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Plant Gene Register

Vacuolar H^+-ATPase 69-Kilodalton Catalytic Subunit cDNA from Developing Cotton (*Gossypium hirsutum*) Ovules[1]

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Vacuoles of higher plant cells are multifunctional organelles that serve as storage and hydrolytic compartments as well as perform key roles in regulating cell turgor and cytoplasmic homeostasis. The transport of many ions and metabolites across the tonoplast is mediated by two electrogenic proton pumps, the H^+-ATPase and the H^+-pyrophosphatase (Rea and Sanders, 1987; Hedrich et al., 1989).

The tonoplast H^+-ATPase of plant vacuoles is a member of the vacuolar, or V-type, ATPase proton pumps residing in acidic compartments of the endomembrane system of plants, animals, and fungi (Forgac, 1989; Gogarten et al., 1992). The H^+-ATPase is a large, multimeric enzyme comprising a hydrophilic V_1 complex located on the cytosolic face of the tonoplast, and a V_0 membrane-bound complex. In plants, as many as 10 different polypeptides contribute to the subunit composition of the V_1 and V_0 complexes (DuPont and Morrissey, 1992; Ward and Sze, 1992). Two nucleotide-binding subunits of 69 and 60 kD, associated with the peripheral V_1 complex, and a V_0 16-kD proteolipid have been identified as the three major subunits common to all V-type ATPases (Forgac, 1989). When the expression of a tonoplast-specific H^+-ATPase 69-kD catalytic subunit is inhibited by antisense mRNA, transgenic carrot plants exhibit altered leaf morphologies resulting from defective cell expansion (Gogarten et al., 1992).

In cotton (*Gossypium hirsutum*), the seed "fibers" so highly prized by the textile industry are specialized single-celled trichomes that differentiate from the outer epidermis of the ovule. Developing trichomes are rapidly elongating cells that can attain lengths in excess of 1 inch within 15 to 20 d postanthesis. Elongation is driven by turgor pressure generated by the influx and accumulation of potassium and malate (Basra and Malik, 1983) in the enlarging central vacuole of developing trichomes. As might be predicted, the differential accumulation of significant vacuolar H^+-ATPase activity on the tonoplast of developing cotton seed trichomes parallels the dramatic increase in the rate of cell elongation (Joshi et al., 1988), indicating the involvement of the vacuolar ATPase in the transport and compartmentalization of these osmoregulatory solutes during cotton fiber elongation.

Table I. *Characteristics of a vacuolar H^+-ATPase 69-kD catalytic subunit from cotton*

Organism:
Gossypium hirsutum L. cv Acala SJ-2 (Upland cotton).
Gene Product:
CVA69; 69-kD catalytic subunit of the vacuolar H^+-ATPase; subunit A.
Method of Isolation:
Cotton cDNA (CVA69.24) isolated from λgt10 cDNA library constructed from immature ovules using the carrot vacuolar H^+-ATPase 69-kD cDNA (Zimniak et al., 1988) as a hybridization probe.
Techniques:
Restriction fragment subcloning into pUC119. Complete dideoxy sequencing of both cDNA strands from single-stranded DNA using successive synthetic oligonucleotide primers or a series of T4 DNA polymerase-derived nested deletions.
Method of Identification:
Comparison to carrot vacuolar H^+-ATPase 69-kD subunit (Zimniak et al., 1988).
Expression Characteristics:
2.5-kb mRNA in developing ovules.
Structural Features of cDNA:
cDNA 2259 bp in length with translation start site at nucleotide 171 and stop codon (TGA) at nucleotide 2039. Cotton and carrot cDNAs share 82.2% nucleotide identity within the coding region.
Codon Usage:
All codons used with preference for codons with T in third position.
(G + C) Content:
44.9% (G + C) content in protein coding region.
Structural Features of Protein:
Open reading frame of 623 amino acids with predicted M_r of 68,522 and isoelectric point of 5.14. ATP-binding site motif (GAFGCGKTV) located between amino acid residues 252 to 259 is absolutely conserved in cotton, carrot, and yeast. Amino acids 449 to 458 (PSVNWLISYS) identified as ATP synthase α and β subunit signatures by computer analysis. Deduced amino acid sequence exhibits 95.7% identify and 2.1% similarity to the carrot polypeptide.
Antibodies:
Not prepared in this laboratory.

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As a focal point to investigating the molecular events of vacuole ontogeny in rapidly elongating plant cells, a cotton cDNA (CVA69.24) encoding the vacuolar ATPase 69-kD catalytic subunit (Table I) was isolated from an ovule λgt10 cDNA library using a carrot cDNA (Zimniak et al., 1988) as a heterologous hybridization probe. Within the coding region, the cotton and carrot cDNA clones exhibit 82.2% nucleotide sequence homology. The hydrophilic cotton polypeptide encoded by the cDNA is 623 amino acids with a predicted M_r of 68,522 and showed extensive homology to the carrot protein. At the amino acid level, cotton and carrot catalytic subunits exhibited 95.7% identity and 2.1% amino acid similarity. When aligned with the analogous sequences from yeast (Hirata et al., 1990), the cotton protein shared only 60.5% amino acid identity and 12.7% similarity to the yeast subunit.

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