Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Physiology and Biochemistry 43 (2005) 947–954
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Original article

The *Arabidopsis* inositol 1,3,4-trisphosphate 5/6 kinase, AtItpk-1, is involved in plant photomorphogenesis under red light conditions, possibly via interaction with COP9 signalosome

Zhi-Xiang Qin [a], Qi-Jun Chen [a], Zhe Tong [b], Xue-Chen Wang [a,*]

[a] *National Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100094, China*
[b] *Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*

Received 13 April 2005; received in revised form 10 June 2005; accepted 12 August 2005

Available online 28 September 2005

Abstract

The COP9 signalosome (CSN) plays important roles in multifaceted cellular processes. Study has shown that inositol 1,3,4-trisphosphate 5/6 kinase (5/6 kinase) interacts with CSN in mammalian cells. However, the biological function of the interaction still remains unknown. Here, we report that the *Arabidopsis* inositol 1,3,4-trisphosphate 5/6 kinase (AtItpk-1) is also associated with CSN and involved in photomorphogenesis under red light (RL) conditions, as demonstrated by co-immunoprecipitation of AtItpk-1 with CSN and characterization of the *atitpk-1* mutants. Expression analysis showed that AtItpk-1 had the same sub-cellular localization and organ expression pattern as CSN. Furthermore, autophosphorylation analysis showed that AtItpk-1 has protein kinase activity. Under RL, the *atitpk-1* mutants exhibited phenotype slightly similar with that of the *csn* mutants, indicating that 5/6 kinase might be involved in the same developmental pathway as CSN. This study suggests that AtItpk-1 may function as a protein kinase that is involved in photomorphogenesis possibly via interaction with COP9 signalosome under red light.

Keywords: Arabidopsis; COP9 signalosome; Inositol 1,3,4-trisphosphate 5/6 kinase; Protein kinase; Red light

1. Introduction

The COP9 signalosome (CSN) was first identified in plants through its role in photomorphogenesis [26,28,29]. Mutation of CSN produced a light-induced phenotype when the mutants were grown in the dark [7,26,27]. Biochemical studies in both plant and animal systems have demonstrated that CSN is a highly conserved nuclear protein complex with eight distinct subunits, designated as CSN1 to CSN8, respectively [3,6]. As research results accumulated, there is increasing evidence suggesting that CSN is similar to the lid sub-complex of the 26S proteasome [12,17,23], and participates in multifaceted cellular processes, ranging from cell cycle progression, signal transduction, transcriptional regulation to the process of protein degradation [10,16,18]. Studies have shown that some protein kinases, which are called CSN-associated kinases, were involved in these cellular processes via interaction with CSN [2,21].

The first identified CSN-associated kinase in mammalian cells was inositol 1,3,4-trisphosphate 5/6 kinase (5/6 kinase) [31]. This enzyme interacts with CSN by the association with CSN1, the largest subunit in the CSN [22]. The 5/6 kinase played a key regulatory role at the branch point for the synthesis of $InsP_4$ isomers, $InsP_5$ and $InsP_6$ in $InsP_6$ biosynthetic pathway [22]. Moreover, 5/6 kinase is conserved from plants to humans and is found even in *Entamoeba histolytica* [8]. In human cells, the 5/6 kinase exhibits pleiotropic activity of inositol kinase; it can not only phosphorylate ins(1,3,4)P_3, but also can function as an ins(3,4,5,6)P_4 1 kinase [32]. In *E. histolytica*, the 5/6 kinase has a novel 3 kinase activity [8]. In calf brains and human cells, the 5/6 kinase exhibits activity

Abbreviations: AtItpk-1, *Arabidopsis* inositol 1,3,4-trisphosphate 5/6 kinase; CaMV, *Cauliflower mosaic virus*; co-IP, co-immunoprecipitation; CSN, the COP9 signalosome; 5/6 kinase, inositol 1,3,4-trisphosphate 5/6 kinase; RL, red light; WL, white light.

* Corresponding author. Tel.: +86 10 6273 2706; fax: +86 10 6273 3450.
E-mail address: xcwang@cau.edu.cn (X.-C. Wang).

0981-9428/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.plaphy.2005.08.006

of protein kinase and can phosphorylate the transcription factor c-Jun, ATF-2 [31] and p53 [22]. Furthermore, the 5/6 kinase is also reported to have the activity of autophosphorylation [22]. Studies have shown that CSN-dependent phosphorylation by protein kinases including 5/6 kinase was essential for the regulation of the ubiquitin-dependent degradation of transcription factors [21], implying that the 5/6 kinase may be also involved in the ubiquitin-dependent signaling pathway.

In 1997, Wilson et al. [30] cloned the 5/6 kinase gene from *Arabidopsis* and expressed the recombinant fusion protein in *E. coli*. The 5/6 kinase gene was later designated as Arabidopsis inositol 1,3,4-trisphosphate 5/6 kinase (*AtItpk-1*) (GenBank accession no. **JC5401**) by Shi et al. [19]. The amino acid sequence of AtItpk-1 has 30% identity to the human homologue and has the activity of inositol kinase [30]. Protein–protein blast (blastp) search showed that there are two other 5/6 kinase-like genes in the *Arabidopsis* genome, both of which were designated as *AtItpk-2* (**T10544**) and *AtItpk-3* (**NP_195103**), respectively [19]. However, so far, it is still not clear whether the plant 5/6 kinase can function as a CSN-associated kinase and what the physiological role of 5/6 kinase is in plants. Here, we report that the *Arabidopsis* 5/6 kinase is also associated with CSN as a protein kinase and involved in photomorphogenesis possibly by association with CSN under red light (RL).

2. Results

2.1. AtItpk-1 can be co-immunoprecipitated with CSN

In mammalian cells, 5/6 kinase is reported to associate with CSN [22]. Whether or not this association also exists in plants remains unknown. It is well-established that CSN is a key regulator in plant photomorphogenesis. Therefore, it is important to test the association of 5/6 kinase with CSN in plants. To address this, the anti-AtItpk-1 polyclonal antibody was raised and the specificity of the purified antibody was examined by Western blot (Fig. 1A). Then co-immunoprecipitation (co-IP) of 5/6 kinase and CSN was performed using the specific anti-AtItpk-1, anti-CSN4 and anti-CSN5 antibodies. The *Arabidopsis* plants, named as *fus6/CSN1-3-4*, contained the Flag-CSN1 full-length transgene in fus6 –/– background [24,25]. The highly specific anti-Flag monoclonal antibody was used to pull down the CSN1 subunit containing a Flag tag in immunoprecipitation. The compositions of the precipitated proteins were analyzed by Western blot experiments. The results with the anti-CSN4 (Fig. 1C, lane 2) and the anti-CSN5 antibodies (Fig. 1D, lane 2) showed that CSN4 and CSN5 subunits were in the precipitation, which indicated that the CSN with Flag-CSN1 fusion protein were successfully pulled down by anti-Flag antibody. The result with anti-AtItpk-1 antibody (Fig. 1E, lane 2) showed that AtItpk-1 was also in the precipitation, which suggested that AtItpk-1 associates with the CSN.

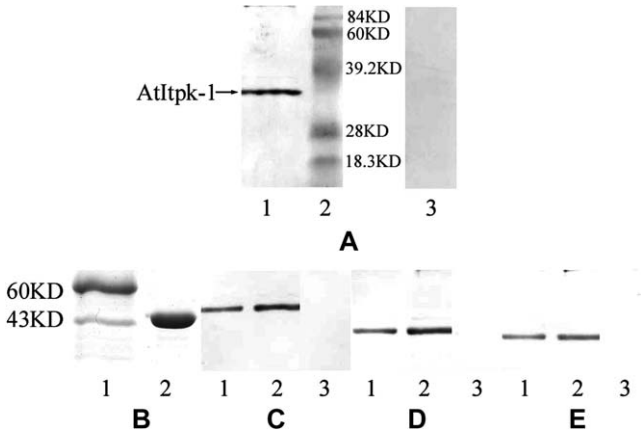

Fig. 1. Co-IP of AtItpk-1 with CSN.
A, Western blot detection of AtItpk-1 protein using the purified anti-AtItpk-1 antibody (titer was 1:1000): A1, total protein of wild-type seedlings (approximately 36.2 kDa); A2, prestained protein marker (PIERCE); A3, Detection using the negative serum. The position of AtItpk-1 protein is indicated by arrow. B, Proteins on 12% SDS-PAGE stained with Coomassie Blue R250: B1, protein marker; B2, positive control of IP (a FLAG-BAP fusion protein, with a molecular weight of 49.3 kDa and migrated as a 45–55 kDa band on SDS-PAGE). C, Detection of CSN protein using anti-CSN4 antibody (1:1500). In co-IP, the highly specific anti-Flag monoclonal antibody was used to precipitate the CSN1 subunit containing a Flag tag. Then the precipitate was parted three aliquots and the interest composition was detected by anti-CSN4, anti-CSN5 and anti-AtItpk-1 antibodies. C1, total protein of *fus6/CSN1-3-4* plants; C2, 1/3 of the precipitate in IP (approximately 46 kDa); C3, negative control (without anti-CSN4 antibody). D, Detection of CSN protein using anti-CSN5 antibody (1:2000). D1, total protein of *fus6/CSN1-3-4* plants; D2, 1/3 of the precipitate in IP (approximately 39.6 kDa); D3, negative control (without anti-CSN5 antibody). E, Detection of AtItpk-1 protein using anti-AtItpk-1 antibody (1:1000). E1, total protein of *fus6/CSN1-3-4*; E2, 1/3 of the precipitate in IP (approximately 36.2 kDa); E3, negative control (without anti-AtItpk-1 antibody).

2.2. AtItpk-1 is localized in the nucleus and is ubiquitous in all organs tested

Since AtItpk-1 can associate with CSN and CSN is a nuclear protein complex [4], it is reasonable to speculate that the AtItpk-1 is also a nuclear protein. To test this hypothesis, a green fluorescent protein (GFP) reporter was fused to the C-terminal of AtItpk-1, and the resulting cassette was introduced into wild-type *Arabidopsis*. Then the sub-cellular localization of AtItpk-1-GFP fusion protein in the guard cells of *Arabidopsis* transgenic plants was detected by confocal laser microscopy. The results showed that AtItpk-1-GFP fusion protein was found mostly in the nucleus of guard cell (Fig. 2B), compared with the control in which the unique GFP protein was distributed in cytoplasm, but not in the nucleus and other organelles (Fig. 2A). This finding confirmed that AtItpk-1 is indeed a nuclear protein.

It has been reported that CSN was found to be ubiquitous in all organs of *Arabidopsis* plants [20,26]. To test whether AtItpk-1 is also expressed in a similar pattern, the accumulation of AtItpk-1 in different organs of both adult plants and seedlings was examined by Western blot analysis. As shown in Fig. 2C, immunoblot analysis of protein samples extracted from a variety of wild-type plants (WT) organs showed that