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NaCl Regulation of Tonoplast ATPase 70-Kilodalton Subunit mRNA in Tobacco Cells[1]

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ABSTRACT

A cDNA clone encoding the 70-kilodalton subunit of the tobacco (*Nicotiana tabacum* var Wisconsin 38) tonoplast ATPase has been isolated. The 1.656 kilobase insert contains only open reading frame that represents more than 80% of the carrot cDNA coding region. The deduced amino acid sequence has greater than 95% sequence identity with the homologous carrot sequence. A transcript of approximately 2.7 kilobase was detected on Northern blots of tobacco poly(A)$^+$ selected or total RNA using labeled probe produced from this clone. The gene was expressed throughout the growth cycle in unadapted and 428 millimolar NaCl adapted cells. Transcription of the 70-kilodalton subunit gene or mRNA stability was induced by short-term NaCl treatment in NaCl adapted cells or by abscisic acid treatment in both adapted and unadapted cells. Southern analysis indicated the presence of up to four genes encoding the 70-kilodalton subunit.

Cellular adaptations that facilitate osmotic adjustment are of critical importance for growth and survival of plants in saline environments. To counter water deficits resulting from high extracellular concentrations of salt, cells accumulate the readily available ions as principal sources of osmotica (3, 11, 14). Because cytosolic physiological and biochemical processes apparently are inhibited by high ion concentrations, mechanisms that permit compartmentation of these ions in the vacuole and regulate the concentrations of these ions in the cytosol are of substantial importance for adaptation and growth (11, 14, 15).

The tonoplast is fundamental to the mediation of ion compartmentation in the vacuole. Mechanisms that influence the transport characteristics and properties of the tonoplast are assumed to be important determinants for vacuolar compartmentation. These include those that are involved in the regulation of both passive solute flux and energy-dependent transport. In plants, it is generally believed that the H^+ electrochemical gradient ($\Delta\mu_{H^+}$) is the principal driving force for the uphill transport of ions and other solutes into the vacuole. The $\Delta\mu_{H^+}$ across the tonoplast is produced by electrogenic H^+ pumps, the H^+-ATPase (22), and the H^+ translocating pyrophosphatase (17, 25). However, the significance of the pyrophosphatase H^+ pump to the generation of the $\Delta\mu_{H^+}$ across the tonoplast under physiological conditions is not clear (17, 25).

H^+-ATPases that have been categorized on the basis of similar biochemical properties as V-type enzymes act as H^+ pumps in the membranes of a wide variety of acidic compartments in eukaryotes (22). The similarities of the protein sequences of these enzymes have recently been confirmed by analysis of cDNA and genomic clones encoding the major ATPase subunits (12, 22). The V-type H^+-ATPase is a multimeric enzyme of about 400 to 600 kD that consists of up to nine subunits (22, 23). The putative catalytic 70-kD subunit forms a hydrophilic complex with a 60-kD subunit that is the principal component of ATPase activity. The 16-kD proteolipid subunits form a hydrophobic complex that functions in H^+ conductance. Additional subunits are postulated to form a stalk connecting these two complexes or to be associated with the proton channel (22).

It is reasonable to assume that the V-type H^+-ATPase is an enzyme that has to be functional at all times during cell growth. Disruption of the genes encoding the 60- or 70-kD subunits of yeast vacuolar H^+-ATPase caused conditional lethality. The mutants grew more slowly than the wild-type cells, failed to grow at neutral pH, and lacked the ability to acidify their vacuoles (18, 28). This indicates that the ability to acidify vacuoles is important for the survival of yeast and probably other eukaryotic cells. The tonoplast H^+-ATPase, therefore, would be essential for higher plants that have to survive under wide fluctuations of growth conditions, particularly in hyperosmotic environments in which solute accumulation and compartmentation are required for osmotic adjustment.

The regulation of the multimeric V-type H^+-ATPase poses a number of interesting questions, especially in relation to salt adaptation. The regulatory aspects of transcription of such genes in plants has received scant attention. The limited information available, however, is intriguing. The genes for the different subunits have been shown to reside on different chromosomes in *Neurospora* (5). The transcription and trans-

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lation of these genes, therefore, should be coordinated in some manner. In *Mesembryanthemum crystallium,* the subunit composition of the enzyme is altered in response to salt (7). In yeast, the 69-kD subunit is obtained from a larger translational product by protein splicing (18).

We were interested in studying the regulation of expression of the 70-kD subunit gene of the tonoplast ATPase of tobacco (*Nicotiana tabacum* L. var Wisconsin 38). We have previously shown that cells adapted to near sea water levels of salinity (428 mM NaCl) accumulate high concentrations of Na^+ and Cl^- and compartmentalize these ions in the vacuole (2, 4). The tonoplast H^+-ATPase from these cells exhibited a higher specific H^+-transport capacity and ATPase activity than the tonoplast ATPase isolated from unadapted cells (26).

In this paper, the isolation of a cDNA clone encoding the catalytic subunit of a V-type H^+-ATPase of tobacco is described. We have investigated the steady-state levels of the tonoplast ATPase mRNA in unadapted and NaCl (428 mM) adapted cells. We present evidence that the levels of the mRNA are regulated, but only moderately, by NaCl, and that this message regulation occurs during the initial period of response to salt but is not evident after cells are adapted to and growing in the saline environment. This moderate up regulation of the mRNA level, in response to a decrease in the external water potential, is what might be expected for a gene that is likely constitutively expressed in cells that are continually adjusting their osmotic potentials during growth.

MATERIALS AND METHODS

Cell Culture

Suspension cultures of tobacco cells (*Nicotiana tabacum* L. var Wisconsin 38) S0,[2] S25, and S25→0 were maintained as described (2). The S25→0 cells have retained the capacity to tolerate NaCl relative to the S0 cells for several hundred generations. Unless mentioned otherwise, cells were grown in the dark.

Isolation of a cDNA Clone

A cDNA clone for the 70-kD subunit of the tonoplast ATPase was isolated using a mixture of three end labeled oligonucleotides (Applied Biosystems model 380A DNA synthesizer, Laboratory for Macromolecular Structure at Purdue University). Probe sequences:5′ ACT ATA GCA GAA TAC TTC AGA GAT 3′5′ GCT GGT AAG GTG AAG TGC CTT GGT GGA 3′5′ GTT CAG GTC TTT TGG GGT TTA GAC 3′representing nucleotides 1157 to 1180, 1328 to 1354, and 1448 to 1472 of the carrot cDNA clone (32) were used. These probes correspond to regions of the carrot cDNA sequence for the 69-kD vacuolar ATPase subunit that are highly homologous to the sequence for the *Neurospora* 67-kD ATPase subunit (12). The probe corresponding to bases 1157 to 1180 is from a region of the carrot cDNA sequence that also exhibits homology with the β subunit of the F_0F_1 ATPases (12).

An *N. tabacum* var Wisconsin 38 λgt11 library consisting of 6×10^5 recombinants had been constructed from poly(A)$^+$ RNA isolated from S25 cells (29). Approximately 4×10^5 recombinants from this library were initially screened with the three probes. The hybridizations were performed in 6 × SSC, (1 × SSC is 150 mM NaCl, 15 mM trisodium citrate, pH 7.0), 5 × Denhardt's solution (1), 0.1% SDS, and 100 μg/mL denatured herring sperm DNA for 18 h at 37°C. Following hybridization, the nitrocellulose filters were washed twice with 6 × SSC and 0.1% SDS, once with 3 × SSC and 0.1% SDS, each for 30 min; and then briefly rinsed once with 3 × SSC at room temperature.

Ten clones were isolated in the first round of screening and six continued to hybridize to the mixture of three probes after plaque purification (three additional rounds of screening). After bacteriophage were isolated via glycerol step gradient purification, the λDNA was isolated from these six clones. The cDNA inserts from three of the clones were then excised with *Eco*RI and subcloned into the plasmid pTZ18U (United States Biochemical). Two of these clones containing the longest inserts (1.656 kb) were identical, based on restriction digest analysis and sequence analysis (data not shown), and one was utilized for all further analyses.

DNA Sequencing

Cesium chloride-purified plasmid DNA was sequenced by Sanger dideoxy nucleotide chain termination using a Sequenase kit (United States Biochemical). Initially, T7 and M13 universal sequencing primers and the oligonucleotides synthesized for screening were used to prime the sequencing reaction. After a partial sequence of the clone was obtained, additional synthetic oligonucleotide primers (representing internal sites) were generated based on the sequence information to obtain the complete sequence of the clone.

Preparation of DNA and RNA

DNA and RNA were isolated from cultured tobacco cells that were collected by filtration through Miracloth and frozen in liquid nitrogen. To extract DNA, ground frozen tissue was lysed by shaking gently with LETS buffer (100 mM LiCl, 10 mM EDTA, 10 mM Tris·HCl, pH 7.5, and 0.5% SDS, 4 mL/g fresh weight). The lysed tissues were then gently extracted with an equal volume of a 1:1 mixture of LETS-equilibrated phenol and chloroform:isoamylalcohol (24:1) for 15 min. The aqueous phase was collected by centrifugation and precipitated with half volume 7.5 M ammonium acetate overnight at 4°C. DNA was precipitated from the ammonium acetate supernatant with an equal volume of isopropanol at 4°C. The DNA was further purified by RNaseA treatment and cetyltrimethylammonium bromide extraction (1). RNA was extracted from cells using the acid guanidinium thiocyanate phenol chloroform method (8). It was further purified by precipitation with 2 M LiCl followed by ethanol precipitation. Poly(A)$^+$ RNA was fractionated from total RNA on an oligo-dT cellulose column (27). Nucleic acids were estimated spectrophotometrically.

[2] Abbreviations: S0, unadapted; S25, adapted to 428 mM NaCl; S25→0, S25 cells returned to medium without salt; kb, kilobase.

DNA and RNA Analyses

DNA was electrophoresed on 0.8% agarose gels and transferred to nitrocellulose (27). RNA was fractionated by electrophoresis through formaldehyde-agarose gels and transferred to nitrocellulose (27). DNA probes were ^{32}P-labeled using a random primed DNA labeling kit (Boehringer Mannheim). The probe for the tobacco 70-kD subunit of the tonoplast ATPase was a 1.4 kb cDNA insert (*Bgl*II 5′ and *Eco*RI 3′), which corresponded to bases 592 to 1996 of the carrot cDNA sequence (32). Northern and Southern blots were hybridized in 50% formamide, 6 × SSPE (1 × SSPE is 150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA, pH 7.4), 5 × Denhardt's solution, 0.1% SDS, and 100 μg/mL herring sperm DNA for 18 h at 45°C. After hybridization, the blots were washed twice for 30 min at room temperature with 1 × SSC and 0.1 × SSC, each containing 0.1% SDS. For Southern analyses, the final washes were at 65°C in 0.1 × SSC containing 0.1% SDS. Autoradiography was performed at −80°C with a single intensifying screen. Films were preflashed whenever a linear response was required (27).

The amount of total RNA loaded per lane was estimated by hybridizing blots with the ^{32}P-labeled *Bam*HI insert of pBG35 (13), which carries genes coding for the 18S and 25S rRNA of flax. The ^{32}P-labeled 18S and 25S rRNA bands in each lane, as located by autoradiography, were excised and radioactivity was determined in a liquid scintillation counter using Complete Counting Cocktail 4a20 (Research Products International Corp.). Counts from equivalent sized regions of the filter that did not contain RNA were used to correct for background radiation. The signal on autoradiograms was quantitated by densitometric scanning with a DU-8 Beckman spectrophotometer Slab Gel Compuset module.

RESULTS

The tobacco cDNA clone that was isolated using oligonucleotides representing regions conserved in the tonoplast ATPase 70-kD subunits of carrot and *Neurospora* had a 1656 base pair insert that was open reading frame and covered the region from Asp-63 to Gly-614 of the carrot cDNA clone (32). This represented about 88% of the entire coding region of the carrot cDNA clone. The deduced amino acid sequence of the tobacco clone was more than 95% identical to the analogous sequence from carrot, with absolute conservation in regions of presumed catalytic function (Fig. 1).

The region shown in Figure 1A has the GXXGXGKT motif that is present in the ATP binding site of the β subunits of F_0F_1 ATPases and the 70-kD subunits of the V-ATPases (22). The lysine residue (indicated by an asterisk) in the β subunit of the F_0F_1 ATPase of beef heart mitochondria irreversibly binds the adenine analog 7-chloro-4-nitrobenzo-2-oza-1,3-diazole in an ATP-protectable manner. The homologous lysine residue in the β subunit of the *Escherichia coli* F_0F_1 ATPase has been shown to be essential for both ATP hydrolysis and synthesis by site-directed mutagenesis. The region shown in Figure 1B is implicated in nucleotide binding and contains the AEYFRD sequence that is highly conserved (12). The region shown in Figure 1C shares homology with the phosphorylation site found in E_1E_2-ATPases (32). It is

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A.
tobacco          TGQRVLDALFPSVLGGTCAIPGAFGCGKTV
carrot           TGQRVLDALFPSVLGGTCAIPGAFGCGKTV  260
Neurospora       VGQRVLDALFPSVQGGTVAIPGAFGCGKTV  254
E. coli β        TGIKVIDLMCPFAKGGKVGLFGGAGVGKTV  158
bovine β         TGDKVVDLLAPYAKGGKIGLFGGAGVGKTV  164
                                              *

B.
tobacco          YTGITIAEYFRD
carrot           YTGITIAEYFRD    342
Neurospora       YTGITVAEYFRD    335
E. coli β        LTGLTMAEKFRD    233
bovine β         LTGLTVAEYFRD    245

C.
tobacco          GSVTIVGAVSPPGGDFSDP
carrot           GSVTIVGAVSPPGGDFSDP  423
Neurospora       GSVSIVGAVSPPGGDFSDP  416
E. coli β        GSITSVQAVYVPADDLTDP  307
bovine β         GSITSVQAIYVPADDLTDP  320
                              **

D.
tobacco          VYVGCGERGNE
carrot           VYVGCGERGNE          285
Neurospora       VYVGCGERGNE          279
E. coli β        VFAGVGERTRE          183
bovine β         VFAGVGERTRE          189
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Figure 1. Homology of amino acid sequences of V-ATPases and F_0F_1 ATPases. The sequences of the carrot 69-kD, *N. crassa* 67-kD, *E. coli*, and beef heart mitochondria β subunits were taken from the paper by Gogarten *et al.* (12) and are compared with the 1.656 kb cDNA insert of NaCl (428 mM) adapted tobacco cells. Identical residues are in boldface type. The oligonucleotide probe 1, which was used for screening of the tobacco cDNA library, corresponds to the sequence in B.

absolutely conserved between carrot and tobacco and has high homology with the corresponding sequences in the β subunit of F_0F_1 ATPases. Tyr-311 (double asterisk, Fig. 1C) of the β subunit of the beef heart mitochondrial enzyme, which binds 7-chloro-4-nitrobenzo-2-oza-1,3-diazole reversibly before it reacts with lysine, is present in this region, although it is replaced by Ser in the 70-kD subunit of the V-ATPases (12, 32). The sequence shown in Figure 1D is highly conserved in the V-ATPase 70-kD subunits. The Glu at the end of this sequence is conserved between the V-ATPase 70-kD subunits and F_0F_1 ATPase β subunits. In *E. coli*, it has been shown to be essential for the structure and assembly of the β subunit (32). It, or sequences close by, may also be involved in Mg^{2+}-protectable *N,N′*-dicyclohexylcarbodiimde binding (32).

The V-type ATPases differ from the F_0F_1 ATPases in their sensitivity to the sulfhydryl reagent *N*-ethylmaleimide (22). This sensitivity is attributed to cysteine residues in the active site. The carrot sequence has five cysteines, three of which are located in the putative active site and two of these are conserved in the *Neurospora crassa* 67-kD subunit (12, 32). The tobacco sequence has an extra cysteine (replaces Leu-385 of the carrot sequence). The significance of this substitution is not known.

Another difference between the F_0F_1 ATPase β subunits and the V-ATPase 70-kD subunits is the absence of tryptophan residues in the former (22). All the tryptophan residues in the carrot sequence are conserved in tobacco. Four of them are also conserved in the *N. crassa* 67-kD subunit (12).

Therefore, it was inferred that the tobacco cDNA clone

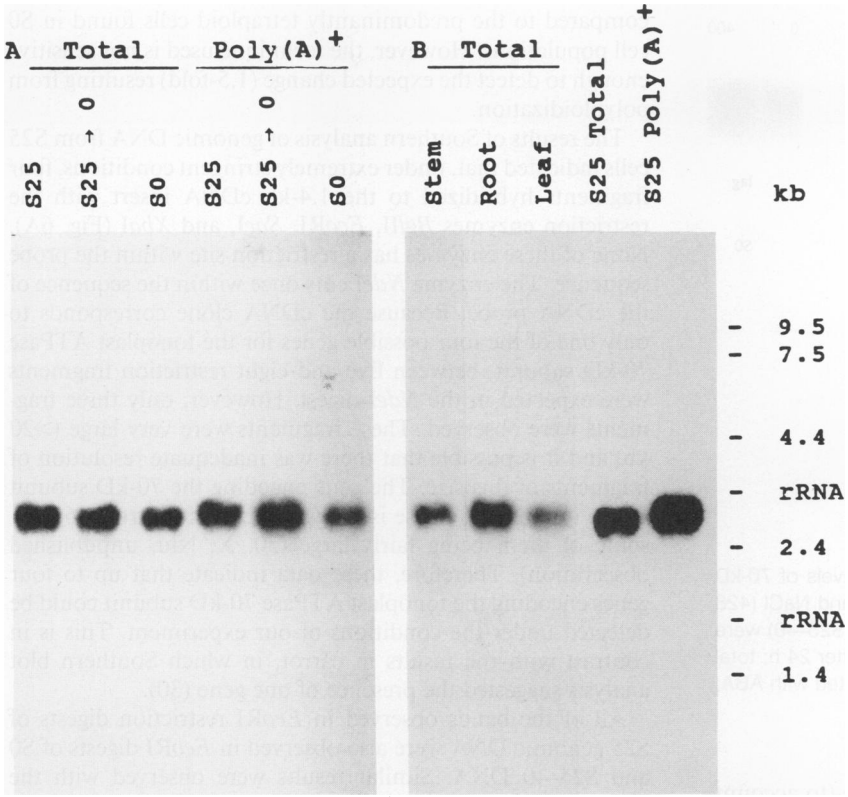

Figure 2. Analysis of the transcript detected by the cDNA insert encoding the 70-kD subunit of the tonoplast ATPase. Total RNA (10 μg) and poly(A)$^+$ RNA (1 μg) were electrophoresed and probed as described in "Materials and Methods." A, Total and poly(A)$^+$ RNA from unadapted (S0) or NaCl (428 mM) adapted cells growing in the presence (S25) or absence (S25→0) of NaCl. B, Total RNA from different plant tissues or total and poly(A)$^+$ RNA from S25 cells. The position of mol wt markers and the rRNA bands, as visualized by ethidium bromide staining, are indicated. The bands in each panel are from the same hybridization reaction and blotting.

encoded a large part of the tobacco tonoplast ATPase 70-kD subunit and a 1.4 kb insert (corresponding to bases 592–1996 of the carrot sequence) was used in these studies.

Northern Analyses of Tonoplast ATPase 70-kD Polypeptide Message

Two isoforms of the 70-kD polypeptide have been detected in tobacco (26) and in barley (9). However, a single band was detected when either total RNA or poly(A)$^+$-selected RNA from unadapted or adapted cells was probed with the cDNA insert from the cloned 70-kD subunit of the tonoplast ATPase (Fig. 2). A single band of equivalent size was also detected in total RNA from different parts of the tobacco plant (Fig. 2). The size of the tonoplast ATPase message was estimated to be 2.75 kb, a somewhat larger message than would be predicted by the carrot cDNA (32).

There did not appear to be major changes in the levels of accumulated message during a culture growth cycle for any of the cell lines examined (Fig. 3). If normalized to levels of ribosomal RNA, the message abundance in S0 and S25 cells that had just entered fresh weight stationary growth phase was comparable (S0:S25 = 1.4:1). However, the amount of immunologically detected 70-kD polypeptide in S0 cells was three- to fourfold greater than that in S25 cells at this growth stage (26).

The level of 70-kD subunit message increased within 24 h in response to NaCl in S25→0 cells at the mid linear phase of growth (Fig. 4). This increase was estimated to be two- to fourfold by two methods. When slopes of the densitometric scanned peak areas *versus* total RNA concentration were compared on slot blots, the induction was found to be between three- and fourfold (average of three determinations, 3.6). When the scanned peak areas of the signal on Northern blots

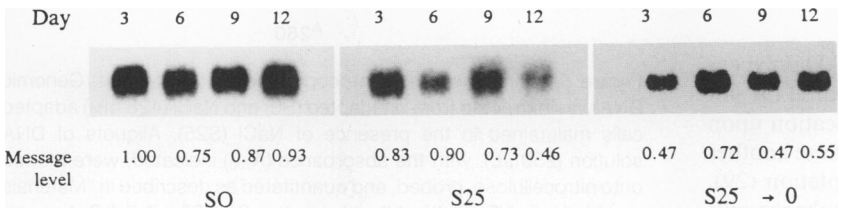

Figure 3. Northern analyses of total RNA (10 μg) through the growth cycles of unadapted (S0) and NaCl (428 mM) adapted cells maintained in the presence (S25) or absence of NaCl (S25→0). The message levels were obtained by normalizing the signals to total rRNA in each lane and taking the S0 day 3 value as 1.0. They are the average of the data from several gels. The lag phase of growth corresponded to day 3 (all cell lines) and day 6 (S0); late lag/early linear growth corresponded to day 6 (S25, S25→0) and day 9(S0). Cells at all other time points were in the linear phase of growth.

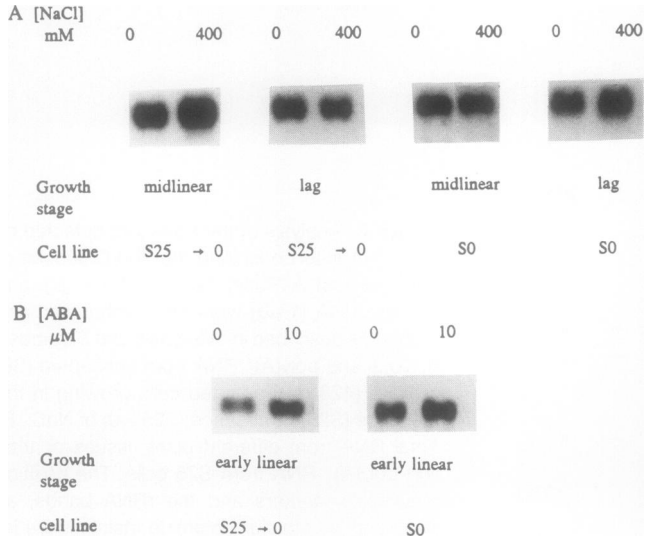

Figure 4. NaCl (A) or ABA (B) regulation of mRNA levels of 70-kD subunit of the tonoplast ATPase. A, Unadapted (S0) and NaCl (428 mM) adapted cells maintained in the absence of NaCl (S25→0) were grown in the dark, treated with NaCl, and harvested after 24 h; total RNA, 10 μg/lane. B, Cells were grown in the light, treated with ABA, and harvested after 24 h; total RNA, 20 μg/lane.

was normalized to the rRNA signal in each lane (to account for differences in transfer and loading), the induction was 1.7 to 3.5 fold (average of six determinations, 2.3). Message levels in S0 cells from the same phase of growth did not show a similar increase in response to salt. S25→0 cells from an earlier stage of growth (lag) did not exhibit the ability to accumulate 70-kD subunit message when challenged with this level of NaCl. These results suggest that S25→0 cells may be more responsive to NaCl. This responsivity is apparently limited to certain stages of the growth cycle and may be related to the enhanced capacity of the cells for NaCl adaptation.

Previous studies (20) have shown that ABA enhances the ability of tobacco cells to osmotically adjust and adapt to NaCl. Message levels in S0 and S25→0 cells increased when the cells were treated with 10 μM ABA for 24 h (Fig. 4). This increase was small and variable; sometimes there was no induction, whereas at other times up to fivefold induction was observed. On an average, the increase was 1.5- to twofold. This suggests that there may be an ABA effect on the tonoplast ATPase 70-kD polypeptide message. The ABA effect was also growth stage-dependent and was usually observed only in the late lag or early linear stage.

Southern Analysis

The copy number of the tonoplast ATPase 70-kD polypeptide gene in the S0 and S25 cell lines was compared on slot blots and there was no evidence for gene amplification upon salt adaptation (Fig. 5). This is not surprising because another stress-induced gene was not amplified after adaptation (29). Salt adaptation is known to induce polyploidy in tobacco cells (19). S25 cell populations are enriched in hexaploid cells compared to the predominantly tetraploid cells found in S0 cell populations. However, the technique used is not sensitive enough to detect the expected change (1.5-fold) resulting from polyploidization.

The results of Southern analysis of genomic DNA from S25 cells indicated that, under extremely stringent conditions, four fragments hybridized to the 1.4-kb cDNA insert with the restriction enzymes *Bgl*II, *Eco*RI, *Sac*I, and *Xba*I (Fig. 6A). None of these enzymes has a restriction site within the probe sequence. The enzyme *Nde*I cuts once within the sequence of the cDNA probe. Because the cDNA clone corresponds to only one of the four possible genes for the tonoplast ATPase 70-kD subunit, between five and eight restriction fragments were expected in the *Nde*I digest. However, only three fragments were observed. These fragments were very large (>20 kb) and it is possible that there was inadequate resolution of fragments of this size. The gene encoding the 70-kD subunit of the tonoplast ATPase is known to contain introns (6, 30), some of them being fairly large (30, X. Niu, unpublished observation). Therefore, these data indicate that up to four genes encoding the tonoplast ATPase 70-kD subunit could be detected under the conditions of our experiment. This is in contrast with the results in carrot, in which Southern blot analysis suggested the presence of one gene (30).

All of the bands observed in *Eco*RI restriction digests of S25 genomic DNA were also observed in *Eco*RI digests of S0 and S25→0 DNA. Similar results were observed with the enzymes *Bgl*II and *Xba*I (not shown). The four *Eco*RI restriction fragments observed in the tobacco DNA were the sum of the fragments observed in the genomic DNA of *N. tomentosiformis* and *N. sylvestris,* the progenitors of *N. tabacum* (Fig. 6B). Similar results were obtained with the enzyme *Bgl*II.

DISCUSSION

The activity of the tonoplast ATPase of several plant species is responsive to salt stress. In *M. crystallinum,* salinity stress

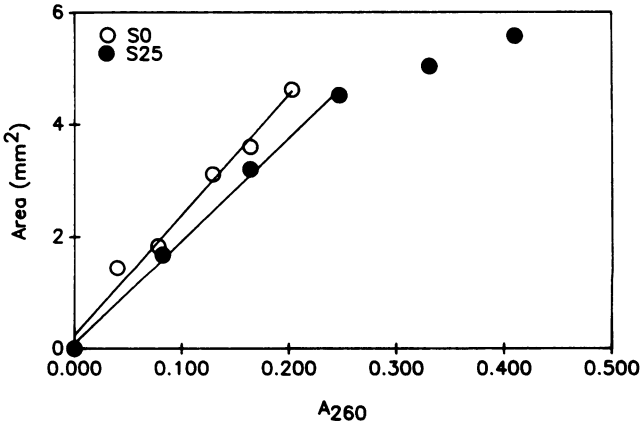

Figure 5. Comparison of gene copy number by slot blot. Genomic DNA was extracted from unadapted (S0) and NaCl (428 mM) adapted cells maintained in the presence of NaCl (S25). Aliquots of DNA solution (250 μL), with the absorbance (A_{260}) indicated, were blotted onto nitrocellulose, probed, and quantitated as described in "Materials and Methods." The ratio of the slopes was S0:S25 = 1.2:1.0. Average of four determinations was S0:S25 = 1.3:1.0.

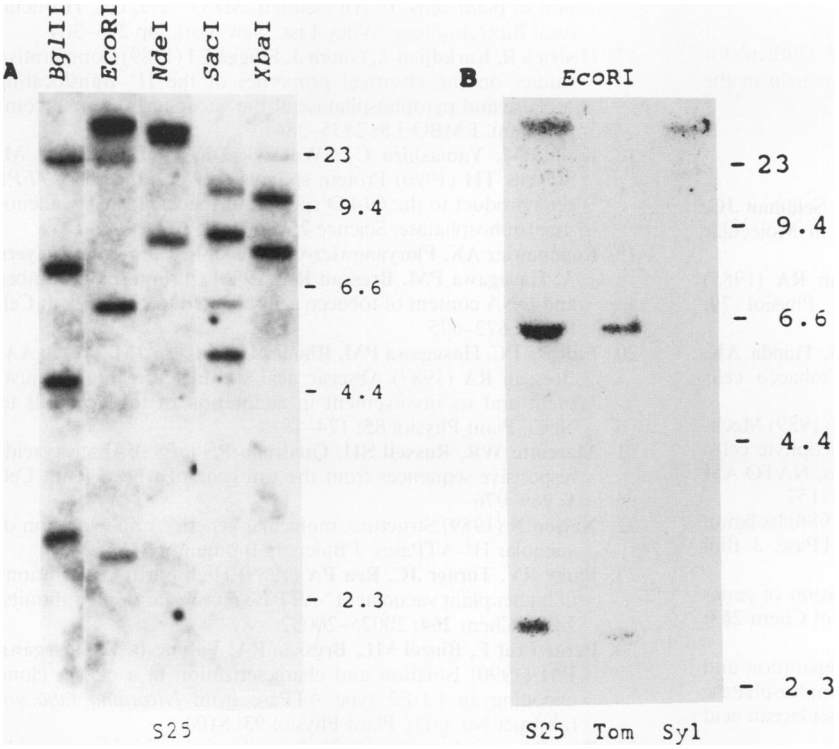

Figure 6. Southern analysis of genomic DNA of *N. tabacum*, *N. tomentosiformis*, and *N. sylvestris*. A, Ten micrograms of genomic DNA from NaCl (428 mM) adapted cells (S25) was digested, electrophoresed on a 1% agarose gel, transferred, and probed as described in "Materials and Methods." The bands are from the same hybridization reaction and blotting. B, Genomic DNA (5–10 μg) from NaCl (428 mM) adapted cells (S25), *N. tomentosiformis* leaves, and *N. sylvestris* leaves was digested with *Eco*RI and electrophoresed on 1% agarose. The bands are from the same hybridization reaction and blotting.

induced tonoplast ATP hydrolysis activity (7). In tobacco cells, the specific H^+ transport activity and ATP hydrolysis activity per unit of 70-kD subunit of the tonoplast ATPase increased upon salt adaptation (26).

The level of the 70-kD polypeptide message was not significantly different in NaCl adapted and unadapted tobacco cells. The observed constitutive expression of the gene encoding the 70-kD polypeptide of the tonoplast ATPase in the absence of either ABA or NaCl is consistent with the need for homeostatic $\Delta\mu_{H^+}$ maintenance. In this context, it is interesting to note that when the β-glucuronidase reporter gene was fused to the promoter of the carrot 70-kD subunit of the vacuolar H^+-ATPase, transformed carrot calli showed no preferential expression in any specific cell type, even though some differentiation occurred during callus growth (30).

Overproduction of plasma membrane H^+-ATPase has been shown to be toxic by gene dosage experiments in yeast (10). In agreement with this observation, the amount of tonoplast ATPase 70-kD polypeptide is less or not significantly altered after salt adaptation in tobacco cells (26). Modulation of activity could occur by altering the subunit composition of the enzyme, as in the case of *M. crystallinum* (7), or the environment. Our observation that the message for the 70-kD subunit of the tobacco tonoplast ATPase is induced by NaCl in salt adapted cells confirms our previously reported observations (16) and suggests for the first time that some elements present on the 70-kD subunit gene participate in the environmental regulation of this enzyme. We have observed similar NaCl induction of the message of two E_1E_2-type ATPases in salt adapted *N. tabacum* var Wisconsin 38 cells (24). One of these appears to be a plasma membrane H^+-ATPase and the other, an endomembrane Ca^{2+}-ATPase. Water deficit induction of a plasma membrane ATPase in soybean seedlings has been reported (31).

Induction of the tonoplast ATPase 70-kD polypeptide message was observed only in NaCl adapted cells that were maintained in the absence of salt, during the period just after reexposure to salt. Presumably, this is a period when the cells are adjusting to stress imposition and are initiating the processes required for solute accumulation and compartmentation. Because the NaCl adapted cells exhibit an enhanced capacity to adapt to salinity relative to the unadapted cells (4), it is probable that these cells have an enhanced ability to invoke mechanisms required for survival and growth in the hyperosmotic environment. The inducibility of the 70-kD polypeptide mRNA in NaCl adapted cells, that exhibit enhanced capacity to adjust to salt imposition, may be a mechanism to provide increased $\Delta\mu_{H^+}$ for increased solute transport necessary during salt adaptation. It is not clear if transcription or stability of the tonoplast ATPase message is enhanced.

ABA has been shown to enhance the ability of tobacco cells to adapt to NaCl (20). The message for the ATPase 70-kD polypeptide was induced moderately by ABA in both cell lines. A putative ABA-type box exists in the published promoter sequence for the gene encoding the tonoplast ATPase 70-kD polypeptide of carrot (30). The carrot ABA-type box (CGAGAAG; base −196 to −190) is similar to the Em2 ABA-responsive element (CGAGCAG) in wheat (21). Preliminary nuclear runoff transcription experiments also indicated that ABA-treated S0 cells have slightly elevated levels of the 70-kD polypeptide message (D. Nelson, unpublished observation).

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