Binding specificity and tissue-specific expression pattern of the Arabdidopsis bZIP transcription factor TGA2

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SHORT COMMUNICATION

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Binding specificity and tissue-specific expression pattern of the *Arabidopsis* bZIP transcription factor TGA2

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Abstract The binding specificity and tissue-specific expression pattern of TGA2 (AHBP-1b), an *Arabidopsis* bZIP transcription factor have been determined. Filter-binding and gel-shift assays showed that TGA2 has high affinity for C-boxes (ATGACGTCAT). In this respect TGA2 is similar to other members of the *Arabidopsis* TGA family (such as TGA1, TGA3 and OBF4) and to tobacco TGA1a. Genomic Southern blot analysis confirmed that *TGA2* is a member of a gene family. Northern blot analysis showed that the gene is expressed at similar levels in root, stem, leaf and flower and at somewhat lower levels in siliques. *TGA3* was also found to be expressed at the same level throughout the plant, whereas genes encoding TGA1 and OBF4 have relatively high RNA expression levels in root. The differential expression of these genes suggests that they have distinct functions.

Key words *Arabidopsis thaliana* · bZIP domain C-box binding protein · TGA family · Transcription factor

Transcriptional regulation of gene expression is mainly mediated through sequence-specific transcription factors that bind to cis-elements in the promoter region. In the pea lectin (*psl*) promoter, we have previously characterized a 22-bp promoter element (W1), that confers seed-specific expression (de Pater et al. 1993). In vitro binding studies with cloned proteins have shown that this element contains a binding site for bZIP proteins (de Pater et al. 1994). In a search for proteins that bind to the W1 sequence, we screened an *Arabidopsis thaliana* (ecotype Columbia, C24) cDNA expression library, made from flowers after fertilization and 0 to 4-day-old siliques, with a trimer of this promoter element (3W1). The sequences of positive clones were compared with those in the databases. One clone was identical to *TGA2* (*AHBP-1b*), isolated by Kawata et al. (1992), except that it was 3 bp longer at the 5′ end and 5 bp at the 3′ end. *TGA2* is a member of the *Arabidopsis TGA* gene family (Miao et al. 1994; Schindler et al. 1992; Zhang et al. 1993).

The sequence specificity of this clone was tested in filter binding assays, in which sections of nitrocellulose filters containing cDNA-encoded protein were incubated with different probes (Fig. 1). The protein interacted with both the monomer and trimer of W1. Binding was also observed with W2, consisting of a tetramer of the 12 bp bZIP binding site present in the W1 element (de Pater et al. 1994). This binding site can be classified as an odd-base C-box (ATGAGTCAT). The binding specificity was further characterized using synthetic C-box and G-box sequences [for definition of (odd-base) C- and G-boxes see (Izawa et al. 1993; de Pater et al. 1994)]. The affinity for the C-box was relatively high. A control cDNA clone encoding a non-specific DNA binding protein from tobacco (BAD; Binds All DNA) showed binding to all probes.

Gel shift assays with crude extracts from *Escherichia coli* expressing TGA2 confirmed and extended the results of the filter binding assays (Fig. 2A). TGA2 bound with high affinity to the synthetic C-box and to a tetramer of the *as-1* site (4A1), which is derived from the cauliflower mosaic virus (CaMV) 35S promoter, where it is located between positions −82 to −62. Gel shift assays with extracts of *E. coli* cells not containing the *TGA2* cDNA insert did not show any DNA-protein complexes with the probes used (results not shown).

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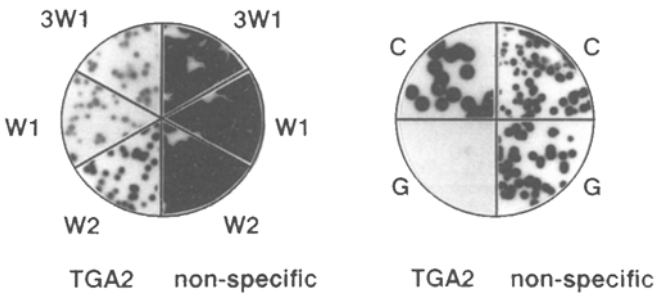

Fig. 1 Filter binding assay with TGA2. Filters containing *Arabidopsis* TGA2 protein (left half of circles) or the non-specific DNA binding protein from tobacco (BAD) (right half of circles) were incubated with the following probes: W1 (tcgagGACACGTAGAATGAGTCATCACgtcga), 3W1, W2 (4 × ATGAGTCATCAC), G-box (agcttaGACACGTGTCactcga) and C-box (agcttaATGACGTCATactcga). Lambda phages were grown on *E. coli* Y1090 until plaques were just visible. Nitrocellulose filters saturated with 10 mM IPTG were placed on the plates and phages were allowed to grow for 10 h. Filters were blocked for 1 h in binding buffer (20 mM HEPES (pH = 7.2), 40 mM KCl, 1 mM EDTA, 0.5 mM DTT, 10% glycerol) supplemented with 5% non-fat dry milk at room temperature. After washing twice in binding buffer, the filters were incubated in binding buffer containing 2 ng/ml probe and 5 μg/ml sonicated calf thymus DNA for 1 h. Probes were end-labeled using the Klenow fragment of DNA polymerase I and [α-^{32}P]dNTPs. Finally, the filters were quickly washed three times and dried before autoradiography

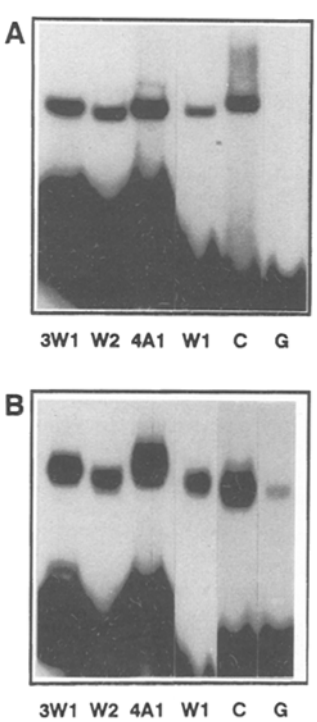

Fig. 2A, B Gel shift assays with (**A**) *Arabidopsis* TGA2 and (**B**) tobacco TGA1a. 3W1, W2, 4A1 (for explanation see text), W1, C-box and G-box were used as probes. Crude TGA2 extract was obtained by in vivo excision of the corresponding pBluescript derivative from the TGA2-containing λZAP clone. A logarithmically growing culture was induced with 0.4 mM IPTG and grown for another 3 h. Bacteria were pelleted, washed, resuspended in 400 μl of binding buffer and sonicated three times for 10 s. Insoluble material was removed by centrifugation. Protein concentration of the extract was 6 mg/ml. Binding reactions were done as described (de Pater et al. 1993) using 5 fmol probe and 0.6 μg protein

The binding specificity of TGA2 was very similar to that of tobacco TGA1a (Katagiri et al. 1989) (Fig. 2B), and of other members of the *Arabidopsis* TGA family, TGA1 (Schindler et al. 1992), TGA3 (Miao et al. 1994) and OBF4 (Zhang et al. 1993).

The mRNAs detected in Northern blot analysis using *Arabidopsis TGA1* (Schindler et al. 1992), *TGA3* (Miao et al. 1994) and tobacco *TGA1a* (Katagiri et al. 1989) cDNAs are more abundant in roots than in other tissues, and the encoded proteins are thought to be involved in root-specific expression. We tested whether *TGA2* had a similar tissue-specific expression pattern. We first performed a Southern blot analysis to determine the number of genes that were detected under our experimental conditions. Figure 3 shows one major band in each lane, indicating that mainly *TGA2* homologous sequences were detected. However, faint additional bands were visible (not well reproduced in Fig. 3) that represent other members of the *TGA* family. Cross-hybridization under more stringent washing conditions was tested with Southern blots containing the cDNA inserts of *TGA1*, *TGA2*, *TGA3* and *OBF4*. These stringent washing conditions showed no cross-hybridization (results not shown) and were used for Northern analysis.

Hybridization with poly(A)$^+$ RNA from root, stem, leaf, flower and siliques (Fig. 4A) showed that TGA2 is expressed at similar levels in all tissues. Compared to leaves the expression in roots was about two-fold higher and in siliques about three-fold lower. The hybridizing mRNA is about 1800 nucleotides long. Therefore the cDNA clones isolated by Kawata et al. (1993) and by us are (close to) full length.

Blots containing poly(A)$^+$ RNA from root, stem, leaf and flower were probed with *TGA1*, *TGA3* and *OBF4*. *TGA1* and *OBF4* are, respectively, expressed at ten-fold and five-fold higher levels in roots than in leaves

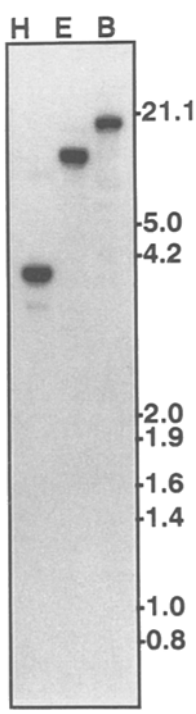

Fig. 3 Southern blot analysis of *TGA2*. One microgram of *Arabidopsis* DNA (ecotype Columbia) was digested with *Hin*dIII (H), *Eco*RI (E), or *Bam*HI (B), electrophoresed on a 0.8% agarose gel, blotted and hybridized as described (Memelink et al. 1994) with *TGA2* cDNA. Positions of *Eco*RI and *Hin*dIII-digested lambda DNA fragments are indicated

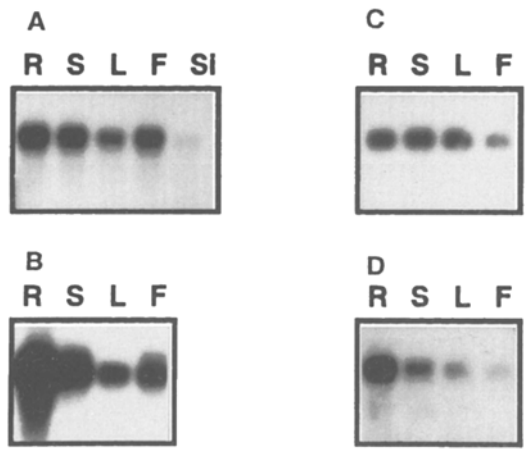

Fig. 4A–D Northern blot analysis of the *TGA* family. One hundred nanograms of poly(A)$^+$ RNA from root (R), stem (S), leaf (L), flower (F) and siliques (Si) of *Arabidopsis* (ecotype Columbia) were loaded on a 1.5% formaldehyde agarose gel, blotted and hybridized as described (Memelink et al. 1994) with *TGA2* (**A**), *TGA1* (**B**), *TGA3* (**C**) or *OBF4* (**D**) cDNA. Washing conditions were: 0.1 × SSPE, 0.5% SDS at 65° C; mRNA size was estimated using an RNA size marker (GIBCO BRL, 0.24–9.5 kb range) run in parallel (not shown)

(Fig. 4B, D), whereas *TGA3* is expressed at the same level in all tissues (Fig. 4C). These results were confirmed using total RNA (results not shown). There is a discrepancy between our results obtained with *TGA3* and the results of Miao et al. (1994), which might be due to less stringent washing conditions used by these authors (1994) resulting in cross-detection of *TGA1* and/or *OBF4*. The different expression patterns of *TGA1*, *TGA2*, *TGA3* and *OBF4* suggest that the different members of the TGA family have distinct functions.

Based on our results, it is unlikely that TGA2 is involved in seed-specific expression mediated by W1. Firstly, the in vitro affinity of TGA2 for W1 is lower than for the synthetic C-box and the *as-1* sequence, which does not confer seed-specific expression (Lam and Chua 1990). Secondly, expression of the *TGA2* gene is relatively low in siliques (Fig. 4). The silique RNA was isolated from a stage of seed maturity at which W1 is active (unpublished results).

Besides ruling out TGA2 as the factor that confers seed-specific expression mediated by W1, the results described in this paper contribute to the characterization of the members of the *Arabidopsis* TGA family and to the elucidation of their roles in gene regulation.

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