Tobacco ZFT1, a transcriptional repressor with a Cys\textsubscript{2}/His\textsubscript{2} type zinc finger motif that functions in spermine-signaling pathway*

Yukiko Uehara\textsuperscript{1,7,†}, Yoshihiro Takahashi\textsuperscript{1,8,†}, Thomas Berberich\textsuperscript{1,2,8}, Atsushi Miyazaki\textsuperscript{1}, Hideki Takahashi\textsuperscript{3}, Kyoko Matsui\textsuperscript{4,5}, Masaru Ohme-Takagi\textsuperscript{4,5}, Hiromasa Saitoh\textsuperscript{6}, Ryohei Terauchi\textsuperscript{6} and Tomonobu Kusano\textsuperscript{1,*}

\textsuperscript{1}Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, 980-8577, Aoba, Sendai, Miyagi, Japan (*author for correspondence; e-mail kusano@ige.tohoku.ac.jp); \textsuperscript{2}Botanisches Institut, Goethe-Universität, Postfach 11 19 32, D-60054, Frankfurt am Main, Germany; \textsuperscript{3}Graduate School of Agricultural Sciences, Tohoku University, 1-1 Tsutsumidori, 981-8555, Amamiyamachi, Aoba, Sendai, Miyagi, Japan; \textsuperscript{4}Gene Function Research Center, National Institute of Advanced Industrial Technology and Science (AIST), Central 4, 305-8562, Tsukuba, Japan; \textsuperscript{5}CREST, Japan Science and Technology Agency, Japan; \textsuperscript{6}Iwate Biotechnology Research Center, 22-174-4 Narita, 024-0003, Kitakami, Iwate, Japan; \textsuperscript{7}RIKEN Plant Science Center, 1-7-22 Suehiro-cho, 230-0045, Tsurumi-ku, Yokohama, Kanagawa, Japan; \textsuperscript{8}Iwate Biotechnology Research Center, 22-174-4 Narita, 024-0003, Kitakami, Iwate, Japan. \textsuperscript{†}The first two authors contributed equally to this work

Received 29 March 2005; accepted in revised form 27 June 2005

Key words: signal transduction, spermine, tobacco, Tobacco mosaic virus, transcription factor, zinc finger protein

Abstract

We previously proposed that a spermine (Spm)-mediated signal transduction pathway is involved in the hypersensitive response induced by Tobacco mosaic virus (TMV) in tobacco plants. To identify regulatory component(s) of this pathway, we surveyed a tobacco cDNA library and found that the ZFT1 gene, which encodes a Cys\textsubscript{2}/His\textsubscript{2} type zinc-finger protein, is Spm-responsive. ZFT1 was not induced by two other polyamines, putrescine and spermidine, or by salicylic acid (SA), jasmonic acid or ethylene. Furthermore, ZFT1 was upregulated in TMV- inoculated tobacco plants in an N gene-dependent manner. Notably, induction of ZFT1 by Spm and by TMV infection was unimpaired in NahG-transgenic tobacco plants, indicating that cross-talk with an SA signaling pathway is not involved in this response. Within the Spm-signaling pathway, we found that ZFT1 functioned downstream of both mitochondrial dysfunction and mitogen-activated protein kinase activation. The ZFT1 protein has two zinc finger motifs and shows a high degree of similarity to ZPT2-3 in petunia and SCOF1 in soybean. However, unlike the latter two proteins, ZFT1 binds to the EP1S sequence and functions as a transcription repressor. Moreover, interestingly, ZFT1 overexpression rendered tobacco plants more tolerant to TMV. Based on the results presented here, we propose that ZFT1 functions as a transcription repressor in a Spm signaling pathway, thereby accelerating necrotic local region formation in tobacco leaves.

Introduction

In higher plants, polyamines (PAs), particularly putrescine (Put), spermidine (Spd) and spermine (Spm), are implicated in cell growth, cell division...
and development (Kumar et al., 1997; Malmberg et al., 1998). In addition, these basic, low molecular weight compounds are purported to have adaptive/defensive roles in response to (a)biotic stresses (Bouchereau et al., 1999; Walters 2003a). Of the many research reports concerning the role of PAs in plant pathogenesis, we paid special attention to one in which Yamakawa et al. (1998) identified Spm as an endogenous inducer of pathogenesis-related (PR) proteins during the Tobacco mosaic virus (TMV)-induced hypersensitive response (HR). The same authors showed that exogenously applied Spm induced the expression of PR proteins and enhanced TMV tolerance in host plants and that two tobacco peroxidase genes are responsive to exogenously applied Spm (Hiraga et al., 2000a, b). Based on that information, we hypothesized that PAs may function as signaling molecules in certain processes and possibly mediate some of the defense signals to pathogen attacks. Initially, we assessed whether PAs modulate phosphorylation/depophorylation events, and found that of the PAs tested, Spm specifically activated two mitogen-activated protein kinases (MAPKs), i.e. salicyclic acid (SA)-induced protein kinase (SIPK, Zhang and Klessig, 1997) and wound-induced protein kinase (WIPK, Seo et al., 1995), with this effect being mediated through mitochondrial dysfunction (Takahashi et al., 2003). Previously, we identified the HIN1 (harpin-induced 1) gene and a subset of HR marker genes, including HSR203J (Pontier et al., 1994), to be Spm-responsive (Takahashi et al., 2004a, b). On the basis of biochemical and molecular biological data, we proposed an Spm-signaling pathway in tobacco whereby in the HR provoked by TMV infection, the synthesis of PAs is enhanced and the end product, Spm, accumulates in the apoplastic space. Due to its cellular toxicity, Spm might be immediately catabolized by amine oxidase and/or polyamine oxidase, with the resultant hydrogen peroxide serving as a downstream signal transducer. In agreement with this hypothesis, Walters (2003b) proposed the involvement of Spm in signaling the HR to pathogen infection, and pointed out the importance of PA catabolism in such processes.

The Cys$_{2}$/His$_{2}$-type zinc finger protein family, also called the TFIIIA-type finger protein family, is one of the best characterized groups of eukaryotic transcription factors (TFs). Plant proteins of this family have one to four finger motif(s) within each molecule (Takatsuji, 1999). Within this family, two-fingered proteins constitute the major class, with examples including the ZPT2 subfamily in petunia, WZF1 in wheat (Sakamoto et al., 1993; Sugano et al., 2003; van der Kroel et al., 1999), soybean SCOF-1, which is involved in cold tolerance (Kim et al., 2001), Arabidopsis STZ/ZAT10, which plays a role in salt tolerance (Lippuner et al., 1996) and drought tolerance (Sakamoto et al., 2004), and petunia ZPT2-3, overexpression of which confers drought tolerance (Sugano et al., 2003). Almost all two-fingered members so far reported have been implicated in the regulation of gene activity during various abiotic stresses and during the development of vegetative and floral organs (Takatsuji, 1999). Takatsuji and his colleagues have extensively investigated DNA interactions of the plant zinc finger proteins of this class (Takatsuji et al., 1994; Takatsuji and Matsumoto, 1996; Kubo et al., 1998; Yoshioka et al., 2001). They observed that the Cys$_{2}$/His$_{2}$-type zinc-finger proteins contain the conserved QALGGH sequence in their zinc finger regions, and that those residues are crucial for DNA binding (Kubo et al., 1998).

Here, we report the identification of a TF, ZFT1, which is involved in the Spm signaling pathway in tobacco plants. This novel tobacco gene, which encodes a zinc finger protein (ZFT1) with two Cys$_{2}$/His$_{2}$-type motifs, is responsive to Spm and is induced during the TMV-triggered HR. Importantly, within the Spm signaling pathway, ZFT1 is positioned downstream of a mitochondrial malfunction event and SIPK/WIPK activation. Moreover, transgenic tobacco plants overexpressing ZFT1 showed enhanced tolerance to TMV infection. On the basis of this combined data, we discuss a role for ZFT1 in Spm-mediated signaling in tobacco.

Materials and methods

Plant materials and treatment

Tobacco (Nicotiana tabacum L. cv. Xanthi nc and NahG transgenic) plants were incubated in a growth chamber at 25 °C with a 14 h light/10 h dark photocycle. NahG transgenic tobacco seeds were provided by Syngenta Co. Six- to eight-week-old plants were used for all experiments. For