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Tissue-Specific Expression of the Gene for a Putative Plasma Membrane H^+-ATPase in a Seagrass

Toshiyuki Fukuhara*, Jun-Yong Pak, Yoshinari Ohwaki, Hidenobu Tsujimura, and Takeshi Nitta

Laboratory of Molecular Cell Biology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwaicho 3–5-8, Fuchu, Tokyo 183, Japan (T.F., H.T., T.N.); Faculty of Education, Korea University, Kodaira, Tokyo 187, Japan (J.-Y.P.); and Japan International Research Center for Agricultural Science, Ishigaki, Okinawa 907, Japan (Y.O.)

A cDNA clone corresponding to the gene (ZHA1) for a putative plasma membrane H^+-ATPase of a seagrass (*Zostera marina* L.) was isolated and sequenced. Comparison of the amino acid predicted sequence from the nucleotide sequence of ZHA1 with those encoded by known genes for plasma membrane H^+-ATPases from other plants indicated that ZHA1 is most similar to the gene (PMA4) for a plasma membrane H^+-ATPase in a tobacco (84.4%). Northern hybridization indicated that ZHA1 was strongly expressed in mature leaves, which are exposed to seawater and have the ability of tolerate salinity; ZHA1 was weakly expressed in immature leaves, which are protected from seawater by tightly enveloping sheaths and are sensitive to salinity. In mature leaves, in situ hybridization revealed that ZHA1 was expressed specifically in epidermal cells, the plasma membranes of which were highly invaginated and morphologically similar to those of typical transfer cells. Therefore, the differentiation of the transfer cell-like structures, accompanied by the high-level expression of ZHA1, in the epidermal cells of mature leaves in particular may be important for the excretion of salt by these cells.

The majority of higher plants are sensitive to a high-salt environment. In particular, almost all crops are unable to tolerate saline conditions. Nevertheless, some unusual angiosperms, such as halophytes and seagrasses, are able to thrive in saline environments. These two classes of plants are morphologically quite different, since halophytes and seagrasses are able to thrive on saline soil and in seawater, respectively. They seem to adapt to salinity by different, independent mechanisms. There are many reports about mechanisms of salt tolerance in halophytes (e.g. *Atriplex nummularia* L. [Braun et al., 1986; Niu et al., 1993] and *Mesembryanthemum crystallinum* L. [Bohnert et al., 1988, 1994]). Most dicotyledonous halophytes adapt to salinity by accumulating inorganic ions in their vacuoles, and the osmotic potential of the cytoplasm is balanced by the synthesis and accumulation of biologically compatible solutes, such as Pro, betaine, sugars, or sugar alcohols (Hanson and Hitz, 1982; Jefferies and Rudmik, 1984; Rhodes and Hanson, 1993). By contrast, mechanisms of salinity tolerance in seagrasses have scarcely been studied to date.

Seagrasses, which consist of fewer than 100 species, are monocotyledonous angiosperms (Phillips and Menez, 1988). It was reported previously that nonspherical protoplasts obtained from mature leaves of a seagrass (*Zostera marina* L.) are highly resistant to a wide range of osmotic potentials and salinities. By contrast, spherical protoplasts isolated from meristematic and immature leaf tissues, which are protected from seawater by tightly enveloping sheaths, as well as protoplasts isolated from terrestrial plants, are more sensitive to salinity (Arai et al., 1991). These results suggest that the structure of plasma membrane of cells in the seawater-resistant mature leaves of the seagrass (*Z. marina*) might be morphologically and physiologically different from those of cells either in the seawater-sensitive immature leaves of the seagrass or in leaves of most terrestrial plants. Invaginated plasma membranes with a transfer cell-like structure and high ATPase activity were found in epidermal cells of the mature leaves by EM and cytochemical techniques, but neither feature was detected in the epidermal cells of the immature leaves of the seagrass (Pak et al., 1995). It is generally accepted that the invagination of plasma membranes of transfer cells increases the capacity for solute flux between the apoplast and symplast by increasing the surface area of the plasma membrane (Pate and Gunning, 1972; Gunning, 1977; Wimmers and Turgeon, 1991). Observations by EM and cytochemical staining, with lead specific for the hydrolysis of ATP, indicate that transfer cells are very active metabolically; their highly invaginated plasma membranes are associated with high ATPase activity, and it is likely that the numerous mitochondria provide the energy (ATP) required for active solute flux (Maier and Maier, 1972; Bentwood and Cronshaw, 1978). It is well established that the enzymes that are primarily responsible for the active transport of ions and nutrients in plants are the plasma membrane H^+-ATPases (Serrano, 1989a, 1989b; Sussman, 1994). Immunocytological procedures coupled with EM have revealed that the plasma membrane H^+-ATPase is more densely distributed in the invaginated plasma membranes of transfer cells than in the normal plasma membranes of other cells (Bouche-Pillon et al., 1994a, 1994b).

* Corresponding author; e-mail fuku@cc.tuat.ac.jp; fax 81–423–60–8830.

Abbreviations: nt, nucleotides; ORF, open reading frame; URF, upstream open reading frame.

In the seagrass Z. *marina*, adaptational (or developmental) changes in the epidermal cells of mature leaves, namely, the invagination of the plasma membrane and the high ATPase activity associated with the plasma membrane, seem to be responsible for the ability of the plant to tolerate high salinity. In this paper, we report that the gene for a putative plasma membrane H^+-ATPase is specifically expressed at a high level in the epidermal cells with transfer cell-like characteristics in the mature leaves. The relationship between the expression of this gene for a putative H^+-ATPase in the transfer cell-like epidermal cells and acquisition by the seagrass of salt tolerance is discussed.

MATERIALS AND METHODS

Plant Materials

Plants of *Zostera marina* L. were collected at Futtsu, in Chiba Prefecture, and washed several times with artificial seawater (Aqua Marine; Yashima Pure Chemical Co. Ltd., Osaka, Japan). The plants were used immediately for experiments.

Cloning of cDNA

Total RNA was purified from mature leaves of Z. *marina* by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987), and then cDNAs were transcribed from the total RNA by the method of Gubler and Hoffman (1983). The cDNA library was constructed in phage λgt10 (Stratagene) with Gigapak II packaging extracts (Stratagene). Forty thousand plaque-forming units were screened with the ^{32}P-labeled, 430-nucleotide fragment of pZM7 that contained the PCR-amplified DNA fragment of a gene for a putative plasma membrane H^+-ATPase gene from Z. *marina* (Pak et al., 1995), and about 50 positive clones were obtained.

DNA Sequencing and Homology Search

Six positive clones were subcloned into pBluescript II SK(+) (Stratagene), and both terminal regions of each insert were sequenced by the dideoxynucleotide chain-terminating method using a 7-deaza Sequenase, version 2.0, DNA-sequencing kit (United States Biochemical). Many deletion clones of one of the cDNA clones (pZM22) were made with an Exo/Mung deletion kit (Takara, Kyoto, Japan), and the complete nucleotide sequence was determined. Analysis of nucleotide and amino acid sequences and a homology search were performed using genetic information-processing programs (SDC-GENETYX; Software Development Co., Tokyo, Japan). A phylogenetic dendrogram was drawn using another genetic information-processing program (DNASIS; Hitachi, Tokyo, Japan).

Probes for Hybridization

Radiolabeled probes for Southern and northern hybridization were prepared from two DNA fragments (P1 and P2, indicated in Figs. 1 and 2) with a random primer-labeling kit (Takara) and [α-^{32}P]dCTP (Amersham). The P1 DNA fragment of 521 nt was obtained by PCR using cDNAs as templates and two 23-mer oligonucleotide primers [5′-CTIGTIATGAA(A/G)GGIGCICCIGA-3′ and 5′-GA(A/G)TC(A/G)TTIACICC-(A/G)TCICCIGT-3′] designed by reference to consensus amino acid sequences of the P-type ATPases (LVMKGAPE and TGDGVNDS). The cDNA region of pZM22 (3250 nt) contained two *Hin*dIII sites (at positions 2355 and 2956 from the 5′ end of the cDNA; Fig. 1). pZM22 was digested with *Hin*dIII, and then the digest was fractionated by agarose gel electrophoresis. The smallest DNA fragment (P2) of 294 nt, which corresponded to the 3′ terminal region of the cDNA and contained the 3′ noncoding region of 247 nt (Fig. 1), was isolated. The P1 probe was used for Southern hybridization, and both the P1 and P2 probes were used for northern hybridization.

A probe for in situ hybridization was labeled with digoxigenin-11-rUTP using a nucleic acid-labeling kit (Boehringer Mannheim), according to the manufacturer's protocol. The P1 fragment was inserted into pBluescript II SK(+), and then a recombinant plasmid was linearized with a restriction enzyme (*Bam*HI or *Eco*RI). Sense and antisense riboprobes were generated from linearized plasmids using T7 and T3 RNA polymerases (Boehringer Mannheim), respectively. It was confirmed by agarose gel electrophoresis and dot-blot hybridization that sense and antisense riboprobes were similar in length and specific activity.

Southern Hybridization

Total DNA from mature leaves of Z. *marina* was purified by the cetyltrimethylammonium bromide method (Rogers and Bendich, 1988) and digested with each of three restriction endonucleases (*Bam*HI, *Eco*RI, and *Hin*dIII). About 5 μg of each digested DNA were subjected to electrophoresis in a 1.0% agarose gel, and then fragments were transferred to a nylon membrane (Zeta-Probe GT, Bio-Rad) by the capillary transfer method. Hybridizations were carried out in hybridization medium (0.25 M sodium phosphate [pH 7.2], 1 mM EDTA, 7% SDS, 1% BSA, 1% Nonidet P-40) for 16 h at 65°C (high-stringency conditions) or 55°C (low-stringency conditions). The membranes were washed twice for 30 min each time with 20 mM sodium phosphate buffer (pH 7.2) that contained 5% SDS and washed twice again for 30 min each time at 65°C (high-stringency conditions) or 45°C (low-stringency conditions) with the same buffer that contained 1% SDS.

Northern Hybridization

Total RNA was prepared from mature leaves, immature leaves, flowers and seeds, and rhizomes of Z. *marina* by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). Five micrograms of each preparation of RNA were fractionated in a 1.2% agarose gel that contained 20 mM Mops (pH 7.0), 5 mM sodium acetate, 2 mM EDTA, 0.66 M formaldehyde, and 500 ng/mL ethidium bromide, and then bands of RNA were transferred to a nylon membrane (Zeta-Probe

GT) by the capillary transfer method. Hybridization and washing of the membranes were carried out under the same high-stringency conditions as used for the Southern hybridization.

In Situ Hybridization

Mature and immature leaves were fixed in 1% glutaraldehyde and 3% paraformaldehyde and embedded in wax, and sections were prepared for in situ hybridization as described by Jackson (1991). The hybridization solution contained 50% formamide, 300 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.25% SDS, 10% sodium dextran sulfate, 1× Denhardt's solution (0.02% Ficoll, 0.02% PVP, 0.02% BSA), and 0.2 μg/mL tRNA. Forty microliters of the hybridization solution containing about 100 ng of digoxigenin-labeled riboprobe were placed on each slide, and the slides were incubated for 16 h at 50°C in a box in an atmosphere of air saturated with 50% formamide. After hybridization, the slides were washed twice with 2× SSC (1× SSC = 150 mM NaCl and 15 mM sodium citrate) and 50% formamide for 30 min at 50°C, and then they were washed again with 500 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA for 10 min at 37°C. The unhybridized riboprobes were degraded by incubation for 30 min at 37°C in 500 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA plus 20 μg/mL RNase A. The slides were washed with 2× SSC and 0.2× SSC for 20 min each at 50°C. Immunological detection of the hybridized probe was carried out with a digoxigenin-nucleic acid detection kit (Boehringer Mannheim). Results shown below were obtained from the same experiment.

RESULTS

Structure of the Gene for a Putative Plasma Membrane H$^+$-ATPase of *Z. marina* (ZHA1)

About 50 positive plaques were obtained from the cDNA library prepared from mature leaves of *Z. marina* (40,000 plaque-forming units) during screening with a PCR-amplified DNA fragment as probe (Pak et al., 1995). Six positive clones were subcloned, and both terminal regions of each insert were sequenced. Since all six clones gave the identical nucleotide sequences, we concluded that they were identical cDNA clones that had been synthesized from mRNAs transcribed from a single gene. The complete nucleotide sequence of one clone (pZM22) revealed that the insert contained an ORF of 2856 nt that encoded 952 amino acid residues (Fig. 1). The amino acid sequence encoded by this ORF was most similar to PMA4 (Fig. 2A; 84.4%), which is a gene for a plasma membrane H$^+$-ATPase from a species of tobacco (*Nicotiana plumbaginifolia*; Moriau et al., 1993). The cDNA clone that we cloned and sequenced was designated ZHA1 (*Z. marina* H$^+$-ATPase), because the corresponding gene can be regarded as a gene for a plasma membrane H$^+$-ATPase from the results of comparisons of encoded sequences. Figure 2B shows a phylogenetic dendrogram deduced from the amino acid sequences encoded by 14 genes for plasma membrane H$^+$-ATPases of angiosperms. Although both seagrass and rice are monocotyledonous plants, ZHA1, OSA1, and OSA2 (rice) belong to different subfamilies of genes for plasma membrane H$^+$-ATPases.

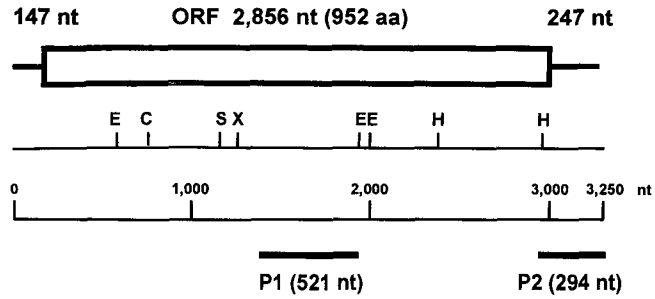

Figure 1. Genetic organization of pZM22 and a map of the DNA fragments used as probes for hybridizations. Restriction sites for selected endonucleases: C, *Cla*I; E, *Eco*RV; H, *Hin*dIII; S, *Sal*I; X, *Xho*I.

The length of the 5′ untranslated region of ZHA1 mRNA (147 nt) is greater than the mean size of a plant leader sequence (40–80 nt; Joshi, 1987). In addition, the leader sequence of ZHA1 includes a small URF (10 amino acid residues) located 39 nt upstream from the ATG codon of the ZHA1 ORF (Fig. 3). A long leader containing one or more URFs is commonly found in genes whose regulation has to be tightly controlled (Kozak, 1991). Such URFs are also found in several genes for plasma membrane H$^+$-ATPases (Fig. 3), and they seem to be involved in regulation of the translation of these genes (Michelet et al., 1994). The ZHA1 gene may be regulated at the translational level by the URF.

Genes for Plasma Membrane H$^+$-ATPases in the Seagrass

Southern hybridization under high- and low-stringency conditions was performed with total DNA from *Z. marina* and the P1 fragment of pZM22 as probe (Fig. 1). Only one or two hybridization bands in each lane were detected under high-stringency hybridization conditions (Fig. 4A), but several bands were detected under low-stringency conditions (Fig. 4B). These results indicate that several genes for plasma membrane H$^+$-ATPases exist in the genome of *Z. marina*. It has been reported that there are at least 4 genes for plasma membrane H$^+$-ATPases in tobacco (Perez et al., 1992), at least 7 genes for H$^+$-ATPases in tomato (Ewing and Bennett, 1994), and more than 10 genes for H$^+$-ATPases in *Arabidopsis thaliana* (Sussman, 1994). These results also indicated that the bands specific for ZHA1 could be detected by the P1 probe under high-stringency hybridization conditions.

The Expression of the Gene for a Plasma Membrane H$^+$-ATPase (ZHA1)

The detection of about 50 positive plaques from a cDNA library of 40,000 clones indicated that the level of expression of the ZHA1 gene was very high. The level of expression of the ZHA1 gene seems to be higher than those of genes for plasma membrane H$^+$-ATPases in other higher plants: only 5 clones of LHA1, for example, were isolated

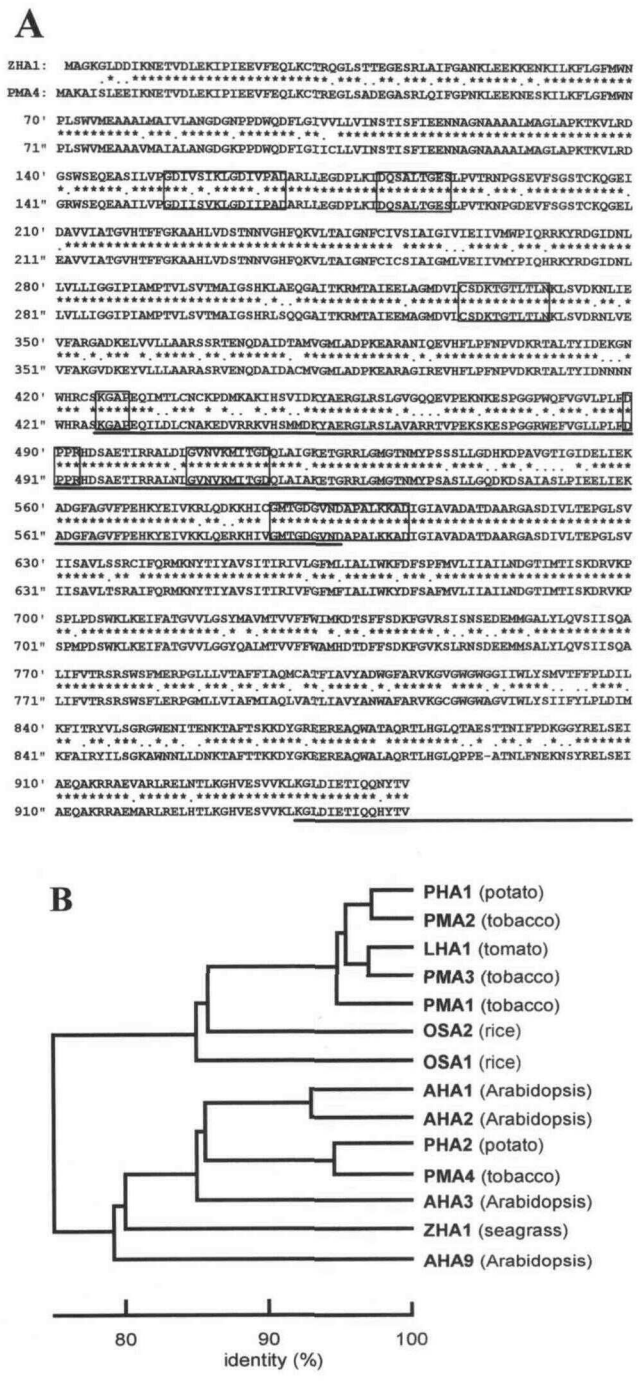

Figure 2. A, Alignment of the predicted amino acid sequences of plasma membrane H^+-ATPases from *Z. marina* (ZHA1; upper lines) and *N. plumbaginifolia* (PMA4; lower lines). Highly conserved regions in P-type ATPases (Serrano, 1989b) are boxed, and the regions to which the P1 and P2 probes (see Fig. 1) were constructed are underlined. B, A phylogenetic dendrogram deduced from the amino acid sequences encoded by 14 genes for plasma membrane H^+-ATPase of angiosperms. Sequences from *A. thaliana* (AHA; Harper et al., 1989, 1990; Pardo and Serrano, 1989; Houlne and Boutry, 1994), *Lycopersicon esculentum* (LHA; Ewing et al., 1990), *Oryza sativa* (OSA; Wada et al., 1992; Ookura et al., 1994), *Solanum tuberosum* (PHA; Harms et al., 1994), *N. plumbaginifolia* (PMA; Boutry et al., 1989; Perez et al., 1992; Moriau et al., 1993), and *Z. marina* (ZHA; this work) were compared.

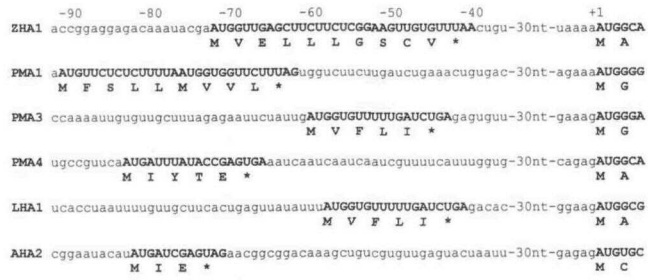

Figure 3. Comparison of the leader sequences of the ZHA1 gene (*Z. marina*), PMA genes (*N. plumbaginifolia*), the LHA1 gene (*L. esculentum*), and the AHA2 gene (*A. thaliana*). The start of the coding sequences in the genes for plasma membrane H^+-ATPases is indicated as +1. The nucleotide and deduced amino acid sequences of the URFs and the ORFs are indicated by boldface letters. The termination codon of the URFs is indicated by an asterisk.

from 250,000 independent clones of cDNA from tomato root (Ewing et al., 1990).

To characterize the expression of the ZHA1 gene in seagrass plants, we performed northern hybridization with total RNA isolated from various organs of *Z. marina* plants using two cDNA fragments (P1 and P2) as probes. Since the P2 probe corresponds to 294 nt in the 3′ terminal region of ZHA1 (pZM22) and includes 247 nt of the 3′ untranslated region (Fig. 1), the expression of ZHA1 seemed to be distinguishable from that of other genes for plasma membrane H^+-ATPases when P2 was used as probe. Figure 5 shows that ZHA1 was strongly expressed in mature leaves, which are exposed to seawater and have the ability to tolerate high salinity. By contrast, ZHA1 was weakly expressed in immature leaves, which are protected from seawater by tightly enveloping sheaths and are sensitive to salinity (Arai et al., 1991). We failed to detect the expression of the ZHA1 gene in rhizomes, flowers, and seeds. Because the result using the P1 probe (Fig. 5B) was very similar to that using the P2 probe (Fig. 5C), the P1 probe also could detect the only ZHA1-specific transcript under the same high-stringency conditions as used for Southern hybridization. The ZHA1 gene was expressed in an or-

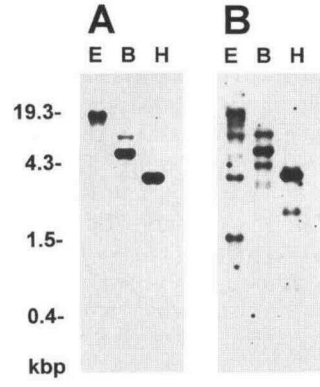

Figure 4. Southern blot analysis of genes for the plasma membrane H^+-ATPases of *Z. marina*. Five micrograms of *Z. marina* genomic DNA digested with *Bam*HI (lane B), *Eco*RI (lane E), or *Hin*dIII (lane H) were subjected to electrophoresis and blotted. A, High-stringency conditions; B, low-stringency conditions. Hybridization conditions and probes are described in "Materials and Methods."

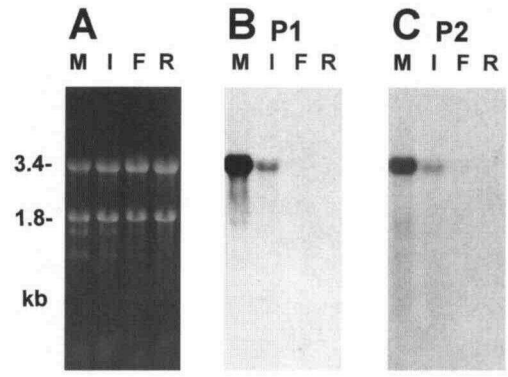

Figure 5. Northern blot analysis of the expression of ZHA1. Five micrograms of total RNA extracted from mature leaves (lane M), immature leaves (lane I), flowers and seeds (lane F), and rhizomes (lane R) were fractionated by agarose gel electrophoresis and stained with ethidium bromide (A). Then RNAs were transferred to nylon membranes and allowed to hybridize with the P1 probe (B) or the P2 probe (C).

gan-specific manner, being specifically expressed in mature leaves. The level of expression of the ZHA1 gene in leaves of *Z. marina* increased markedly when leaves were exposed to seawater and acquired the ability to tolerate high salinity. Thus, the expression of the ZHA1 gene was adaptationally (and/or developmentally) regulated in response to salinity.

The expression of the ZHA1 gene in mature and immature leaves of *Z. marina* was further investigated by in situ hybridization. Since the P1 probe was used under high-stringency conditions for in situ hybridization, the results shown in Figure 6 must be specific for the ZHA1 transcript. In mature leaves, the ZHA1 gene was specifically expressed in epidermal cells (Fig. 6, A and C), the plasma membranes of which were highly invaginated and morphologically similar to those of typical transfer cells in the vascular systems of various higher plants (Pak et al., 1995). In immature leaves, by contrast, the ZHA1 gene was expressed in the cells that formed vascular bundles but not in epidermal cells (Fig. 6, B and E) that did not have an invaginated plasma membrane (Pak et al., 1995). The expression of the ZHA1 gene in *Z. marina* leaves was tissue specific (cell specific), and the expression in epidermal cells was apparently correlated with the invagination of the plasma membrane. It is likely that the invagination of the plasma membrane of the epidermal cells increases the capacity for excretion of salts by increasing the surface area of the plasma membrane and allows adaptation to the marine environment. Hence, it is reasonable that the high-level expression of the ZHA1 gene was found in the epidermal cells of mature leaves (Fig. 6, A and C), which have invaginated plasma membranes and in which high ATPase activity was detected by EM and cytochemical techniques (Pak et al., 1995).

DISCUSSION

Almost all contemporary terrestrial plants lost the ability to tolerate salt during the course of their evolution from marine algae. However, a few species of angiosperms, namely, seagrasses, have reacquired salt-tolerance mechanisms that allow them to thrive in seawater. Thus, ability to thrive in seawater is one of the most prominent examples of adaptation in higher plants. Such adaptation must be accompanied by morphological and physiological changes in seagrass plants, and these changes must be genetically regulated.

It was reported previously that the epidermal cells of mature leaves of *Z. marina* change morphologically and physiologically in response to salinity; cells isolated from immature leaf tissues that are protected from seawater by tightly enveloping sheaths are sensitive to salinity, whereas mature leaf cells with the morphological characteristics of transfer cells are resistant to salinity (Arai et al., 1991). It is generally accepted that transfer cells are highly active in transporting various ions and nutrients and the invaginated plasma membranes of typical transfer cells are associated with high levels of ATPase activity (Maier and Maier, 1972; Gunning, 1977; Bentwood and Cronshaw, 1978; Wimmers and Turgeon, 1991). Plasma membrane H^+-ATPases are responsible for creating an electrochemical proton gradient (proton-motive force), which is used for the transport of ions and nutrients that is mediated by specific carriers and channels (secondary transport; Sussman, 1994). The high degree of invagination of the plasma membrane of transfer cells increases the surface area of the membrane and the number of these H^+-ATPases, carriers, and channels (Bouche-Pillon et al., 1994a, 1994b). Therefore, the differentiation of the transfer cell-like structures, accompanied by high-level expression of the gene for a putative plasma membrane H^+-ATPase (ZHA1), in the epidermal cells of mature leaves (Figs. 5 and 6) may be important for the excretion of various ions by these cells. These specifically differentiated epidermal cells with their novel features in mature leaves may play a crucial role in the survival of the seagrass in seawater.

There are two types of H^+-translocating ATPase in higher plants, namely, the tonoplast H^+-ATPase and the plasma membrane H^+-ATPase. They have quite different structures, and their genes belong to different families. When many terrestrial plants, including halophytes, are subjected to salt stress, the tonoplast ATPase seems to play a more important role in tolerance to salinity than the plasma membrane ATPase, because the tonoplast ATPase is responsible for sequestering various deleterious ions in the large vacuoles (Binzel et al., 1988; Reuveni et al., 1990; Narasimhan et al., 1991). Such plants synthesize and accumulate biologically compatible solutes in the cytoplasm to balance the osmotic potential of the vacuole (Hanson and Hitz, 1982; Rhodes and Hanson, 1993). In the epidermal cells of mature leaves of *Z. marina*, by contrast, an invaginated plasma membrane with high ATPase activity was observed by EM (Pak et al., 1995), and large amounts of the mRNA for a putative plasma membrane H^+-ATPase (ZHA1) were detected by in situ hybridization (Fig. 6). Therefore, the seagrass seems to have acquired a different mechanism for salt tolerance from those of most terrestrial halophytes, and the plasma membrane H^+-ATPase may play a more important role than the tonoplast enzyme in the survival and growth of the seagrass in seawater.

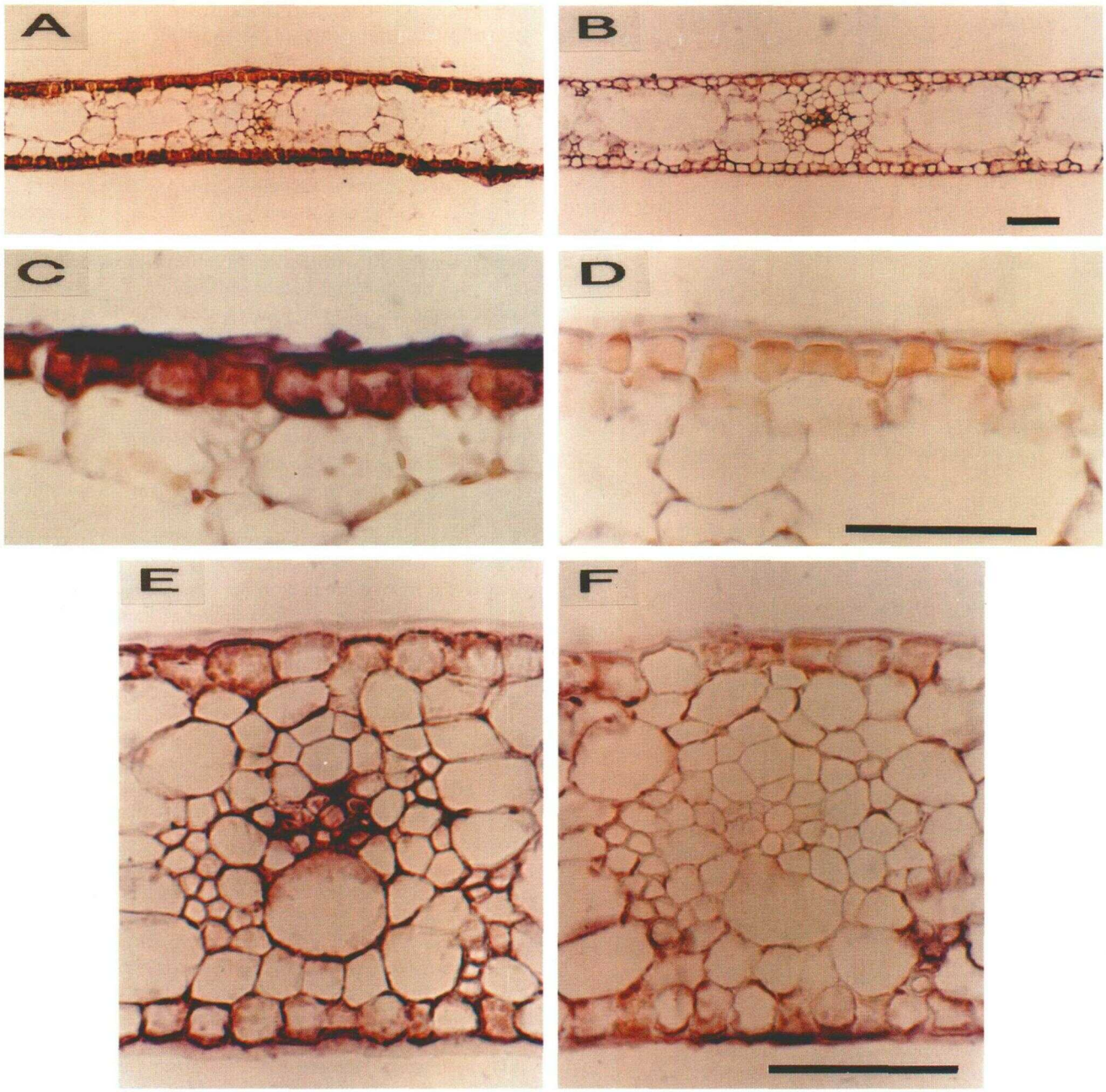

Figure 6. In situ localization of ZHA1 transcripts in cross-sections of *Z. marina* leaves. A, Mature leaf hybridized with the ZHA1 antisense RNA probe. B, Immature leaf hybridized with the ZHA1 antisense RNA probe. C, Epidermal cells in a mature leaf hybridized with the ZHA1 antisense RNA probe. D, Epidermal cells in a mature leaf hybridized with the ZHA1 sense RNA probe. E, Immature leaf hybridized with the ZHA1 antisense RNA probe. F, Immature leaf hybridized with the ZHA1 sense RNA probe. Bars = 50 μm. The scale in B is the same for A; the scale in D is the same for C, E, and F.

It is now clear that the plasma membrane H^+-ATPases of higher plants are encoded by a multigene family. In *A. thaliana*, for example, an approach using PCR has suggested the existence of more than 10 genes (Sussman, 1994). It has been reported that multiple genes for H^+-ATPases in plants allow the expression of tissue-specific isozymes in specialized cell types. In *A. thaliana*, it has been reported that the AHA3 and AHA9 genes are specifically expressed in the phloem of vegetative tissue (DeWitt et al., 1991) and in anther tissue (Houlne and Boutry, 1994), respectively. Sussman (1994) proposed the hypothesis that each of the AHA genes is expressed in a specific type of cell with unique transport functions. In the seagrass *Z. marina*, one gene (ZHA1) among several genes for plasma membrane H^+-ATPases was strongly expressed in epidermal cells of mature leaves, which are

morphologically similar to transfer cells, but it was not expressed in rhizomes, flowers, and seeds. The product of the ZHA1 gene may be an unusual plasma membrane H^+-ATPase whose function allows the seagrass to thrive in seawater, and its structure and activity may be different from those of plasma membrane H^+-ATPases in terrestrial higher plants. Other genes for plasma membrane H^+-ATPases must be expressed in other tissues and organs (e.g. rhizomes and flowers), and the isolation of other genes for H^+-ATPases in the seagrass is now in progress.

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