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Nuclear transport receptors underpin plastidial retrograde signaling

The nucleus integrates cytosolic and organellar signals to regulate gene expression during plant stress responses, and this process is highly dependent on shuttling of signaling molecules in and out of the nucleus. The nucleocytoplasmic transport of macromolecules is facilitated by nuclear transport receptors (NTRs). NTRs are broadly categorized into exportins, which mediate the export of cargo from the nucleus to the cytoplasm, and importins, which facilitate the import of cargo into the nucleus. The importin family comprises importin-α (IMPα) and importin-β (IMPβ). IMPα proteins are adaptor proteins and composed of three functional domains: an N-terminal IMPβ-binding (IBB) domain, ten Armadillo (ARM) repeats responsible for recognizing and interacting with cargo, and an exportin-interacting domain crucial for recycling via nuclear export (Wing et al., 2022). During a nuclear transport event, IMPα recognizes cargo containing a classical nuclear localization signal (cNLS) through its ARM repeats and engages IMPβ via its IBB domain, forming the cargo-IMPα-IMPβ ternary complex. IMPβ facilitates translocation of this complex through the nuclear pore. Once inside the nucleus, RanGTP competes with IMPα for binding to IMPβ, promoting disassembly of the transport complex and release of cargo.

While budding yeast encodes a single *IMPα* gene, the genomes of *Drosophila*, humans, and *Arabidopsis* contain 3, 7, and 9 *IMPα* isoforms, respectively (Figure 1A). This expansion of IMPα isoforms in multicellular eukaryotes likely reflects an adaptation to facilitate more complex regulation of nuclear import across different cell types, tissues, and diverse environmental conditions. Taking *Arabidopsis* as an example, *IMPα* genes exhibit distinct spatiotemporal expression patterns (Figure 1B, data source: Yu et al., 2022). Specifically, *IMPα-1*, *IMPα-2*, *IMPα-3*, and *IMPα-4* are highly expressed in all tissues, except in pollen where *IMPα-1* and *IMPα-2* are not detectable. These paralogs are presumed to play major roles in cargo import in plant cells under normal conditions and appear highly redundant in regulating plant growth and development, evidenced by their single and higher-order mutant phenotypes (Ludke et al., 2021). In contrast, the expression of *IMPα-5* and *IMPα-7* is only detected in pollen, while *IMPα-8* is barely detectable in any tissue under normal conditions. *IMPα-9*, on the other hand, exhibits weak expression in all tissues except seeds, where its expression is comparable to other highly expressed *IMPα* isoforms. Phylogenetically distant from other IMPαs, IMPα-9 contains a glutamine (Q) to arginine (R) substitution in the conserved "RRRR" motif within the IBB domain. Additionally, a second conserved "KKRR" motif in the IBB region is altered to "AKRL." Those two motifs are responsible for blocking the minor and major cNLS-binding site in the ARM domain of IMPα, respectively, forming an important autoinhibitory mechanism that prevents cargo binding of IMPα in the absence of IMPβ (Miyamoto et al., 2016; Wing et al., 2022). Together with a few short stretches of amino acid insertions between ARM repeats, those changes in IMPα-9 may potentially result in weak autoinhibition and distinct binding dynamics for substrates.

Notably, different biotic stresses, including bacterial, fungal, viral, and insect infections, could significantly augment the expression of *IMPα* genes, especially those that express at low levels under normal conditions, including *IMPα-5*, *IMPα-6*, *IMPα-7*, and *IMPα-8* (Figure 1C), suggesting that those IMPα isoforms may play a role in plant biotic stress responses. In particular, IMPα-6 and its closely related IMPα-3 have been reported to promote the nuclear transport of MYB3R4, a transcription factor regulating cytokinin-mediated cell division. Transported MYB3R4 can bind to the promoters of *IMPα-3* and *IMPα-6*, further activating their expression. This positive feedback loop significantly enhances the nuclear transport during cell division (Yang et al., 2021). In addition, IMPα-3 has been implicated in transporting the NLR (nucleotide-binding domain leucine-rich repeat containing) type of immune receptor SNC1 (SUPPRESSOR OF npr1-1, CONSTITUTIVE 1) and is associated with another NLR protein TIR-NBS13 to regulate plant immune activation (Roth et al., 2017; Ludke et al., 2021). These findings support a significant and specialized role of IMPα isoforms in regulating plant responses to developmental and environmental stimuli. Nevertheless, the functions of most IMPα isoforms remain largely unexplored in plants.

Recently, Zeng et al. (2024) reported the discovery of a gain-of-function mutant of IMPα-9, characterized by a mutation in a conserved arginine residue in the first ARM repeat (R105Q). This mutation substantially suppresses the phenotypes of the *ceh1* mutant, which accumulates high levels of 2-C-methyl-d-erythritol-2,4-cyclopyrophosphate (MEcPP), a plastid-derived retrograde signal essential for regulating stress-related gene expression in the nucleus (Xiao et al., 2012). In the *ceh1* mutant, MEcPP accumulation leads to dwarfism, elevated phytohormone salicylic acid (SA), and increased expression of stress response genes. Interestingly, Zeng et al. found that the *IMPα-9(R105Q)* mutation does not affect MEcPP accumulation but rather suppresses stress gene expression, including SA-dependent immune activation, indicating that IMPα-9 functions downstream of MEcPP in retrograde signaling.

Subsequent yeast-two-hybrid screen and immunoprecipitation followed by mass spectrometry analysis using IMPα-9 as bait identified two interactors: ASK1, a core component of an SCF

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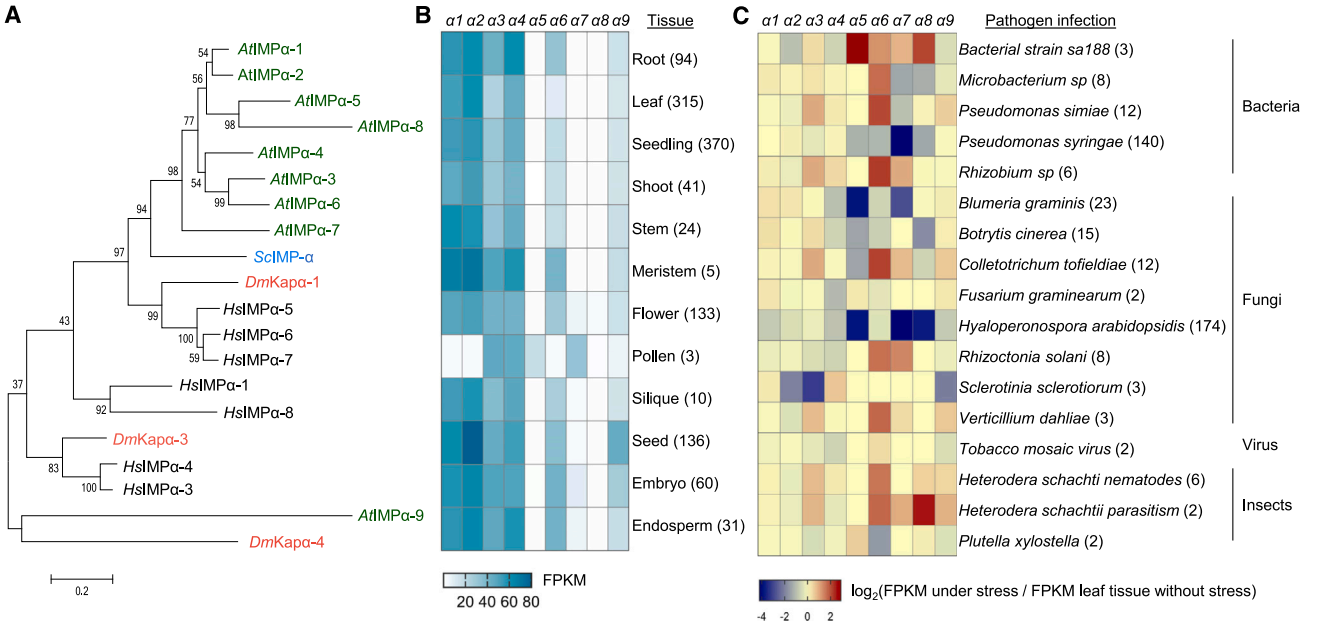

Figure 1. Phylogenetic and expression analyses of *Arabidopsis* importin-αs

(A) Phylogenetic tree of importin-αs in *Arabidopsis thaliana* (*At*), *Saccharomyces cerevisiae* (*Sc*), *Homo sapiens* (*Hs*), and *Drosophila melanogaster* (*Dm*). Protein sequences were aligned using ClustalW, and the dendrogram was constructed using maximum likelihood method of MEGA version 6.0. Bootstrap = 1,000; scale bar represents amino acid substitutions per position.

(B) Heatmap of tissue-specific expression of *importin-α* genes in *Arabidopsis thaliana.*

(C) Heatmap of relative expression levels of *importin-α* under biotic stresses compared to expression in leaf without stresses. FPKM: fragments per kilobase of transcript per million mapped reads. Numbers in parentheses represent the number of independent libraries surveyed. Data source: Arabidopsis RNA-seq Database (Yu et al., 2022).

E3 ubiquitin ligase complex, and TPR2, a transcription corepressor that negatively regulates stress-related gene expression. Zeng et al. demonstrated that MEcPP overaccumulation stabilizes ASK1, although the exact mechanism remains unclear. Additionally, overexpression of ASK1 reduces IMPα-9 protein levels without altering its transcript abundance, suggesting that IMPα-9 is a substrate of ASK1 for degradation. This finding is consistent with the observation that IMPα-9 levels are reduced in the *ceh1* mutant with high MEcPP and elevated ASK1 level. Notably, IMPα-9^{R105Q} loses the interaction with ASK1, thereby escaping regulation by ASK1.

In contrast to AKS1, TPR2 interacts with both the wild-type and mutant IMPα-9 protein, and the TPR2 protein level appears to be stabilized by this interaction. Intriguingly, ChIP-seq analysis revealed that both TPR2 and IMPα-9 bind to stress-related genes with largely overlapping binding profiles, suggesting a collaborative role in controlling MEcPP-dependent stress gene expression. Supporting this hypothesis, overexpressing *TPR2* also partially suppresses *ceh1* mutant phenotypes, similar to *IMPα-9^{R105Q}*. Collectively, this study suggests a model in which IMPα-9 binds to the transcriptional repressor TPR2, potentially stabilizing it and consequently facilitating the repression of stress-related genes. Concurrently, the stability of IMPα-9 itself is regulated by ASK1, whose stability is modulated by MEcPP-dependent retrograde signaling. Consistent with this model, under high light conditions, plastidial MEcPP is induced and promotes ASK1-mediated degradation of IMPα-9, thereby potentially alleviating the repressive effect on nuclear TPR2 and enabling transcriptional responses to the high light stress.

The nuclear export of TPR proteins is coordinated by Exportin-4, which regulates the amplification of SA-dependent immune responses (Xu et al., 2021). Although the current data do not provide direct evidence that IMPα-9^{R105Q} mediates nuclear import of TPR2, it is likely that TPR2 is a cargo of IMPα-9. This would suggest that IMPα-9 functions not only in nuclear transport but also in preventing its cargo from proteasome-dependent degradation, and these two functions may occur simultaneously and could potentially be interconnected processes. Recent findings in both animal and plant systems have revealed that certain IMPβ proteins possess an unconventional chaperone-like activity, facilitating the disaggregation of molecular condensates formed by their cargo proteins (Mboukou et al., 2021; Jia et al., 2023). These findings together highlight the diverse mechanisms by which NTRs might regulate the activities of their cargoes beyond simple nuclear transport.

Further research is warranted to address remaining gaps and uncover more exciting molecular mechanisms involved in MEcPP-mediated retrograde signaling regulation. These include but are not limited to elucidating the mechanisms by which ASK1 protein is stabilized upon MEcPP accumulation, understanding how IMPα-9 contributes to the stabilization of TPR2 protein, and evaluating whether the nucleocytoplasmic transport of TPR2 is integral to this regulatory process. Additionally, it would be also intriguing to determine whether the cargo stabilization function is unique to IMPα-9 or also applicable to other IMPα proteins. Considering the fundamental, diverse, and expanding roles that NTRs play in plant stress responses, further investigation of these

proteins holds significant potential for engineering strategies to enhance crop resilience in the face of global climate change.

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