Plant Science 277 (2018) 11–19

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Technical Perspectives

Molecular and functional characterization of *Arabidopsis thaliana VPNB1* gene involved in plant vascular development

Varvara Podia[a], Dimitra Milioni[b], Efthimia Katsareli[a], Chryssanthi Valassakis[a], Andreas Roussis[a], Kosmas Haralampidis[a,*]

[a] National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, 15784 Athens, Greece
[b] Agricultural University of Athens, Department of Agricultural Biotechnology, Iera Odos 75, 11855 Athens, Greece

ARTICLE INFO

Keywords:
Arabidopsis
Armadillo domain
Cambium
Nuclear bodies
T-DNA mutants
Vascular system

ABSTRACT

Armadillo (ARM) repeat containing proteins constitute a large family in plants and are involved in diverse cellular functions, like signal transduction, proliferation and differentiation. In animals, ARM repeat proteins have been implicated in cancer development. In this study, we aimed in characterizing the *VPNB1* gene from *Arabidopsis thaliana* and its role in plant development, by implementing a number of genetic and molecular approaches. *AtVPNB1* encodes for an ARM repeat protein of unknown function, exclusively expressed in the cambium as well as in the differentiating xylem and phloem cells of the vascular system. Subcellular localization experiments showed that VPNB is confined in nucleoplasmic speckle-like structures unrelated to cajal bodies. Transgenic *VPNB*-impaired plants exhibit a slower growing phenotype and a non-canonical pattern of xylem tissue. On the contrary, *VPNB* overexpression lines display an inverted phenotype of increased growth, accompanied by an increased deposition of phloem and xylem cell layers. In line with the above data, qPCR analysis revealed a deregulation of several key master genes of secondary wall biosynthesis, underlining the involvement of VPNB1 in the regulation and differentiation of the root and shoot vascular tissue.

1. Introduction

Plants and animals manifest their spatiotemporal organization through complex networks of regulatory interactions, mediated by a wide range of protein interaction domains present in the protein sequences [1]. These domains may be assembled by multiple repeats of the same motif to form a larger binding surface, enabling considerable sequence divergence and interaction with multiple binding partners, to form functionally diverse protein complexes. These scaffolding proteins play a crucial role in intracellular molecular interactions that regulate different cellular processes [2].

One such motif is the Armadillo (ARM) repeat, approximately 42 amino acids long. Each ARM repeat has three ahelices, while multiple tandem ARM repeats assemble in a superhelical structure to form an ARM repeat domain [3]. The ARM domain was originally identified in the Drosophila armadillo protein (bcatenin), which constitutes a component of Wingless/Wnt signal transduction pathway [4]. Its mammalian homolog was found to play dual role in structural and transcriptional regulation during embryonic development [5]. Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular and colorectal carcinoma, lung cancer, malignant breast tumors, as well as ovarian and endometrial cancer [6–8]. Furthermore, alterations in the localization and expression levels of beta-catenin have been associated with various forms of heart disease, including dilated cardiomyopathy [9]. However, ARM domaincontaining proteins are found in the proteomes of all eukaryotic organisms, including plants [10–13]. The existence of ARM domain family of proteins across species suggests its ancient evolutionary origin and a functional conservation of these proteins in all organisms, including bacteria and archaea [14,15]. It is suggested that ARM proteins participate in creating scaffolds with other proteins and thus, enabling the assembly of functional complexes that mediate a number of different cellular processes including disease resistance, signal transduction, cytoskeletal regulation, nuclear import, transcriptional regulation, ubiquitination and cell differentiation [16–22].

Due to their functional involvement in diverse and significant processes, considerable effort has been given in characterizing ARM proteins also from plant species, including Arabidopsis [14,18,19].

* Corresponding author at: National and Kapodistrian University of Athens, Biology Department, Division of Botany, Molecular Plant Development Laboratory, 15701 Athens, Greece.
E-mail addresses: vapodia@hotmail.com (V. Podia), dmilioni@aua.gr (D. Milioni), ekatsa@hotmail.com (E. Katsareli), xrysva@gmail.com (C. Valassakis), aroussis@biol.uoa.gr (A. Roussis), kharalamp@biol.uoa.gr (K. Haralampidis).

https://doi.org/10.1016/j.plantsci.2018.09.006
Received 23 July 2018; Received in revised form 31 August 2018; Accepted 5 September 2018
Available online 11 September 2018
0168-9452/

Indicative of their functional diversity, the Arabidopsis genome contains about 124 ARM repeat domain proteins. Nevertheless, only few of them have associated biological functions to date. The most frequent domain arrangement observed is the Ubox/ARM (PUB-ARM) combination, suggesting a role of these proteins as components of ubiquitin-proteasome system [16,23]. The ARM-BTB (Broad-Complex, Tramtrack and Bric a brac) protein ABAP1 (ARMADILLO BTB ARABIDOPSIS PROTEIN 1) and AIP1 (ACTIN INTERACTING PROTEIN 1) have been shown to interact with pre-replication complex (pre-RC) subunits, regulating DNA replication, transcription and chromatin remodeling [24,25], while the UPL3/KAKTUS (HECT-containing UBIQUITIN-PROTEIN LIGASES 3/KAKTUS) protein is involved in ubiquitination of one or more activators that regulate branching and endoreplication of Arabidopsis trichomes [26,27]. Ngo et al. [28] have also characterized the *ZIX* (*ZAK IXIK*) genes, which encodes for an ARM repeat protein that promotes Arabidopsis early embryo and endosperm development. The authors show a reproductive function of the ZIX protein in promoting early seed growth, which is achieved through a distinct gametophytic maternal effect. The ARM repeat proteins MOS6 (MODIFIER *OF* SNC1, 6) encodes for a putative nuclear-import-receptor subunit homologous to human nucleoporin 96, and has been shown to play an important role in plant innate immunity [29]. Furthermore, some ARM repeat proteins have also been implicated in abscisic acid (ABA) signaling [12,30,31], and the regulation of plant secondary metabolism comprising abiotic and biotic stress stimuli [32–35].

However, reports of solely ARM repeat domain proteins, associated purely with the plant vascular system are to the best of our knowledge not available. Here we report on the characterization of the *VPNB1* gene from Arabidopsis, which encodes for a low molecular weight ARM repeat protein with no other protein domains. *VPNB1* is expressed exclusively in the vascular system, while the protein is localized in nucleoplasmic speckles unrelated to cajal bodies. *VPNB*-impaired plants exhibit a delayed germination and slower growing phenotype, while the overexpression lines display an inverted phenotype of increased growth. Q-PCR analysis revealed a deregulation of several genes involved in the transcriptional network of secondary cell wall biosynthesis.

2. Materials and methods

2.1. Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh. (ecotype Col-0) plants were used in this study. The *AtVPNB1* T-DNA transgenic line, SALK_121622.21.70.x, was obtained from ABRC Arabidopsis Stock Center. Seeds from individual T1, T2 and T3 generation lines were imbibed at 4 °C for 24 h, surface-sterilized and germinated on selective halfstrength Murashige and Skoog (MS) medium containing Cefotaxime (200 mg L^{-1}) and Kanamycin (50 mg L^{-1}). Transgenic plants were transferred to soil for further development. For phenotypic analysis, seeds were sown directly on soil. *A. thaliana* wild-type (WT) and transgenic plants were grown under long-day conditions, 16 h light/8 h dark, at 21 °C with 60–65% humidity and illumination of 110 µE m^{-2} s^{-1} PAR supplied by cool-white fluorescent tungsten tubes (Osram, Berlin, Germany). Growth parameters of WT and transgenic plants were scored on half-strength MS medium plates or soil-grown plants at different developmental stages.

2.2. Plant treatments and seed germination assays

Seed germination assays were carried out in petri dishes with two layers of watersaturated filter paper (Whatman) or supplemented with 0, 1, or 3 µM of ABA (SigmaAldrich). Germination on 0 µM ABA was scored in 6-h time intervals for up to 42 h, while comparative germination on 0, 1, and 3 µM ABA was scored after 48 h and the germination frequency (%) was calculated. ABA (stock solution 20 mg/ml) was diluted in methanol and the equivalent amount of methanol was included into the medium of the control plates. Plates with 1 µM of ABA contained a final percentage of 0005% methanol and plates with 3 µM ABA contained a final percentage of 0015% methanol. Each experiment was repeated three times using 100,150 seeds per genotype. Before sowing, all seeds were imbibed at 4 °C for 48 h in darkness. The statistical package SPSS was used to perform Duncan's multiple test in order to determine statistical differences between genotypes and treatments at a 5% significance level.

2.3. Computational analysis and phylogenetic tree construction

The DNA sequence of the *VPNB1* gene (*At5g14510*) was obtained from TAIR (http://www.arabidopsis.org). Protein sequences used for the phylogenetic analysis were collected from NCBI (http://www.ncbi.nlm.nih.gov/pmc/) using the BLASTp software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple protein alignments were performed using CLUSTAL Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) or TCoffee software (https://www.ebi.ac.uk/Tools/msa/tcoffee/), hosted at EMBL-EBI. Homology detection and structure prediction of At*VPNB1* was carried out by using the HHpred online analysis software (https://toolkit.tuebingen.mpg.de/#/tools/hhpred). Armadillo repeats motifs were identified using NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and InterPro (https://www.ebi.ac.uk/interpro/). For all routine bioinformatics the ExPaSy software suite was used (http://www.expasy.org/).

The phylogenetic tree was constructed by using the neighbor-joining method as implemented in the NEIGHBOR program of PHYLIP 3.65 software (University of Washington, Seatle, WA, USA). Amino acid distances were calculated by using the Dayhoff PAM matrix method of the PROTDIST program. Statistical significance was tested by bootstrap analysis for 1000 replicates and the consensus tree was visualized with TreeView 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). The protein parsimony method (PROTPARS program of PHYLIP) produced trees with essentially identical topologies.

2.4. Nucleic acid extraction, cDNA synthesis and gene expression analysis

DNA and total RNA was extracted from *A. thaliana* plant tissues with the NucleoSpin® Plant II and NucleoSpin® RNA Plant kits, respectively, according to the manufacturer's instructions (Macherey Nagel, Düren, Germany). First-strand cDNA synthesis was performed using 1 µg of DNase I-treated RNA as template and the PrimeScript Reverse Transcriptase (Takara-Clontech, Kyoto, Japan), according to the manufacturer's protocol. PCR products for cloning were amplified with Phusion® High-Fidelity DNA Polymerase (New England Biolabs, Beverly, MA, USA). For semiquantitative RT-PCR, Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) was used, according to manufacturer's instructions. As an internal control for RNA calibration, the transcripts of *GAPDH* (At3g26650) were monitored in all PCRs. All experiments were performed in triplicate. PCR products were separated by electrophoresis on 0,9% agarose gels and visualized under UV light after staining with 100 µg L^{-1} ethidium bromide. For quantitative (q) RT-PCR, the KAPA SYBR® FAST qPCR Kits (Kapa Biosystems, Woburn, MA, USA) was used, according to manufacturer's instructions. The primers used in this study are listed in Supplementary Table A1.

2.5. Construction of vectors for plant transformation

For generating the *AtVPNB1*promoter::*GUS* construct a 1352 bp fragment upstream of the ATG translational start codon, representing the promoter sequence *A. thaliana VPNB1* gene (*At5g14510*, Accession No. NC_003076.8), was amplified by PCR from genomic DNA using primer pair ARM510pdaF1 and ARM510pdaR1. Forward and reverse primers were designed with a SalI and BamHI restriction site on the 5′ and 3′ ends, respectively. The amplified fragment was digested, gel purified and cloned into the SalI/BamHI-linearized pBI101 binary