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Arabidopsis proline-rich extensin-like receptor kinase 4 modulates the early event toward abscisic acid response in root tip growth

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Exogenous application of plant hormone abscisic acid (ABA) can inhibit root growth. We recently reported that the proline-rich extensin-like receptor kinase 4 *(PERK4)* functions at an early stage of ABA signaling to inhibit primary root cell elongation by perturbing Ca^{2+} homeostasis.[1] Transcription analysis indicated that *PERK4* modulates the expression of the genes related to cell elongation and ABA signaling in root growth, such as polygalacturonases, *AtExt1, AtMYC2* and *ABR1*. Under ABA treatment, the transcript level of *ZAT10*, a Ca^{2+}-responsive gene, increased in *perk4* plants compared to that of wild-type. Based on both present data and the previous evidence, we propose a probable model for PERK4-mediated ABA-regulated primary root cell growth.

Key words: abscisic acid, PERK4, root cell elongation, polygalacturonases, extensin, Ca^{2+}, Arabidopsis

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Although the intrinsic genetic program determines the development pattern of the organs, a sophisticated developmental plasticity allows plants to optimize the size and shape of their new organs in order to shrink away from unfavorable conditions or grow near nutrients or light to maximize resource acquisition.[2,3] For example, the architecture of the root system was strongly influenced by environmental and endogenous signals including soil type, moisture and nutrients and phytohormones,[3-6] which result in highly variable numbers, placement and direction of growth of each root in the morphologies, even among genetically identical plants. Morphological changes in the root system as an adaptive response result from complicated development pathways, that require plants to both recognize constantly changing environmental signals and to convert such signals into appropriate actions.[3]

The plant hormone abscisic acid (ABA) has multiple functions in regulating plant development and stress responses.[7,8] For example, ABA inhibits the well-watered seedlings primary root growth and the formation of lateral roots and root hairs.[9-12] Therefore, the diversity of ABA responses may require multiple sites of ABA perception.[13] Two G-protein-coupled receptors (GTG1 and GTG2) have been implicated in ABA response and can directly bind ABA in vitro.[14] Two other soluble ABA receptors, PYRABACTIN RESISTANCE 1 (PYR1) and PYR1-LIKE 9 (PYL9, RCAR1), have been identified and both interact with ABI1 in an ABA dependent manner and inhibit its activity.[15,16] We recently demonstrated a member of the proline-rich extensin-like receptor kinase family, *PERK4*, functions at an early stage of the ABA signaling pathway to inhibit root growth through intracellular calcium signaling.[1] The decreased sensitivity to ABA in primary root tip growth of *perk4* mutant was due to enhanced cell elongation.

To understand how *PERK4* modulates the ABA response at the gene expression level, we investigated the expression patterns of the genes related to ABA signaling in root growth in wild-type and *perk4* mutant by quantitative real-time polymerase chain reaction (qRT-PCR). We first examine the transcription levels of genes encoding cell wall hydrolytic enzymes which loosen the structure of the wall and control the process of cell growth. These enzymes mainly include

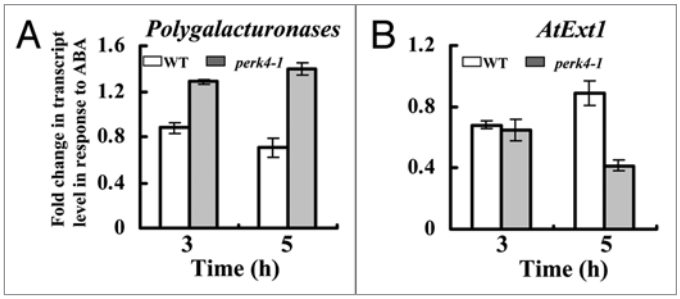

Figure 1. PERK4 regulates the expression of genes related to cell wall loosening. Total RNA was isolated from the 8-d-old mutant and wild-type seedling roots with or without 100 μM ABA treatment with the TRIzol^{R} reagent (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed on a Rotor-Gene 3000 (Corbett Research, Australia). Reaction contained 10 μL SYBR Premix Ex Taq (TaKaRa), 0.6 μL primer (to 0.3 μM), 2 μL cDNA, and 6.8 μL deionized water to make a total volume of 20 μL. The gene-specific primers were 5'-TAG CCT CAC CAG CCA CCT CAC TC-3' and 5'-GCA ATT CCA TAT TCA TCC CAA CC-3' for the polygalacturonases gene (At3g06770, A), 5'-CCG CAA ATT ACT TCT ACT CTT CC-3' and 5'-GTA TTC GTA GTG CTT CTT GGG TG-3' for *AtExt1* (B). *Actin2* gene was used as a standard control. For relative quantification the method of Pfaffl (2001)[27] was used to determine the relative expression ratio. Means ± SD are shown.

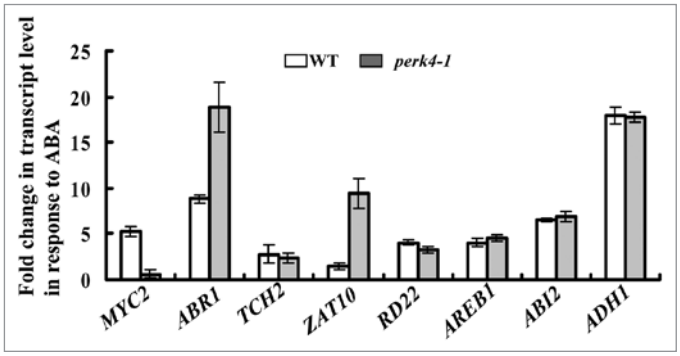

Figure 2. Expression of ABA- and stress-responsive genes in wild-type and *perk4* mutants. qRT-PCR was performed in the same conditions as in Figure 1.

polygalacturonases (pectinase), xyloglucan endo-transglycosylases (XTHs) and expansins.[17-20] Extensins are a family of hydroxyproline-rich glycoproteins (HRGPs) found in the cell walls of higher plants,[21] which have been implicated in nearly all aspects of plant growth and development including the cessation of cell elongation.[22,23] Transcript levels of one or two genes of these protein families were tested. The qRT-PCR data showed that the expression levels of a polygalacturonases gene (At3g06770) decreased in the wild-type, while increased in the mutant after ABA treatment (**Fig. 1A**). Approximately 2-fold expression in the *perk4* plants was observed compared to wild-type plants. After treatment with ABA for 5 h, the level of *AtExt1* transcripts was slightly affected in the wild-type, but significantly decreased in the mutant (**Fig. 1B**). However, no significant differences were found in the transcript levels of the selected *XTH* and expansin genes. As mentioned above, pectinase and extensins could loosen the structure of the wall and control the process of cell growth.[17,18,20] Our result suggested that these two genes might have some role in the PERK4-mediated ABA response. Physiological substrates of the PERK4 kinase are not known. The similar expression patterns of the selected genes encoded XTHs or expansins in *perk4-1* and wild-type plants did not rule out expansins or XTHs would be the PERK4 target. Further work is needed to determine which the direct target of the PERK4 kinase is and what event exactly occurs mediated by PERK4 in response to ABA.

We also examined the transcription levels of other well-known genes regulated by ABA in wild-type and *perk4* mutant. These genes include a basic helix-loop-helix (bHLH) transcription factor gene (*AtMYC2*) and an APETALA2 (AP2)-domain protein (*ABR1*). Among these, the transcription level of *MYC2* was reduced 8.3-fold after 5 h ABA treatment in *perk4* mutants, less induced than that in wild-type after ABA treatment (**Fig. 2**). By contrast, two-fold more expression of *ABR1* in the *perk4* plants was observed compared to wild-type plants (e.g., approximately 19-fold induction by ABA in *perk4* mutants compared approximately nine-fold induction in wild-type). Since *ABR1* is strongly responsive to ABA and functions as a negative transcription factor of ABA responses, disruption of *ABR1* led to hypersensitive response to ABA in root growth assay.[24] The result here coincides with the fact that root growth of *perk4* mutants is less sensitive to ABA compared to that of wild-type plants. Interestingly, under ABA treatments, expression levels of other stress-responsive genes, such as *Alcohol Dehydrogenase 1* (*ADH1*), *Dehydration-Responsive 22* (*RD22*), *ABA-Responsive Element Binding Protein 1* (*AREB1*) and *ABI2,* were found at similar levels in the wild-type and *perk4* plants (**Fig. 2**).

Both *ZAT10* and *TCH2* were Ca^{2+}-responsive upregulated genes by which ABA regulates the physiological responses.[25,26] For *ZAT10* gene, the transcript level in *perk4* plants was increased for nine-fold after ABA treatment, while only 1.5-fold induction by ABA was observed in wild-type plants (**Fig. 2**). However, transcripts of a potential Ca^{2+} sensor gene *TCH2* were found at similar levels in the wild-type and *perk4* mutant plants. The observation that PERK4 activity appears to be increased by ABA or Ca^{2+} in vitro implies a possibility of direct interaction with ABA.[1] That PERK4 might be (part of) an ABA receptor is very exciting, because alternative pathways provide flexibility for evolutionary changes and are an important mechanism to prevent undesirable mutations causing plant death.

Based on both present data and the previous findings that PERK4 localized to the plasma membrane and is bound

to the pectin by the pectinase digestion of the cell wall,[1] we present a simplified model describing the ABA signal transduction pathway in root morphological changes associated with *PERK4* (**Fig. 3**). This model provides new insights into root development plasticity mediated by ABA. In the presence of ABA, *PERK4* as a positive regulator of ABA signaling is activated, which lead to activation of Ca^{2+} channel and stimulation expression of genes related with cell growth. Calcium functions as a downstream messenger that is essential for ABA responses inhibiting root cell elongation. And through a positive feedback loop, Ca^{2+} regulated the kinase activity of PERK4. Finally, the wild-type root tip growth was inhibited. PERK4 protein-mediated event would thereby represent a kind of ABA perceiving step, and PERK4 imposes a positive role toward the ABA response.

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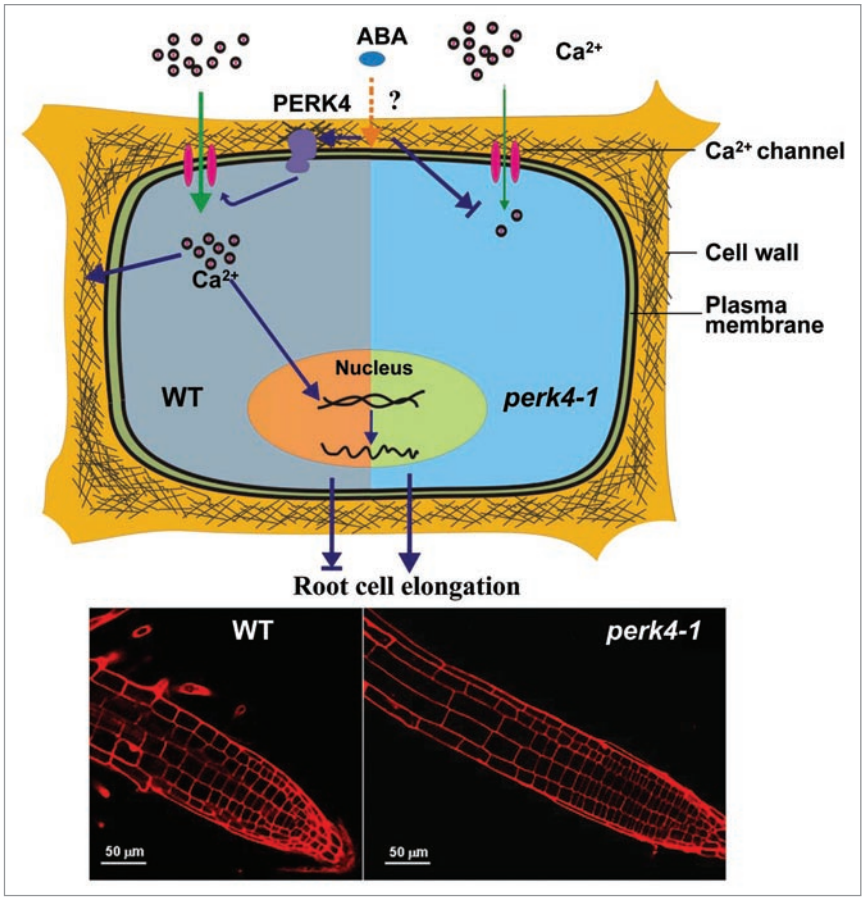

Figure 3. A simplified model showing ABA-regulated root cell elongation through *PERK4* in the wild-type (WT) and *perk4-1* seedlings. Arrows indicate positive interactions; line with a bar at the end denotes negative effect.

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