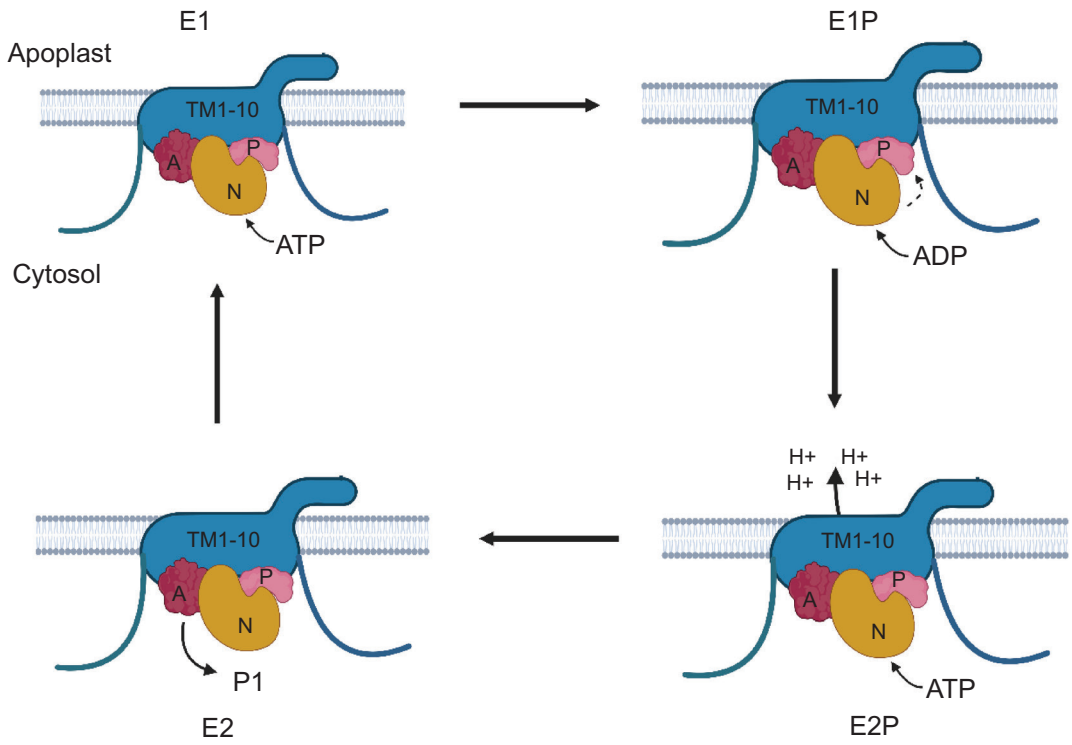

Fig 2 | Cycle of plant PM H⁺-ATPase following the Albers-Post scheme. The pump oscillates between two principal conformational states: (1) the E1 state with high-affinity proton-binding sites facing the cytoplasm, and (2) the E2 state with low-affinity sites oriented toward the apoplast. Reversible phosphorylation of the conserved aspartate residue (Asp329 in AHA2) within the P-domain's DKTGT motif drives these conformational transitions. The cycle involves four distinct intermediates (E1↔E1P↔E2P↔E2), with phosphorylation occurring during ATP hydrolysis at the N-domain and dephosphorylation mediated by the A-domain. Structural rearrangements during state transitions facilitate vectorial proton transport across the membrane. See the main text for detailed mechanistic explanations and regulatory aspects

Structural insights into the proton transport mechanism of AHA2 in Arabidopsis thaliana. Crystallographic studies of AHA2, the PM H⁺-ATPase in A. thaliana, identified a highly conserved aspartic acid residue (Asp684) in the M6 helix that serves as the primary proton donor/acceptor.[41] The adjacent Asn106 (located in the M2 domain) stabilizes Asp684 and is essential for directional proton transport against the electrochemical gradient.[42] These residues are functionally conserved across plant and fungal H⁺-ATPases, underscoring their critical role in energy transduction.

The catalytic cycle of plant PM H⁺-ATPase operates through the well-established Albers-Post model, which involves the coordinated action of three cytoplasmic domains: the nucleotide-binding (N), phosphorylation (P), and actuator (A) domains.[41,43,44] Central to this mechanism is the reversible phosphorylation of a conserved aspartate residue (Asp329 in AHA2) within the P-domain's DKTGT motif, which serves as the molecular switch driving conformational transitions between high-affinity (E1) and low-affinity (E2) states. The cycle initiates when the N-domain (containing the KGA motif) binds and hydrolyses ATP, transferring the γ-phosphate to Asp329 to form the phosphorylated E1P intermediate. This triggers a conformational shift to the E2P state, facilitating the release of protons to the apoplast. Subsequently, the A-domain (featuring the TGE motif) catalyzes aspartate dephosphorylation, returning the pump to the E2 state before spontaneous relaxation to the E1 ground state completes the cycle. The precise spatiotemporal coordination between these domains is critical, as both N- and A-domains must alternately access the same aspartate residue while maintaining strict coupling between ATP hydrolysis and proton transport. This intricate interplay ensures efficient proton translocation against electrochemical gradients while allowing rapid regulation of pump activity in response to cellular demands.

The C- and N-terminal domains are critical regulatory elements of plant PM H⁺-ATPases. The C-terminal domain, comprising approximately 100 amino acid residues, functions as an autoinhibitory domain suppressing pump activity in its non-phosphorylated state. This inhibition occurs through two distinct mechanisms: (i) steric blocking of proton access to transmembrane binding sites, and (ii) restriction of the actuator (A) domain's mobility.[41,45] Phosphorylation of the penultimate threonine residue within this domain triggers 14-3-3 protein binding, displacing the autoinhibitory region and consequent enzyme activation.[46,47] Recent structural studies have revealed an additional layer of regulation through intermolecular interactions - the C-terminal domain of one AHA2 monomer can engage with amino acid residues in the A-domain of an adjacent monomer.[48] This finding suggests that PM H⁺-ATPases may function

as homooligomeric complexes in native membranes, potentially enabling cooperative regulation of proton transport activity (Figure 2).

The N-terminal domain serves as a critical modulator of 14-3-3 protein binding to the C-terminal autoinhibitory domain. Structural studies have demonstrated that deletion of the first 10 N-terminal residues leads to enhanced phosphorylation of the penultimate threonine and consequent 14-3-3 protein association, suggesting the N-terminus normally exerts a restraining influence on this regulatory interaction.[42] This finding reveals an intricate interdomain communication network within the H^+-ATPase molecule. The functional architecture of P-type ATPases reflects a remarkable evolutionary optimization for active transport, featuring precise coupling between transmembrane helices and cytoplasmic domains. This coordinated arrangement enables thermodynamically unfavorable proton translocation against steep electrochemical gradients. Current understanding of PM H^+-ATPase regulation primarily centers on reversible phosphorylation mechanisms, with extensive characterization of stimulatory phosphorylation at the C-terminal penultimate threonine, inhibitory phosphorylation at other regulatory sites, and coordinated actions of specific protein kinases and phosphatases.[37]

In Arabidopsis thaliana, the predominant H^+-ATPase isoforms AHA1 and AHA2 undergo critical phosphorylation at their penultimate threonine residues (Thr948 and Thr947, respectively) within the C-terminal autoinhibitory domain. This post-translational modification creates a binding platform for 14-3-3 regulatory proteins, inducing a conformational change that releases autoinhibition and activates proton transport. The resulting 14-3-3/H^+-ATPase complex exhibits remarkable stability, becoming essentially irreversible upon binding of the fungal toxin fusicoccin.[46,49]

This phosphorylation event serves as a central hub for auxin-mediated signaling. Recent mechanistic studies have demonstrated that the transmembrane kinase TMK1, activated by auxin, directly phosphorylates Thr947 in AHA2, triggering apoplastic acidification through pump activation.[50,51] Conversely, in the absence of auxin, protein phosphatases PP2C-D maintain pump inactivation by dephosphorylating this critical threonine residue. The auxin-responsive SAUR19 proteins counteract this inhibition by suppressing PP2C-D activity, thereby promoting extracellular acidification and subsequent root growth responses.[52]

Notably, auxin signaling also influences the spatial organization of these components within the PM. As demonstrated by Pan,[53] auxin induces sterol-dependent nanoclustering of TMK1 kinase, which is essential for establishing polarized membrane domains during auxin-mediated pavement cell morphogenesis. This finding reveals an additional layer of regulation, where membrane microdomain organization modulates the efficiency of H^+-ATPase activation.

The PM H^+-ATPase is activated by blue light through a signaling cascade initiated by the photoreceptors phototropic 1 and 2 (phot1 and phot2), which possess serine-threonine kinase activity.[54,55] In guard cells, blue light perception by phototropins triggers phosphorylation of the serine-threonine kinase BLUS1, which subsequently activates the Raf-like kinase MAP3K-BHP. This kinase cascade ultimately phosphorylates Thr881 and Thr947 in AHA2, leading to H^+-ATPase activation.[55] The resulting proton extrusion induces membrane hyperpolarization, driving K^+ uptake into the cytosol and promoting stomatal opening.[56] Notably, blue light also enhances the interaction between phot1 and AtRem1.3, a marker of sterol-enriched membrane nanodomains.[57] This suggests that phot1 recruitment to sterol-rich microdomains may facilitate efficient signal transduction during blue light responses.

In contrast to light-mediated activation, abscisic acid (ABA) signaling exerts complex regulation over H^+-ATPase activity through the PYR/PYL/RCAR receptor-PP2C-SnRK2 signaling module. Under low ABA conditions, the protein phosphatase PP2C-A (ABI1) dephosphorylates Thr947 in AHA2 (and Thr948 in AHA1), maintaining the pump in an inactive state.[58] Upon ABA binding, PYR/PYL/RCAR receptors inhibit PP2C-A, allowing phosphorylation of the penultimate threonine, 14-3-3 protein binding, and subsequent H^+-ATPase activation.[59] Additionally, ABA signaling may involve the SnRK2 kinase, though its direct phosphorylation of H^+-ATPase in vivo remains unclear.[60] These regulatory mechanisms highlight the intricate balance between light- and ABA-mediated pathways in modulating H^+-ATPase activity for stomatal movement and stress adaptation.

The activity of PM H^+-ATPase is finely tuned through phosphorylation at distinct sites by various protein kinases. The receptor-like kinase PSY1R phosphorylates Thr881 in AHA2 in response to the peptide hormone PSY1, resulting in pump activation that is independent of 14-3-3 protein binding.[61,62] Conversely, phosphorylation at Thr924 or Ser931 by PKS5 inhibits H^+-ATPase activity and prevents 14-3-3 association, demonstrating the opposing effects of site-specific phosphorylation.[61,62] The FERONIA kinase pathway provides another layer of regulation. Upon interaction with the RALF peptide, FERONIA phosphorylates Ser889 in AHA2, thereby suppressing proton transport and causing extracellular alkalization that inhibits cell growth.[63] Notably, FERONIA localizes to DRMs, and its abundance in these domains increases following flg22 treatment, coinciding with AHA2 recruitment.[64] While this suggests potential co-localization of regulator and target, the functional implications require further investigation.

Recently, QSK1 was identified as a nitrate-responsive kinase that phosphorylates AHA2 at Ser889 under low-nitrate conditions, through a complex with the nitrate transporter NRT1.1, linking nutrient sensing to proton transport regulation.[65] The diverse phosphorylation-mediated regulation of H^+-ATPase involves kinases associated with DRMs and nanodomain organizers like flotillins and remorins.[53,57,66] This raises important questions about the lipid microenvironment

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preferences for H^+-ATPase and its regulators. In yeast, altered membrane composition (sterols, sphingolipids, anionic phospholipids) affects protein clustering and PMA1 H^+-ATPase activity.[67] Similarly, reduced anionic phospholipids and sterols in plants decrease H^+-ATPase clustering, suggesting both electrostatic and hydrophobic interactions govern its lateral segregation. These findings highlight how membrane lipid composition may spatially organize H^+-ATPase with its regulatory kinases to facilitate precise, stimulus-responsive control of proton transport.

Phospholipid Composition of Membrane and Its Regulation of P-type ATPase Activity

The functional activity of P-type ATPases is critically dependent on membrane phospholipid composition and the physicochemical properties of the lipid bilayer.[10] Reconstitution studies with plant H^+-ATPase from mung bean roots revealed that phosphatidylcholine (PC) and phosphatidylserine (PS) stimulate hydrolytic activity, while phosphatidylinositol (PI) exerts an inhibitory effect.[68] Similar lipid specificity has been observed for animal P-type ATPases, where the Na^+/K^+-ATPase shows optimal activity in membranes containing PS and PC with unsaturated fatty acids.[10] Structural analyses have identified specific lipid-binding sites in several P-type ATPases, including SERCA and PM Ca^{2+}-ATPase, suggesting a conserved regulatory mechanism across this enzyme family.[10,69]

A key breakthrough in understanding lipid regulation came with the discovery that lysophosphatidylcholine (LPC), a signaling lipid produced by phospholipase A2 activity, directly activates plant H^+-ATPase at micromolar concentrations.[70] This activation occurs through interactions with the N- and C-terminal regulatory domains and is independent of phosphorylation or 14-3-3 protein binding.[71] The finding that LPC increases the pump's affinity for ATP suggests it may act as a physiological regulator during stress responses, such as pathogen defence.[72] The P4-ATPase subfamily, including yeast Drs2p and Arabidopsis ALAs, shares structural homology with other P-type ATPases but transports phospholipids rather than ions. These flippases are activated by specific anionic lipids; for example, Drs2p requires phosphatidylinositol-4-phosphate (PI4P) binding to a cavity formed by transmembrane domains M7, M8, and M10.[73] Cryo-EM structures reveal that electrostatic interactions between positively charged residues and PI4P's phosphate group induce activating conformational changes.[74] This mechanism likely extends to plant flippases, where similar lipid-binding pockets may regulate activity.

The differential responses of P-type ATPases to membrane composition (e.g., unsaturated PC/PS activation vs. PI inhibition) suggest evolutionary adaptation to distinct membrane environments. This lipid sensitivity enables cells to fine-tune pump activity in response to membrane remodeling during stress or development. The conservation of lipid-binding sites across P-type ATPase families highlights the fundamental importance of lipid-protein interactions in regulating these essential membrane transporters. Biochemical studies of plant PM H^+-ATPase have demonstrated distinct lipid dependencies in different species. In maize roots, partial solubilization with deoxycholate revealed that PS and phosphatidylglycerol (PG), but not PC, enhanced ATP hydrolysis rates without significantly altering K_m values.[75] Similar experiments with Vigna radiata root membranes showed PC, PS, and PG stimulated activity, while PI, phosphatidylethanolamine (PE), and phosphatidic acid (PA) were inhibitory.[76] These effects depend critically on lipid acyl chain properties, with unsaturated fatty acids generally promoting higher activity,[77] mirroring observations in animal Na^+/K^+-ATPase.

While reconstitution experiments using detergents, such as dodecyl maltoside, enable controlled studies of lipid-protein interactions,[34] they may introduce artifacts by disrupting native membrane organization. Recent work with AHA2-containing proteoliposomes demonstrated PS-specific activation independent of the C-terminal regulatory domain, suggesting the presence of transmembrane lipid-binding sites.[78] Comparative sequence analysis revealed conserved anionic lipid interaction motifs in P-type ATPases, supporting this hypothesis. Structural modulation of the N-terminus electrostatically interacts with PS-containing membranes, potentially influencing conformational transitions between E1 and E2 states.[8] Molecular dynamics (MD) simulations confirm that the N-terminus reaches membrane-anionic lipids.[79] Membrane organization of the kinase FERONIA, which inhibits H^+-ATPase via Ser899 phosphorylation, also regulates PS distribution and nanodomain formation,[80] suggesting potential coupling between lipid microenvironment and phosphoregulation. Future directions involve phospholipids creating a permissive environment that facilitates H^+-ATPase conformational dynamics.[10,78] These findings highlight the need for native membrane studies complementing reconstitution approaches, to understand the lipid-mediated regulation of plant H^+-ATPases.

Recent cryo-EM studies of AHA2 in proteoliposomes[71] not only confirm PS-dependent stimulation but also reveal oligomeric interfaces stabilized by anionic lipids, mirroring the nanoscale clustering of H^+-ATPases observed in plant PMs.[8] These oligomers, likely facilitated by lipid-mediated electrostatic interactions (e.g., between the N-terminus and PS), suggest a dual regulatory mechanism: lipid binding primes individual pumps for activation while promoting higher-order assemblies that could enhance cooperative activity or spatial coordination in membrane microdomains. Notably, this aligns with in planta super-resolution data, which show that H^+-ATPase nanoclusters are enriched in sterol/sphingolipid rafts,[72] where a localized lipid composition may template oligomerization. The convergence of structural[71] and cellular[8,72] evidence implies that lipid-dependent oligomerization is a conserved feature across P-type ATPases. However, its functional consequences (e.g., for signal amplification or lipid homeostasis) remain to be tested in living plants.

Glycerophospholipids Dependent Regulation of H^+-ATPase Localization

Growing evidence suggests that sphingolipids play crucial roles in P-type ATPase biogenesis and membrane targeting. In yeast, PMA1 H^+-ATPase requires ceramide association for proper endoplasmic reticulum (ER) exit and PM delivery, with sphingolipid depletion leading to vascular degradation.[81] This dependence extends to functional oligomerization, as hexameric PMA1 complexes incorporate sphingolipids with very-long-chain (C26) fatty acids during Golgi processing before domain formation in the PM.[82,83] Similar mechanisms may operate in plants, where H^+-ATPases and sphingolipids co-fractionate in detergent-resistant membranes.[17,84] Genetic and pharmacological studies reveal that sphingolipids critically influence H^+-ATPase activity. *Saccharomyces cerevisiae* mutants with impaired sphingolipid biosynthesis show reduced proton transport and stress sensitivity. *Candida albicans* exhibits decreased CaPma1p activity and altered membrane domain association when sphingolipid synthesis is disrupted.[85]Maize embryos treated with the ceramide synthase inhibitor fumonisin B1 exhibit a 20–30% reduction in H^+-ATPase activity, which is reversible by exogenous ceramides.[86] These effects may involve both direct lipid-protein interactions and indirect consequences of altered membrane properties. Notably, fumonisin's structural mimicry of long-chain bases suggests potential competitive binding at regulatory sites.[86] While yeast studies provide a compelling model for sphingolipid-dependent H^+-ATPase regulation,[83] plant-specific mechanisms remain unclear. Key unknowns include. Whether plant H^+-ATPases require sphingolipids for ER export and oligomerization. How 14-3-3 protein binding[87] interfaces with potential sphingolipid interactions. The relative contributions of glycosylceramides vs. free ceramides in modulating activity. Future directions for resolving these questions will require in planta tracking of H^+-ATPase trafficking in sphingolipid mutants, high-resolution structural studies of lipid-protein interactions and characterization of plant-specific sphingolipid species in membrane microdomains. Current evidence suggests that sphingolipids likely influence plant H^+-ATPases through both conserved (trafficking, membrane organization) and possibly unique (signaling metabolite) mechanisms compared to fungal systems.

H^+-ATPase and the Role of Sterols in the PM

Over the past decades, numerous experimental studies have investigated the influence of membrane sterols on the transport and catalytic activities of P-type ATPases.[9,88–91] While these studies have established a correlation between sterol content and enzyme functionality, the findings remain inconsistent, and the underlying molecular mechanisms remain poorly understood.

The variability in experimental outcomes may stem from the differential effects of sterols on specific P-type ATPases. For instance, studies on animal Na+/K+-ATPase reconstituted into liposomes demonstrated that cholesterol depletion significantly reduced hydrolytic activity, with optimal function observed at 20 mol% cholesterol relative to total lipids.[9,92] This enhancement coincided with increased phosphointermediate formation, elevated total protein phosphorylation, and a decreased K_m value. Conversely, H^+/K^+-ATPase exhibited opposing behavior: elevated cholesterol levels partially inhibited ATP hydrolysis and completely abolished proton transport.[91] Surprisingly, SERCA activity remained unaffected by sterol content in proteoliposomes.[93] While some studies suggest cholesterol indirectly modulates catalytic parameters via bilayer physicochemical properties,[94] SERCA's insensitivity may reflect its localization in the ER, where sterol concentrations are substantially lower than in the PM.

Early studies on DRMs from yeast cells identified the presence of the yeast PM H^+-ATPase, PMA1.[95] Initially, it was hypothesized that disruption of lipid rafts impaired PMA1 association with the PM. However, fluorescence microscopy later revealed that PMA1 forms its distinct clusters termed the membrane compartment of Pma1, which are ergosterol-independent.[96] Furthermore, Permyakov in 2012 demonstrated that ergosterol levels in Saccharomyces cerevisiae did not affect PMA1 biosynthesis, localization, or glucose-dependent activation.[97] Notably, yeast cells can adapt their membrane composition in response to sterol biosynthesis perturbations by modulating sphingolipid content. Guan in 2009 proposed that membrane proteins may recognize sterol-sphingolipid complexes, suggesting crosstalk between sterol and sphingolipid biosynthesis pathways.[98] Consequently, interpreting H+-ATPase activity in mutants with defective lipid biosynthesis remains a challenge.

Similarly, plant PM H^+-ATPase activity in reconstituted liposomes depends on sterol composition.[90] Cholesterol and stigmasterol enhance proton transport and pump activity, particularly at low concentrations, whereas campesterol, sitosterol, 24-methylcholesterol, and ergosterol exhibit inhibitory effects across all concentrations.[99] Intriguingly, sterol content affects proton transport more significantly than ATP hydrolysis.[90] The authors speculated that the pump discriminates between sterol types, as sitosterol and stigmasterol differ only by a double bond at C-22. These findings imply that "direct, structurally specific lipid-protein interactions" may underlie these effects. The activation of PM H^+-ATPase by cholesterol and stigmasterol, even at trace concentrations, further supports this hypothesis, as such minimal lipid levels are unlikely to alter bulk membrane physicochemical properties (e.g., fluidity) and thus must act via specific molecular interactions.

In a previous study, Lapshin et al.[100] demonstrated that sterol extraction from PM vesicles of Pisum sativum following MβCD treatment led to an increase in both the maximal rate of ATP hydrolysis (V_{max}) and active proton transport by H^+-ATPase.[100] However, the stimulatory effect on H^+ pumping was transient and did not correlate with the enzyme's hydrolytic

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activity. The authors proposed that sterol depletion not only modulates H^+-ATPase activity but also increases the passive proton permeability of the plasmalemma, possibly due to the differential sensitivity of secondary transporters to membrane sterol content.

Sterols are thought to regulate PM H^+-ATPase through multiple mechanisms. The most straightforward involves their direct interaction with transmembrane domains of the protein. For instance, crystallographic studies of animal Na^+/K^+ ATPase and SERCA have identified specific lipid and sterol-binding sites that enhance the stability of these pumps.[9,10] Experimental evidence suggests that the association of cholesterol with PC at the cytoplasmic interface stabilizes transmembrane domains (TM8–10) and maintains the equilibrium between E1 and E2 conformational states during catalysis.[9,101] Additionally, certain membrane transporters and neurotransmitter receptors possess well-defined sterol-binding motifs (e.g., CRAC/CARC domains.[14,102] While cholesterol recognition sequences (CARC domains) have been identified in animal PM Ca^{2+}-ATPase, they are absent in SERCA.[103] Given the structural conservation among P-type ATPases, plant PM H^+-ATPase may similarly harbor sterol/lipid-binding sites that modulate its activity.

Sterols may also influence protein function indirectly by altering the physicochemical properties of the lipid bilayer, thereby affecting conformational dynamics.[104] For example, cholesterol-induced changes in bilayer properties can regulate protein partitioning between sterol-rich and sterol-poor membrane domains.[105] Notably, proteins with short transmembrane α-helices are energetically disfavored in cholesterol-rich regions. Furthermore, the impact of cholesterol on H^+ permeability in artificial membranes varies with the phospholipid composition.[106] Cholesterol-mediated modulation of bilayer thickness has been implicated in Na^+/K^+-ATPase activity.[107] As highlighted by Clarke,[8] membrane proteins dynamically adjust their lipid environment during catalytic cycles to mitigate hydrophobic mismatch, with sterols playing a key role in maintaining bilayer thickness.[8,12] Intriguingly, SERCA adopts a distinct mechanism: rather than inducing membrane deformation, its transmembrane helices tilt to accommodate the surrounding bilayer.[39]

Future Direction and Conclusion

The influence of the lipid environment on the functional regulation of P-type ATPases is a complex and multifaceted process involving diverse molecular and cellular mechanisms. Although experimental studies on lipid-mediated modulation of ATPase activity began over three decades ago[68,88,93] and high-resolution structures of major P-type ATPases have since been elucidated,[43,108] key questions remain regarding the precise molecular mechanisms governing protein-lipid interactions within their native membrane environment. The plant PM exhibits a unique lipid composition, enriched in phytosterols and sphingolipids.[109,110] Significant advances have been made in understanding the role of sphingolipids in the yeast PMA1 proton pump, where they are critical for ER biosynthesis, oligomerization, and subsequent trafficking to the PM.[82,83] While the involvement of sphingolipids in the oligomerization and vesicular transport of plant H^+-ATPases remains unexplored, their functional contribution to these processes is highly plausible.

Sterols appear to regulate PM H^+-ATPase activity through both specific lipid-protein interactions and general membrane effects. However, whether PM H^+-ATPases possess dedicated lipid-binding sites[9] or conserved motifs such as CARC/CRAC domains[14] remains unresolved. MD simulations could provide valuable insights into these mechanisms. A particularly intriguing avenue for future research is the potential colocalization of PM H^+-ATPases with their regulatory proteins, as well as the role of membrane lipids in facilitating these interactions. The detection of PM H^+-ATPases and their binding partners in DRMs enriched in sterols and sphingolipids has fueled speculation about their localization in membrane microdomains.

Lipid-mediated regulation of H^+-ATPase activity forms a mechanistic bridge between membrane dynamics and plant adaptation to abiotic stress. These insights are fueling biotechnological innovations such as the design of engineered lipid microdomains to enhance pump performance, nutrient uptake, and crop sustainability under challenging environments.[27,111,112]

However, detergent-based fractionation is now recognized as an outdated and unreliable method for assessing protein lateral organization in membranes. Instead, emerging evidence suggests that direct phosphorylation or dephosphorylation of PM H^+-ATPases by partner proteins implies close physical proximity, possibly within functionally active membrane complexes. A critical unresolved question is the subcellular site of complex formation: (i) during pump biosynthesis in the ER, (ii) during transit through the trans-Golgi network, or (iii) at the PM following ligand-induced recruitment of receptor-like kinases/phosphatases. Addressing this will require advanced high-resolution imaging techniques, such as super-resolution fluorescence microscopy or cryo-electron tomography.

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