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Short communication

Ca^{2+} is involved in phytochrome A-dependent regulation of the succinate dehydrogenase gene *sdh1-2* in Arabidopsis

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ABSTRACT

The mechanism of transduction of the phytochrome signal regulating the expression of succinate dehydrogenase in Arabidopsis has been investigated. Using the phytochrome mutants of Arabidopsis, it is demonstrated that the inhibition of succinate dehydrogenase in the light may result from the phytochrome A-dependent modulation of Ca^{2+} amount in the nuclear fraction of leaves. This leads to the activation of expression of the gene *pif3* encoding the phytochrome-interacting factor PIF3, which binds to the promoter of the gene *sdh1-2* encoding the SDHA subunit of succinate dehydrogenase and suppresses its expression. It is concluded that Ca^{2+} ions are involved in the phytochrome A-mediated inhibition of succinate dehydrogenase activity in the light.

Introduction

Plants possess different photoreceptors perceiving visible light, the most important of which are phytochromes (Quail, 1991). There are five phytochromes in Arabidopsis encoded by different genes and participating in various light-dependent processes from plant development to metabolism (Furuya, 1993). Understanding the mechanism of signal transduction events from light activation of the phytochrome molecule to different cellular responses is an important goal in plant biology (Deng, 1994). Several pathways of signal transduction exist in plant cells through which phytochromes can act at the level of gene expression (Bowler et al., 1994). They include phytochrome-dependent changes in the concentration of cAMP, of Ca^{2+} levels, and the joint action of cAMP and Ca^{2+} (Horn et al., 1983). The effect of Ca^{2+} in regulation of transcription involves its subcellular redistribution between the cytosol and nucleus (Galon et al., 2010). This redistribution of Ca^{2+} can lead to activation or suppression of activity of Ca^{2+}-dependent proteins such as protein kinases and phosphatases; it can also suppress or enhance the binding of transcriptional factors and thus regulate expression of target genes (Galon et al., 2010). The effect of Ca^{2+} as a messenger for the regulation of expression of genes is shown, in particular, for proteins induced under stress conditions when Ca^{2+} level becomes elevated (Nie et al., 2006).

The phytochrome-interacting factors (PIFs) are located in the nucleus (Lorrain et al., 2006) and function as transcription factors regulating positively or negatively the expression of several genes (Oh et al., 2009). PIF contains in its structure the specific G-site (CACGTG) responsible for binding with the elements of G-box sites of promoters of target genes (Martínez-García et al., 2000; Shin et al., 2007; Hornitschek et al., 2009) controlling the level of their transcription and thus transferring the phytochrome signal in the nucleus.

Besides the nuclear compartment, the significant changes in response to irradiation by red light are observed in the cytosol, e.g. it has been reported that the cytoplasm fluidity and ion currents in it are affected (Brownlee and Kendrick, 1979; Takagi et al., 2003). After irradiation by red light, the accumulation of the active form of phytochrome A takes place in different parts of the cytosol and a slow increase of the phytochrome B is observed in the perinuclear space (Yamaguchi et al., 1999).

In this connection, understanding of the mechanisms of transduction and realization of the phytochrome signal in the nuclear compartment appears an important and actual task. One of the key reactions of the tricarboxylic acid cycle catalyzed by succinate dehydrogenase (SDH) can be a point of interconnection of energetic and biosynthetic processes in the cell. SDH (EC 1.3.99.1) is located in the inner mitochondrial membrane and its operation is linked

Abbreviations: EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid; PIF, phytochrome-interacting factor; SDH, succinate dehydrogenase.

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both to the tricarboxylic acid cycle and electron transport chain where it functions as a succinate: ubiquinone reductase (complex II; EC 1.3.5.1). Previously we demonstrated that light directly influences the expression of SDH in photosynthetic tissues and that phytochrome A is involved in this process (Popov et al., 2010), which can be considered as an important mechanism for regulation of the mitochondrial respiration in the light.

The goal of this study was to clarify the mechanisms of transduction of the phytochrome signal regulating the expression of SDH. The preliminary data have shown that Ca^{2+} is involved in the expression of SDH in maize (Eprintsev et al., 2012). The current study aims to reveal the mechanism of Ca^{2+} involvement in phytochrome signal transduction during regulation of expression of SDH in Arabidopsis. We have shown that the observed inhibition of SDH in the light results from modulation of Ca^{2+} level in the nucleus. This can cause the activation of expression of the gene *pif3* encoding the phytochrome-interacting factor PIF3 which binds to the promoter of the gene *sdh1-2* encoding the SDHA subunit of SDH and suppresses its expression.

Materials and methods

Plants of *Arabidopsis thaliana* L. were grown hydroponically at 20–22 °C and 12 h light–12 h dark cycle with light intensity 300 μmol quanta m^{-2} s^{-1}, 24 days old plants were used. In the experiments with white light illumination, all plants were harvested between 11 and 12 h of the period of illumination provided by luminescent lamps. The irradiation of plants by red and far-red light was performed after 24 h of darkness using light diodes of 640–680 nm KIPD40M40-K-P6 and 710–750 nm 3L127A5 (Proton, Russia) correspondingly. In these conditions, the light intensity was 0.2 μmol quanta m^{-2} s^{-1} and the samples were taken after three hours of illumination. The experiments were carried out using three lines of *A. thaliana* plants: the background wild type (WT CoL-0), the mutant of the phytochrome A gene (PhyA-201), and the mutant of the phytochrome B gene (PhyB-1), all obtained from the Max-Planck-Institute of Molecular Plant Physiology (Golm, Germany).

Isolation of the nuclear fraction from Arabidopsis leaves was performed according to Lee and Lin (2005). Ca^{2+} was measured spectrophotometrically by the difference between absorption at 540 and 506 nm (isobestic point) in 50 mM MOPS buffer, pH 7.5, containing 10% glycerol and 100 μM murexide (modified from Scarpa, 1972). Since the permeability of murexide through membranes is limited, the pellet containing nuclei was lysed by resuspending it in distilled water, followed by freezing at −20 °C and defrosting. Contamination of the nuclear fraction by the cytosol was tested by determination of activities of the cytosolic enzymes alcohol dehydrogenase and lactate dehydrogenase and the contamination did not exceed 6%. Alcohol dehydrogenase was measured by reduction of 0.5 mM NAD^+ at 340 nm in the presence of 150 mM ethanol in 50 mM HEPES buffer, pH 8.0 (Pathuri et al., 2011). Lactate dehydrogenase was determined at 340 nm with 0.15 mM NADH and 1 mM sodium pyruvate in 100 mM Tris–HCl (pH 7.3) buffer (Yang et al., 2010).

The inhibitors of calcium transport and metabolism ruthenium red (ammoniated ruthenium oxychloride, 25 μM) and EGTA (5 mM) were supplied by incubating upper parts of plants (roots detached) in their water solution for 1 h at the end of dark period. EGTA chelates Ca^{2+} ions and thus suppresses calcium uptake by the cells, while ruthenium red modulates intracellular calcium by interacting with intracellular ion channels or Ca^{2+}-ATPases (Trewavas and Knight, 1994). The total RNA from Arabidopsis leaves was extracted by guanidine isothiocyanate method using LiCl as a precipitating agent (Chomczynski and Sacchi, 1987). The analysis and visualization of RNA after electrophoresis in 1% agarose gel was performed by staining with 0.1% ethidium bromide in ethanol. Real-time PCR was conducted on the RT-PCR machine Chromo 4 (MJ Research, Bio-Rad, USA), using SYBR Green I. The cDNA was obtained from 100 ng of total cellular RNA. The fluorescence of the elongation factor 1 (EF-α1) gene product during RT-PCR was used to normalize the mRNA content in the samples (Nicot et al., 2005). For studying the rate of expression of *pif3* the following primers were used: direct – 5′-GGTTACTATCTGCCACCGGCG-3′; reverse – 5′-TGCTAACAAATAAACAATACATC-3′. The expression of *sdh1-2* was determined as described earlier (Popov et al., 2010). The PCR cycle, after the initial denaturation at 95 °C for 5 min, consisted of 20 s denaturation at 95 °C, 20 s of primer annealing at 57 °C (for *sdh1-2*) and 60 °C for *pif3*, 15 s of elongation at 72 °C, 15 s for detection at 72 °C. The total RNA without the stage of reverse transcription was used as a negative control. Determination of the relative level of expression of the studied genes was performed by the $2^{-\Delta\Delta C_T}$-method (Livak and Schmittgen, 2001) using the Opticon Monitor™ Software (BioRad, USA).

The experiments were repeated four times and each sample was analyzed three times. The statistical significance of the differences between the values was evaluated by Student's *t* test. The values significantly different at $P < 0.05$ are discussed in the paper, the error bars represent standard deviations.

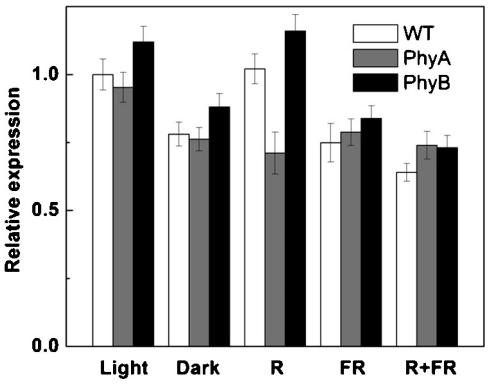

Fig. 1. Relative level of transcription of the gene *pif3* in the wild type (WT), phytochrome A mutant (PhyA) and phytochrome B mutant (PhyB) of Arabidopsis. R – red light; FR – far-red light. The averages (four biological replicates, three technical replicates) ±SD are presented.

Results

Expression of pif3 in Arabidopsis plants with mutated phytochrome A and B genes

To define the role of each component of the phytochrome system in regulation of expression of the target genes encoding SDH, we have studied the level of transcription of *pif3* gene in Arabidopsis plants with the knocked-out genes *phya* and *phyb*. As shown in Fig. 1, the highest level of *pif3* expression in the wild type and PhyB plants is observed under white light and red light irradiation. The irradiation of wild type and PhyB plants by far-red light applied either after darkness or after red light resulted in the decrease of transcription level of *pif3* by 25–30% while the PhyA plants do not exhibit the increase of *pif3* expression after irradiation by red light.

This suggests that the *pif3* gene is a signal transduction component of the pathway initiated by activation of phytochrome A. Earlier data presented in Fig. 2 of Popov et al. (2010) show that the succinate dehydrogenase genes *sdh1-2* and *sdh2-3* are also regulated by the active form of phytochrome A.