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An *Arabidopsis* syntaxin homologue isolated by functional complementation of a yeast *pep12* mutant

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ABSTRACT **The syntaxin family of integral membrane proteins are thought to function as receptors for transport vesicles, with different isoforms of this family localized to various membranes throughout the cell. The yeast Pep12 protein is a syntaxin homologue which may function in the trafficking of vesicles from the trans-Golgi network to the vacuole. We have isolated an *Arabidopsis thaliana* cDNA by functional complementation of a yeast *pep12* mutant. The *Arabidopsis* cDNA (*aPEP12*) potentially encodes a 31-kDa protein which is homologous to yeast Pep12 and to other members of the syntaxin family, indicating that this protein may function in the docking or fusion of transport vesicles with the vacuolar membrane in plant cells. Northern blot analysis indicates that the mRNA is expressed in all tissues examined, although at a very low level in leaves. The mRNA is found in all cell types in roots and leaves, as shown by *in situ* hybridization experiments. The existence of plant homologues of proteins of the syntaxin family indicates that the basic vesicle docking and fusion machinery may be conserved in plants as it is in yeast and mammals.**

Soluble plant vacuolar proteins are synthesized with an N-terminal signal sequence on endoplasmic reticulum-bound ribosomes and cotranslationally translocated into the lumen of the endoplasmic reticulum. They then traverse the secretory pathway to the trans-Golgi network, where they are sorted from secreted proteins and transported to the vacuole in membrane-bound vesicles (1). Vacuolar targeting requires sorting information, additional to the signal sequence, within the amino acid sequence of the protein. Plant vacuolar targeting signals have been found in a number of proteins and three classes of signal have been identified: N-terminal propeptides (2, 3), C-terminal propeptides (4, 5), and regions of the mature protein (6, 7).

While a substantial amount of information is now available about the sorting signals contained in these proteins, very little is known about the mechanisms by which they are sorted from the default pathway of secretion and transported to the vacuole. A potential sorting receptor for proteins with N-terminal propeptides has been isolated, which is able to bind to synthetic peptides corresponding to N-terminal propeptides but not to a C-terminal propeptide (8). Also, the vacuolar targeting of proteins containing a C-terminal propeptide is sensitive to the fungal metabolite wortmannin, whereas sorting of those containing an N-terminal propeptide is relatively insensitive to this inhibitor (41). This indicates that different classes of sorting signals probably utilize different receptor proteins and possibly different transport mechanisms. However, N-terminal-propeptide- and C-terminal-propeptide-containing proteins have been shown to reside in the same vacuole in transgenic tobacco plants (9). It is unknown whether different classes of proteins are transported in the same vesicle, or whether subsets of vesicles exist, each of which is responsible for carrying certain proteins. In addition, plant vacuolar targeting signals do not function in yeast (10), indicating that there are differences in the mechanisms of sorting to the vacuole between these organisms.

Recently, families of proteins have been identified in various organisms which are involved in transport vesicle targeting and fusion with their target membrane. Many of these proteins were identified as components of synaptic vesicles and the presynaptic membrane of mammalian neuronal cells and are thought to be involved in synaptic vesicle fusion during neurotransmitter release (11, 12). These include the presynaptic membrane proteins syntaxin and 25-kDa synaptosomal-associated protein (SNAP-25) and the synaptic vesicle protein synaptobrevin/vesicle-associated membrane protein. These proteins were shown to form a 20S complex which also included the soluble proteins *N*-ethylmaleimide-sensitive factor (NSF) and soluble NSF-attachment protein (SNAP), and a model (the SNARE hypothesis) was proposed in which this complex mediates the docking and fusion of the vesicle with the plasma membrane (13). Homologues of these proteins are present in yeast and are found at several stages of the secretory pathway. The soluble factors NSF and SNAP seem to be common components found at each stage of the pathway, whereas a different isoform of syntaxin and synaptobrevin is thought to exist for each vesicle fusion step. The membrane components could thus ensure the fidelity of the vesicle targeting and fusion reaction by the specific interaction between a syntaxin isoform (t-SNARE) on the target membrane and a synaptobrevin isoform (v-SNARE) on the vesicle. The synaptobrevin isoform on a vesicle destined for the plasma membrane, for example, should therefore interact with the syntaxin at the plasma membrane and not with syntaxins at any other membrane of the cell.

The yeast *pep12* (for peptidase) mutant was identified as lacking vacuolar carboxypeptidase Y (CPY) activity (14) and found to contain a mutation in a gene required for vacuolar protein targeting. This mutation causes secretion of the CPY protein, thus preventing its activation, as activation occurs in the vacuole (15). The *PEP12* gene encodes a protein, Pep12, which is homologous to mammalian and yeast syntaxins (16) and resides on the yeast endosomal or vacuolar membrane (ref. 11; E. Jones, personal communication). Pep12 is therefore thought to be a receptor for vesicles targeted to the yeast vacuole.

By functional complementation of the yeast *pep12* mutant, we have isolated an *Arabidopsis thaliana* cDNA which encodes a protein homologous to Pep12 and to other members of the syntaxin family. This protein may thus be a component of the

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Abbreviations: APE, *N*-acetyl-DL-phenylalanine β-naphthyl ester; CPY, carboxypeptidase Y; NSF, *N*-ethylmaleimide sensitive factor; SNAP, soluble NSF-attachment protein; SNAP-25, synaptosomal-associated protein of 25 kDa.

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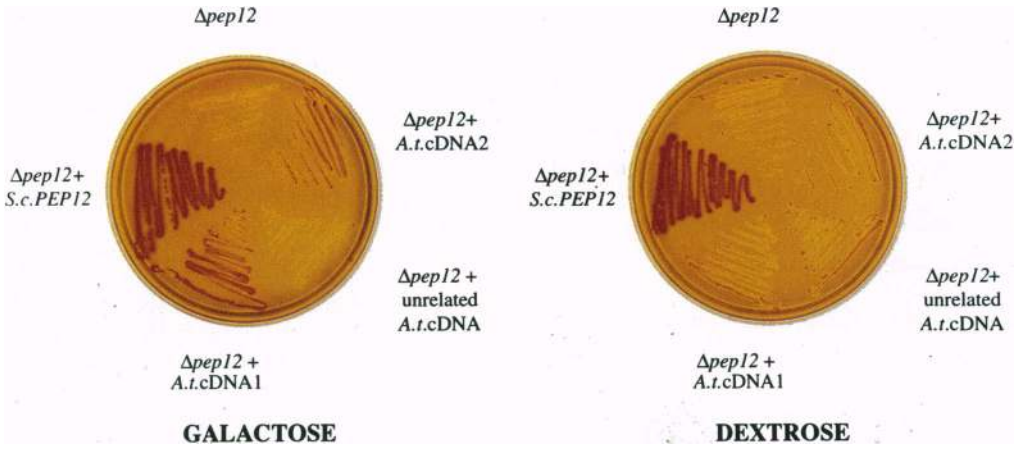

FIG. 1. Complementation of a yeast *pep12* mutant by *A. thaliana* cDNAs. The Δ*pep12* mutant was transformed with the yeast *PEP12* gene or *A. thaliana* cDNAs (*A.t.*cDNA1 and -2). CPY activity was assayed by the APE overlay test. Colonies containing CPY activity are red, whereas colonies lacking CPY activity remain yellow.

recognition and/or fusion apparatus for vesicles targeted to the plant vacuole.‡

MATERIALS AND METHODS

Yeast Strain, cDNA Library, and Growth Conditions. The *A. thaliana* λYES cDNA expression library, containing cDNAs under the control of the *GAL1* galactose-inducible promoter (17), was obtained from R. W. Davis (Stanford University, Stanford, CA). The library was used to transform (18) the yeast strain BJ4932 (*MATα, trp1, ura3-52, Δpep12::TRP1*; provided by E. Jones, Carnegie Mellon University, Pittsburgh). The yeast *PEP12* gene in the YCp50 shuttle vector (ref. 19; provided by E. Jones) was used as a control.

Isolation of an *A. thaliana* cDNA Which Complements the Yeast *pep12* Mutation. Yeast transformants were grown on SC (synthetic complete medium) plates minus uracil and transferred to YP (yeast extract/peptone medium) plates (20) containing dextrose or galactose. Colonies were then assayed for CPY activity by the *N*-acetyl-DL-phenylalanine β-naphthyl ester (APE) overlay test (21). APE (Sigma) at 1 mg/ml in dimethylformamide was mixed with 1.5 volumes of molten 0.6% agar, poured over the plates, and allowed to set. The surface of the agar was then covered with a solution of fast garnet GBC (Sigma; 5 mg/ml in 0.1 M Tris·HCl, pH 7.5) for ≈10 min. Colonies containing CPY activity were identified by their dark red color, compared with yellow Cpy⁻ colonies. DNA was isolated from these transformants and the cDNA insert was subcloned into pBluescript KS(−) plasmid (Stratagene). The cDNA was sequenced with Sequenase (United States Biochemical) by the method of Sanger *et al.* (22). Amino acid sequence alignments were generated with the PILEUP program (Genetics Computer Group).

Nucleic Acid Blots. *A. thaliana* ecotype RLD were grown as described (23). Genomic DNA was isolated from mature leaves (24). RNA was isolated from leaves, inflorescence stems, flowers, and roots from *A. thaliana* ecotype RLD as described (23). Southern and Northern blots were prepared (25) and probed with a ^{32}P-labeled *Hin*dIII DNA fragment containing the coding region of the cDNA. The probe was synthesized with [α-^{32}P]dATP (Amersham) by using the Klenow fragment of DNA polymerase (Boehringer Mannheim) and random hexanucleotide primers.

RNA *in Situ* Hybridization. To synthesize *aPEP12* antisense and sense RNA probes by *in vitro* transcription, the 3′ untranslated region and the poly(A) tail of the full-length cDNA clone were deleted. The probe labeling was performed as described in the DIG RNA labeling kit from Boehringer Mannheim. The protocol for *in situ* hybridization described by De Block and Debrouwer (26) was used with minor modifications (23, 27). Plants were grown as described for nucleic acid blots.

RESULTS

Isolation of an *A. thaliana* cDNA by Complementation of a Yeast *pep12* Mutant. Mutations in the yeast *PEP12* gene cause the secretion of protease precursors such as CPY which are normally located in the vacuole (14, 15). Secreted CPY is inactive and restoration of CPY activity thus provides a convenient assay for complementation of the *pep12* mutant. A yeast strain containing a *PEP12* deletion (Δ*pep12*) was transformed with an *A. thaliana* cDNA yeast expression library (17). The colonies generated were transferred to plates containing galactose to induce expression of the cDNAs from the *GAL1* promoter (17) and assayed for CPY activity by the APE plate assay (21). In this assay, colonies are overlaid with agar containing both dimethylformamide to permeabilize the cells and APE, which is a substrate for CPY. The β-naphthol produced by CPY reacts with fast garnet GBC, producing an insoluble red dye. Colonies containing CPY activity are therefore red, whereas colonies lacking CPY activity are yellow.

After screening 60,000 colonies, we obtained two independent transformants which complemented the CPY-deficient phenotype of the Δ*pep12* mutant (Fig. 1). Isolation of the cDNA from these colonies and retransformation into the *pep12* mutant confirmed that complementation of the *pep12* phenotype was due to the presence of the cDNA. Restoration of CPY activity was confirmed in these transformants by their ability to cleave *N*-benzoyl-L-tyrosine *p*-nitroanilide (ref. 21; data not shown), which is a substrate for CPY. Restriction enzyme analysis of the two complementing cDNAs demonstrated that they were derived from the same gene.

Sequence Analysis of a Complementing cDNA. One of the cDNAs (cDNA1 in Fig. 1) was chosen for further analysis. The sequence of the cDNA was determined by the method of Sanger *et al.* (22). An open reading frame which potentially codes for a 31-kDa protein containing 279 aa was identified. An alignment of the predicted amino acid sequence with the yeast Pep12 protein sequence (Fig. 2) indicated that the proteins share 29% identity over their entire length and 44% similarity when conservative substitutions are allowed. The *Arabidopsis* cDNA was designated *aPEP12*. The C-terminal

‡The sequence reported in this paper has been deposited in the GenBank database (accession no. L41651).

```
      1                                                          50
aPep12 MSFQDLEAGT RSPAPNRFTG GRQQRPSSRG DPSQEVAAGI FRISTAVNSF
       ||  :   |          : |  |             ||||  : | |    : :
yPep12 MSEDEFFGGD NEGV...WNG SRFSDSPEFQ TLKEEVAAEL FEINGQISTL

      51                                                         100
aPep12 FRLVNS.... IGTPKDTLEL RDKLQKTRL. ...QISELVK ..NTSAKLKE
          :     |      :  :  ::::|  :       |  |:|    ||| |  :
yPep12 QQFTATLKSF IDRGDVSAKV VERINKRSVA KIEEIGGLIK KINTSVKKMD

      101                                                        150
aPep12 ASEADLHGSA SQIKKIADAK LAKDFQSVLK EFQKAQRLAA EREITYTPV.
       | |     |        ||  |  | :|         |||  ||          :| |
yPep12 AIE...EASL DKTQIIAREK LVRDVSYSFQ EFQGIQR...  ....QFTQVM

      151                                                        200
aPep12 ..VTKEIPTS YNAPELDTES LRISQQQALL LQSRRQEV.. ..........
         |       |   | |:  ::         |||     ||
yPep12 KQVNERAKES LEASEMANDA .......ALL DEEQRQNSSK STRIPGSQIV

      201                                                        250
aPep12 ....VFLDNE ITFNEAIIEE REQGIREIED QIRDVNGMFK DLALMVNHQG
               |  :::  :||  |:| |  ||   | ::| :|| ||  :|: ||
yPep12 IERDPINNEE FAYQQNLIEQ RDQEISNIER GITELNEVFK DLGSVVQQQG

      251                                                        300
aPep12 NIVDDISSNL DNSHAATTQA TVQLRKAAKT QRSNSSL.TC LLILIFGIVL
        :|| | :|:   :   |  |  :  |||| :  |:  |      |||::  ::|
yPep12 VLVDNIEANI YTTSDNTQLA SDELRKAMRY QKRTSRWRVY LLIVLLVMLL

      301
aPep12 LIVIIVVLV
        | :|: |
yPep12 FIFLIMKL.
```

FIG. 2. Sequence comparison between *Arabidopsis* and yeast Pep12 proteins. Identical residues are indicated by vertical lines, and conservative substitutions by colons. Dots mark gaps introduced for maximum homology; numbering includes gaps. aPep12 and yPep12 (ref. 14; Swiss-Prot accession no. P32854) are the *Arabidopsis* and yeast Pep12 proteins, respectively. Sequences were aligned with the Genetics Computer Group (Madison, WI) sequence analysis software package (version 7.0).

domains are the most highly conserved between different syntaxins (28) and have been shown to be involved in interactions with synaptobrevin and SNAP-25 (29). An amino acid sequence alignment between the C-terminal 100 aa of aPep12 and various other members of the syntaxin family is shown in Fig. 3. The aPep12 protein displays 36% identity and 59% similarity to yeast Pep12 over this region, with similarities to other syntaxins of around 45%. This identifies aPep12 as a member of the syntaxin protein family, with the highest homology to the yeast Pep12 protein.

Hydropathy plots of the yeast and *Arabidopsis* Pep12 proteins were generated by the method of Kyte and Doolittle (ref. 33; data not shown) and similarities were apparent between the two proteins. Both proteins are hydrophilic, with the exception

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aPep12 NEITFNEAI.IEEREQGIREIEDQIRDVNGMFKDLALMVNHQGNIVDDISSNLD
yPep12 E|F:::QN:.||Q|:|E|SN||RG|T::|E:|||GS:|:Q||V:||N|E:|:Y
Sso1   ..........:QA|H|E:LK:|KS:A::TQ:|N|:EE:|IE|QEN||V|DK|::
Sed5   E|GQLSNN:Y:Q||NRA:ET||ST|Q:|GN:|QQ||S||:E||E::QR|D:|:|
Epim   ..........||S|HKD|MK:|TS|||::HE||M|:|:F|ET||E::NN|ER|:M
Syn1a  ..........||T|HSE|IK:|NS|||::HD||M|:|::|ES||E::|R|EY|::

aPep12 NSHAATTQATVQLRKAAKTQRSNSSL.TCLLILIFGIVLLIVIIVVLV......
yPep12 T:SDN|QL|:DE|||M:Y|:RT|RWRVY|||::LV::|F|F:|:K|.......
Sso1   D:QLDVE|GVGHTD||V|:A|KARKNKIRCW:::|A|::::|::||:PAVVKTR
Sed5   DIDLNI:G|QRE|L|YFDRI:||RW|A:K:FF:||VFF::W|::N.........
Epim   |:TDYVEH|KEET:||I|Y|SKARRKKWI..|:AVS:||:::|::::GLSVGK.
Syn1a  H:VVDYER|VSDT:||V|Y|SKARRKKIM..|:|CC::|G|:|AST:GGIFG..
```

FIG. 3. Sequence comparison between the C-terminal regions of syntaxin homologues. The last 100 aa of aPep12 are compared with the equivalent regions of other proteins. Vertical lines represent residues identical to aPep12; colons represent conservative substitutions. Dots mark gaps introduced into the sequence to allow maximum homology. aPep12 and yPep12 are as in Fig. 2. Sso1 (ref. 28; Swiss-Prot accession no. P32867) and Sed5 (ref. 30; Swiss-Prot accession no. Q01590) are from yeast. Epim represents human epimorphin (ref. 31; Swiss-Prot accession no. P32856) and Syn1a represents rat syntaxin 1a (ref. 32; Swiss-Prot accession no. P32851). The sequences were aligned as described in Fig. 2.

of a short hydrophobic region at their extreme C termini which may act as a membrane anchor. These are general features of members of the syntaxin family (16).

Southern Analysis of the *A. thaliana aPEP12* Gene. To investigate the number of *aPEP12*-related genes in *Arabidopsis*, Southern analysis was performed with a *Hin*dIII fragment of the *aPEP12* cDNA containing the coding region as a probe (Fig. 4). The pattern of hybridizing bands was found to be similar in the Columbia and RLD ecotypes of *A. thaliana*, and RLD was used for the work described here. *Eco*RI, *Hin*dIII, *Hin*cII and *Xba* I do not cut within the sequence of the probe. After digestion of *A. thaliana* (ecotype RLD) genomic DNA with *Eco*RI, *Hin*dIII, or *Hin*cII, a single band was observed upon hybridization with a ^{32}P-labeled probe containing the *aPEP12* coding region, as would be expected for a single gene copy. However, after digestion using *Xba* I, a very weakly hybridizing band was present as well as a strongly hybridizing higher molecular weight band. There is one *Xho* I site within the probe sequence but it is only 6 bp from the 5′ end of the cDNA and therefore the probe would be expected to hybridize with only one band. However, two bands of equal intensity were observed. There is one *Eco*RV site within the probe sequence, and two hybridizing bands were seen upon digestion of the genomic DNA with this enzyme, as expected for a single-copy gene. These results suggest that the *aPEP12* gene contains introns (*Xba* I and *Xho* I digests), although we cannot rule out the possibility of a second related gene.

mRNA Distribution of *aPEP12*. To determine the pattern of *aPEP12* expression in different organs of *Arabidopsis*, an RNA hybridization analysis was performed. A Northern blot of total RNA from leaves, roots, inflorescence stems, and flowers was hybridized with a radiolabeled probe containing the *aPEP12* coding region. A single band of ≈1.2 kb was detected in all tissues tested, although the intensity of the band varied between tissues. The level of *aPEP12* mRNA was highest in roots, with a very low level observed in leaves (Fig. 5). A more detailed analysis of the amount of *aPEP12* mRNA in leaves indicated that the transcript levels remain low at different stages throughout leaf development (data not shown).

The expression of *aPEP12* throughout all plant tissues (Fig. 5), although at a low level in leaves, is consistent with a general function for this syntaxin homologue in plant cells. To characterize further the expression pattern of *aPEP12* in vegetative tissues and to determine whether the low level in leaves was the result of expression in only certain cell types, RNA *in situ*

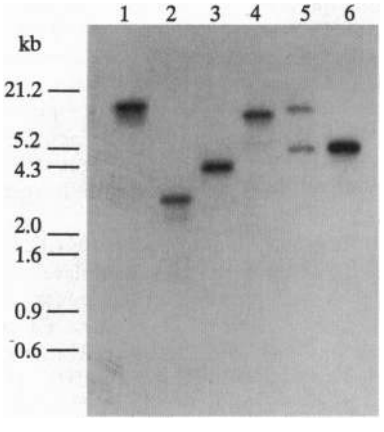

FIG. 4. Southern blot analysis of the *aPEP12* gene. Genomic DNA (10 μg) from *A. thaliana* was digested overnight with *Eco*RI (lane 1), *Eco*RV (lane 2), *Hin*dIII (lane 3), *Xba* I (lane 4), *Xho* I (lane 5), or *Hin*cII (lane 6). The digestion products were separated by electrophoresis in an agarose gel and transferred onto a nylon membrane. The membrane was hybridized with a ^{32}P-labeled *Hin*dIII fragment of cDNA containing the *aPEP12* coding region. Positions of DNA size markers are indicated at left.

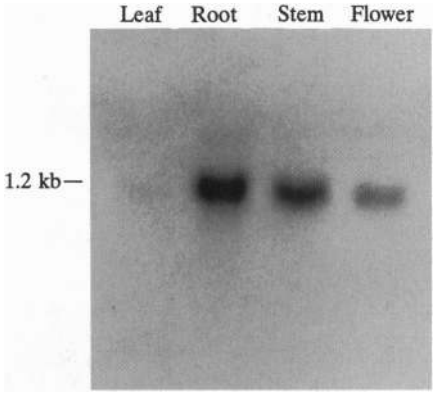

FIG. 5. Northern blot analysis of *aPEP12* transcripts. Total RNA (30 μg) from leaves, roots, stems, and flowers was separated in a 1.2% agarose/6% formaldehyde gel and blotted onto nylon membrane. The membrane was hybridized with a ^{32}P-labeled *Hin*dIII fragment of cDNA containing the *aPEP12* open reading frame. The estimated size of the hybridizing band is indicated.

hybridizations were performed. Paraffin-embedded sections of rosette leaves of plants growing in soil and roots of plants grown in liquid culture were hybridized with antisense or sense RNA probes. As shown in Fig. 6, the aPEP12 transcript was uniformly distributed throughout the leaf and root tissues. This result demonstrates that the *aPEP12* mRNA, in addition to being transcribed and accumulated in all *Arabidopsis* tissues tested, is also constitutively expressed at the cellular level.

DISCUSSION

The yeast *PEP12* gene product is thought to be involved in vesicular transport from the trans-Golgi network to the vacuole. We have isolated a cDNA from *A. thaliana* encoding a 31-kDa protein by complementation of a yeast *pep12* mutant. This protein shows homology to the yeast Pep12 protein and to various syntaxins, indicating that it is a member of the syntaxin/epimorphin family of proteins. Syntaxins are involved in vesicular transport through the endomembrane system and have been proposed to act as receptors for transport vesicles by forming specific docking complexes with other target membrane proteins, vesicle membrane proteins, and soluble proteins (13). Epimorphin was identified as a protein required for mouse epithelial morphogenesis (34) and, while it shows considerable sequence homology to syntaxins (35), the functional relationship between these two proteins is unclear.

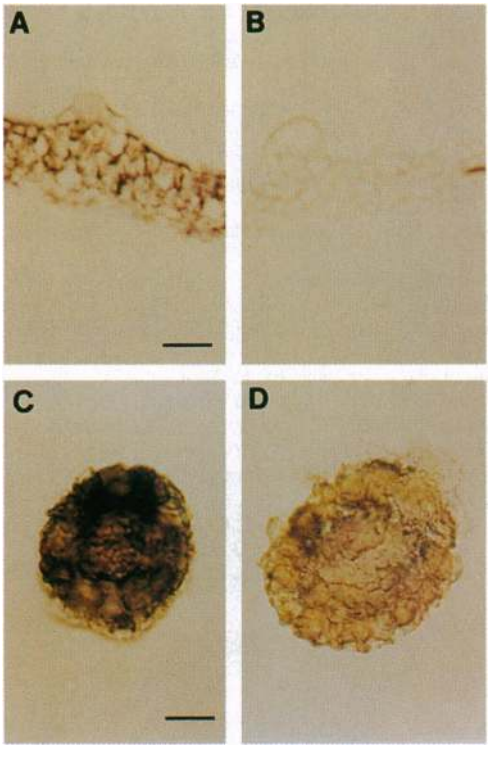

FIG. 6. RNA *in situ* localization of the *aPEP12* transcript. Paraffin sections of *Arabidopsis* tissues were hybridized with digoxigenin-labeled RNA probes, transcribed either in the antisense (*A* and *C*) or sense (*B* and *D*) orientation. (*A* and *B*) Transverse sections of young rosette leaves. (Bar = 32 μm.) (*C* and *D*) Transverse sections of roots. (Bar = 128 μm.)

The most highly conserved region of syntaxins is the C-terminal third of the proteins and this region of aPep12 also displays the highest homology to the syntaxin family. Its extreme C-terminal 22 aa are highly hydrophobic and predicted to form a membrane-anchoring domain, consistent with the structure of other syntaxins. Most syntaxins are C-terminally membrane-anchored with the bulk of the protein at the cytosolic face and few or no amino acid residues extending from the opposite side of the membrane (28). This orientation would be required for function as transport vesicle receptors in order to interact with components on the vesicular membrane. In contrast, it has been suggested that the hydrophilic portion of epimorphin faces the extracellular space (34).

The ability of *aPEP12* to complement the yeast *pep12* mutant may indicate that the protein is present in the vacuolar membrane in a similar orientation to Pep12, at least when expressed in yeast. It is probable that the localization of aPep12 in plant cells is similar to that in yeast, although it is possible that aPep12 functions at a different stage of the secretory pathway and that its overexpression in yeast causes mislocalization to the vacuolar membrane, which is the default destination of membrane proteins in this organism (36, 37). We consider this to be unlikely, however, as the protein would not be expected to interact with other components of the docking and fusion machinery (such as SNAP-25 and synaptobrevin homologues) at an incorrect location. This point could be clarified by immunolocalization and membrane fractionation studies in *Arabidopsis*. The homology between the *PEP12* genes from yeast and *Arabidopsis* is relatively low, in light of the ability of *aPEP12* to functionally replace the yeast *PEP12* gene. The interactions of Pep12 with other proteins may be more related to structural features of the syntaxin than to amino acid sequence, particularly over the C-terminal region as discussed below. The low homology between the yeast and *Arabidopsis* proteins in this case may not be surprising. In addition, it is likely that the interaction between syntaxin and synaptobrevin is not the only determinant of fusion specificity, and other components known to be involved in vesicular transport, such as small GTP-binding proteins, may also be involved in ensuring correct targeting and fusion (11). Even a weak interaction between aPep12 and yeast vacuolar targeting components may therefore be sufficient to allow vesicle transport.

The region immediately preceding the membrane-spanning domain of the syntaxin family contains a heptad repeat structure which potentially can form an amphiphilic α-helix. This has been proposed to form a coiled-coil domain in several syntaxin homologues (28). As this region is the most highly conserved between different syntaxins, including aPep12, it may be involved in interactions which are critical for protein function. The yeast Sed5 protein, which is required for endoplasmic reticulum-to-Golgi transport (30), has been proposed to form homodimers, with the coiled-coil domain mediating dimerization (38). The coiled-coil region of mammalian syntaxin 1, a protein found at the presynaptic membrane, is required for interaction with synaptobrevin and SNAP-25 and, therefore, for fusion of synaptic vesicles with the presynaptic membrane (29). The conservation of this protein region

indicates that aPep12 may interact with homologues of synaptobrevin and/or SNAP-25 to permit vesicle fusion with the vacuolar (or prevacuolar) membrane. Homologues of synaptobrevin have been identified in *Arabidopsis*, although their subcellular location and function are not yet known (ref. 39; M. Schena and R. W. Davis, GenBank accession No. M90418). The aPep12 protein will therefore be an important tool in the identification and isolation of other components of the vacuolar protein transport machinery. Proteins which directly interact with aPep12 can now potentially be isolated on the basis of their affinity for this syntaxin homologue. In addition, the interaction of aPep12 with membrane proteins of vacuolar transport vesicles should facilitate the purification of these vesicles. This will allow the study of vesicle proteins and thus aid the identification of vesicular components important for protein sorting such as receptors for vacuolar targeting signals. Analysis of the cargo of vesicles isolated by this method may indicate whether all proteins can be transported in the same type of vesicle or whether distinct vesicle subclasses exist for the transport of different proteins.

The *aPEP12* gene is expressed in all tissues examined by Northern analysis, but at widely varying levels. In particular, the amount of mRNA detected in leaves is very low, although the gene is expressed in all cell types. This has also been shown for other genes which function in the plant secretory pathway (23, 40). It is not known whether this is reflected in differences in the amount of aPep12 protein in leaves compared with other tissues, or whether secretory pathway activity is lower in leaves than in other tissues.

The isolation of the *Arabidopsis* Pep12 homologue will thus allow elucidation of the role of aPep12 in protein trafficking through the plant secretory pathway and provide a tool to study the mechanism of vesicle trafficking through the secretory pathway.

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