Plant Physiol. (1994) 104: 287–288

Plant Gene Register

Two cDNA Clones Encoding Isoforms of the B Subunit of the Vacuolar ATPase from Barley Roots

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The vacuolar H^+-ATPase of higher plants is a member of the V-ATPase family, which comprises complex, multisubunit ATPases found in all eukaryotes. The electrochemical gradient created by the V-ATPase is thought to provide the driving force for the secondary transport of other ions and metabolites (Taiz, 1992). In barley (*Hordeum vulgare* L.) roots this enzyme may be involved in the sequestration of Na^+ and Ca^{2+} ions in the vacuole, because the proton gradient produced by the ATPase is used by Na^+/H^+ and Ca^{2+}/H^+ antiports to drive the uptake of Na^+ and of Ca^{2+} (Garbarino and DuPont, 1989; DuPont et al., 1990). The quaternary structure of the ATPase from barley roots is very similar to that from other organisms, with approximately 10 different subunits (DuPont and Morrissey, 1992).

Two of the best-characterized subunits of the V-ATPases are the A and B subunits of the large, knob-like head group on the cytoplasmic side of the vacuolar membrane. The A subunit is between 67 and 70 kD, and the B subunit is between 53 and 60 kD in various organisms. Both A and B subunits are present in a stoichiometry of three per enzyme complex and contain ATP binding sites, although the B subunit is not directly involved in ATP hydrolysis (Puopolo et al., 1992). The primary structures of the A and B subunits are highly conserved among widely divergent organisms, making them useful for evolutionary studies. Isoforms of the B subunit have been described for mammals, and the distribution of the isoforms seems to be tissue specific (Puopolo et al., 1992). It was of some interest to learn whether there are also isoforms of the B subunit in plants.

Two different cDNA clones for the B subunit of the barley V-ATPase, designated HTB1 and HTB2, were selected from a barley root cDNA library. The two clones were very similar to each other, having higher sequence identity to each other than to any other B subunit sequence in the GenBank data base (see Table I for details). Since barley is an inbred diploid organism, it is likely that HTB1 and HTB2 are encoded by different genes, rather than alleles of the same gene. The next most similar sequence was that for the *Arabidopsis*

Table I. *Characteristics of two cDNAs encoding the 54-kD subunit (B subunit) of the vacuolar ATPase from roots of Hordeum vulgare*

Organism:
Hordeum vulgare L. (barley) cv CM72.
Gene Function:
Major noncatalytic ATP-binding subunit of vacuolar proton pump.
Clone Type:
Two cDNA clones containing the entire coding sequence, designated pHTB1 and pHTB2.
Source:
cDNA library in λgt11Sfi-Not (Promega) prepared from mRNA isolated from roots of 7-d-old hydroponically grown barley plants. The clones were selected by screening at moderate stringency with a ^{32}P-labeled fragment of a cDNA encoding the *Arabidopsis thaliana* B subunit (gift from M. Manolson).
Sequencing Techniques:
Both cDNAs were subcloned into Bluescript and sequenced by the dideoxy method using exonuclease III deletion clones and custom-made oligonucleotide primers. Both strands of pHTB1 were sequenced entirely. One strand of pHTB2 was sequenced entirely and the second strand was sequenced only in regions where the sequence diverged from pHTB1.
Method of Identification:
Comparison to other B subunit sequences.
Structural Features of the cDNA Clones:
HTB1 is 1927 nucleotides in length. HTB2 is 1818 nucleotides in length. Sequence identity between the two clones is 81%, and 87% in the coding region. The 5′ sequences prior to the first in-frame start codon (83 bp in HTB1 and 60 bp in HTB2) are over 50% identical, but the 3′ untranslated regions (350 bp for HTB1 and 280 bp for HTB2) have little similarity to each other. The motif of CTCCAC is repeated three times at the beginning of the 5′ untranslated region of HTB1, and the motif CCCAA is repeated three times at the beginning of the 5′ untranslated region of HTB2.
Structural Features of the Deduced Polypeptides:
The open reading frame of HTB1 encodes a polypeptide of 488 amino acids with a predicted M_r of 54,026. The open reading frame of HTB2 encodes a polypeptide of 483 amino acids and a predicted M_r of 53,726. The deduced amino acid sequences are 98% identical. In both clones, the first in-frame ATG codons are flanked by sequences that match the reported consensus sequence for plant initiation codons (ANN ATG G) (Heidecker and Messing, 1986). Both clones, however, have other in-frame ATG codons within 120 nucleotides of the 5′ end that cannot be ruled out as possible initiation codons.

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Abbreviation: V-ATPase, vacuolar ATPase.

thaliana B subunit (Manolson et al., 1988), for which the predicted amino acid sequence is 94% identical to HTB1 and 96% identical to HTB2. Therefore, it is likely that the two barley isoforms arose by gene duplication subsequent to the divergence between monocots and dicots.

To date, there are no other reports of multiple B subunit isoforms in plants. Sequence comparison did not reveal any correspondence between either of the barley isoforms and any of the reported mammalian isoforms (Suedhof et al., 1989; Bernasconi et al., 1990; Nelson et al., 1992; Puopolo et al., 1992).

Received August 16, 1993; accepted August 26, 1993.

The GenBank accession numbers for HTB1 and HTB2 are L11862 and L11873, respectively.

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