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Structural Aspects of Multistep Phosphorelay-Mediated Signaling in Plants

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ABSTRACT

The multistep phosphorelay (MSP) is a central signaling pathway in plants integrating a wide spectrum of hormonal and environmental inputs and controlling numerous developmental adaptations. For the thorough comprehension of the molecular mechanisms underlying the MSP-mediated signal recognition and transduction, the detailed structural characterization of individual members of the pathway is critical. In this review we describe and discuss the recently known crystal and nuclear magnetic resonance structures of proteins acting in MSP signaling in higher plants, focusing particularly on cytokinin and ethylene signaling in *Arabidopsis thaliana*. We discuss the range of functional aspects of available structural information including determination of ligand specificity, activation of the receptor via its autophosphorylation, and downstream signal transduction through the phosphorelay. We compare the plant structures with their bacterial counterparts and show that although the overall similarity is high, the differences in structural details are frequent and functionally important. Finally, we discuss emerging knowledge on molecular recognition mechanisms in the MSP, and mention the latest findings regarding structural determinants of signaling specificity in the *Arabidopsis* MSP that could serve as a general model of this pathway in all higher plants.

Key words: multistep phosphorelay, structure, histidine kinase, phosphotransfer protein, response regulator

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INTRODUCTION

To adapt to a constantly changing environment, plants have evolved numerous signal perception and transduction pathways that allow them to sense, process, and respond to diverse external stimuli. Plant signaling pathways can operate independently, but cooperation and cross-talk between them is crucial for the integration of various signals and to effect the proper adaptive responses. Multistep phosphorelay (MSP) is a backbone signaling pathway in plants, which appears to have evolved from the bacterial two-component signaling (TCS) system. Like most of the known signaling pathways in all organisms, both MSP and TCS systems are based on protein (trans)phosphorylation. The TCS system consists of two conserved proteins: a homodimeric sensor histidine kinase (HK) and a response regulator (RR). Recognition of an external signal activates HK, leading to its autophosphorylation at a conserved histidine (His) residue. The subsequent phosphotransfer from HK to the receiver domain (RD) of RR activates the output domain of the RR (West and Stock, 2001; Skerker et al., 2008). The final response is mediated by interaction of the phosphorylated RR with gene or protein targets that induces cellular responses, mainly via transcriptional activation. *Vice versa*, dephosphorylation of RR attenuates TCS signaling (Casino et al., 2009). MSP is a more elaborate system mediating signaling in bacteria, fungi, algae, and plants, but not in animals (Schaller et al., 2011). Similar to TCS, a typical MSP signaling pathway commonly includes a stimulus activation of a membrane-associated HK, which triggers phosphorelay to nuclear RR, mainly resulting in MSP-mediated control of gene expression. However, in contrast to TCS, MSP includes two additional domains: (1) an RD similar to that of RR and (2) a His-containing phosphotransfer (HPt) domain. In bacterial MSP systems, both of these additional modules may be single-domain proteins or reside within multidomain proteins. In plants, hybrid HKs consist of the RD and HK domain in a single polypeptide chain. Following signal perception and

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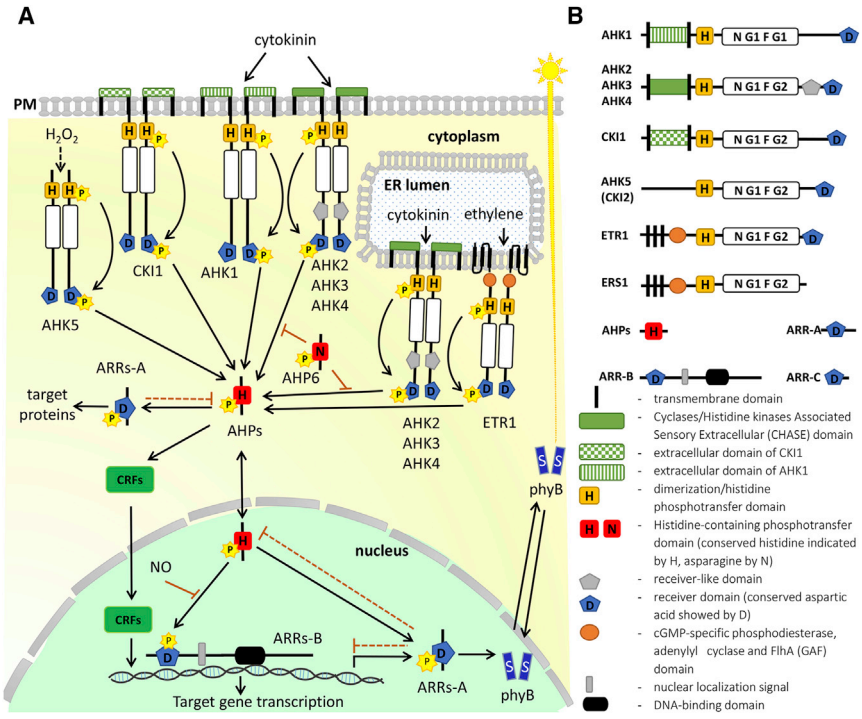

Figure 1. Multistep Phosphorelay in Plants.
(A) Model of MSP signaling in *Arabidopsis*. Signal perception induces autophosphorylation of the histidine residue in the kinase domain of sensor histidine kinases localized in the cytoplasm or in the ER lumen. The phosphoryl group is subsequently transferred intramolecularly to a conserved aspartate residue of the C-terminal receiver domain. AHP proteins accept the signal from histidine kinases. The signal is transferred either to the nucleus, where the signal output is realized via regulation of target gene expression through ARRs-B or via control of effector proteins through ARRs-A. Alternatively, AHPs may remain in the cytoplasm and allow transphosphorylation of ARRs-A, thereby further transferring the signal to target proteins. CRFs (depicted as green horizontal rectangles) are transcription factors, and their function is similar to that of ARRs-B as they work in parallel. One of the ARRs-A target effector proteins is phytochrome B (phyB), here shown as a blue rectangle. ARRs-A also mediate negative feedback regulation by attenuating the signaling. AHP6 and nitric oxide (NO) also negatively regulate cytokinin signaling. Dashed lines show regulation via an unclear molecular mechanism or proposed regulatory interactions. Phosphoryl group is shown as a yellow star. Light is depicted as a yellow sun, and hydrogen peroxide as H_2O_2. Domain coding is the same as in **(B)**.
(B) Schematic representation of the protein domains.

autophosphorylation, the first transphosphorylation occurs intramolecularly from a phosphorylated His of the catalytic domain of HK to a conserved aspartate (Asp) of the C-terminal RD. The single-domain HPt protein accepts the signal from the RD of the membrane-associated hybrid HK and transfers it to the RD of RRs in nuclei, which either induce transcriptional changes or interact with effector proteins, e.g., light receptors (for a recent review see Schaller et al., 2011).

Here, we overview the recent research into structural aspects of plant MSP signaling using the MSP of *Arabidopsis thaliana* which, to date, provides the only system with high-resolution structures available for typical examples of most of its components. First, we briefly highlight the most important structural and functional aspects of the MSP pathway allowing integration of multiple signaling (particularly cytokinin and ethylene) inputs. Next, we review the high-resolution crystal and nuclear magnetic resonance (NMR) structures of plant MSP components resolved to date, starting with the description of individual domains of hybrid HKs, the sensory domain of ARABIDOPSIS HISTIDINE KINASE 4 (AHK4), cytosolic domains of HKs (the dimerization domain of ETHYLENE RESPONSE SENSOR 1 [ERS1], the catalytic domain of ETHYLENE RESPONSE 1 [ETR1], the RD of CYTOKININ-INDEPENDENT 1 [CKI1], AHK5, and ETR1), and discuss the proposed model of the cytosolic part of ETR1. We then compare the known structures of plant HPts and continue to the end of the pathway by describing the DNA-binding domain of ARABIDOPSIS RESPONSE REGULATOR 10 (ARR10). Wherever relevant, the structures of bacterial counterparts are compared. Finally, we discuss possible structural determinants of the MSP specificity in *Arabidopsis* and characterize a molecular recognition mechanism involved in interactions between RD and HPt domains in *Arabidopsis*, and compare it with the related bacterial mechanisms.

MULTISTEP PHOSPHORELAY SIGNALING IN PLANTS

Cytokinin Signaling

The plant MSP plays a major role in cytokinin perception and signaling (Figure 1A). Cytokinins are phytohormones that have important roles in several aspects of plant growth and development, including control over cell division and differentiation and the modulation of the response to biotic and abiotic stresses (for a recent review see Kieber and Schaller, 2013). The cytokinin signal is perceived by three membrane-spanning cytokinin receptors, the sensor HKs AHK2, AHK3, and AHK4, which are localized mostly in the endoplasmic reticulum (ER) (Caesar et al., 2011; Lomin et al., 2011; Wulfetange et al., 2011). Binding of the cytokinin ligand triggers kinase activity in the receptor. Interestingly, of the three cytokinin receptors only AHK4 has dual activity. In the presence of cytokinins, AHK4 phosphorylates AHPs, while in the absence of cytokinins, AHK4 acts as a phosphatase, targeting the dephosphorylation of AHPs (Mahonen et al., 2006b). Five AHPs (AHP1–5), the downstream intermediates of cytokinin signaling in *Arabidopsis*, function as redundant positive regulators of cytokinin signal transduction (Hutchison et al., 2006; Hutchison and Kieber, 2007), whereas the pseudo-HPt, AHP6, in which the His residue essential for the phosphorelay is replaced by asparagine, attenuates cytokinin