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Serine/threonine protein phosphatases type 2A and their roles in stress signaling

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Serine/threonine protein phosphatases are ubiquitous enzymes in all eukaryotes but many of their physiological roles in plants remain unknown. The available results have demonstrated critical functions for these enzymes in the regulation of adaptive stress responses, and recent studies have directed attention to the functional roles of Ser/Thr phosphatases type 2A (PP2A) as components of stress signaling pathways. This review is focused primarily on plant PP2As and their participation in the control of biotic and abiotic stress responses.

Introduction

Phosphorylation and dephosphorylation of key regulatory proteins serve as an "on-off" switch in the control of cellular activities in eukaryotic cells.[1] While the large family of protein kinases and their functions have been recognized for many years, the understanding of the physiological roles of protein phosphatases, which are obligate partners of protein kinases in cellular control circuitry, has been developed only recently. Phosphatases can be grouped by substrate specificity into serine/threonine (Ser/Thr), tyrosine (PTPs) and dual specificity (DSPs) classes. Ser/Thr phosphatases are classified into Ser/Thr-specific protein phosphatases (PPP, which include subfamilies of PP1, PP2A, PP2B, PP4, PP5, PP6, PP7 and protein phosphatases with kelch-repeat domains) and the metal ion-dependent protein phosphatases (PPM, also known as PP2Cs). Members of Ser/Thr phosphatases of all subfamilies (except PP2B) have been identified in plants.[2-4]

PP2A holoenzyme consists of a catalytic (C) subunit, that occurs either in association with a regulatory A subunit or together with a third variable B subunit.[5,6] The B subunits, which determine the substrate specificity and subcellular localization of PP2As, are classified into B, B' and B" families. In *Arabidopsis thaliana*, five genes encoding catalytic PP2Ac subunits, three genes encoding A subunits and seventeen genes encoding B subunits have been identified.[4] Catalytic subunits can be grouped into two subfamilies, I and II.[4,7,8] PP2As have been implicated in auxin transport,[9,10] abscisic acid and ethylene signaling,[11-13] growth and development[14,15] and control of cytoskeletal structure.[16]

Over the recent years, considerable effort has been devoted to the study of PP2A roles in plant stress responses. Stress signal transduction pathways start with signal perception, followed by the activation of protein phosphorylation cascades involving protein kinases and phosphatases, that finally target proteins involved in cellular protection or transcription factors controlling sets of stress-regulated genes.[17] This review focuses on plant PP2As and their participation in biotic and abiotic stress signaling pathways.

Several research papers discussed in the present review encompass the determination of stress responses (generally induction of stress-regulated genes) in the presence or absence of okadaic acid at nM concentrations. This compound has been widely used as PP2A and PP1 inhibitor, which are the most prevalent phosphatases in plants, being responsible for more than 85% of the total cellular phosphatase activity,[18] however, the less abundant Ser/Thr phosphatases PP4, PP5 and PP6, are also affected.[19] Okadaic acid has been useful to identify the stress responses that are mediated by Ser/Thr phosphatases. Other reports are based on expression profiles of PP2A subunits under stress conditions and provide suggestive information about the roles of PP2As in stress signaling pathways, while further studies using loss-of-function mutants or overexpression/silencing of specific PP2A subunits isoforms provide more conclusive data.

Biotic Stress

Participation of Ser/Thr phosphatases as negative regulators of plant defense responses against pathogens has been evidenced by the use of okadaic acid. The inhibitor activates anti-fungal defense responses even in the absence of infection or elicitors in several plant species, such as soybean (*Glycine max*),[20] potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*),[8] tobacco (*Nicotiana tabacum*)[21] and opium poppy (*Papaver somniferum*),[22] suggesting the existence of active signaling pathways in the absence of stress that require continuous Ser/Thr phosphatase activity to remain inhibited.

Silencing of the subfamily I catalytic subunits *NbNPP4-1* and *NbNPP4-2* in *Nicotiana benthamiana* results in constitutive activation of plant defense responses and localized cell death.[7] In addition, these plants are resistant to a virulent strain of the bacteria pathogen *Pseudomonas syringae*, and show an accelerated hypersensitive response to effector proteins from both *Pseudomonas*

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syringae and the fungal pathogen *Cladosporium fulvum*, indicating that catalytic subunits of PP2Ac subfamily I act as negative regulators of plant defense responses.

Surprisingly, transcripts of the catalytic subunits of PP2A subfamily I are upregulated by fungal elicitors in potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*).[8] Likewise, a PP2A catalytic subunit expression is induced in sunflower (*Helianthus annus*) after inoculation with the necrotrophic fungus *Phoma macdonaldii*.[23] Activation of defense-related protein phosphorylation cascades leads to oxidative burst and localized cell death, therefore, upregulation of potential negative modulators by pathogens may be a protective mechanism. PP2As may act by desensitizing protein phosphorylation cascades, tightly regulating the defense response to prevent extensive damage to host tissues. In this regard, ectopic expression of the Arabidopsis calcium-dependent protein kinase AK1-6H in tomato protoplasts enhances plasma membrane-associated NADPH oxidase activity (responsible for the oxidative burst), while the Arabidopsis PP2A catalytic subunit PP2A-5 abolishes this enhancement.[24]

Water Deficit Stress

Water deficit condition can be caused by drought or osmotic stress imposed by high salt concentration in the soil. Suggestive data about the participation of PP2A in water deficit stress was found in several plant species. In rice (*Oryza sativa*), all catalytic subunit genes (*OsPP2A-1-5*) are upregulated in response to high salinity in leaves.[25,26] In the same way, salt stress increases mRNA levels of potato *StPP2Ac1*, *StPP2Ac2a*, *StPP2Ac2b* and *StPP2Ac3* in leaves.[8] Furthermore, okadaic acid inhibits salt stress response in potato, indicating a positive regulation by Ser/Thr phosphatases.[8]

The wheat (*Triticum aestivum*) *TaPP2Ac-1* catalytic subunit transcripts accumulate in seedlings in response to water deficit. Transgenic tobacco plants overexpressing *TaPP2Ac-1* exhibit enhanced drought tolerance,[27] indicating that this PP2A catalytic subunit acts as positive regulator of salt stress adaptive response.

The studies discussed so far are focused on PP2A catalytic subunits, however, emerging evidence suggest that A and B subunits may also play important roles in the regulation of water deficit stress responses. Arabidopsis loss-of-function mutants for *RCN1,* encoding a regulatory A subunit, exhibit increased sensitivity to osmotic stress, indicating that RCN1-regulated PP2A activity positively regulates the response to water deficit stress.[15] Expression of alfalfa (*Medicago sativa*) *MsPP2A B*β subunit is induced by the phytohormone abscisic acid, the major player in mediating the adaptation of plants to water deficit, suggesting a specific function for this protein in the water deficit stress response.[28]

Cold Stress

Studies based on the use of okadaic acid indicate that Ser/Thr phosphatases are involved in cold stress signaling as negative modulators of adaptive responses. The inhibitor enhances the cold response in wheat[29] and tomato.[8] Moreover, treatment of *Brassica napus* leaves with okadaic acid increases freezing tolerance.[30] It has been demonstrated that chilling decreases PP2A mRNA levels and/or activity. The catalytic subunits *LePP2Ac1*, *LePP2Ac2* and *LePP2Ac3* mRNA levels decreases in response to low temperatures in tomato.[8] PP2A activity is inhibited by cold in alfalfa cell suspension cultures.[31] All the available data suggest that PP2As act as negative regulators of low temperature responses, however, more work is required to confirm this hypothesis and to identify which PP2A isoforms are components of the cold signal transduction pathways.

An A subunit of PP2A (PP2AA3) was identified as an interaction partner and substrate of Arabidopsis AtCHIP, which functions as an E3 ubiquitin ligase.[32] Chilling inhibits PP2A activity in Arabidopsis. *AtCHIP*-overexpression overrides this inhibition, leading to higher PP2A activity under low-temperature conditions and increased cold sensitivity, when compared to wild-type plants. This data indicates that AtCHIP may function as an upstream regulator of PP2A in the low temperature signaling pathway and supports the hypothesis that PP2A act by inhibiting the cold adaptive response.

Arabidopsis TAP46 (2A Phosphatase Associated Protein of 46 kDa) shows co-immunoprecipitation with a PP2A catalytic subunit. Transcription of *TAP46* is induced by chilling, which suggests a role for a TAP46-PP2A complex in cold stress signaling.[33]

Heat Stress

Evidence on the participation of PP2As in heat stress responses is limited and merely suggestive. In Arabidopsis, transcripts of the B' subunit gene *AtB'*γ accumulate in seedlings after exposure to high temperature,[34,35] suggesting that PP2A heterotrimers containing this subunit are involved in heat stress responses. In rice, mRNA levels of PP2A catalytic subunits are regulated by heat-shock treatments. Members of PP2Ac subfamily I (*OsPP2A-1* and *3*) show opposite regulation during heat stress:[25] *OsPP2A-1* is repressed in stems while *OsPP2A-3* is induced in all organs of plants exposed to heat. In contrast, expression levels of all three members of PP2Ac subfamily II (*OsPP2A-2, 4* and *5*) are downregulated in leaves and stems in response to combined heat stress and drought, although they are unaffected by either heat stress or drought.[26] Altogether, these results suggest that PP2As may be involved in adaptive responses to counteract heat stress in plants, however, more studies are required to confirm this hypothesis.

Mechanical Wounding

Evidence on the participation of Ser/Thr phosphatases in wound signaling pathways was formerly obtained using okadaic acid. In *Arabidopsis thaliana*, this inhibitor blocks the induction of wound-responsive genes by jasmonic acid, the main regulator of gene activation in response to mechanical damage.[36] Similar experiments performed in potato showed that okadaic acid severely reduces the jasmonic acid-induced expression of wound-related genes in leaves.[37] These results indicate that Ser/Thr phosphatases are required for transmission of the jasmonic acid signal after wounding. Recently it was found that mechanical damage increases mRNA levels of potato *StPP2Ac2b* and tomato *LePP2Ac1* and *LePP2Ac2* in leaves,[8] supporting the hypothesis that PP2As play a role in wound signaling.

Concluding Remarks and Future Perspectives

Although there are many aspects about the roles of PP2As in stress responses that remain unknown, recent advances in the functional analysis of PP2As revealed that they are key components of stress signal transduction pathways, balancing the action of protein kinases. Even though it is believed that phosphatases are uninteresting enzymes that simply turn off signaling cascades activated by kinases, PP2As have also been shown to play positive and dynamic roles in stress signaling.

Several studies are based on the use of okadaic acid and/or expression profiles and provide only suggestive data, that must be confirmed, in the future, by specific functional analysis encompassing loss-of-function mutants or overexpression/silencing of individual PP2A subunit isoforms. Much effort needs to be devoted to the identification of target substrates on which these enzymes act in vivo to better understand PP2A action in stress signaling. It is also worthy to enhance the study of A and B subunit functions in the regulation of stress responses, since the association of regulatory subunits with the catalytic subunit controls its specificity, activity and subcellular targeting and are thus critical to the proper function of this enzyme.

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