Biochem Genet (2013) 51:722–727
DOI 10.1007/s10528-013-9601-1

NOTE

Cloning and Tissue-Specific Expression of Predicted *Pisum sativum* Actin Isoform PEAc14-1

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Received: 15 April 2012 / Accepted: 21 December 2012 / Published online: 28 May 2013

Introduction

Actin is an essential component of the cytoskeleton in eukaryotes and participates in many important subcellular processes, making it critical in plant development and morphogenesis (Pei et al. 2012). Under the control of actin-binding proteins, the length, polarity, stability, and three-dimensional structure of the actin microfilament are dynamic (Henty et al. 2011; Fan et al. 2013). The actin microfilament is linked to other organelles in cells to play important roles in a variety of subcellular processes, such as cell division, cell elongation, endocytosis, cell signaling, gravity sensing, apical growth, organelle movement, and programmed cell death (Pei et al. 2012; Li et al. 2012; Smertenko and Franklin-Tong 2011). First isolated from muscle cells, actin constitutes approximately 20% of total cell protein. The existence of plant actin was proved for the first time by Yan and Shi (1963). In plants, actin plays an essential role in many processes; many genes of actin isoforms have been cloned from plants and characterized (Zhang and Liu 2006; Zhao et al. 2012). In general, plants express several isoforms of actin encoded by a family of related genes. In addition, the actin isoforms are often differentially expressed in many tissues and organs, and their functions are also different (Jiang and Zhao 2002; Díaz-Camino et al. 2005); for example, the *Arabidopsis thaliana* actin gene family contains 10 isovariants and can be divided into reproductive and vegetative classes (Pei et al. 2012). Cao et al. (1994) constructed a cDNA library of *Pisum sativum* tendrils and obtained 18 highly conservative actin genes divided into three classes: PEAcI, PEAcII, and PEAcIII. These isoforms tend to be expressed in rapidly developing organs, and their activity

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obviously decreases as tissues become older, suggesting that they play an important role in seedling morphogenesis. Besides, the expression levels of PEAcI and PEAcII are very similar, showing that they should be essential for tendril movement. The function and tissue-specific expression characteristics of PEAcIII are still unknown.

In this study, a predicted *P. sativum* actin isoform was identified from a *P. sativum* cDNA library; it may belong to class PEAcIII. Since the biological significance of this actin isoform is unclear, the tissue-specific expression pattern of the actin isoform would suggest its potential role. Therefore, semiquantitative PCRs and quantitative real-time PCRs were carried out to quantify the relative transcription levels in various tissues. In addition, the evolutionary relationships of the predicted *P. sativum* actin isoform and the known actins from *P. sativum* and *A. thaliana* are discussed.

Materials and Methods

Escherichia coli DH5α was purchased from Invitrogen Corp. Plasmid pET-30 was purchased from Novagen Inc. Plasmid pGEM-T, AMV reverse transcriptase, and HRPI were purchased from Promega Corp. The dNTPs, *Taq* polymerase, DNA fragment purification kit, and TRNzol reagent were purchased from Takara Co. Oligonucleotide primers were synthesized and purified by Sangon Co. (Shanghai, China).

Roots, stems, leaves, tendrils, flowers, seeds, and fruits of 9-week-old *P. sativum* plants were collected and stored at −80°C before use. Total RNA was isolated using the Trizol reagent. RNA qualities were checked by agarose/formaldehyde gel electrophoresis, and RNA yields were determined by measuring absorbance at 260 nm. Total RNAs from the tissues were reverse-transcribed into cDNAs.

For each reaction, 1 μg total RNA, 1 μg Oligo-d(T) 16, and 0.5 μL HRPI were first kept at 65°C for 10 min, put on ice for 2 min, and then reverse-transcribed into cDNA in a 15 μL reaction mixture including 1× AMV buffer, 15 U AMV reverse-transcriptase, and 1 mmol/L dNTPs. The mixture was incubated at 42°C for 90 min, followed by 94°C for 5 min. Using each cDNA as template, we performed semiquantitative PCR using PEAc14-specific primers 5′-GCGAATTCAT GGCAGAATCCGAAGATAT-3′ (*Eco*RI site underlined) and 5′-GCGGATCCGA AGCATTTCCTGTGTAC-3′ (*Kpn*I site underlined). As an internal control, 18S rRNA was amplified by semiquantitative PCR using primers 5′-CCAGGTCCA GACATAGTAAGGATTG-3′ and 5′-CGGTGTGTACAAAGGGCAGG-3′. For each PCR, the reaction mixture contained 1× *Taq* polymerase buffer, 2 μL cDNA product, 0.5 μmol/L each primer, 0.2 mmol/L each dNTP, and 2 U *Taq* DNA polymerase in a total volume of 50 μL. The reaction conditions were 95°C for 2 min, 35 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 2 min, and a final incubation at 72°C for 10 min. After purification using a DNA fragment purification kit, the product was digested with *Eco*RI and *Kpn*I, cloned into the corresponding restriction enzyme sites of pGEM-T, and transformed into *E. coli* DH5α. The resulting recombinant plasmid was extracted and used to perform DNA sequencing.

Quantitative real-time PCR was performed following the protocol of the Perfect Real-Time PCR kit (Takara) on the Applied Biosystems 7500 Real-Time PCR

system. Aliquots of the RT reaction products were used as templates for quantitative real-time PCR. PEAc14-1 gene-specific primers 5′-GAGGACCAGCTCATCCG TG-3′ and 5′-GACATGGTTGTTCCCTCTGAAAGG-3′ were used for amplification. Amplification products were visualized using SYBR Green. For relative quantification, the 18S rRNA gene was detected as an internal reference, and the $2^{-\Delta\Delta C}{}_{T}$ method (Livak and Schmittgen 2001) was used.

The sequence of PEAc14-1 cDNA was compared with other actin sequences deposited in GenBank using the online Blast tool of the National Center for Biotechnology Information (NCBI). DNAman 6.0 software (Lynnon Biosoft, USA) was used to identify the open reading frame (ORF), deduce the amino acid sequence, perform multiple sequence alignment, and build a phylogenetic tree. The amino acid sequences of PEAc14-1 and human F-actin (with 89% identity) were aligned for secondary structure analysis. The program ProtScale (http://web.expasy.org/protscale) was used to predict hydrophobic regions. The program Sosui (http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html) was used to predict protein solubility. BlastP (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare and predict the protein conserved domains. SignalP (http://www.cbs.dtu.dk/services/SignalP) was used to predict the protein signal peptide. TargetP 1.1 (http://www.cbs.dtu.dk/services/TargetP) was used for subcellular localization. The PSIPred Protein Structure Prediction Server (http://bioinf.cs.ucl.ac.uk/psipred/submit) was used to analyze protein transmembrane topological structure.

Results and Discussion

An ORF was identified from a *P. sativum* cDNA library. The cDNA sequence is 1,134 bp in length, encoding a polypeptide of 377 amino acids. In a previous study, *P. sativum* actin proteins were subjected to restriction endonuclease analysis and sequencing and divided into three classes, PEAcI, PEAcII, and PEAcIII (Hu and Yan 1999). Among the known *P. sativum* actin isoforms, PEAc1 and PEAc17 belong to PEAcI; PEAc3, PEAc9, and PEAc12 belong to PEAcII; and PEAc14 belongs to PEAcIII. All the actin isoforms belonging to PEAcI and PEAcII have 377 amino acids. PEAc14, however, has two more sequences than the other *P. sativum* actin isoforms, one of six amino acid residues inserted at site 111 and another of three residues at site 212 (Jiang and Zhao 2002). The initial purpose of the PCR in this work was to amplify PEAc14 with primers specific to PEAc14. As a result, the cDNA obtained is identical to the PEAc14 sequence, except that the two inserts are absent. The PCR and sequencing were repeated to get the same results. Therefore, we speculate that PEAc14 and the cloned cDNA sequence may originate from the same DNA, but the mRNA splicing process during transcription may be different. Therefore, the sequence was predicted to encode a new *P. sativum* actin isoform, and it was named PEAc14-1. PEAc14-1 was compared with protein sequences from the GenBank database using the Blast tool, and all the known proteins having more than 80% identity to PEAc14-1 were found to be actins. In addition, all the proteins having more than 95% identity to PEAc14-1 are plant-originated actins (data not shown). Multiple sequence alignments revealed that the PEAc14-1 sequence has the

highest identity (98%) to PEAc14, along with 82–98% identity to the actin protein homologs from *P. sativum*. In addition, restriction endonuclease analysis of DNA sequences shows that both PEAc14-1 and PEAc14 belong to the PEAcIII family. The cDNA sequence of PEAc14-1 was registered in GenBank (accession no. HQ231775.1). Another phylogenetic tree was constructed based on the amino acid sequence comparisons of PEAc14-1 and eight highly expressed actins from *A. thaliana*. The selected actins from *A. thaliana* can be divided into two reproductive and vegetative classes according to their tissue-specific expression patterns. PEAc14-1 is classified in the reproductive group and is most closely related to ACT11, which is a member of the reproductive class.

The G+C content of the ORF is 46%. The predicted molecular weight of the deduced protein is 41.8 kDa, and the isoelectric point is 5.23. SignalP was used online to predict the protein signal peptide; no obvious signal peptide sequence was detected, so the PEAc14-1 may exist as a mature protein. The analysis with the TargetP 1.1 server also proved that the protein may not have a signal peptide. In addition, no mitochondrial targeting peptide was detected, and therefore the PEAc14-1 may exist outside the mitochondrion. Our analysis of the protein transmembrane topological structure (using PSIPred Protein Structure Prediction Server) detected only one transmembrane domain, aa 144–159 (Fig. 1), with the

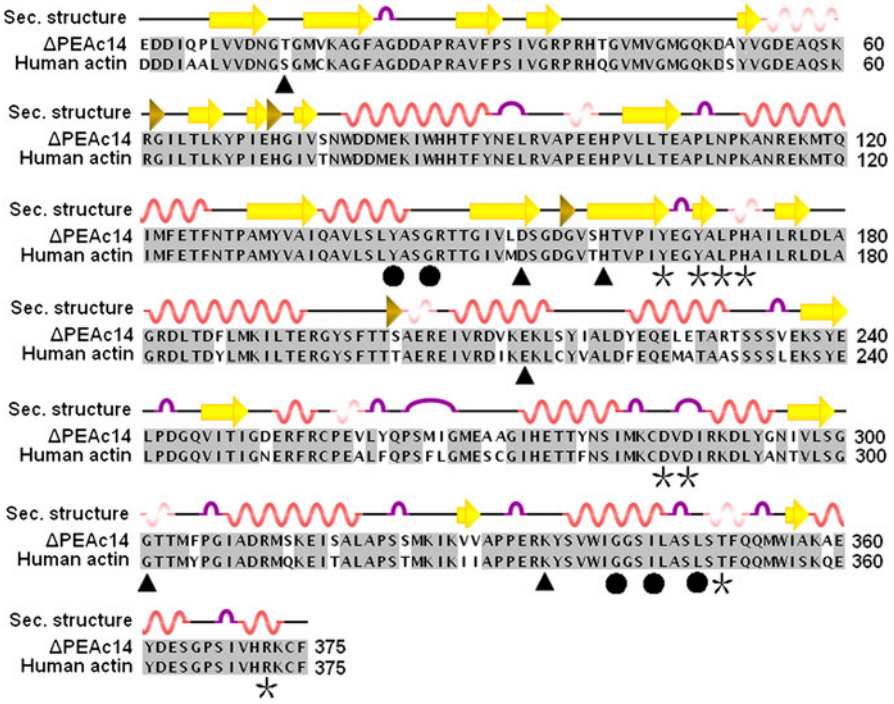

Fig. 1 Sequence alignment of PEAc14-1 obtained in this work and human F-actin based on secondary structure and sequence homology. Identical residues in all aligned sequences are shaded *dark gray*. *Black triangle* ATP binding site. *Asterisk* (*) profilin binding site. *Black circle* gelsolin binding site

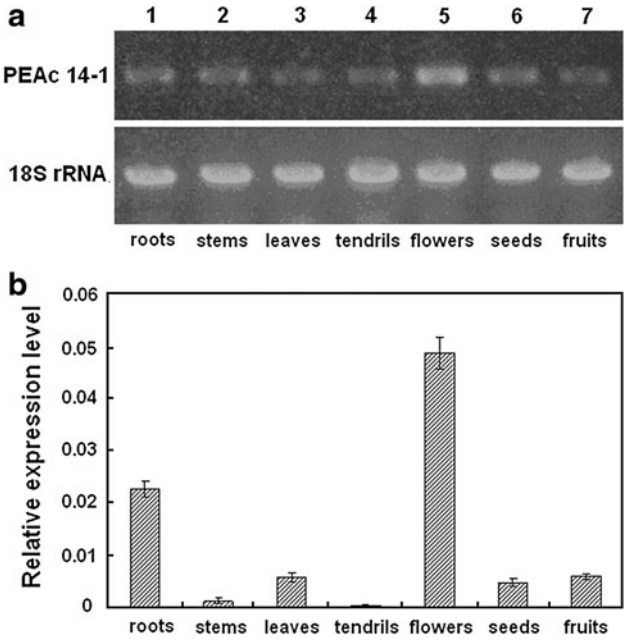

Fig. 2 Expression patterns of the PEAc14-1 gene. Electrophoretic results **a** of semiquantitative PCR in seven tissues, using a gene-specific primer pair for the *P. sativum* PEAc14 gene. Quantitative real-time PCR **b** using a gene-specific primer pair to analyze expression levels for the *P. sativum* PEAc14 gene in seven tissues. The 18S rRNA gene was used as an internal reference

C-terminal in cytoplasm and the N-terminal in extracellular space. The amino acid sequences of human F-actin and PEAc14-1 (with 89% identity) were aligned for secondary structure analysis. The conservative regions, including ATP binding sites, profilin binding sites, and gelsolin binding sites, are all conserved except for a substitution of T14S (Fig. 1). It is well known that both serine and threonine are nonpolar amino acids and have similar properties. These results further suggest that PEAc14-1 may belong to the actin family.

Semiquantitative RT-PCRs and quantitative real-time PCRs were used to detect and quantify PEAc14-1 gene expression levels in various tissues, including roots, stems, leaves, tendrils, flowers, seeds, and fruits (Fig. 2). The results showed that the PEAc14-1 gene was present in all the tissues tested, indicating that it should be a constitutive gene. Its product plays important roles in various tissues. The highest mRNA level was found in flowers (at least twice the level in other tissues), suggesting that the gene should be tissue-specific and may play an important role during the formation of flowers. This is consistent with a previous study showing that actin isoforms cannot substitute for each other and that the highly conserved actins are functionally specialized for the tissues in which they predominate (Khaitlina 2001).

In summary, an ORF encoding a predicted *P. sativum* actin isoform was identified from a *P. sativum* cDNA library and its function was predicted by

bioinformatics tools. In addition, the tissue-specific expression of the predicted actin isoform was analyzed, revealing that the highest mRNA level is in flowers and that the gene may play an important role during the formation of flowers. Functional verification and time-specific expression should be further studied to facilitate the understanding of its exact role in the cell.

Acknowledgments This work was supported by the Open Research Foundation of China State Key Laboratory of Plant Physiology and Biochemistry (No. PPB08003) and the National Natural Science Foundations of China (No. 31100045 and No. 31270114).

References

Cao X, Wang R, Yan L, Lu Z, Pan N, Chen Z (1994) Construction of a pea tendril cDNA library and sequence analysis of a pea actin cDNA, PEAc1. Chin Sci Bull 39:332–337

Díaz-Camino C, Conde R, Ovsenek N, Villanueva MA (2005) Actin expression is induced and three isoforms are differentially expressed during germination in *Zea mays*. J Exp Bot 56:557–565

Fan T, Zhai H, Shi W, Wang J, Jia H, Xiang Y, An L (2013) Overexpression of profilin 3 affects cell elongation and F-actin organization in *Arabidopsis thaliana*. Plant Cell Rep 32(1):149–160

Henty JL, Bledsoe SW, Khurana P, Meagher RB, Day B, Blanchoin L, Staiger CJ (2011) *Arabidopsis* actin depolymerizing factor 4 modulates the stochastic dynamic behavior of actin filaments in the cortical array of epidermal cells. Plant Cell 23:3711–3726

Hu S, Yan L (1999) Sequence analysis of pea tendril actin cDNA, PEAcII. Chin J Biochem Mol Biol 15:857–860

Jiang Y, Zhao W (2002) Expression and phylogenetic analysis of pea actin isoforms. Acta Bot Sin 44:1456–1461

Khaitlina SY (2001) Functional specificity of actin isoforms. Int Rev Cytol 202:35–98

Li J, Henty-Ridilla JL, Huang S, Wang X, Blanchoin L, Staiger CJ (2012) Capping protein modulates the dynamic behavior of actin filaments in response to phosphatidic acid in *Arabidopsis*. Plant Cell 24(9):3742–3754

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_T$ method. Methods 25:402–408

Pei WK, Du F, Zhang Y, He T, Ren HY (2012) Control of the actin cytoskeleton in root hair development. Plant Sci 187:10–18

Smertenko A, Franklin-Tong VE (2011) Organisation and regulation of the cytoskeleton in plant programmed cell death. Cell Death Differ 18:1263–1270

Yan L, Shi D (1963) The presence of a contractile protein in higher plants. Acta Biochim Biophys Sin 3:90–96

Zhang S, Liu G (2006) Research advances in plant actin isoforms. Chin Bull Bot 23:242–248

Zhao D, Tao J, Han C, Ge J (2012) An actin gene as the internal control for gene expression analysis in herbaceous peony (*Paeonia lactiflora* Pall.). Afr J Agric Res 7:2153–2159