Differential Expression within a Three-gene Subfamily Encoding a Plasma Membrane H⁺-ATPase in *Nicotiana plumbaginifolia*  

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Differential Expression of a gene subfamily (*pma*) encoding a plasma membrane H⁺-translocating ATPase in *Nicotiana plumbaginifolia* were isolated and sequenced. They are between 95 and 96% identical at the deduced amino acid sequence level. Sequence comparisons with the corresponding tomato genes (Ewing, N. N., Wimmers, L. E., Meyer, D. J., Chetelat, R. T., and Bennett, A. B. (1990) *Plant Physiol.* 94, 1874–1881) indicate that divergence among the three *N. plumbaginifolia* *pma* genes occurred before the development of the Solanaceae family. Here, determination of *pma1* transcription initiation sites reveals several 5' boundaries located 266 to 120 nucleotides upstream from the plasma membrane H⁺-ATPase translation initiation codon. The 5'-untranslated region contains a small open reading frame, 9 residues long, *pma3* has a single, 264-nucleotide long 5' leader containing a 5-residue open reading frame. The latter is completely conserved in the corresponding tomato gene. These features suggest the possibility of translational regulation of plant *pma* genes.

S1 nuclease protection assays on total cellular RNA isolated from different organs reveals that all three genes are expressed in leaf, stem, flower, and root tissues, albeit at different levels according to the organ and gene. The different genes for the plant H⁺-translocating ATPase are thus subject to differential regulation of transcription, possibly related to specific aspects of enzyme function.

Biochemical and electrophysiological studies have established that the major transport ATPase activity associated with the plasma membrane of plants and fungi is an H⁺-translocating ATPase. This enzyme is responsible for creating an electrochemical proton gradient (proton motive force) which is used for ion and nutrient transport mediated by specific carriers and channels (secondary transport). The plant plasma membrane H⁺-ATPase (PMA) has been reported to respond to hormonal signals (auxins, cytokinins, gibberellic acid), to pathogenic agents (fusococcin), and to physical factors such as light (for a review, see Marré and Ballarin-Denti (1985), Serrano (1989), and Sussman and Harper (1989)).

The proton pump of the plant plasma membrane belongs to the P-type ATPases, a class of cation-translocating ATPases that form a phosphorylated intermediate during the catalytic process (Briskin and Leonard, 1982; Clément et al., 1985). These ATPases are inhibited by vanadate, contrary to the ATPases of the V-type (vacuolar) or F (mitochondrial F, O ATPase) types (for a review, see Bowman and Bowman (1986) and Pedersen and Carafoli (1987)).

A partial protein sequence was obtained from tryptic fragments of the oat plasma membrane H⁺-ATPase (Schaller and Sussman, 1988). The complete protein sequence has been deduced from the corresponding genes in *Arabidopsis thaliana* (Harper et al., 1989; Pardo and Serrano, 1989; Harper et al., 1990), in *Nicotiana plumbaginifolia* (Boutry et al., 1989), and in *Lycoerpis esculentum* (Ewing et al., 1990). These studies have shown that the plant plasma membrane H⁺-ATPase is encoded by a multigenic family. However, functional data are scarce concerning the different genes. Because their products may play different physiological roles in distinct tissues during plant growth and development, the genes encoding the different PMA isoforms may be subject to differential regulation. In mammals, for instance, it has been shown that the Na⁺/K⁺- and Ca⁺⁺-ATPase isoforms are differentially expressed according to the tissue (Orlowski and Lingrel, 1988; Burk et al., 1989).

We have previously reported the isolation and preliminary characterization of three different clones from a root cDNA library, encoding distinct isoforms of the H⁺-ATPase of *N. plumbaginifolia* (Boutry et al., 1989; Michelet et al., 1989). We present here the isolation and characterization of genomic and cDNA clones constituting a three-gene subfamily. Analysis of their relative mRNA levels in different organs shows that they are differentially expressed.

**MATERIALS AND METHODS**

*Plant Material—* *N. plumbaginifolia* plants were grown in soil at 24 °C with a 16-h light period until they had two or three developed leaves. They were then either transplanted in soil or hydroponically grown in MS medium (Murashige and Skoog, 1962).

*Screening of the Genomic Library—* A genomic library of *N. plumbaginifolia* constructed in λ EMBL4 (Boutry and Chus, 1985) was screened with a cDNA clone for the *N. plumbaginifolia* *pma1* gene (Boutry et al., 1989). Hybridizations were carried out overnight at 42 °C in 6 × SSC, 50% formamide. Final washes were performed at 50 °C in 2 × SSC, 0.1% sodium dodecyl sulfate.

*Screening of the cDNA Library—* A Agt11 root cDNA library of *N. plumbaginifolia* generously provided by Tingey and Coruzzi (1987) was screened by the hybridization plaque method (Maniatis et al., 1982). The 109-bp nonsense *pma3* probe was synthesized with the
Klenow fragment of DNA polymerase I from the synthetic primer 5' AGCACCTCGGCTTC 3' to the EcoRI site upstream from the initiation codon. Single-stranded DNA obtained from the pma3 genomic clone was used as a template. Hybridization was performed at 42°C in 6x SSC, 50% formamide, and final washes were performed at 65°C in 0.1% sodium 3-sulfate.

Sequencing: The DNA fragments were subcloned in pBluescript (Stratagene) or pTZ18 (U.S. Biochemical Corp.) vectors. Overlapping deleted clones were obtained using the progressive deletion strategy described by Barnes et al. (1983) or the unidirectional deletion strategy according to the Stratagene instruction manual. The dioxygenase chain termination method was used to sequence single-stranded DNA (Biggs et al., 1983). The complete sequence was obtained from both strands.

RNA isolation: Plant material was ground in a mortar in liquid nitrogen, and total cellular RNA was extracted by the guanidine chloride method (Maniatis et al., 1982).

Mapping Experiments: For RNA quantitation and pma3 5' end mapping, 10 μg of total RNA was hybridized overnight with 20,000 cpm of the labeled probe in hybridization buffer (65% formamide, 400 mM NaCl, 40 mM Pipes, pH 6.8, 1 mM EDTA) at 50°C for the pma2 gene or at 37°C for pma1 and pma3 genes. S1 nuclearase reactions were then performed at 37°C with 2 units of enzyme/μl of reaction medium. After ethanol precipitation, the samples were loaded on denaturing urea polyacrylamide gels of various concentrations depending on the expected size of the protected fragments. For pma1 5' end mapping, 20 μg of total RNA were hybridized as above except that hybridization was conducted at 42°C in the presence of 50% formamide.

Probe Labeling: All probes were labeled by primer extension (Maniatis et al., 1982). Their locations are indicated on the restriction map displayed in Fig. 1 (probes used for RNA quantitation) or in Fig. 3 (probes used for determining 5' transcript boundaries).

For the pma3 probe used for RNA quantitation and mapping, single-stranded DNA from a genomic subclone containing the HindIII fragment surrounding the initiation codon was used as a template with the primer 5' AGCACCTCGGCTTC 3'. The 859-nucleotide labeled probe extended to the HindIII site located upstream from the ATG codon.

The probe was used to determine the 5' boundary of the pma1 transcript was obtained from the synthetic primer 5' AGCACCTGAAA-GAACCCATG 3' and a genomic clone containing the EcoRI fragment surrounding the 5' end of pma1. The 3' end of the probe corresponded to the HindIII site located 289 nucleotides upstream from the initiation ATG. Since several 5' pma1 boundaries were observed (see "Results"), a 3' probe giving a single signal was designed to qualify transcripts. A genomic subclone containing the EcoRI fragment surrounding the 3' end of pma1 was used to synthesize a 244-nucleotide noncoding strand probe extending from the universal primer located in the pBluescript cloning vector to the EcoRV site located 52 nucleotides downstream from the HpaII site located upstream from the ATG codon.

RESULTS

Isolation and Characterization of pma Genomic and cDNA Clones: The existence of several genes for the plasma membrane H+-ATPase in N. plumbaginifolia has been previously reported, and three complete or partial cDNA clones have been isolated, corresponding to the genes previously named pma1, pma2, and pma3 (Boutry et al., 1989; Michelet et al., 1989). In this work, we screened a genomic library of N. plumbaginifolia with a genomic DNA clone and obtained three positive clones Np-a, Np-b, and Np-f, shown by restriction analysis to contain different genes. Southern blot analyses with 5' and 3' regions of pma cDNAs (not shown) indicated that Np-b contains a complete pma gene, whereas Np-a and Np-f respectively contain the 5' and 3' parts of two other pma genes (Fig. 1). The hybridizing fragments were subcloned and sequenced. The complete pma nucleotide sequence of the Np-b clone is presented in Fig. 2. Nucleotide sequence comparisons of the genomic clones with the previously reported cDNA clones of N. plumbaginifolia (Boutry et al., 1989) showed that Np-b corresponds to pma1 and Np-f to pma2. As no cDNA corresponding to Np-a was yet available, we screened a genomic library of N. plumbaginifolia with a probe derived from the upstream region of the gene. A cDNA clone with a 3.3-kilobase pair EcoRI insert was isolated, sequenced and shown to be identical to Np-a. This gene was named pma3 and, together with pma1 and pma2, completes a pma subfamily predicted, on the basis of a Southern blot analysis of N. plumbaginifolia genomic DNA, to contain three closely related genes (Boutry et al., 1989; Michelet et al., 1989) (see below). As a previous cDNA clone named cpm3 (Boutry et al., 1989) appears to be quite different from those described above (data not shown), it belongs to another pma subfamily and will be renamed when further characterized.

Amino Acid Sequence Analysis: Long open reading frames of 956 (pma2 and 3) and 987 (pma1) amino acids were predicted. A three-base insertion located in the 5' region of the coding sequence is responsible for the additional amino acid encoded by pma1. Translation initiation codons could be identified unambiguously since, in each case, the ATG codon was preceded by an in-frame stop codon.

The three pma genes are very closely related. Many amino acid substitutions are conservative (Table I). The deduced amino acid sequences of pma1 and pma3 display 95.9% identity with that of pma2, whereas pma1 and pma3 show 96.4% amino acid identity (Table II). Comparison with the analogous genes of A. thaliana (aha) (Table II) indicates an overall identity of 80-82%. More recently, sequences from a complete and a partial cDNA clone for analogous tomato genes (lha) were made available (Ewing et al., 1990). The protein encoded by the complete clone (lha1) is 97.5% identical to N. plumbaginifolia PMA3. This figure, higher than the identity observed among the three N. plumbaginifolia PMAs, indicates
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that the latter three diverged before *N. plumbaginifolia* and tomato (*L. esculentum*) emerged within the Solanaceae family. A similar reasoning is supported by analysis of the partial cDNA clone for LHA2 which is closer to the *N. plumbaginifolia* PMA2 than to LHA1 (not shown).

The major sequence divergence between the various plant plasma membrane ATPases is located in the C-terminal third of the proteins (not shown) which is the least conserved region among all the P-type ATPases. A 33-residue stretch (918-950) constitutes an exception; it is found to be almost invariant at the C terminus of all plant H$^+$-ATPases sequenced so far. It could correspond to a regulatory sequence since Palmgren et al. (1990) have reported an activation of the oat plasma membrane H$^+$-ATPase by a proteolytic removal of the C-terminal region and have suggested that this region could be involved in the regulation of proton pumping. Moreover, the C-terminal regions of the yeast H$^+$-ATPase (Portillo et al., 1989) and of the mammalian Ca$^{2+}$-ATPase (James et al., 1989) also contain regulatory elements.

**Determination of the Transcription Starts**—The genomic clones of *pma1* and *pma3* have enabled us to carry out S1 nuclease protection experiments to determine the 5′ termini of their transcripts. Single-stranded probes were synthesized from a primer complementary to a sequence located near the first ATG. The results revealed a complex pattern of 5′ boundaries for *pma1* (Fig. 3A). One was located 120 nucleotides upstream from the start codon, and a cluster of four additional sites were located 212–266 nucleotides upstream from the initiation ATG codon. They do not correspond to different splicing events of a single primary transcript since no 3′ intron border is found just in front of the various leaders. A fifth protected fragment seen in Fig. 3A was also observed with yeast tRNA used as a control and was not considered a *pma1* 5′ boundary. No other transcription start is located further upstream, since no additional fragment was observed when the probe was extended to the HindIII site located approximately 1 kilobase pair upstream from the initiation ATG (not shown).

A single 5′ boundary was observed for *pma3* at position -264 of the initiator ATG (Fig. 3B). The similar 5′ boundaries were obtained for *pma1* and *pma3* with total RNA from different tissues (not shown). Thus the mRNA leader sequences of these two *pma* genes are unusually long for a plant species. Moreover, they contain a small, upstream open reading frame (uORF) of 9 (*pma1*) or 5 (*pma3*) amino acid residues located, respectively, 61 or 42 nucleotides upstream from the ATG of the PMA coding sequence (see Fig. 5).

Because a genomic clone for the 5′ region of the *pma2* gene was not available, we could not conduct S1 nuclease mapping of the *pma2* mRNA. However, this gene seems to possess a long leader as well, since the longest cDNA clone found so far contains a 5′-untranslated leader sequence 86 nucleotides long.

**Differential Expression of pma1, 2, and 3 in Various Organs**—Quantitation of the *pma* transcripts could not be achieved by Northern blot analysis, since the three genes are too similar to enable us to design probes long enough to specifically hybridize with each of the *pma* transcripts separately. However, the high divergence of the three *pma* genes outside their coding sequence enabled us to design specific 5′ or 3′ probes from genomic clones and to conduct S1 nuclease mapping in order to analyze the transcript level in different organs. The probes were designed to contain in part an intron or plasmid vector sequence which should not hybridize with the transcript. Thus, the expected protected fragments are shorter than the probes which therefore do not interfere with the quantitation. For each *pma* gene, we obtained the expected protected fragment (Fig. 4). A broader signal was obtained for *pma1*, probably because of the AT-rich region apparent in the nucleotide sequence at the boundary of the protected fragment. Transcripts for the three *pma* genes were found in all organs analyzed, namely roots, stems, leaves from both vegetative and flowering plants, and flowers at early and late developmental stages. Very high expression of the three *pma* genes was found in flowers. In addition, each gene showed a different pattern of expression. For instance, the mRNA level difference between stems and flowers is more marked for *pma2* and *pma3* than for *pma1*. Since all the experiments were conducted using the same RNA preparation, these results reveal real differences in *pma* mRNA levels between the organs tested.

**DISCUSSION**

The three genes analyzed here form a *pma* subfamily predicted to contain three members (Boutry et al., 1989). Restriction maps of the available genomic clones are in agreement with a Southern blot analysis of genomic DNA (Boutry et al., 1989). However, the previous report also mentioned the isolation of a cDNA clone whose partial sequence indicated a
Fig. 2. Nucleotide sequence of the pmal-3 genes. Lane 1 displays the genomic nucleotide sequence of pmal. Exons are in boldface upper case and are topped by the deduced amino acid sequence in one-letter code and numbered (underlined). The nucleotide sequence is numbered from the first nucleotide of the translation initiation codon. Lanes 2 and 3 report the nucleotide sequence of the largest cDNA clones for pmu2 and 3 respectively. In addition, lane 3 (pma3) displays a 400-nucleotide sequence upstream from the translation initiation codon obtained from the genomic clone. Transcription starts are indicated by arrows. uORFs are in upper case. Polyadenylation sites are shown by an asterisk.
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| Expression of Plant Plasma Membrane H⁺-ATPase Genes |
|------------------------------------------------------|

**FIG. 2** Continued
Expression of Plant Plasma Membrane H⁺-ATPase Genes

The first column indicates the amino acid residues of PMAl which are modified in PMAB (column 2) or PMAB (column 3). Numbering of residues corresponds to the PMAl sequence.

Table I
Amino acid modifications between the deduced protein sequences of the three genes pma1, pma2, and pma3

| PMA1 | PMA2 | PMA3 | PMA1 | PMA2 | PMA3 |
|------|------|------|------|------|------|
| Glu-4 | Absent | Absent | Ser-552 | Ala |
| Ala-16 | Thr | Thr | Ala-553 | Ser |
| Thr-39 | Ser | Ile-556 | Val |
| Ala-40 | Gly | Lys | Leu-719 | Val |
| Thr-41 | Pro | Ile | Val |
| Ala-48 | Ser | Lys | Glu-738 | Gln |
| Asp-60 | Glu | Glu | Asn-740 | Asp |
| Leu-63 | Phe | Phe | His-744 | Arg |
| Leu-64 | Ser | Glu | Leu-752 | Gln |
| Ile-109 | Val | Ile-772 | Thr |
| Arg-144 | Lys | Val | Val-789 | Met |
| Lys-146 | Asp | Phe | Phe-794 | Leu |
| Glu-148 | Gln | Ile | Val-798 | Val |
| Val-152 | Ile | Val | Val-801 | Leu |
| Ile-215 | Val | Val | Ser-817 | Ala |
| Ala-274 | Lys | Lys | Leu-834 | Leu |
| Asn-349 | Tyr | Tyr | Leu-837 | Ile |
| Ile-351 | Val | Val | Ile-841 | Phe |
| Lys-358 | Arg | Ile-845 | Leu |
| Met-360 | Thr | Thr | Phe-849 | Ile |
| Ala-381 | Thr | Arg | 857 | Lys |
| Ala-388 | Ser | Phe | Phe-863 | Leu |
| Asn-436 | His | Arg | Arg-865 | Gln |
| Ala-450 | Ser | Thr | Thr-869 | Leu |
| Gly-464 | Ala | Leu | Leu-901 | Ile |
| Gly-478 | Thr | Ala | Ala-905 | Thr |
| Gly-486 | Ala | Leu | Leu-927 | Gln |
| Val-515 | Ile | Ile | Val-954 | Ser |

Table II
Percentages of amino acid identity between the deduced amino acid sequences

| PMA1 | PMA2 | PMA3 | LHA1 | AHAl | AHAl | AHAl | AHAl |
|------|------|------|------|------|------|------|------|
|      |      |      |      |      |      |      |      |
| Glu-4 | Absent | Absent | Ser-552 | Ala |
| Ala-16 | Thr | Thr | Ala-553 | Ser |
| Thr-39 | Ser | Ile-556 | Val |
| Ala-40 | Gly | Lys | Leu-719 | Val |
| Thr-41 | Pro | Ile | Val |
| Ala-48 | Ser | Lys | Glu-738 | Gln |
| Asp-60 | Glu | Glu | Asn-740 | Asp |
| Leu-63 | Phe | Phe | His-744 | Arg |
| Leu-64 | Ser | Glu | Leu-752 | Gln |
| Ile-109 | Val | Ile-772 | Thr |
| Arg-144 | Lys | Val | Val-789 | Met |
| Lys-146 | Asp | Phe | Phe-794 | Leu |
| Glu-148 | Gln | Ile | Val-798 | Val |
| Val-152 | Ile | Val | Val-801 | Leu |
| Ile-215 | Val | Val | Ser-817 | Ala |
| Ala-274 | Lys | Lys | Leu-834 | Leu |
| Asn-349 | Tyr | Tyr | Leu-837 | Ile |
| Ile-351 | Val | Val | Ile-841 | Phe |
| Lys-358 | Arg | Ile-845 | Leu |
| Met-360 | Thr | Thr | Phe-849 | Leu |
| Ala-381 | Thr | Arg | 857 | Lys |
| Ala-388 | Ser | Phe | Phe-863 | Leu |
| Asn-436 | His | Arg | Arg-865 | Gln |
| Ala-450 | Ser | Thr | Thr-869 | Leu |
| Gly-464 | Ala | Leu | Leu-901 | Ile |
| Gly-478 | Thr | Ala | Ala-905 | Thr |
| Gly-486 | Ala | Leu | Leu-927 | Gln |
| Val-515 | Ile | Ile | Val-954 | Ser |

Fig. 2—Continued

The amino acid sequences deduced from the sequences of the pma1–3 genes are about 96% identical (Table II). All three genes are transcribed, since corresponding cDNA clones were isolated (Boutry et al., 1989 and this paper). These results raise the question of whether the encoded polypeptides have the same function and the same pattern of expression. Because of their high level of similarity, it is most likely that the three genes encode H⁺-translocating ATPases with similar functions. However, we must consider the possibility of different regulations at the enzyme level. H⁺-ATPases are thought to play a major role in tissues or cells, such as root epidermal or sieve element-companion cells, where intense cellular transport occurs. It is conceivable that the enzymes expressed in different tissues evolved differently to respond to regulatory factors specifically occurring in those tissues. This hypothesis cannot be verified easily since it appears difficult to isolate a single isoform on which biochemical analyses can be performed. On the other hand, we were able to assess the transcriptional regulation of the pma genes by analyzing their respective mRNA distribution in the different organs of the plant. pma transcripts were found in all organs analyzed: roots, leaves from both vegetative and flowering plants, stems, and flowers at two developmental stages. Thus, the PMA enzyme as a whole may be considered a housekeeping enzyme at the organ level at least.

Although the use of different probes prevented us from high divergence from the pma1–3 subfamily. This indicates the existence of a second pma subfamily whose composition and function are still unknown.
making a quantitative comparison of transcript levels between the different genes in a same organ, the distribution of mRNAs for pmal, pma2, and pma3 in the different organs clearly indicated differential expression. Besides the high RNA level observed in flower tissues for the three genes, pmal was mainly active in the stem, pma2 showed a similar transcript level in vegetative leaves and in the stem, and the highest activity for pma3 was observed in root tissues (Fig. 4). A. thaliana genes aha1 and aha2 have also been shown to be differently expressed in root and shoot tissues (Harper et al., 1990). In animal cells, it has been demonstrated that the genes encoding different isoforms of the a subunit of the Na+/K+·ATPase or the plasma membrane Ca2+-ATPase are differentially expressed in a tissue-specific manner (Orlowski and Lingrel, 1988; Burk et al., 1989; Greeb and Schull, 1989). Although, to our knowledge, the level of H+-ATPase in flower tissues has not been documented to date, the high pma transcript level in this organ is not really unexpected. The flower is a fast-developing sink organ which must receive metabolites from other parts of the plant. Thus H+-ATPase-dependent active transport may be well-developed in this organ. The comparative study of promoter regions linked to reporter genes such as uidA (β-glucuronidase), whose expression can be monitored in situ, will reveal the specific expression of the different pma genes at the tissue or cell level.

The size of the untranslated 5’ region of the pma messengers is much larger (120–270 nucleotides) than the mean size for a plant leader sequence (40–80 nucleotides, reviewed in Joshi (1987)). This observation is not specific to N. plumbaginifolia pma, since the determined length of the leader sequence of the A. thaliana aha2 gene is 133 nucleotides (Harper et al., 1990).

In addition, the pmal1 and pma3 leaders contain a small uORF, 5 (pma3) or 9 (pmal) amino acids long, located 42 or 61 nucleotides upstream from the ATG of the PMA ORF. These uORFs are in a good context for translation as defined by Kozak’s model, there being an A-3 and G+1 in pma3 and an A-3 and T+1 in pmal (Kozak, 1986; Kozak, 1989). The presence of a short uORF has also been observed in the leader sequences of the A. thaliana aha2 gene (Harper et al., 1990) and of the L. esculentum lha1 gene (Ewing et al., 1990) (Fig. 5). The latter case is interesting since the high sequence similarity between the L. esculentum lha1 and the N. plumb-
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FIG. 5. Sequence comparison of the leader sequences of N. plumbago-
aginifolia pma3 genes, A. thaliana aha2 and L. esculentum tha1 genes.
Gaps have been introduced in the lha1 sequence to optimize its alignment with
the pma3 sequence. Numbers indicate the position of the transcription start
sites (pma1 only the shortest is dis-
played), pma3, aha2 or (in parentheses) the 5’ boundary of the largest cDNA
clones (pma2, lha1). The 14-bp sequence
similar to both pma1, pma3, and lha1 is
double-underlined. Start of the PMA
coding sequence is indicated (+1).

uORFs are in boldface upper case and are
topped by the deduced amino acid se-
quence.

baginifolia pma3 (97.5 % identical at the protein level) clearly
indicates a recent common origin. Analysis of the two leader
sequences shows that the uORF is 100% identical, while the
surrounding sequence is less conserved except for a 14-bp
conserved sequence which is found upstream of the pma1,
pma3, and lha1 uORFs (Fig. 5). This observation suggests the
possible existence of regulation of the
pma genes at the
translational level, in which the uORF nucleotide or amino
acid sequence could be involved. The presence of long leaders
containing small uORFs has also been detected in the leader
of a few other plant genes and their possible involvement in
translational regulation has been proposed (for instance, see
Snustad et al. (1988), Hartings et al. (1989), Singh et al. (1990),
Schmidt et al. (1990)).

In conclusion, our data suggest both transcriptional and
translational regulation of plant pma genes. Regulation at the
protein level has also been documented (for review, see Suss-
man and Harper (1989)). Consequently, understanding how
the expression of the different pma genes is regulated within
a plant organism and how they participate in the physiology
of transport will require developing appropriate tools or
probes which are specific for each gene and for each regulatory
level.

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