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Plant Gene Register

Isolation of Multiple cDNAs Encoding the Vacuolar H^+-ATPase Subunit B from Developing Cotton (*Gossypium hirsutum* L.) Ovules[1]

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V-ATPases are multimeric enzyme complexes that reside in the endomembrane system of eukaryotes. These enzymes are responsible for generating proton-chemical gradients across the membrane, thereby facilitating the maintenance of proper organellar pH and secondary transport of ion and metabolites into subcellular compartments (Taiz, 1992). In plants, cell expansion is driven by turgor pressure generated by the influx of osmoregulatory solutes into the vacuole. This influx of solutes is mediated in part by the V-ATPases (Rea and Sanders, 1987; Sze et al., 1992). Cotton (*Gossypium hirsutum* L.) fiber cells are specialized single-celled seed trichomes that undergo rapid turgor-driven cell expansion to attain a final length-to-diameter ratio of 3000 to 4000 within 15 to 20 d postanthesis. Histochemical localization of significant ATPase activity on the tonoplast of developing cotton trichomes (Joshi et al., 1988) suggests that V-ATPases may be instrumental in providing energy for the active transport of osmoregulatory solutes into the vacuole during cotton fiber elongation (Wilkins, 1993).

V-ATPases are composed of 8 to 10 different subunits, which are assembled into a peripheral sector (V_1) located on the cytosolic face of the membrane and an integral membrane sector (V_0). ATP hydrolysis is catalyzed in the V_1 sector by the 69-kD nucleotide-binding catalytic subunit (A subunit), whereas the other nucleotide-binding, B subunit is noncatalytic and is believed to perform regulatory functions. Although the mechanism is not clear, phosphorylation of the B subunit may play a regulatory role in V-ATPase activity (Martiny-Baron et al., 1992). Clones encoding the V-ATPase B subunit isolated from yeast, plants, and mammals show a high degree of conservation at the amino acid level (Gill and Ross, 1991). However, the variation in molecular mass of V-ATPase subunit B polypeptides observed among species is attributable to differences in the length and composition of the N- and C-terminal regions of the protein. Although the presence of multiple V-ATPase B subunit isoforms are reported in bovine and barley (Puopolo et al., 1992; Berkelman et al., 1994). V-ATPase B subunit isoforms have been shown

Table I. *Characteristics of a cDNA clone encoding the V-ATPase subunit B from cotton ovules*

Organism:
Cotton (*Gossypium hirsutum* L. cv Acala SJ-2).
Gene Product:
CVA55; vacuolar H^+-ATPase subunit B.
Cloning Techniques:
A λgt10 cDNA library constructed from immature ovules was screened with an *Arabidopsis* V-ATPase 57-kD cDNA (Manolson et al., 1988) as a hybridization probe. The cDNA was subcloned into pUC118 and both strands of the cDNA were sequenced by the dideoxy-chain termination method from single-stranded DNA using T4 polymerase-derived nested deletions.
Method of Identification:
Comparison of the nucleotide and the deduced amino acid sequences with the *Arabidopsis* V-ATPase 57-kD subunit (Manolson et al., 1988).
Structural Features of the cDNA Sequence:
Cotton V-ATPase subunit B cDNA consists of 2047 bp upstream of the poly(A)$^+$ tail, which includes a 140-bp 5′ leader sequence followed by 1464 bp of open reading frame and a 443-bp 3′ untranslated region. Cotton and *Arabidopsis* cDNAs share 81.4% nucleotide identity within the coding region.
Codon Usage:
Preference for codons with T in the third position.
(G + C) Content:
45.2% (G + C) content in protein-coding region.
Gene Copy Number:
Southern blot analysis of genomic DNA indicated that the V-ATPase subunit B is organized as a multigene family consisting of at least three genes.
Structural Features of the Predicted Amino Acid Sequence:
The 1464-bp open reading frame encodes a polypeptide of 488 amino acids with a calculated M_r of 54,205 and an isoelectric point of 4.75. There are 15 potential phosphorylation sites dispersed in the polypeptide. Amino acids 368 to 377 (PPINVLPSLS) were identified as ATP synthase α and β subunit signatures by computer analysis. The deduced amino acid sequence exhibits 95.5% identify and 1.8% similarity to the *Arabidopsis* polypeptide.
Expression Characteristics:
Single mRNA of 2.1 kb in developing ovules, petals, and embryos.
Antibody:
Not available.

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Abbreviations: RT-PCR, reverse transcriptase-polymerase chain reaction; V-ATPase, vacuolar H^+-ATPase.

to be expressed in a tissue-specific manner only in bovids (Puopolo et al., 1992).

A full-length cotton cDNA (CVA55.29) encoding the B subunit of the V-ATPase was isolated from an ovule λgt10 library using an *Arabidopsis* cDNA (Manolson et al., 1988) as a heterologous hybridization probe. The characteristic features of the complete cDNA clone are described in Table I. The cotton V-ATPase subunit B clone, designated CVA55.29, shares 73 to 76% nucleotide identity and 95 to 96% amino acid identity with the *Arabidopsis* (Manolson et al., 1988) and two barley (Berkelman et al., 1994) proteins. A second cotton cDNA clone of 1551 bp, designated pAT3P, encodes a 54,205-D polypeptide of 386 amino acids that shares 98.7% amino acid homology to the C terminus of the polypeptide deduced from CVA55.29. CVA55.29 and pAT3P share 82.4% nucleotide identity overall, with 93 and 55% identity in the coding and 3′ untranslated region, respectively. Another partial cotton V-ATPase subunit B cDNA clone (pAT33R) of 606 bp encoding the N-terminal portion was identified following reverse transcriptase PCR amplification of total RNA from ovules, using V-ATPase B subunit sequence-specific primers. Sequence comparison of pAT33R with CVA55.29 showed 57% nucleotide identity in the 5′ untranslated region, and the deduced amino acid sequences revealed only 60% identity within the first 10 residues at the amino terminus. Overlapping sequence information confirmed that pAT33R represents a third highly homologous gene identified in the genome of cotton (*Gossypium hirsutum* L.).

Genomic DNA blot analysis showed three hybridization fragments, which suggests that at least three genes encoding the B subunit of cotton V-ATPase are present in the cotton genome (data not shown). These results are in agreement with the multigene family organization of V-ATPase B subunits found in other plants. To date, V-ATPase B subunits are known to be encoded by at least two genes in barley (Berkelman et al., 1994) and four genes in *Arabidopsis* (D. Gunasekera and T.A. Wilkins, unpublished data).

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