Scientific Research Publishing

Journal of Biosciences and Medicines, 2026, 14(9), 425-439
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Arabidopsis thaliana Type One Protein Phosphatases: Context-Dependent Regulators of Development, Stress Responses, and Immunity

Kai Chen, Jianzhong Huang*

Department of Basic Medicine, Fuzhou Medical College, Fuzhou, China
Email: *huangjz@whu.edu.cn

How to cite this paper: Chen, K. and Huang, J.Z. (2026) *Arabidopsis thaliana* Type One Protein Phosphatases: Context-Dependent Regulators of Development, Stress Responses, and Immunity. *Journal of Biosciences and Medicines*, **14**, 425-439.
https://doi.org/10.4236/jbm.2026.149026

Received: July 29, 2026
Accepted: September 18, 2026
Published: September 21, 2026

Abstract

Reversible protein phosphorylation enables plants to coordinate growth, stress responses, and immunity. *Arabidopsis thaliana* TYPE ONE PROTEIN PHOSPHATASES (TOPPs), homologs of eukaryotic protein phosphatase 1 catalytic subunits, exemplify how a highly conserved phosphatase family can generate distinct signaling outputs. The nine TOPPs are partially redundant, yet their functions are shaped by regulatory partners, substrate recruitment, expression patterns, and subcellular localization. TOPP4 dephosphorylates DELLA repressors, PIN1, and PIF5; TOPP1-associated complexes regulate ABA signaling through SnRK2s; and TOPP1, TOPP4, and TOPP5 dephosphorylate EIN2 at Ser655 to reinforce ethylene signaling. TOPP4 directly dephosphorylates ATG13a, while TOPP1, TOPP3, and TOPP9 also reduce ATG13a phosphorylation in planta; higher-order topp mutants support broader family-level redundancy during fixed-carbon starvation-induced autophagy. In immunity, TOPPs restrain basal defense, mutant TOPP4 states are monitored by the CNL receptor SUT1, and the *Pseudomonas syringae* effector AvrE targets TOPPs to enhance ABA-dependent water soaking. Collectively, current evidence supports a model in which catalytic redundancy is constrained by protein interactions and spatially restricted substrate access. Defining these TOPP complexes and their phosphosites may enable more selective manipulation of plant signaling than altering bulk phosphatase activity.

Keywords

Type One Protein Phosphatases, *Arabidopsis thaliana*, Protein Phosphatase 1, Phosphorylation, Hormone Signaling, Autophagy, Immunity, Stress

*Corresponding author.

Response

1. Introduction

Reversible phosphorylation regulates protein activity, stability, interactions, and localization, enabling plants to respond rapidly to developmental and environmental cues [1] [2]. Protein phosphatases therefore do more than terminate signals: by removing specific phosphate groups, they can alter signaling thresholds, duration, and pathway output. For many phosphatases, specificity depends less on divergence of the catalytic pocket than on regulatory proteins, substrate docking, and subcellular localization [3]-[5]. This principle is particularly relevant to the *Arabidopsis* TYPE ONE PROTEIN PHOSPHATASES (TOPPs), homologs of eukaryotic protein phosphatase 1 (PP1) catalytic subunits. *Arabidopsis* contains nine TOPP genes, TOPP1-TOPP9, with highly conserved catalytic domains [6]-[8]. Genetic evidence, however, supports neither complete redundancy nor strict isoform specificity. Single mutants often display modest phenotypes, whereas higher-order mutants reveal shared roles in ABA responses, immunity, autophagy, and ethylene signaling [9]-[12]. This combination of conservation and selectivity is best explained by the molecular context in which a TOPP operates. AtI-2 directly binds and inhibits TOPPs, whereas PP1R3 regulates TOPP activity and localization in ABA signaling [9]-[13]. TOPPs are therefore better viewed as catalytic components of regulated phosphatase complexes than as enzymes with fixed substrate repertoires.

Several experimentally validated substrates now anchor this model. TOPP4 dephosphorylates DELLA repressors in gibberellin signaling, PIN1 during pavement-cell morphogenesis, and PIF5 in red-light responses [14]-[16]. TOPP1 and its regulatory proteins modulate SnRK2-mediated ABA signaling [9] [17]. In contrast, TOPP1, TOPP4, and TOPP5 directly dephosphorylate EIN2 at Ser655, thereby stabilizing EIN2 and promoting nuclear accumulation of its C-terminal domain [12]. TOPPs also function in autophagy and immunity. During fixed-carbon starvation, TOPP4 directly dephosphorylates ATG13a *in vitro*, while TOPP1, TOPP3, and TOPP9 also reduce ATG13a phosphorylation in planta; higher-order mutant phenotypes demonstrate broader TOPP-family redundancy in autophagy [11]. Higher-order topp mutants display enhanced defense responses to *Pseudomonas syringae*, whereas the dominant-negative topp4-1 allele ($TOPP4^{T246M}$) activates SUT1-dependent autoimmunity [10] [18]. In heterologous assays, SUT1 activation requires mutant rather than wild-type TOPP4 and depends on plasma-membrane localization [19]. Conversely, the bacterial effector AvrE binds and inhibits TOPPs, thereby enhancing ABA signaling and water soaking [20].

This article is a narrative review focused primarily on experimental studies of TYPE ONE PROTEIN PHOSPHATASES in *Arabidopsis thaliana* (**Table 1**). We prioritized primary studies providing genetic, biochemical, protein-protein inter-

action, phosphosite, or subcellular-localization evidence for TOPP function, with particular emphasis on experimentally supported substrates, regulatory partners, and signaling outputs. Throughout this review, "direct substrate" is reserved for proteins for which TOPP-dependent dephosphorylation has been demonstrated biochemically or in planta; "physical association" denotes experimentally demonstrated interaction or colocalization without direct proof of dephosphorylation; and "genetic association" denotes pathway involvement inferred primarily from mutant, overexpression, or epistasis analyses. These categories are applied throughout to avoid conflating family-level genetic redundancy with isoform-specific catalytic activity.

Table 1. Experimentally supported TOPP-centered signaling modules in *Arabidopsis*.

Pathway/process	TOPP component (s)	Target/evidence level	Regulatory effect	Biological output	Key reference
Gibberellin signaling	TOPP4	RGA, GAI (DELLAs): direct substrates	Direct dephosphorylation; promotes DELLA turnover	GA-responsive growth	[14]
Auxin-dependent morphogenesis	TOPP4	PIN1: direct substrate	Direct dephosphorylation; controls polarity and trafficking	Pavement-cell interdigitation	[15]
Photomorphogenesis	TOPP4	PIF5: direct substrate	Direct dephosphorylation; limits red-light-induced ubiquitin-mediated degradation	Red-light seedling development; tunes hypocotyl elongation and hook/cotyledon opening	[16]
ABA signaling	TOPP1; multiple TOPPs	SnRK2s: physical association with TOPP1 and biochemical suppression of kinase activity; PP1R3-regulated complexes	Suppresses SnRK2 activity; localization-dependent regulation	ABA and salt responses	[9] [17]
Ethylene signaling	TOPP1, TOPP4, and TOPP5	EIN2 Ser655: direct substrate	Site-specific dephosphorylation; stabilizes EIN2 and promotes CEND nuclear accumulation	Ethylene response and salt tolerance	[12]
Fixed-carbon starvation	TOPP4; TOPP1, TOPP3, and TOPP9; higher-order topp mutants	ATG13a: direct dephosphorylation by TOPP4 *in vitro*; additional in planta isoform support	ATG13a dephosphorylation promotes ATG1a-ATG13a assembly; higher-order mutants support broader redundancy	Autophagy and starvation tolerance	[11]

Continued

Basal immunity	Multiple TOPPs	MAPK-associated defense network: physical/genetic association	Restrains defense signaling	SA-dependent defense and bacterial resistance	[10]
NLR surveillance	TOPP4^{T246M}/mutant TOPP4 states	SUT1: physical and genetic association	Aberrant TOPP4 activates SUT1-dependent cell death	Autoimmune surveillance	[18] [19]
Pathogen virulence	Multiple TOPPs	AvrE effector: physical association with TOPPs	AvrE binds and inhibits TOPPs, enhancing ABA signaling	Stomatal closure and water soaking	[20]

Here, we synthesize these findings around three recurring features of TOPP biology: partial redundancy, partner-dependent substrate selection, and spatial control. We also outline the major experimental gaps and consider how pathway-specific TOPP interactions might be exploited without broadly disrupting phosphatase function.

2. Catalytic Conservation, Partial Redundancy, and Context-Dependent Specificity

At the catalytic level, TOPPs belong to the phosphoprotein phosphatase (PPP) superfamily of serine/threonine phosphatases [6]-[8]. Their catalytic cores contain highly conserved residues required for metal coordination and phosphoester hydrolysis, placing strong evolutionary constraints on variation within the active site [21] [22]. Functional diversification is therefore more likely to arise from differences in expression, localization, and interaction surfaces. This view is consistent with canonical PP1 systems, in which regulatory proteins control localization, substrate access, and enzyme activity [8] [22]. In *Arabidopsis*, AtI-2 inhibits multiple TOPPs, whereas PP1R3 associates with TOPPs and affects both their activity and nuclear localization [9] [13]. Thus, catalytic sequence alone is unlikely to predict TOPP substrate specificity. Although AtI-2 inhibits TOPP catalytic activity toward a generic phosphatase substrate *in vitro*, it can enhance TOPP1-mediated suppression of SnRK2s by promoting TOPP1-SnRK2 association. Thus, the apparently divergent effects are best interpreted as substrate- and assay-context dependent, while the contribution of complex composition and localization *in vivo* remains unresolved [17].

Consistent with this conserved catalytic framework, functional overlap is evident across several pathways. Septuple and octuple topp mutants were required to reveal pronounced defects in fixed-carbon starvation-induced autophagy [11], and the *topp1 topp4 topp5 triple mutant*, unlike the corresponding single mutants, displays partial ethylene insensitivity [12]. Higher-order mutants likewise uncovered roles in ABA signaling and immunity [9] [10]. Redundancy is therefore

an intrinsic feature of the family and likely provides robustness when individual isoforms are limiting. Nevertheless, this redundancy is incomplete: TOPP4 repeatedly appears in DELLA-, PIN1-, PIF5-, and SUT1-associated pathways. The available evidence is thus best described as shared catalytic capacity combined with context-dependent specialization.

Catalytic similarity, however, does not translate into indiscriminate activity *in vivo*. Instead, three mechanisms appear to narrow TOPP activity. Regulatory proteins can alter enzyme activity or localization; substrate binding can recruit TOPPs to particular phosphoproteins; and compartmentalization restricts substrate accessibility. DELLA proteins, PIN1, PIF5, and EIN2 have direct biochemical dephosphorylation evidence, while ATG13a is a direct TOPP4 substrate with additional isoform-level support as detailed below [11] [12] [14]-[16]. SnRK2s and immune MAPK components are described separately where the evidence is primarily physical, functional, or genetic. AtI-2 and PP1R3 illustrate regulatory-protein control [9] [13] [17]. Spatial regulation is best documented in ABA signaling. PP1R3 and TOPPs are present in both the nucleus and cytoplasm; ABA increases the cytoplasmic pools of TOPP1 and TOPP4; and altering TOPP4 nuclear localization changes ABA-related phenotypes [9]. Whether comparable stimulus-dependent redistribution operates in other TOPP pathways remains to be tested directly.

3. Developmental and Hormonal Functions of TOPPs

Among developmental and hormonal pathways, gibberellin signaling provides a clear example of TOPP function. Gibberellins promote seed germination, stem elongation, flowering, and other developmental processes largely by reducing the abundance and activity of DELLA growth repressors. GA-bound GID1 receptors facilitate recruitment of DELLAs to SCF ubiquitin ligase complexes, leading to proteasome-dependent degradation. TOPP4 adds a phosphorylation-dependent layer to this canonical pathway. It physically interacts with the DELLA proteins RGA and GAI and directly dephosphorylates them. The phosphorylation state of these repressors is associated with their stability, and impaired TOPP4 activity causes DELLA accumulation and reduced GA responsiveness [14] (**Figure 1(A)**). Thus, TOPP4-mediated dephosphorylation is linked to DELLA turnover rather than simply terminating GA signaling. The kinases and individual phosphosites that establish this balance remain incompletely resolved.

Beyond gibberellin signaling, TOPP4 also influences auxin-dependent morphogenesis. Auxin transport depends on the polarized localization of PIN-FORMED efflux carriers. In *Arabidopsis* leaf pavement cells, TOPP4 functions in the same developmental context as PIN1 and directly influences its phosphorylation state. Genetic, interaction, colocalization, and biochemical analyses showed that TOPP4 antagonizes PINOID-dependent phosphorylation of PIN1, thereby regulating PIN1 polarity and endocytic trafficking [15] (**Figure 1(B)**). The resulting changes in auxin distribution and ROP-dependent cytoskeletal organization

alter pavement-cell interdigitation. In this context, the principal consequence of dephosphorylation is a change in where PIN1 functions rather than a simple change in protein abundance.

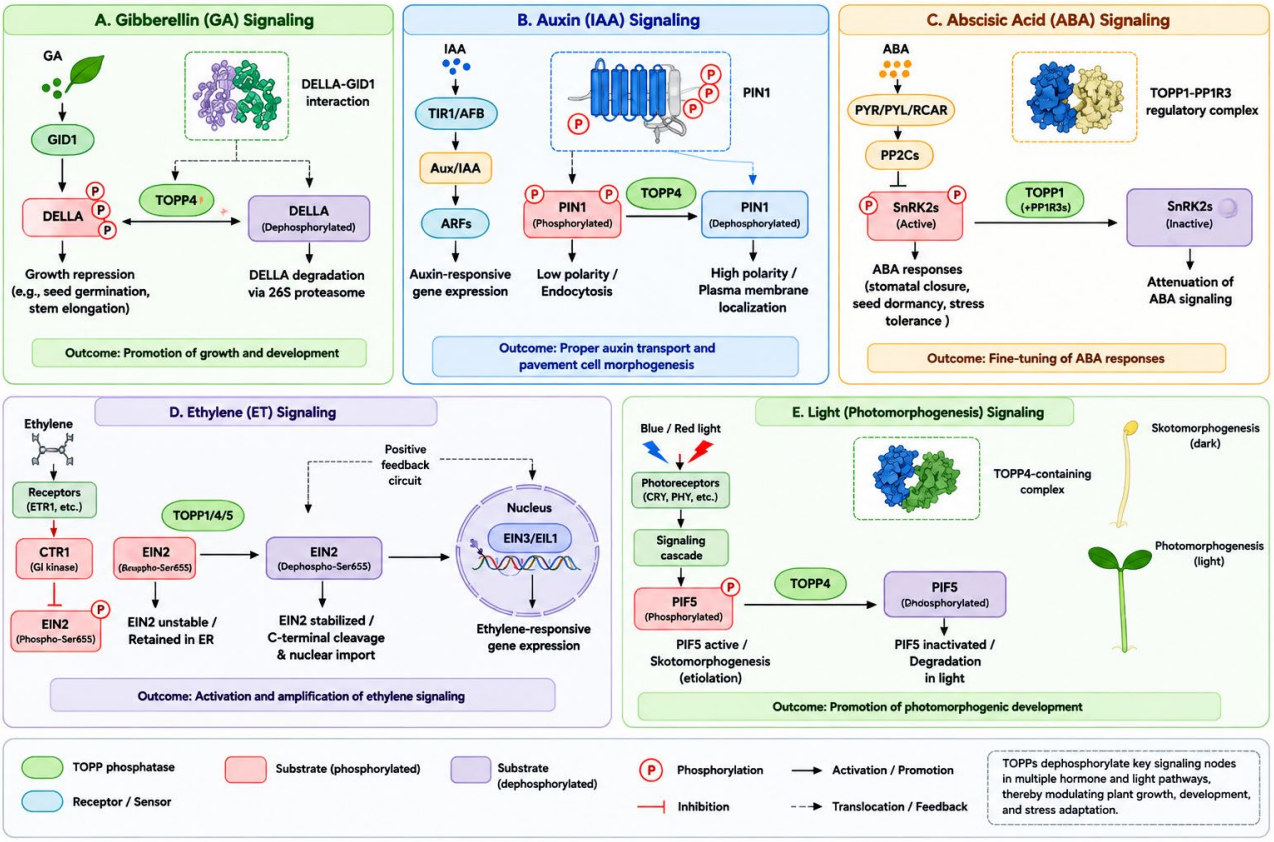

Figure 1. TOPP-mediated regulation of hormone and light signaling in *Arabidopsis thaliana*. (A) TOPP4 dephosphorylates DELLA proteins to promote their degradation and GA-dependent growth. (B) TOPP4 dephosphorylates PIN1, regulating its polarity and auxin-dependent pavement-cell morphogenesis. (C) TOPP1-containing complexes physically associate with SnRK2s and suppress their kinase activity to attenuate ABA signaling. (D) TOPP1, TOPP4, and TOPP5 dephosphorylate EIN2 at Ser655, stabilizing EIN2 and promoting EIN3/EIL1-dependent ethylene signaling and positive feedback. (E) TOPP4 dephosphorylates and stabilizes PIF5, counterbalancing phyB-dependent PIF5 turnover and attenuating red-light responses during photomorphogenesis.

TOPP regulation also extends to ABA signaling. Although PP2C phosphatases are canonical components of ABA perception, TOPP-dependent mechanisms provide an additional phosphatase layer. TOPP1 interacts with SnRK2 kinases and PYL ABA receptors and suppresses SnRK2 kinase activity. AtI-2 associates with TOPP1 and, although it reduces TOPP1 activity toward a generic phosphatase substrate *in vitro*, it enhances TOPP1-mediated suppression of SnRK2s, likely by promoting TOPP1-SnRK2 interaction [17]. This apparent difference is therefore best interpreted as assay- and substrate-context dependent; whether complex composition or localization further modulates this effect *in vivo* remains unresolved. Consistent with this biochemical activity, topp1 and ati-2 mutants display enhanced sensitivity to ABA and salt, together with increased expression of ABA-responsive genes [17]. PP1R3 further illustrates how regulatory subunits can di-

versify TOPP behavior. PP1R3 interacts with TOPPs, inhibits their enzymatic activity, and promotes nuclear localization of TOPP4. Genetic analyses indicate that PP1R3 and TOPPs jointly regulate ABA responses, whereas localization experiments show that nuclear versus cytoplasmic distribution contributes to pathway output [9] (**Figure 1(C)**). These findings support the view that the relevant signaling unit is a regulated TOPP complex rather than an isolated catalytic subunit.

Ethylene signaling provides another example of coordinated TOPP action. In *Arabidopsis*, ethylene induces the expression of several TOPP genes, with particularly strong functional evidence for TOPP1, TOPP4, and TOPP5. The *topp1 topp4 topp5 triple mutant* displays partial ethylene insensitivity and reduced EIN3 protein accumulation, whereas TOPP overexpression enhances ethylene responses. TOPP1, TOPP4, and TOPP5 interact with the EIN2 C-terminal domain and directly dephosphorylate EIN2, primarily at Ser655. This modification stabilizes EIN2 and promotes nuclear accumulation of its C-terminal signaling domain, thereby strengthening EIN3/EIL1-dependent transcription. In turn, EIN3/EIL1 binds TOPP promoters and increases TOPP expression, forming a positive-feedback loop [12] (**Figure 1(D)**). A phospho-deficient EIN2^{S655A} allele also enhances ethylene output and salt tolerance, linking this phosphosite to stress adaptation.

A mechanistically distinct role emerges in red-light signaling. Red-light-activated phytochrome B promotes the phosphorylation and degradation of phytochrome-interacting factors, including PIF5. TOPP4 physically interacts with PIF5, directly dephosphorylates it, and reduces its red-light-induced ubiquitination and degradation [16] (**Figure 1(E)**). By stabilizing PIF5, TOPP4 attenuates phyB-dependent red-light responses, promoting hypocotyl elongation and modulating apical-hook and cotyledon opening. Thus, TOPP4 counterbalances rather than contradicts phyB-dependent PIF5 turnover, tuning the magnitude and duration of PIF5 depletion during photomorphogenesis. This contrasts with the DELLA pathway, in which TOPP4-dependent dephosphorylation is associated with destabilization, underscoring that the consequence of phosphate removal depends on the substrate and its surrounding regulatory machinery.

4. TOPPs in Metabolic Adaptation and Autophagy

Metabolic adaptation further illustrates the importance of TOPP redundancy. Autophagy supports nutrient recycling during starvation. In *Arabidopsis*, ATG13 is highly phosphorylated under nutrient-rich conditions but is rapidly dephosphorylated after carbon deprivation. Higher-order topp mutants exhibit impaired autophagy and reduced tolerance to fixed-carbon starvation, identifying TOPPs as major regulators of this transition [11] (**Figure 2(A)**). The requirement for septuple and octuple mutants provides strong evidence for functional overlap among TOPP isoforms in autophagy and illustrates why single-gene analyses can underestimate family-level phosphatase functions. Mechanistically, this starvation response converges on ATG13a. TOPP4 was directly shown to dephosphorylate ATG13a *in vitro*, while coexpression assays showed reduced ATG13a phosphor-

ylation with TOPP1, TOPP3, and TOPP9 *in planta* [11]. These isoform-specific data should be distinguished from the septuple and octuple mutant phenotypes, which establish broader family-level genetic redundancy but do not demonstrate direct ATG13a dephosphorylation by every TOPP isoform. At least 18 phosphorylation sites have been identified in ATG13a. An alanine-substituted phospho-dead ATG13a variant enhances autophagy and starvation tolerance. TOPP-dependent ATG13a dephosphorylation promotes formation of the ATG1a-ATG13a complex and is accompanied by increased ATG1a phosphorylation, although TOPP4 does not directly dephosphorylate ATG1a [11] (**Figure 2(B)**). Thus, phosphate removal from ATG13a activates rather than suppresses the pathway by favoring assembly of the autophagy-initiation complex. The upstream signal that recruits or activates TOPPs during carbon starvation remains unknown.

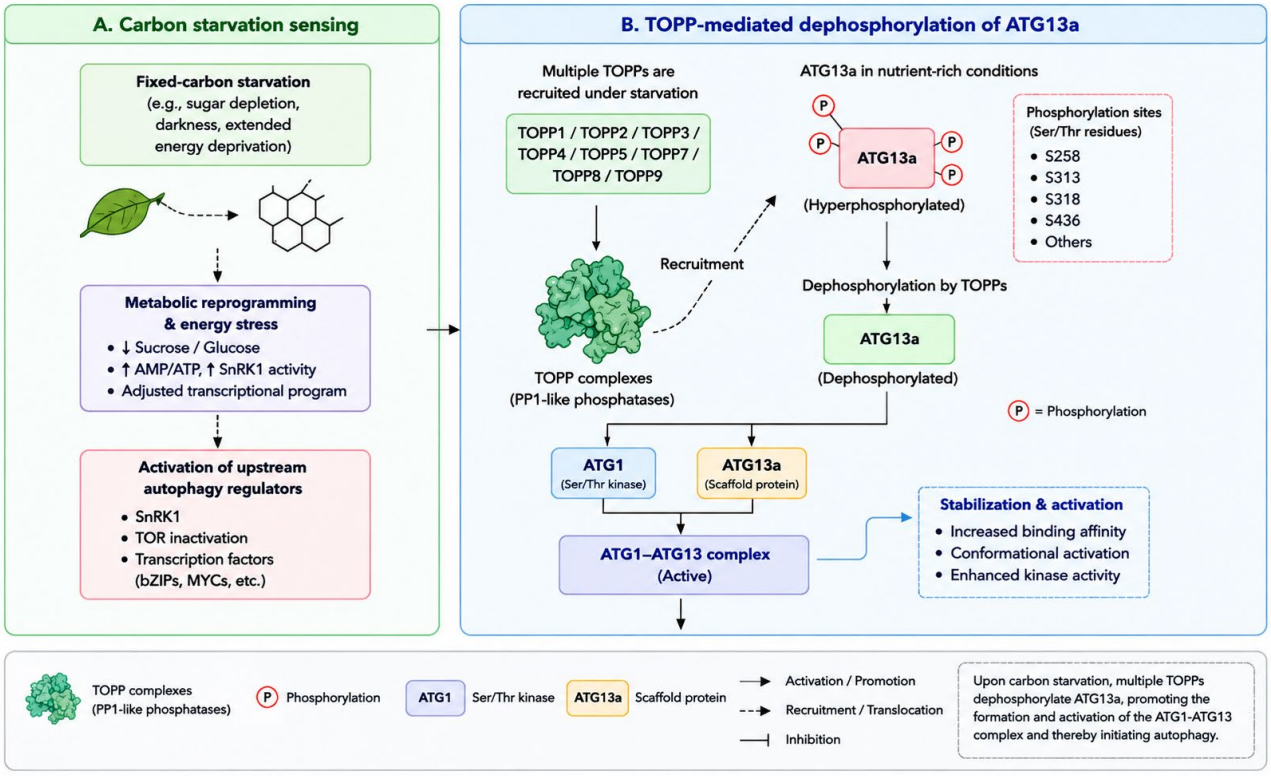

Figure 2. Carbon starvation-induced TOPP-mediated activation of the ATG1-ATG13 complex. (A) Carbon starvation triggers metabolic reprogramming and activates upstream autophagy regulators. (B) Starvation engages TOPP-dependent ATG13a dephosphorylation; TOPP4 has direct *in vitro* evidence, while TOPP1, TOPP3, and TOPP9 are additionally supported by in planta coexpression assays, promoting ATG1 - ATG13 complex activation.

5. TOPPs in Immune Homeostasis and Pathogen Interaction

In immune homeostasis, *Arabidopsis* TOPPs generally restrain basal immune activity. The dominant-negative topp4-1 mutant exhibits constitutive defense-associated phenotypes, whereas a *topp1 topp4 topp5 topp6 topp7 topp8 topp9 septuple mutant* shows elevated defense-gene expression and enhanced resistance to *Pseudomonas syringae* pv. *tomato* DC3000. TOPPs also physically interact with

MAPKs and influence MAPK-associated downstream defense responses [10] (**Figure 3(A)**). Suppressor screening identified SUT1, a coiled-coil nucleotide-binding leucine-rich-repeat receptor (CNL), as essential for topp4-1-induced autoimmunity. SUT1 interacts with both wild-type and mutant TOPP4, but genetic evidence indicates that the aberrant TOPP4 state activates SUT1-dependent immunity [18] (**Figure 3(B)**). These observations are consistent with a guard-type mechanism in which SUT1 monitors TOPP4 or a TOPP4-associated complex. They do not demonstrate that normal fluctuations in TOPP4 catalytic activity are sufficient to trigger SUT1, and the molecular feature of mutant TOPP4 that is sensed remains unresolved. Importantly, SUT1-dependent surveillance is also spatially constrained. Heterologous assays further showed that SUT1 induces strong cell death in the presence of TOPP4^{T246M} and other mutant TOPP4 proteins, but not wild-type TOPP4. SUT1 associates with the plasma membrane; substitutions at Gly2, Cys4, or Ser6 disrupt membrane localization and abolish cell-death activity, whereas artificial membrane anchoring restores activity to localization-defective variants [19] (**Figure 3(B)**). These results establish localization as a functional component of SUT1 signaling. Whether *Arabidopsis* SUT1 directly senses a membrane-associated TOPP4 complex, an altered guardee, or a downstream consequence of TOPP4 perturbation remains unknown.

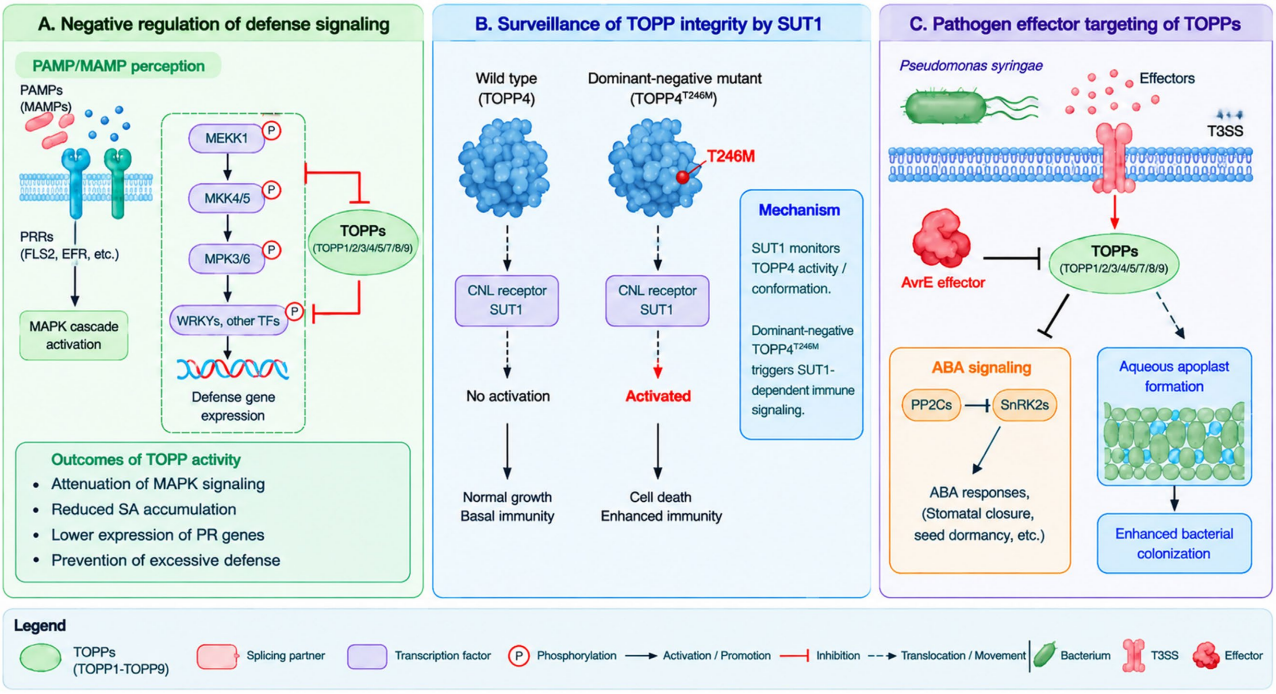

Figure 3. Proposed model of TOPP-mediated regulation of plant immunity. (A) TOPPs negatively regulate MAPK-dependent defense signaling. (B) SUT1 monitors TOPP4 integrity and activates immunity upon TOPP4 impairment. (C) Pathogen effectors target TOPPs to manipulate host ABA signaling and promote bacterial colonization.

Conversely, TOPPs can be exploited by pathogens. The *Pseudomonas syringae* type III effector AvrE binds *Arabidopsis* TOPPs and inhibits their function. Be-

cause TOPPs negatively regulate ABA signaling through SnRK2-associated mechanisms [9] [17], AvrE-mediated TOPP inhibition increases ABA responses, promotes stomatal closure, and contributes to the formation of an aqueous apoplast that favors bacterial proliferation [20] (**Figure 3(C)**). This finding lends broader biological plausibility to the SUT1 guard model [18] [19]. If microbial effectors manipulate TOPP-centered signaling, host surveillance of altered TOPP states could provide a means of detecting virulence activity. However, direct mechanistic links between AvrE-dependent TOPP inhibition and SUT1 activation have not been established and should not be assumed.

6. Discussion

Although considerable progress has been made in defining the biological functions of *Arabidopsis* TOPPs, current evidence also highlights several unresolved questions. A recurring theme of this review is that catalytic conservation alone cannot explain the substantial functional diversity of TOPP phosphatases. More broadly, studies of plant PPP-family phosphatases indicate that signaling specificity can be generated through regulatory subunits, protein-protein interactions, substrate recruitment, and subcellular compartmentalization rather than through extensive diversification of the catalytic core [8] [9] [13] [17] [23]. In the TOPP family, this principle is supported by the distinct involvement of TOPP-containing complexes in gibberellin, auxin, light, ABA, ethylene, autophagy, and immune signaling [9] [11] [12] [14]-[17]. At the same time, the strong phenotypes of higher-order topp mutants demonstrate extensive genetic redundancy, whereas the recurrent involvement of TOPP4 in multiple developmental and immune processes points to context-dependent specialization [10]-[12]. This framework therefore reconciles the apparent contradiction between catalytic conservation and functional diversification. Nevertheless, whether individual TOPP isoforms possess intrinsic substrate preferences or function primarily through distinct regulatory complexes remains largely unresolved.

A second major challenge is the limited identification of direct substrates and phosphorylation sites. Several proteins, including DELLA repressors, PIN1, PIF5, and EIN2, have direct biochemical support as TOPP substrates, whereas ATG13a has direct *in vitro* dephosphorylation evidence for TOPP4 together with additional in planta support for TOPP1, TOPP3, and TOPP9 [11] [12] [14]-[16]. Nevertheless, many TOPP-associated signaling pathways remain supported primarily by genetic interactions or physical associations rather than by direct biochemical demonstration of phosphosite dephosphorylation. This limitation is particularly evident in plant immunity, where TOPPs interact with MAPKs and influence MAPK-associated defense outputs, but the corresponding direct immune substrates and target phosphosites remain largely undefined [10]. Quantitative phosphoproteomics provides a powerful framework for monitoring dynamic phosphorylation changes and identifying candidate regulatory sites, although biochemical validation remains necessary to distinguish direct phosphatase sub-

strates from downstream signaling effects [24] [25]. Accordingly, comprehensive phosphoproteomic profiling combined with TOPP perturbation, substrate-trapping approaches, phosphosite-specific mutagenesis, and *in vitro* dephosphorylation assays should help define pathway-specific TOPP substrates and distinguish direct enzymatic targets from secondary signaling consequences.

The emerging relationship between TOPPs and immune surveillance represents another important conceptual advance. The requirement of SUT1 for topp4-1-associated autoimmunity, together with the preferential induction of cell death by mutant rather than wild-type TOPP4 proteins, supports a model in which SUT1 monitors an aberrant TOPP4 state or a TOPP4-associated signaling complex [18] [19]. Such a model is conceptually consistent with the broader guard paradigm of plant NLR immunity, in which intracellular immune receptors detect perturbations of host proteins or signaling nodes targeted by pathogen effectors rather than necessarily recognizing the effectors directly [26]-[28]. Meanwhile, the *Pseudomonas syringae* effector AvrE directly targets TOPPs, enhancing ABA-associated responses and promoting formation of an aqueous apoplast favorable for bacterial proliferation [20]. Because TOPPs themselves negatively regulate ABA signaling through SnRK2-associated mechanisms [9] [17], these findings position TOPPs at an intriguing interface between host signaling and pathogen virulence. However, AvrE-mediated TOPP inhibition has not been demonstrated to activate SUT1, and the two mechanisms should therefore remain explicitly separated. Determining whether additional pathogen effectors converge on TOPP-containing complexes, whether SUT1 senses TOPP4 itself or a modified associated component, and which molecular alterations constitute the activating signal will be important for establishing the mechanistic basis of phosphatase-centered immune surveillance.

Finally, increasing evidence indicates that TOPPs should be viewed not as isolated catalytic enzymes but as components of dynamic signaling modules whose biological outputs depend on interacting proteins, substrate availability, phosphorylation state, and intracellular localization [8] [9] [13] [17]. Resolving these modules will require approaches that integrate structural biology with quantitative phosphoproteomics, protein-interaction analysis, and spatially resolved measurements. Modern phosphoproteomic approaches allow large-scale and quantitative analysis of phosphorylation dynamics [24] [25], whereas live-cell imaging can resolve stimulus-dependent protein localization and dynamics at subcellular scales [29]. Spatial proteomics, particularly when combined with quantitative mass spectrometry and imaging, offers an additional means of defining the compartment-specific composition and dynamics of signaling complexes [30]. Integrating these approaches with genetic and biochemical analyses should clarify how TOPP regulatory partners determine substrate accessibility and signaling output. Such knowledge may ultimately enable selective manipulation of individual TOPP complexes without broadly disrupting the conserved catalytic activity shared across the family, thereby creating opportunities to modify growth, stress tolerance, and disease resistance with reduced pleiotropic effects.

7. Conclusions

Arabidopsis TOPPs constitute a highly conserved phosphatase family whose biological diversity is generated largely through context-dependent regulation rather than catalytic divergence. Across hormone signaling, photomorphogenesis, autophagy, stress adaptation, and immunity, current evidence consistently supports a model in which regulatory proteins, substrate recruitment, and subcellular localization determine TOPP specificity. Although substantial functional redundancy exists among TOPP isoforms, selected members, particularly TOPP4, repeatedly emerge as central regulators in multiple signaling pathways. These observations indicate that conserved catalytic activity is progressively refined by molecular context to generate distinct physiological outputs.

Recent advances have further expanded the biological significance of TOPPs beyond classical signal attenuation. The identification of SUT1-mediated surveillance of aberrant TOPP4 states and the exploitation of TOPPs by the bacterial effector AvrE demonstrate that TOPPs are integral components of plant immune homeostasis as well as important targets during host-pathogen interactions. Together, these findings position TOPPs at the intersection of developmental regulation, environmental adaptation, and immune signaling. Despite this progress, important questions remain regarding substrate recognition, phosphosite specificity, regulatory-complex assembly, and spatial control of TOPP activity. Addressing these issues through integrated biochemical, structural, genetic, and phosphoproteomic approaches will provide a more complete mechanistic framework for TOPP function. Such studies are expected to facilitate the development of pathway-specific strategies for improving crop growth, stress resilience, and disease resistance while minimizing the pleiotropic effects associated with global phosphatase manipulation.

Author Contributions

Conceptualization, K. C. and J. H.; Methodology, K. C. and J. H.; Formal Analysis, K. C. and J. H.; Investigation, K. C. and J. H.; Resources, K. C. and J. H.; Writing—Original Draft Preparation, K. C.; Writing—Review and Editing, J. H.; Supervision, K. C. and J. H. All authors have read and agreed to the published version of the manuscript.

Funding

This study was funded by the teaching and research start-up funds from Kai Chen, and Jiangxi Provincial Natural Science Foundation (Grant No. 20252BAC200384) from Jianzhong Huang.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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