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Sugar Regulates mRNA Abundance of H^+-ATPase Gene Family Members in Tomato[1]

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The plant plasma membrane H^+-ATPase energizes the secondary uptake of nutrients and may facilitate cell expansion by acidifying the cell wall. In yeast, Glc stimulates the accumulation of H^+-ATPase mRNA, and the growth rate supported by various sugars is correlated with H^+-ATPase protein abundance. Expression of three H^+-ATPase genes, *LHA1*, *LHA2*, and *LHA4*, was previously detected in tomato (*Lycopersicon esculentum*). We have characterized the sequence of the *LHA4* gene and examined the expression of these three tomato H^+-ATPase genes in growing tissues and in response to exogenous sugars. *LHA4* is a member of the H^+-ATPase subfamily, including the *Arabidopsis thaliana* genes *AHA1*, *AHA2*, and *AHA3*. The 5′ untranslated region of the deduced *LHA4* cDNA contains a short, open reading frame very similar to that in the *Nicotiana plumbaginifolia* gene PMA1. *LHA4* transcript abundance in seedlings is correlated with cell growth, being 2.5 times greater in hypocotyls of dark- versus light-grown plants. The accumulation of both *LHA4* and *LHA2* mRNAs is induced by the addition of exogenous sugars and this induction appears to be dependent on sugar uptake and metabolism, because mannitol and 3-*O*-methylglucose do not stimulate mRNA accumulation. These results suggest that the induction of expression of H^+-ATPase genes by metabolizable sugars may be part of a generalized cellular response to increased cell growth and metabolism promoted by the availability of an abundant carbon source.

H^+-ATPases in the PM plays a critical role in the physiology of plants at both the cellular and organismal levels. They establish an electrical potential and pH gradient across the PM, which provides the force for the secondary transport of anions, cations, amino acids, and sugars (Serrano, 1989; Sussman and Harper, 1989). These secondary transport systems control physiological processes such as nutrient uptake by roots, phloem transport, and stomatal function. H^+-ATPase in the PM may have other physiological roles, because the enzyme is subjected to control by physiological effectors such as hormones, light, and pathogens (Serrano, 1989; Sussman and Harper, 1989). Furthermore, it has been proposed to play a direct role in the control of the cell cycle by the regulation of cytosolic pH (Pichon and Desbiez, 1994) and by driving the auxin-induced cell expansion by cell-wall acidification (Rayle and Cleland, 1992).

Total H^+-ATPase levels in the PM may be directly correlated with growth. Hager et al. (1991) demonstrated by immunodetection that H^+-ATPase levels in the PM increased 2-fold during auxin-induced maize coleoptile elongation. Rao et al. (1993) showed that the growth rate of liquid cultures of yeast supported by different sugars is closely correlated with PM H^+-ATPase activity. Furthermore, the expression of the primary yeast PM H^+-ATPase isoform PMA1 was shown to be induced by the addition of growth-inducing exogenous sugars.

Considerable progress has recently been made in the understanding of the molecular biology of plant PM H^+-ATPases. Multiple genes encoding PM H^+-ATPases have been cloned in tomato (*Lycopersicon esculentum*; Ewing et al., 1990; Ewing and Bennett, 1994), *Nicotiana plumbaginifolia* (Boutry et al., 1989; Perez et al., 1992; Moriau et al., 1993), *Arabidopsis thaliana* (Harper et al., 1989, 1990, 1994; Pardo and Serrano, 1989; Houlne and Boutry, 1994), potato (Harms et al., 1994), rice (Wada et al., 1992; Ookura et al., 1994), and *Vicia faba* (L.E. Wimmers, unpublished data). Each of the genes that have been characterized are expressed differentially in the organ and cell types analyzed, and highly specific expression patterns have been reported for several isoforms. For example, mRNA analysis revealed that Arabidopsis *AHA9* is expressed primarily in anther tissue (Houlne and Boutry, 1994), and tobacco *PMA1* and *PMA2* are most highly expressed in flowers (Perez et al., 1992). Analysis of promoter-GUS fusions has localized *AHA3* expression to phloem tissue (DeWitt et al., 1991) and *PMA1* to root epidermis, stem cortex, anthers, pistils, and guard cells (Michelet et al., 1994).

Despite the progress in the molecular cloning and tissue-specific expression patterns of PM H^+-ATPase genes, relatively little attention has been paid to the regulation of the genes' expression by physiological factors. Increases in total PM H^+-ATPase mRNA levels have been reported in response to salt in *Atriplex nummularia* (Niu et al., 1993a, 1993b) and in soybean exposed to low water potential (Surowy and Boyer, 1991). However, the only report of

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Abbreviations: BAP, benzylaminopurine; H^+-ATPase, proton-translocating ATPase; MS, Murashige-Skoog; OMG, 3-*O*-methylglucose; PM, plasma membrane; URF, upstream open reading frame; UTR, untranslated region.

regulation of a specific isoform by endogenous physiological factors is the stimulation of *PMA1* expression in guard cells by hydroponic growth conditions (Michelet et al., 1994). Considering that H^+-ATPases are encoded by multigene families, it is reasonable to speculate that individual H^+-ATPase isoforms possess unique functions and that the expression of each gene may be differentially regulated by distinct physiological stimuli..

Previously, we identified seven H^+-ATPase genes in tomato, three of which, *LHA1*, *LHA2*, and *LHA4*, are expressed at detectable levels (Ewing and Bennett, 1994). The goal of this study was to further investigate the expression of H^+-ATPase isoforms in tomato and to assess the potential physiological role of specific isoforms, especially as related to cell growth and metabolic activity. In this paper, we report the characterization of the genomic clone of the fourth isoform, LHA4, and the regulation of *LHA2* and *LHA4* mRNA levels by sugars.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum*) plants were grown hydroponically in aerated 1× Hoagland solution at 25°C. Hypocotyls were collected from plants grown in the dark or in the light for 4 d. Cotyledons were collected from plants grown in the light for 5 d. Mature leaves were collected from plants grown in the light for 21 d. Auxin treatment was conducted using dark-grown hypocotyls, as described elsewhere (Mito and Bennett, 1995). MS medium and sugar treatment was conducted using fully expanded cotyledons. One-millimeter strips were made from 12 cotyledons and incubated in 50 mL of 100 mM sugar solutions or 1× MS medium with 1× Nitsch and Nitsch vitamins. The MS medium was supplemented with the hormones NAA (2 mg/mL) and BAP (0.5 mg/mL) as indicated.

Sequencing of LHA4 Genomic Clone

The genomic clone of *LHA4*, 30A, was previously isolated by our laboratory (Ewing and Bennett, 1994). The 10-kb insert of this clone was isolated by *Eco*RI digestion and subcloned into the pBS II vector (gLHA4-30). A 6-kb *Hin*dIII/*Eco*RI fragment of gLHA4-30A was digested by *Kpn*I and *Xba*I and subcloned as five pieces. Both strands of each subclone were analyzed by dideoxy sequencing (Sequenase kit, United States Biochemical) with double-stranded plasmid DNA templates and specific primers.

Anchored PCR Amplification of LHA4 5′ Sequence

Anchored amplification of the 5′ region of LHA4 was conducted using a commercial 5′ rapid amplification of cDNA ends system (BRL), according to the supplier's instructions. Briefly, 200 ng of hypocotyl poly(A) RNA was reverse-transcribed using the gene-specific primer 4-c (5′-GCAAGACCAGCCATAAGAGC-3′) and tailed with dCTP. A fraction (10%) of the first-strand-tailed cDNA was amplified using the gene-specific primer 4-d (5′-CCAGCAAGCAAACAATACCAAC-3′) and the anchor primer supplied with the system. Amplification was performed in an automated thermal cycler (Perkin-Elmer/Cetus). Samples were heated to 94°C for 5 min to denature the template and then maintained at 80°C for 5 min while DNA polymerase (Amplitaq, Perkin-Elmer/Cetus) was added. Amplification included a single cycle of 2 min of annealing at 43°C (5°C below the Tm of the G/I tract of the anchor primer), and 10 min of extension at 72°C, followed by 40 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 56°C (2°C above the Tm of the gene-specific primer), and 3 min of extension at 72°C. The single product of approximately 500 bp was subcloned into the vector PCR II (pLHA4 5′) (Vitrogen, San Diego, CA), and sequenced as described above.

PCR Amplification of Exon 1 3′ Junction

Genomic DNA was isolated from tomato leaves as described previously (Ewing et al., 1990), and 100 ng was used as template for PCR amplification. Gene-specific primers used were 4-e (5′-TGCGATCGATGAGTCTATTAGTCG-3′) and 4-f (5′-CAAAGCATCTCGTTTCTCATACTATTC-3′). Samples were amplified, subcloned, and sequenced as described above, except all annealings were performed at 50°C.

Total RNA Extraction

Frozen tissue (200 mg) was ground in 0.5 mL of buffer A (4 M guanidine thiocyanate, 1% sarcosyl, 0.1 M sodium acetate, pH 5.2, and 0.7% 2-mercaptoethanol) premixed with 0.4 mL of water-equilibrated phenol. After the sample was centrifuged at 15,000*g*, the upper phase was precipitated with 1 volume of isopropanol. The precipitated RNA was dissolved in 100 μL of diethylpyrocarbonate-treated water. After polysaccharide precipitation by 20 μL of ethanol, the RNA in the supernatant was precipitated by adding lithium chloride to 2 M on ice. The precipitate was resuspended in water and quantified spectrophotometrically. Polyadenylated mRNA was selected using an mRNA purification system (Oligotex, Qiagen, Chatsworth, CA) following the manufacturer's instructions.

Preparation of Competitor RNA

Competitive PCR for the quantification of *LeAux* and H^+-ATPase mRNA was conducted according to the method described by Siebert and Larrick (1992). Competitor RNA was prepared as follows: For *LeAux* mRNA, a genomic DNA sequence corresponding to LeAux cDNA was isolated by PCR amplification from genomic DNA. The PCR product, which is approximately 100 bp larger than cDNA because of the presence of a small intron, was subcloned into PCR II (gLeAux). Sequence analysis of about 100 bp of the 3′ and 5′ ends of gLeAux confirmed identity to the LeAux cDNA. Competitor RNA was synthesized by in vitro transcription of gLeAux using SP 6 RNA polymerase (Promega) to yield sense RNA. For *LHA1*, *LHA2*, and *LHA4* mRNA, C-terminal regions of the corresponding genomic DNA sequences were isolated by PCR amplification from corresponding genomic clones (Ewing and Bennett, 1994) using the following primers: 1-a (5′-GCTTTCACCCGGAAGAAGG-3′) and an antisense

1-b (5′-CTTACAACTTCAGGGGGTCC-3′) for LHA1, 2-a (5′-GCTTTCACCAGGAAGAAGG-3′) and an antisense 2-b (5′-CTAGTCATACTT-GCTCCCAC-3′) for LHA2, and 4-a (5′-CTTGACATAATGAAATTCGC-3′) and an antisense 4-b (5′-CAGAGCCTGCCATT-CCCTTTTC-3′) for LHA4. The products, which contained small introns, were approximately 100 bp (LHA1), 200 bp (LHA2), and 80 bp (LHA4) larger than the PCR product from total RNA. Each PCR product was subcloned into the PCR II vector and competitor RNA was synthesized by in vitro transcription of these clones to yield sense RNA.

Quantification of mRNA by Competitive PCR

One microgram of total RNA was heated to 65°C for 5 min, placed on ice, and incubated with Moloney leukemia virus reverse transcriptase (100 units, BRL) for 30 min at 42°C in a total volume of 10 μL, containing 1× first-strand buffer (50 mM Tris-HCl [pH 8.3], 37.5 mM KCl, 1.5 mM $MgCl_2$), 0.5 mM each deoxyribonucleotide triphosphate, 10 mM DTT, 10 units of RNasin (Promega), and 100 pmol of random hexamer primers (Pharmacia). Following reverse transcription the mixture was heated at 94°C for 5 min to denature reverse transcriptase and used for PCR amplification. AS1 (5′-CCAGGGAGGACAGATGA-3′) and AS2 (5′-CAAGCATCAAGTCACCA-3′) primers for LeAux, corresponding to nucleotides 21 to 39 and 418 to 436 of a previously published partial sequence (Mito and Bennett, 1995), and 1-a, 1-b, 2-a, 2-b, 4-a, and 4-b primers for H^+-ATPase amplification were used. Amplifications were carried out in a final volume of 50 μL containing PCR buffer (50 mM KCl, 10 mM Tris-HCl [pH 9], 1.5 mM $MgCl_2$ 0.01% [w/w] gelatin, 0.1% [v/v]) Triton X-100, 0.2 mM each deoxyribonucleotide triphosphate, 25 pmol of each primer, and 0.25 unit of DNA polymerase for 30 cycles with denaturation for 1 min at 94°C, annealing for 2 min at 55°C for LeAux and 58°C for H^+-ATPases and extension for 2 min at 72°C. The specificity of the competitive PCR assay was assessed by challenging, including nontarget competitor species in a series of reactions (e.g. LHA4 competitor RNA included with LHA2 PCR primers), and in these experiments the nontarget competitor was not amplified. In addition, in the case of LHA2 amplification a number of PCR products were cloned and sequenced and all corresponded to LHA2. Each competitive PCR experiment produced only two products of the size corresponding to the target and competitor RNA species and quantification of these products was by the method of Guiliano et al. (1993). Products were separated by electrophoresis on a 1.5% agarose gel containing 0.2 mg/mL ethidium and photographed (Polaroid type 55 film), and the intensity of the bands was quantified by scanning the negative on the BioImage analysis system (Millipore). All of the quantifications were conducted at a minimum of two competitor concentrations and each experiment was repeated at least twice, with similar results.

RNA Gel Blot Hybridization

Total RNA (20 μg) was size-separated by electrophoresis on a 1.2% agarose formaldehyde denaturing gel, transferred to a nylon membrane (Hybond, Amersham), and cross-linked to the membrane by baking at 80°C for 2 h. Hybridization was carried out in 50% formamide, 6× SSC, 5× Denhardt's solution, 0.5% SDS, 100 μg/mL base-denatured salmon sperm DNA, and ^{32}P-labeled probe at 37°C. The ^{32}P-labeled probe was prepared by random priming of *LeAux* cDNA.

RESULTS

Sequence Analysis

The nucleotide sequence of the tomato H^+-ATPase isoform designated LHA4 was determined (accession no. U72148). However, the genomic clone did not contain the entire sequence of exon 1. To identify the entire exon 1 sequence, hypocotyl poly(A) RNA was amplified by anchored PCR using *LHA4* gene-specific primers for reverse transcription and as the 3′ primer during amplification. Amplification yielded a single product and sequence analysis of the resulting clone confirmed its identity as *LHA4* and defined the 5′ coding sequence. The 5′ UTR was long (168 bp) and contained a short URF similar to the one reported for the tobacco *PMA1* H^+-ATPase sequence (Fig. 1, underlined). Because the genomic clone included only 6 bp of exon 1, the 3′ junction of exon 1 was confirmed by PCR amplification and sequencing. Genomic DNA was amplified using gene-specific primers corresponding to the first exon and intron. Sequence analysis of the single product confirmed the predicted splice junction.

Intron positions (Fig. 1A) were assigned to the *LHA4* genomic sequence by the identification of the consensus splice site sequence dN/GT. . . . . AG/dN and by comparison with the coding sequence of AHA2 (Harper et al., 1990). The position of each intron was exactly the same as those of *AHA2*, except introns 2, 3, 4, and 7 of *AHA2* were not found in *LHA4*. Intron 1 was exceptionally large, comprising 2710 bp (Fig. 1A).

The deduced amino acid sequence of LHA4 shares 81% identity with LHA1 and 75 to 98% identity with PM H^+-ATPases from other plants. The greatest similarity is with the potato isoform PHA2 (98%) and the *N. plumbaginifolia* isoform PMA4 (95%). Comparisons made over the complete sequences of PM H^+-ATPases have led to the suggestion that it is appropriate to differentiate at least two subfamilies within the PM H^+-ATPase gene family, with LHA1, PMA1, PMA2, and PMA3 constituting one family, and AHA1, AHA2, AHA3, and PMA4 forming the second family (Moriau et al., 1993). We have extended this analysis to include LHA4 and other recently published sequences (Fig. 2). LHA4 belongs to the second subfamily, along with PHA2.

Validation of Competitive PCR Assays

PCR-based mRNA assays have the advantage of being simple and rapid (Becker-Andre and Hahlbrock, 1989; Guiliano et al., 1993) but are difficult to optimize for quantitative results because of the exponential nature of PCR (Siebert and Larrick, 1992). Competitive PCR assays provide quantitative data comparable to RNA blot techniques

B

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catacctcacaatttctctcttcacaaagaggccttaaaaacctctcttcattccctcc   60
gccgttctcaattttgcgatcgatgagtctattagtcgttatactcactgaaatcaatcc  120
                  M  S  L  L  V  V  I  L  T  E  I  N  P
gttttctgcttaattttgtattgacttgtttgctgctggtttaccgacATGGCGAAAGCT  180
  F  S  A                                    M  A  K  A
ATAAGCCTCGAAGAGATCAAAAATGAGACTGTTGATCTGGAGAAAATCCCCATTGAAGAA  240
  I  S  L  E  E  I  K  N  E  T  V  D  L  E  K  I  P  I  E  E
GTGTTTGAACAACTGAAATGTTCGCGAGAGGGTCTGACTTCGGACGAAGGAGCCAACAGG  300
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  A  L  A  N  G  D  G  K  P  P  D  W  Q  D  F  V  G  I  V  C
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atggcaggctctgatatatctcatgttaattattaattagagaagaagagagagacaagc 3120
ttttggccttaaccacatataaacaaaaaaacattgtcttttaatataaaggggaaaacat 3180
tgtcttgtgttgtctttccccctctcatctatttttctttaacttcgttatgttatattt 3240
tgaagcaccacccattgaaagggattgtgtccatgtttttttccaaaatttttaatggtgaa 3300
gtgacatttccttgtaacaacagttttttcttacaatttcttcacctctttttttctttct 3360
tcatttgatgttgacgataacgtctctctctctctgctttttttgcacattcactgttgta 3420
tgtgaccctgctgaaaatatattactc                                    3449
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Figure 1. A, Gene structure of *LHA4*. Wide bars represent exons and thin bars represent introns. B, Nucleotide and predicted amino acid sequence of the deduced LHA4 cDNA. The URF is underlined.

(Siebert and Larrick, 1992). To confirm the effectiveness of a competitive PCR assay of RNA levels in tomato, we used the auxin-regulated *LeAux* gene as a standard with an expression pattern that had previously been studied in tomato (Mito and Bennett, 1995). Competitor RNA was synthesized from a partial genomic clone of *LeAux*, which was distinguishable from the *LeAux* mRNA because of its larger size, due to the presence of an intron. Figure 3A shows the results of a titration experiment with total RNA prepared from tomato hypocotyl with and without NAA treatment. As shown in Figure 3A, a single concentration of total RNA was co-amplified with a dilution series of a known amount of competitor RNA by reverse transcriptase-PCR. Nearly equal amounts of PCR products were produced from the total RNA of NAA-treated hypocotyl and competitor RNA at 10 pg, whereas only a small amount of PCR product was produced from the total RNA of untreated hypocotyl in the presence of 10 pg of competitor RNA. The induction of *LeAux* mRNA accumulation by NAA treatment is consistent with our previous results (Mito and Bennett, 1995).

The validity of the competitive PCR method for the quantification of plant mRNA was further confirmed by comparison of the quantification of *LeAux* mRNA accumulation by both northern hybridization and competitive PCR. Northern hybridization analysis demonstrated that *LeAux* mRNA accumulation was induced at 90 and 180 min after NAA treatment (Fig. 3C). Similar induction of *LeAux* mRNA induction was observed when LeAux mRNA was quantified by competitive PCR (Fig. 3B), indicating that this assay reports relative levels that are comparable to that detected by northern hybridization.

Expression of Tomato H^+-ATPase Isoforms

Organ-specific expression of three tomato H^+-ATPase mRNAs was studied using the competitive PCR assay (Fig.

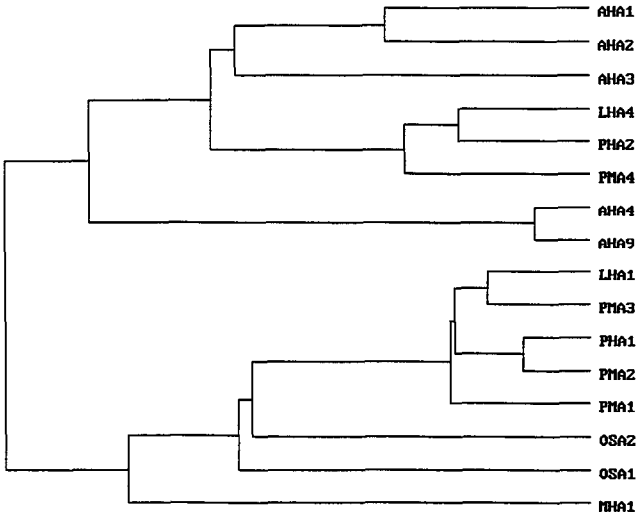

Figure 2. Predicted phylogenetic relationships among H^+-ATPases. Comparisons were made with the CLUSTAL program on PC/GENE software (Intelligenetics, Mountain View, CA). Program parameters were: k-tuple value, 1; gap penalty, 5; window size, 10; filtering level, 2.5; open gap cost, 10; and unit gap cost, 10.

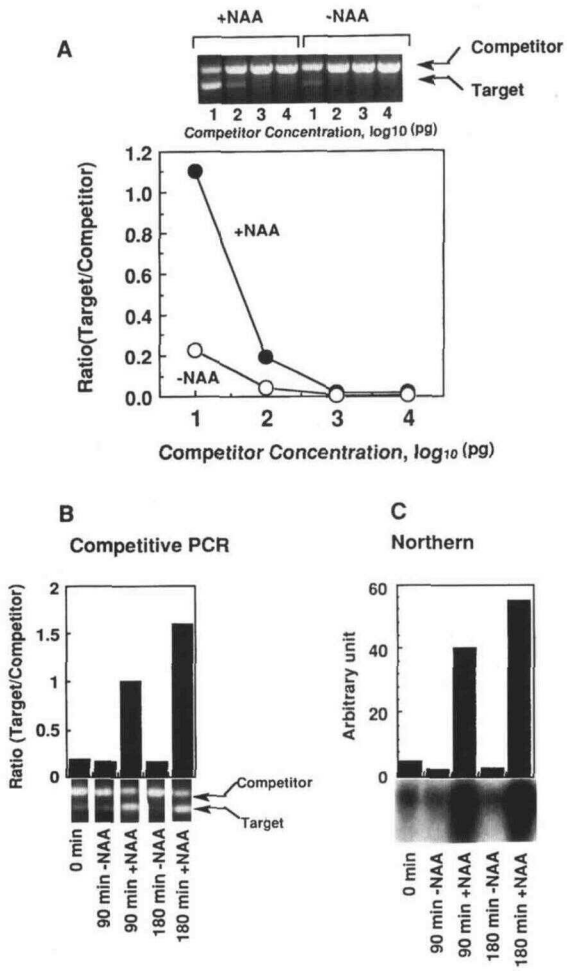

Figure 3. A, Quantification of *LeAux* mRNA level in total RNA from untreated and NAA-treated tomato hypocotyls. Samples containing 1 μg of total RNA and different amounts of competitor RNA were subjected to competitive reverse transcriptase-PCR. After 30 cycles, 12 μL of each sample was analyzed on a 1.5% agarose gel. The agarose gel was photographed and the intensity of the bands was quantified by scanning of the negatives on a BioImage analysis system. The ratio of the target to competitor band was then plotted. B and C, Changes in the LeAux mRNA levels in total RNA from untreated and NAA-treated tomato hypocotyls was quantified by competitive reverse transcriptase-PCR (B) or by northern hybridization (C).

4). Expression of *LHA1* and *LHA2* was rather constitutive, with higher mRNA levels in the hypocotyl and slightly lower expression in the root, cotyledon, and mature leaf. *LHA4* mRNA was most abundant in the hypocotyl and root, with substantially lower levels in the leaf and cotyledon. *LHA1*, *LHA2*, and *LHA4* expression patterns in various organs was consistent with our previous observations (Ewing and Bennett, 1994). The effect of light was analyzed in the hypocotyl and root. *LHA4* mRNA abundance was approximately 3-fold higher in the hypocotyls of plants grown in the dark relative to that of light-grown plants; however, no similar effect was observed in roots. No difference was observed in *LHA1* or *LHA2* message levels in dark- versus light-grown plants.

Induction of H+-ATPase mRNA Accumulation by MS Medium and Sugar

To assess the possible influence of exogenous sugars, nutrients, and hormones on PM H+-ATPase expression, *LHA1*, *LHA2*, and *LHA4* mRNA levels were determined in cotyledons incubated in MS medium alone or with additions. Relative to buffer alone, incubation of tomato cotyledons in MS medium induced the accumulation of *LHA4* and *LHA2* mRNA, whereas the abundance of *LHA1* mRNA was not influenced (Fig. 5). The inclusion of NAA (2 mg/mL) and BAP (0.5 mg/mL) in the MS medium did not significantly increase the mRNA level of *LHA1*, *LHA2*, or *LHA4*, indicating that these H+-ATPase genes are not regulated by auxin or cytokinin.

The MS medium used contained Suc, inorganic salts, and 1× Nitsch and Nitsch vitamins. To specifically address the role of sugars in regulating H+-ATPase gene expression, cotyledons were incubated in 100 mM Suc, Glc, Fru, and mannitol (Fig. 6). The metabolizable sugars Glc, Fru, and Suc induced *LHA2* and *LHA4* mRNA accumulation, whereas the relatively impermeant sugar mannitol did not. To determine whether the metabolism of sugar is necessary

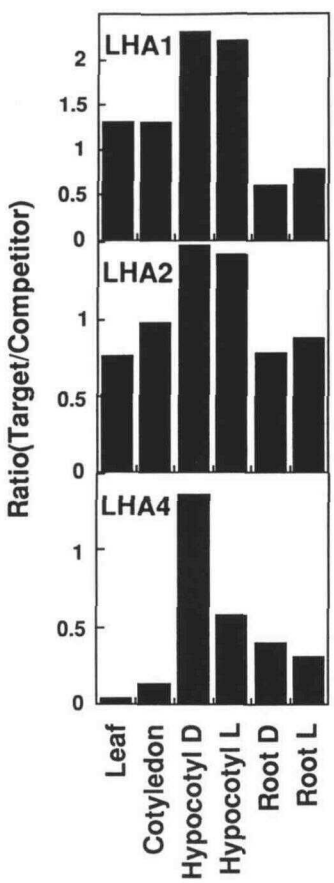

Figure 4. Organ-specific expression of *LHA1*, *LHA2*, and *LHA4*. H+-ATPase mRNA was quantified by competitive reverse transcriptase-PCR and the relative amount of mRNA was determined by the ratio of the PCR product from the competitor RNA to that from target mRNA. D, L, From seedlings grown in continuous dark/from seedlings grown in continuous light.

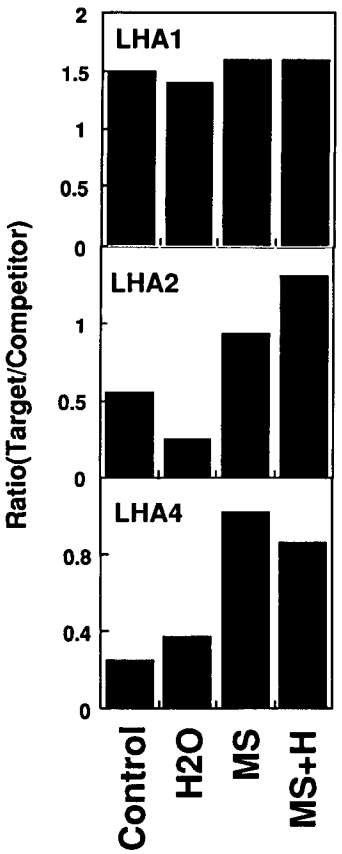

Figure 5. Induction of H^+-ATPase mRNA accumulation by incubation in MS medium was quantified by competitive reverse transcriptase-PCR. Total RNA was isolated from tomato cotyledons following incubation in water, MS medium, or MS medium with NAA and BAP (MS + H) for 15 h. The control was RNA from freshly harvested cotyledons.

for the induction of *LHA2* and *LHA4*, the effect of the nonmetabolizable Glc analog OMG was investigated. Incubation in OMG did not induce the accumulation of *LHA1*, *LHA2*, or *LHA4* mRNA (Fig. 7).

DISCUSSION

The PM H^+-ATPase is thought to play a critical role in a number of cellular processes that require high metabolic activity, including cell expansion and solute transport. Immunological studies have revealed high PM H^+-ATPase protein levels in cell types and tissues thought to have high PM solute flux rates. For example, in leaves PM H^+-ATPase abundance appears to be greatest in guard cells and phloem (Parets-Soler et al., 1990; Villalba et al., 1991; Lohse and Hedrich, 1992; Becker et al., 1993). In the roots of barley and oat (Parets-Soler et al., 1990; Samuels et al., 1992) PM H^+-ATPase protein is most abundant at sites of active nutrient uptake, the epidermis near the root tip and the endodermis and vascular parenchyma of mature tissue. In growing tissues it is thought that PM H^+-ATPase-driven acidification of the cell wall facilitates turgor-driven cellular expansion.

In tomato the PM H^+-ATPase genes *LHA1*, *LHA2*, and *LHA4* are expressed at detectable levels and the cDNA sequence encoding LHA1 and LHA2 have been characterized. To fully characterize the expression of all three isoforms the genomic sequence corresponding to LHA4 was determined. The deduced amino acid sequence of LHA4 shares 75 to 98% identity with PM H^+-ATPases from other plants, with greatest identity to the potato isoform PHA2 (98%) and the *N. plumbaginifolia* isoform PMA4 (95%). We found that LHA4 groups with the second subfamily of PM H^+-ATPase genes, which also includes AHAI, AHA2, AHA3, and PMA4 (Moriau et al., 1993).

The 5′ UTR of several PM H^+-ATPases contains a short URF. The LHA4 cDNA includes a 5′ URF of 16 codons beginning at position −86. Although the LHA4-coding sequence is most similar to tobacco PMA4, the deduced amino acid sequence of the LHA4 5′ URF is similar to that reported for the 5′ URF of PMA1. It is thought that the 5′ URF plays a role in translational regulation. Michelet et al. (1994) demonstrated that the 5′ URF of the *N. plumbaginifolia* isoform PMA1 may regulate translational efficiency. Deletion of the PMA1 5′ URF resulted in a 2.7-fold increase in translation in an in vitro assay. It may be significant that the two PM H^+-ATPases, PMA1 and LHA4, for which expression has been shown to be regulated by physiologi-

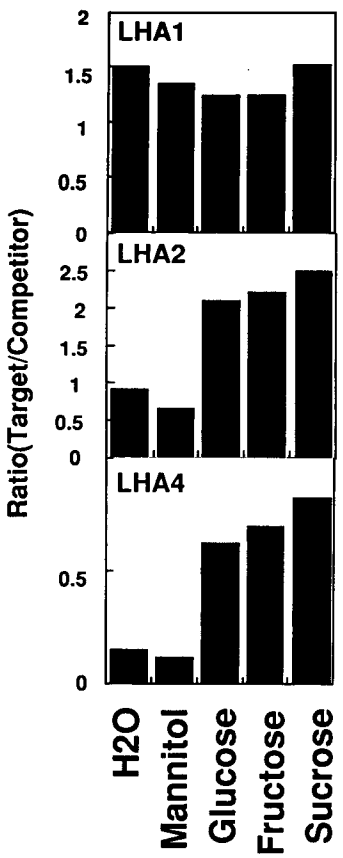

Figure 6. Sugar induction of H^+-ATPase mRNA accumulation was quantified by competitive reverse transcriptase-PCR. Total RNA was isolated following the incubation of tomato cotyledons in water or in 100 mM Suc, Glc, Fru, or mannitol for 15 h.

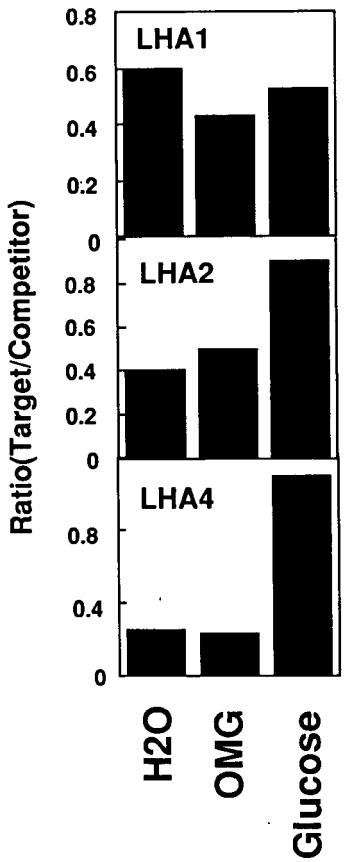

Figure 7. Effect of OMG on the induction of H+-ATPase mRNA accumulation was quantified by competitive reverse transcriptase-PCR. Total RNA was isolated following the incubation of tomato cotyledons in water, 100 mM OMG, or Glc for 15 h.

cal or environmental stimuli, have similar URF sequences. Another feature of the PM H+-ATPase 5′ UTR recognized by Ookura et al. (1994) is the presence of polypyrimidine tracts of 22 and 23 bp in *OSA2* and *AHA2*, respectively. The 5′ URF of *LHA4* is pyrimidine-rich but does not contain a polypyrimidine tract greater than 11 bp in length. The significance of these tracts is not known.

LHA4 expression appears to be correlated with growth rate in tomato seedlings. The mRNA levels of *LHA1*, *LHA2*, and *LHA4* were determined in various organs of light- and dark-grown tomato seedlings. All three isoforms are expressed in each of the organs examined (Fig. 5). *LHA1* and *LHA2* mRNA are present at somewhat different levels in each organ but are not affected by light exposure. *LHA4* expression is induced by approximately 2.5-fold in hypocotyls of dark-grown plants, compared with light-grown plants, and hypocotyl elongation rates are significantly greater in dark-grown plants. This apparent correlation of *LHA4* expression with growth and the similar finding of growth-related, sugar-inducible expression of *PMA1* in yeast prompted us to investigate the effect of growth media and various sugars on the accumulation of *LHA* mRNAs.

Exogenous metabolizable sugars induce the accumulation of both *LHA4* and *LHA2* mRNA. The induction of *LHA4* and *LHA2* expression is essentially the same in cotyledons incubated in the relatively complex MS medium or in simple solutions of Glc, Fru, or Suc. mRNA abundance may be regulated both by rates of gene transcription and by rates of RNA turnover. The experiments reported here did not distinguish whether one or both of these processes contributed to the observed increase in *LHA2* and *LHA4* mRNA levels. Addition of the hormone NAA or BAP, the nonmetabolizable sugar OMG, or the relatively impermeant sugar mannitol had no significant effect on the accumulation of any *LHA* mRNA. This indicates that the effect of sugar is likely to be independent of hormonal and osmotic influences and may be transduced through cellular metabolism. Although we did not observe any effect of hormone treatment on *LHA1*, *LHA2*, or *LHA4* mRNA abundance, these experiments were carried out at only one time and hormone concentration: it is possible that hormonal effects may be observed under other hormone regimes.

Studies of sugar-regulated gene expression have generally focused on storage tissue, for example, in potato tubers and sweet potatoes, in which the physiological process associated with starch and protein accumulation is predominant (Rocha-Sosa et al., 1989; Hattori et al., 1991). In these systems sugar induction of storage protein genes has been proposed to be associated with the induction of a storage function when excess carbon is available (Nakamura et al., 1991). In addition to storage proteins, Suc synthase is also regulated by sugar. The maize Suc synthase gene *Sh1* is up-regulated by metabolizable sugar, and the elevation of *Sh1* mRNA level has been implicated as a mechanism for the biosynthesis of cell-wall material to accompany an enhanced supply of sugar (Koch et al., 1992).

Although the regulation of *LHA2* and *LHA4* mRNA expression by sugar is similar to other sugar-regulated genes, which are induced only by metabolizable sugar, the physiological implication of *LHA2* and *LHA4* induction by sugar is unclear at present. It is interesting to note the similar induction of a PM H+-ATPase gene by sugar in yeast. Rao et al. (1993) reported the correlation between *PMA1* expression and growth rate and suggested that the induction of *PMA1* mRNA and the polypeptide is involved in the regulation of cell growth. *LHA4* mRNA is most abundant in rapidly growing tissue such as hypocotyls growing in the dark. It is possible that *LHA4* is involved in the regulation of cell growth, as was suggested in the studies in yeast. The induction of *LHA4* mRNA by sugar may be part of a generalized cellular response to induced cell growth and metabolism when an abundant carbon source is available.

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