Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 834–838, February 1991
Cell Biology

Propeptide of a precursor to a plant vacuolar protein required for vacuolar targeting

(plant vacuoles/targeting signal/secretory pathway/storage protein)

KEN MATSUOKA AND KENZO NAKAMURA*

Laboratory of Biochemistry, School of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan

Communicated by Shang Fa Yang, October 15, 1990 (received for review September 4, 1990)

ABSTRACT **Sporamin is a protein without glycans that accumulates in large quantities in the vacuoles of the tuberous root of the sweet potato. It is synthesized as a prepro precursor with an N-terminal extension composed of a 21-amino-acid signal peptide and a 16-amino-acid propeptide. A total of 48 base pairs, corresponding to the nucleotide sequence that encodes the propeptide, was deleted from a cDNA clone for sporamin. This Δpro mutant sequence, as well as the sequence of the wild-type sporamin cDNA, was placed downstream from the promoter of the 35S transcript from cauliflower mosaic virus and introduced into the genome of suspension-cultured tobacco cells by *Agrobacterium*-mediated transformation. In contrast to the vacuolar localization of sporamin in cells that expressed the wild-type precursor, sporamin was secreted into the culture medium from cells in which the Δpro precursor was expressed. The secreted form of sporamin was shorter by two amino acids at its N terminus than authentic sporamin; it migrated anomalously during electrophoresis on SDS/polyacrylamide gel as a result of the presence of intramolecular disulfide bridges, as does authentic sporamin. The kinetics of secretion of sporamin from the cell were similar to those of proteins normally secreted by the host tobacco cells. These results indicate that the propeptide of the precursor to sporamin is required for correct targeting of sporamin to the vacuole and that proteins can be secreted from plant cells by a bulk-flow default pathway in the absence of a functional sorting signal.**

The vacuole is the largest organelle in many plant cells, and it plays various important roles in the maintenance of cell organization and function, being involved in the intracellular digestion of various materials, the generation of turgor, and the accumulation of nutrients, metal ions, and secondary metabolites. In addition to various enzymes, vacuoles in certain cells specifically accumulate large amounts of enzyme inhibitors, lectins, and storage proteins. The correct targeting of these proteins to the vacuole is a prerequisite for the expression of the various functions of the vacuole.

Precursors to the matrix proteins of the plant vacuole enter the lumen of the endoplasmic reticulum (ER) in a process directed by the N-terminal signal peptide, and they are transported to the vacuole via the Golgi complex (1, 2), as are the precursors to the matrix proteins of animal lysosomes (3) and yeast vacuoles (4). The transport of proteins to animal lysosomes and yeast vacuoles requires the presence of specific signals in the precursor, in addition to the signal peptide, so that they can be sorted from proteins with other destinations (3, 4). Mannose 6-phosphate in the oligosaccharide side chain of certain proteins serves as a signal for targeting to lysosomes (3). By contrast, the vacuolar targeting signals in precursors to two vacuolar proteins in yeast, namely, carboxypeptidase Y (5, 6) and proteinase A (7), have been shown to be associated with the structures of the propeptides. These propeptides are located between the signal peptide and the mature polypeptide of the prepro precursor, and they are cleaved off after the arrival of the protein in the vacuole (8).

The translocation, mediated by the signal peptide, of plant (9) and bacterial (10–12) cytosolic proteins into the lumen of the ER in plant cells results in the secretion of these proteins, suggesting that proteins can be secreted from cells by a bulk-flow default pathway and that transport of proteins to the vacuole in plant cells also requires a positive signal (2). Precursors to several plant vacuolar proteins have been expressed in heterologous plant cells and their targeting to the vacuole has been demonstrated (13–18). However, no direct evidence to indicate the presence of a positive vacuolar targeting signal in the precursors to plant vacuolar proteins has been presented. Although it has been proposed that the glycan side chains of proconcanavalin A may play a role in the transport process (19), analysis by *in vitro* mutagenesis of precursors to several plant vacuolar proteins (16, 20, 21) indicates that glycosylation is not essential for targeting of these proteins to the vacuole in plant cells.

Sporamin is a storage protein found in the tuberous roots of sweet potato (*Ipomoea batatas*) (22), and it is a mixture of closely related polypeptides encoded by a multigene family composed of two major subfamilies, A and B (22, 23). It is accumulated in the vacuoles of parenchymatous cells as a nonglycosylated monomeric protein. Sporamin is synthesized by membrane-bound polysomes as a prepro precursor (24, 25). The most N-terminal part of the precursor is a signal peptide, and the adjoined propeptide of 16 amino acid residues is removed posttranslationally (25).

We have previously shown that, when the precursor to sporamin is expressed in transformed tobacco calli, the precursor is correctly targeted to the vacuole (18). Now we report that deletion of the propeptide from the precursor to sporamin results in secretion of sporamin into the culture medium from transformed tobacco cells in suspension culture with kinetics similar to those of the secretion of proteins that are normally secreted by the host cells.

MATERIALS AND METHODS

Materials. Tran^{35}S-label (43 TBq/mmol; a mixture of [^{35}S]methionine and [^{35}S]cysteine), ^{125}I-labeled staphylococcal protein A (1.1 GBq/mg), and potassium dextran sulfate were obtained from ICN, Amersham, and Meito Sangyo (Nagoya, Japan), respectively. Oligonucleotides were synthesized on a DNA synthesizer (model 381A, Applied Biosystems).

Construction of the Δpro Mutant of Sporamin cDNA. The *Bam*HI–*Hin*dIII fragment from pCSAD (18), containing cDNA for sporamin A (pIMO23; ref. 24) was cloned in the *Bam*HI and *Sma* I sites of pNUT7 with a *Hin*dIII linker to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Δpro, a mutant of the precursor to sporamin that lacks the propeptide; ER, endoplasmic reticulum.

*To whom reprint requests should be addressed.

generate pMAT103. pNUT7 is derived from the pNUT4 vector (26), which contains *tac*-SP6 promoters, by the insertion of the M13 intergenic region and the *lacI* gene, which makes it possible to obtain single-stranded DNA with a helper phage and an RNA copy of the cDNA by using SP6 RNA polymerase.

Using single-stranded DNA from pMAT103, we introduced a unique *Pvu* II site near the C terminus of the coding sequence for the signal peptide by site-directed mutagenesis (27), using the oligonucleotide CAATCCAGCTGATTCCAGGTTCA, to yield pMAT104. A *Stu* I site was introduced near the C terminus of the coding sequence for the propeptide, using the oligonucleotide CCACACATGAACCCGCCTCCTCTCCGAA, to generate pMAT105. The *Eco*RI–*Pvu* II fragment of pMAT104, which contained the coding sequence for the signal peptide, was introduced into the *Eco*RI and *Stu* I sites of pMAT105 to yield a derivative of the cDNA for sporamin in which the 48-base-pair (bp) sequence coding for the propeptide was deleted (Δpro mutant; pMAT107).

Binary Plasmids for the Expression of Wild-Type and Δpro cDNAs for Sporamin in Tobacco Cells. A plant binary expression vector, pMAT037 (Fig. 1*B*), was constructed from pCSAD, which contained the promoter of the cauliflower mosaic virus 35S transcript (18), by tandem duplication of the enhancer sequence of the 35S promoter (28). The *Bam*HI–*Hin*dIII fragments of pMAT103 and pMAT107 were cloned in the *Bgl* II and *Hin*dIII sites of pMAT037 to yield the plasmids pMAT110 and pMAT108, respectively. These plasmids were transferred to *Agrobacterium tumefaciens* EHA101 (29), and then they were used to transform suspension-cultured cells of tobacco (*Nicotiana tabacum*) line BY-2 (30) essentially as described by An (31). Approximately 3 × 10^3 kanamycin-resistant colonies were pooled, reintroduced into suspension cultures, and maintained as BY-2 cells (32). Cells transformed with pMAT110 and pMAT108 are referred to below as wild-type transformants and Δpro transformants, respectively.

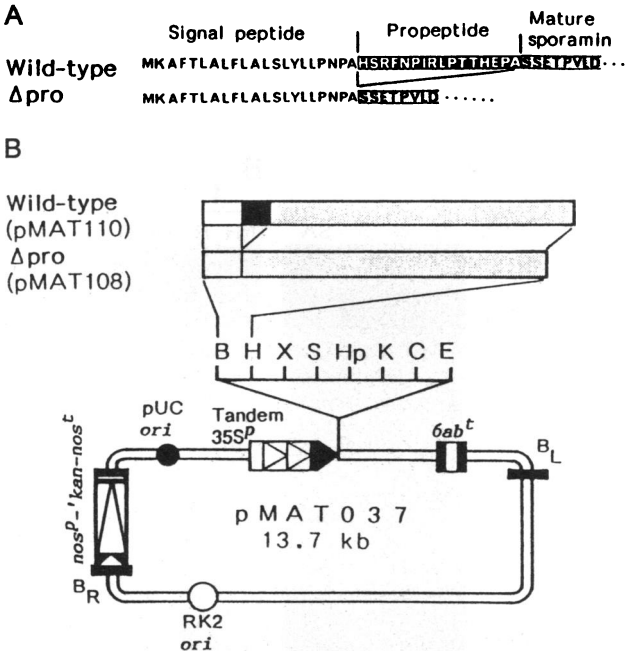

FIG. 1. The Δpro mutant of the precursor to sporamin. (*A*) Amino acid sequence of the N-terminal part of the wild-type and Δpro precursors to sporamin deduced from the nucleotide sequence. (*B*) Structure of binary Ti plasmids. Tandem 35S^P, the promoter of the 35S transcript from cauliflower mosaic virus with a tandemly duplicated enhancer; B$_R$ and B$_L$, T-DNA right and left borders, respectively; 6ab^t, transcription terminator region of T-DNA transcripts 6a and 6b; *nos*^{P}-'*kan-nos*^{t}, *nos* promoter-driven kanamycin-resistance gene; B, *Bgl* II; H, *Hin*dIII; X, *Xba* I; S, *Sac* II; Hp, *Hpa* I; K, *Kpn* II; C, *Cla* I; E, *Eco*RI.

Fractionation of the Culture. The medium and the cell, protoplast, and vacuole fractions were prepared from 4-day-old cultures. Culture medium was separated from cells by filtration and concentrated with a centrifugal ultrafiltration unit (Centricell; Polysciences). The concentrated medium was buffered with 50 mM Tris·HCl, pH 7.5/1 mM EDTA and used as the medium fraction. Cells harvested by filtration were suspended in 2 vol of extraction buffer (18) and lysed by sonication. After centrifugation at 1000 × *g* for 10 min, the supernatant was used as the cell fraction.

Protoplasts were prepared from the cells by the method of Nagata *et al.* (32). The protoplast pellet was homogenized with 2 vol of extraction buffer (18) in a Teflon homogenizer. The homogenate was centrifuged at 1000 × *g* for 10 min, and the supernatant was used as the protoplast fraction. Vacuoles were prepared from the protoplasts by the modification of methods described previously (18). The purity of the vacuoles in this fraction was checked under the light microscope and by the assay of specific marker enzymes, as described (18).

Labeling *in Vivo*. A 0.5-ml sample of a 3-day-old culture of cells was incubated with 2.8 MBq of Tran^{35}S-label for 15 min, and then 50 μl of 50 mM methionine and 50 mM cysteine was added for indicated time periods. The cell and medium fractions were prepared and sporamin-related polypeptides were precipitated with sporamin-specific antiserum and analyzed by SDS/PAGE (18). Standards of precursors with and without the signal peptide were prepared by translation *in vitro* of SP6 transcripts of the wild-type and Δpro cDNAs in a rabbit reticulocyte lysate in the presence of dog pancreas membranes (23).

SDS/PAGE and Immunoblotting. SDS/PAGE on 12.5% polyacrylamide gels and immunoblotting with antiserum against SDS-denatured sporamin and ^{125}I-labeled protein A were performed as described (18).

Secreted Form of Sporamin. The secreted form of sporamin in the medium fraction from a culture of the Δpro transformants was purified by Mono Q column (Pharmacia–LKB) chromatography and SDS/PAGE and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore). The N-terminal amino acid sequence was determined with a protein sequencer (model 470A, Applied Biosystems) (33).

RESULTS

Construction of Plasmids and Transformation of Suspension-Cultured Tobacco Cells. A cDNA from plasmid pIMO23, encoding a precursor to A-type sporamin (24), was modified by deletion of a 48-bp nucleotide sequence that corresponds to the propeptide of the precursor to sporamin. The resulting Δpro mutant of sporamin cDNA codes for a precursor polypeptide in which the mature part of sporamin is immediately adjacent to the signal peptide (Fig. 1*A*). To obtain high-level expression of the precursors to sporamin in tobacco cells, we constructed the binary Ti-plasmid vector pMAT037 (Fig. 1*B*), which contains the 35S promoter of the cauliflower mosaic virus with a tandemly linked enhancer region (28).

The wild-type and the Δpro mutant of sporamin cDNA were inserted into the multiple cloning site of pMAT037 to generate plasmids pMAT110 and pMAT108, respectively (Fig. 1*B*). These plasmids were introduced into *Agrobacterium* and used to transform suspension-cultured tobacco cells of line BY-2 (30). About 3 × 10^3 kanamycin-resistant colonies were obtained 3 weeks after coculture of 2 × 10^6 BY-2 cells with *Agrobacterium*. Expression of polypeptides that were immunoreactive with sporamin-specific antibodies was detected in each of the 48 independent colonies analyzed for each transformant, although the level of expression varied significantly among individual transformants. To eliminate

the possible effect of differences in the level of expression, mixtures of about 3000 colonies were reintroduced into suspension culture in the case of both the wild-type and the Δpro transformants and used for further analyses. The levels of sporamin-related polypeptides detected by immunoblotting in cultures of equal numbers of cells from these mixed cultures were similar between the wild-type and the Δpro transformants. No immunoreactive polypeptides were detected in cultures of the control BY-2 cells.

Sporamin Is Secreted into the Culture Medium from the Δpro Transformants. To examine the distribution of sporamin-related polypeptides, cultures of the wild-type and the Δpro transformants were separated into culture medium and cells. Then protoplasts and vacuoles were prepared from the cells. Specific activities of two vacuolar marker enzymes, phosphodiesterase and α-mannosidase, in the vacuole fraction were 32- to 37-fold higher than those in the protoplast fraction. By contrast, specific activities of marker enzymes for other organelles were significantly lower in the vacuole fraction than those in the protoplasts (data not shown; see ref. 18 for marker enzymes), indicating that the purity of the vacuoles in this fraction was fairly high. On average, 1 ml of the 4-day-old culture contained about 13 μg of medium proteins and 300 μg of cellular proteins, of which about 8.7 μg was recovered in the vacuole fraction.

The relative levels of sporamin-related polypeptides in these fractions were examined by immunoblotting of equal amounts of proteins (2 μg) fractionated by SDS/PAGE (Fig. 2). In the cultures of the wild-type transformants, sporamin was concentrated in the vacuole fraction of the cells and could not be detected in the culture medium. A band of sporamin in the cell and in the protoplast fractions could be detected only in autoradiographs exposed for a relatively long time (360 hr compared to 10 hr in the case of Fig. 2; data not shown). A comparison of the intensities of bands revealed that sporamin is concentrated about 40-fold in the vacuole fraction as compared to the protoplast fraction, indicating that sporamin is almost exclusively localized in the vacuoles. By contrast, a heavy band of sporamin-related polypeptide was detected in the case of the culture medium and only a very faint band was detected in the vacuole fraction, when cultures of the Δpro transformants were analyzed (Fig. 2). Although longer exposure of the filter revealed a clear band of sporamin in the vacuole fraction, the concentration of sporamin was estimated to be less than 1/20th of that in the vacuole of the wild-type transformants. It is estimated that more than 90% of the sporamin-related polypeptides expressed in the Δpro transformants were present in the culture medium.

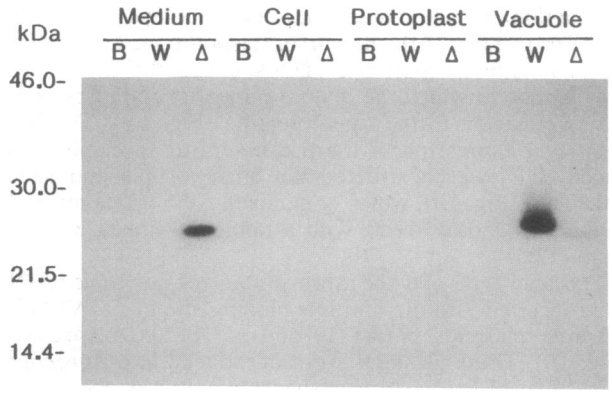

FIG. 2. Secretion of sporamin from the Δpro transformant cells. Two micrograms of protein in various fractions from cultures of nontransformed BY-2 cells (B), the wild-type transformant (W), and the Δpro transformant (Δ) were separated by SDS/PAGE and sporamin-related polypeptides were detected by immunoblotting with sporamin-specific antiserum and ^{125}I-protein A. The film was exposed for 10 hr.

Secreted Form of Sporamin. The sporamin-related polypeptide detected by immunoblotting of proteins in both the culture medium and the cell fractions of cultures of the Δpro transformants migrated with an apparent molecular mass of 23.8 kDa, which is slightly less than that of the sporamin-related polypeptide detected in the vacuole of the wild-type transformants. We have previously shown that sporamin accumulated in the vacuoles of tobacco callus in which the wild-type precursor to sporamin is expressed is longer by three amino acids at its N terminus than authentic sporamin purified from the sweet potato (18).

A 23.8-kDa protein was detected as one of the major proteins in the culture medium of the Δpro transformants (Fig. 3*A*). This 23.8-kDa protein was absent from the culture medium of nontransformed BY-2 cells and of the wild-type transformants (Fig. 3*A*), suggesting that this protein is the secreted form of sporamin. The 23.8-kDa protein in the culture medium of the Δpro transformants was purified and its N-terminal amino acid sequence was determined. The sequence,

ETPVLDINGDEVRAGGNYYMVSAIXGAG,

shown in the one-letter code with X representing unidentified amino acids, corresponds to the amino acid sequence of mature sporamin from positions +3 to +31 (ref. 22; see Fig. 1*A*). From these results, the 23.8-kDa protein in the culture medium of the Δpro transformants was identified as the secreted form of sporamin, which is shorter by two amino acids than authentic sporamin at its N terminus.

Sporamin A treated in a solution of SDS without a reducing reagent migrates on SDS/polyacrylamide gels more slowly than the reduced form (22). This anomalous migration is probably due to intramolecular disulfide bridges, since the amino acid sequence of sporamin shows homologies to the Kunitz-type trypsin inhibitor from soybean, and the four cysteine residues involved in the formation of two intramolecular disulfide bridges in the inhibitor are conserved in

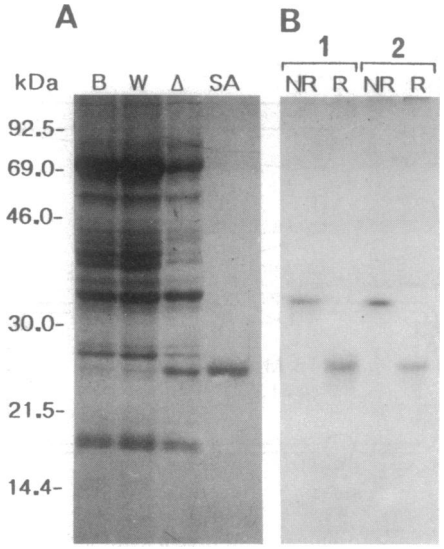

FIG. 3. The secreted form of sporamin. (*A*) Staining with Coomassie brilliant blue of proteins (20 μg) in the culture medium of nontransformed BY-2 cells (B), the wild-type transformant (W), and the Δpro transformant (Δ). Lane SA, 2 μg of sporamin A purified from sweet potato. (*B*) SDS/PAGE of authentic sporamin A (lanes 1) and the secreted form of sporamin (lanes 2) after heating in a 1% solution of SDS in the absence (NR) or presence (R) of 2-mercaptoethanol.

sporamin (ref. 23; unpublished results). When treated with a solution of SDS in the absence of a reducing reagent, the secreted form of sporamin migrated at a position that corresponded to an apparent molecular mass of 32.5 kDa, which is almost identical to the position to which authentic sporamin A migrates under the same conditions (Fig. 3*B*). These results suggest that appropriate intramolecular disulfide bridges are formed in the absence of the propeptide.

Kinetics of Secretion of Sporamin into the Culture Medium. The kinetics of the processing and transport of precursors to sporamin in the wild-type and the Δpro transformants were examined by pulse-labeling the cultures with [^{35}S]methionine and [^{35}S]cysteine for 15 min, with a subsequent chase with excess unlabeled methionine and cysteine for various periods of time. Sporamin-related polypeptides in the cell and medium fractions of the culture were precipitated with sporamin-specific antiserum and analyzed by SDS/PAGE (Fig. 4).

In the case of the wild-type transformants, pulse-labeled cells gave a major band that corresponded to the pro form of the precursor to sporamin (Fig. 4*A*). The pro form was converted into the apparently mature-sized form of sporamin during the chase by at least two processing steps. In transformed tobacco calli, the propeptide is first cleaved between Thr^{-6} and Thr^{-5} and then the dipeptide Thr^{-5}-His^{-4} is removed (18). The nature of the minor bands is not clear. The half-time for the first step of posttranslational processing was 21 min, which is similar to the value obtained with the callus (18). No ^{35}S-labeled sporamin was detected in the culture medium even after a chase for 4 hr (Fig. 4*A*).

In the case of the pulse-labeled Δpro transformants, a single band was found which migrated slightly faster than the Δpro precursor to sporamin processed *in vitro* by dog membranes (Fig. 4*B*). The amount of ^{35}S-labeled sporamin in the cell decreased during the chase, and the secreted form of sporamin started to appear in the medium after 30 min of chase (Fig. 4*B*). About 95% of the sporamin labeled during the pulse was secreted into the medium within 4 hr during the chase period.

We did not detect any changes in the size of pulse-labeled, sporamin-related polypeptides in the Δpro transformants during the chase. Thus, it appears that the Δpro precursor is cotranslationally cleaved at Ser^{2}-Glu^{3} in tobacco cells. The site of cotranslational cleavage by the dog membranes has not been examined.

The culture medium of the Δpro transformants, after a 60-min chase, contained at least 14 ^{35}S-labeled bands of polypeptides. Among these was the 23.8-kDa band, which was identified as the secreted form of sporamin since this band was absent from the culture medium of nontransformed

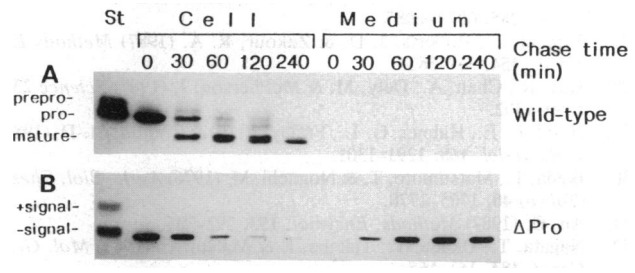

FIG. 4. Pulse–chase analysis of secretion of sporamin. The wild-type (*A*) and the Δpro (*B*) transformants labeled with Tran^{35}S-label for 15 min were incubated with unlabeled methionine and cysteine for the indicated periods of time and sporamin-related polypeptides in the cell and medium fractions from equal volumes of cultures were immunoprecipitated and analyzed by SDS/PAGE. Lane St shows size standards for the precursors; SP6 transcripts of the wild-type (*A*) and the Δpro mutant (*B*) cDNA were translated *in vitro* in the presence of dog microsomal membrane to allow partial processing of the signal peptide and were then immunoprecipitated.

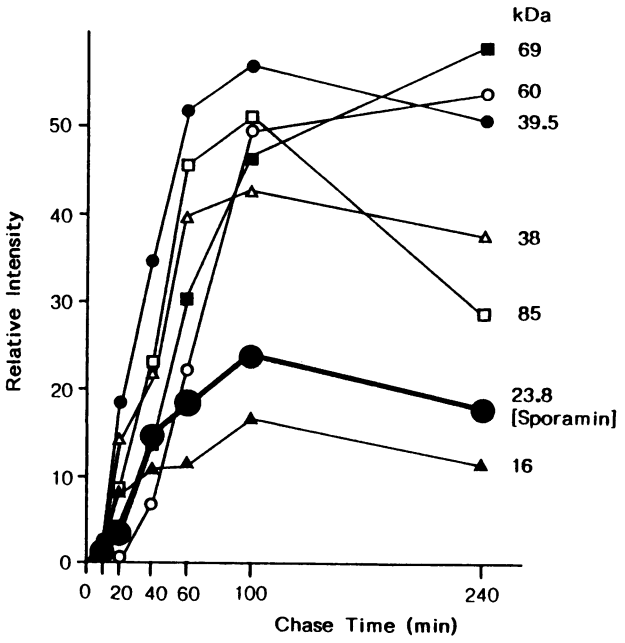

FIG. 5. Kinetics of secretion of sporamin. Seven major ^{35}S-labeled polypeptides in the culture medium of the Δpro transformants, indicated by their apparent molecular mass, were analyzed in terms of their kinetics of secretion. Changes in relative radioactivities of polypeptides in equal volumes of culture medium after the chase are shown.

cells and of the wild-type transformants. Furthermore, the polypeptide could be specifically precipitated with sporamin-specific antiserum (data not shown).

The rate of secretion of sporamin from the Δpro transformants was compared with rates of secretion of endogenous secretory proteins from the host tobacco cells, ^{35}S-labeled polypeptides in identical volumes of culture medium at various times during the chase were separated by SDS/PAGE, and changes in relative radioactivities of seven major polypeptides were determined by densitometric scanning of the fluorogram (Fig. 5). The kinetics of secretion of the various polypeptides differed slightly from one another. We calculated the lag time to the appearance and the half-time for the increase in radioactivity of these seven polypeptides in the medium by computer-assisted best-fit analysis of the data, assuming that the kinetics of secretion of protein are first order. In the case of six endogenous polypeptides, the lag time fell within a range from 2 to 33 min, and the half-time was between 20 and 37 min. The lag time and the half-time for the secreted form of sporamin were 15 and 23 min, respectively, indicating that the kinetics of secretion of sporamin from the Δpro transformant cells are similar to those of the secretion of endogenous proteins.

DISCUSSION

The absence of glycan side chains in the precursor to sporamin and the presence of a short propeptide between the signal peptide and the mature region in the precursor, which is probably removed after the arrival of the polypeptide in the vacuole (18), prompted us to search for the vacuole targeting signal in the sporamin precursor. The use of suspension-cultured cells of the BY-2 line of tobacco (30) facilitated our analysis, since BY-2 cells grow rapidly, can be transformed with *Agrobacterium* very efficiently, and are well suited for the preparation of pure vacuoles.

In sharp contrast to the efficient transport of sporamin to the vacuole in the wild-type transformants, almost all the sporamin synthesized was secreted into the culture medium

in the case of the Δpro transformants (Figs. 2 and 4). Furthermore, the kinetics of secretion of sporamin from the Δpro transformant cells were similar to those of the endogenous proteins secreted by the host BY-2 cells (Fig. 5), suggesting that the secretion of sporamin follows the general pathway for secretion of polypeptide from these cells. From these results, we conclude that the propeptide of the precursor to sporamin contains the information required for targeting of sporamin to the vacuole in tobacco cells. However, our results do not preclude the possibility that other regions of prosporamin also play a role in vacuolar targeting, since a small amount of sporamin was detected in the vacuole fraction of the Δpro transformants. Furthermore, it is not known at present whether the propeptide of the sporamin precursor, together with the signal peptide, is sufficient for the targeting of alien proteins to the vacuole. The results presented in this paper, together with results reported previously (9–12), also suggest that proteins can be secreted from the plant cells by a bulk-flow default pathway in the absence of positive topogenic information, as in the case of animal and yeast cells (3, 4).

The propeptides in two precursors to yeast vacuolar proteins, carboxypeptidase Y (5, 6) and proteinase A (7), have been shown to contain vacuolar targeting signals, and the propeptide of rat preprosomatostatin (34) has been shown to contain the information necessary to target the somatostatin to storage secretory vesicles for subsequent participation in the regulated secretory pathway. Propeptides of these precursors are much longer than the propeptide of the precursor to sporamin. The propeptides of precursors for yeast vacuolar and animal lysosomal hydrolytic enzymes are also required to keep the enzyme in a latent form (6, 8). Some of the propeptides may play a role in the folding of polypeptides (35). The secreted form of sporamin seems to contain intramolecular disulfide bridges similar to those in authentic sporamin from the sweet potato (Fig. 3*B*), suggesting that the correct folding of the sporamin polypeptide takes place in the absence of the propeptide. The short propeptide of the precursor to sporamin may function primarily in vacuolar targeting.

The 16 amino acid-long propeptide of the precursor to sporamin is enriched with hydrophilic and charged amino acid residues (Fig. 1*A*), and it does not contain a sequence similar to the vacuole-targeting domain of the propeptide of the precursor to carboxypeptidase Y (Gln-Arg-Pro-Leu; refs. 4 and 36). The propeptide of the precursor to yeast proteinase A also does not contain this sequence (4, 7). It is not known whether the two yeast vacuolar proteins are sorted by different recognition systems or whether some unrecognized structural feature shared by the two propeptides is recognized by a common "receptor." It also remains to be determined whether topogenic signals in proteins for transport to acidic compartments of the cell are evolutionaly conserved between yeast and plants. Recently, Tague *et al.* (37) have shown that N-terminal portion of a precursor to phytohemagglutinin from bean can efficiently target invertase to the yeast vacuole and the Leu-Gln-Arg sequence close to the N terminus of the mature phytohemagglutinin is important for this targeting. However, when the same fusion construct was expressed in plant cells it directed the secretion of invertase from the cells (2).

We thank Dr. Yasunori Machida and Mr. Yasuo Niwa (School of Science, Nagoya University) for valuable suggestions on the transformation of BY-2 cells, Dr. Eugene W. Nester (University of Washington, Seattle) for *Agrobacterium tumefaciens* EHA101, Dr. Atsushi Oshima (Takara Shuzo, Kyoto) for pNUT7, Dr. H. Hattori and Ms. H. Kajiura (National Institute for Basic Biology, Okazaki) for analysis of the N-terminal amino acid sequences, and Dr. Maarten J. Chrispeels (University of California, San Diego) for a copy of ref. 2 before publication. This work was supported in part by a Grant-in-Aid for Special Project Research and a Grant-in-Aid for Cooperative Research from the Ministry of Education, Science and Culture, Japan, to K.N., and K.M. is a recipient of a Japan Society for the Promotion of Science Fellowship for Japanese Junior Scientists.

1. Müntz, K., Bassüner, R., Lichtenfeld, C., Scholz, G. & Weber, E. (1985) *Physiol. Veg.* **23**, 75–94.
2. Chrispeels, M. J. (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, in press.
3. Pfeffer, S. R. & Rothman, J. E. (1987) *Annu. Rev. Biochem.* **56**, 829–852.
4. Rothman, J. H., Yamashiro, C. T., Kane, P. M. & Stevens, T. H. (1989) *Trends Biochem. Sci.* **14**, 347–350.
5. Johnson, L. M., Bankaitis, V. A. & Emr, S. D. (1987) *Cell* **48**, 875–885.
6. Valls, L. A., Hunter, C. P., Rothman, J. H. & Stevens, T. H. (1987) *Cell* **48**, 887–897.
7. Klinoski, D. J., Banta, L. M. & Emr, S. D. (1988) *Mol. Cell. Biol.* **8**, 2015–2116.
8. Woolford, C. A., Daniels, L. B., Park, F. J., Jones, E. W., Van Arsdell, J. N. & Innis, M. A. (1986) *Mol. Cell. Biol.* **6**, 2500–2510.
9. Dorel, C., Voelker, T. A., Herman, E. M. & Chrispeels, M. J. (1989) *J. Cell Biol.* **108**, 327–337.
10. Lund, P., Lee, R. Y. & Dunsmur, P. (1989) *Plant Physiol.* **91**, 130–135.
11. Iturriaga, G., Jefferson, R. A. & Bevan, M. W. (1989) *Plant Cell* **1**, 381–390.
12. Denecke, J., Botterman, J. & Deblance, R. (1990) *Plant Cell* **2**, 51–59.
13. Greenwood, J. S. & Chrispeels, M. J. (1985) *Plant Physiol.* **79**, 65–71.
14. Sturm, A., Voelker, T. A., Herman, E. M. & Chrispeels, M. J. (1988) *Planta* **175**, 170–183.
15. Sonnewald, U., Sturm, A., Chrispeels, M. J. & Willmitzer, L. (1989) *Planta* **179**, 171–180.
16. Wilkins, T. A., Bednarek, S. Y. & Raikhel, N. V. (1990) *Plant Cell* **2**, 301–313.
17. De Clercq, A., Vandewiele, M., De Rycke, R., Van Damme, J., Van Montagu, M., Krebbers, E. & Vandekerckhove, J. (1990) *Plant Physiol.* **92**, 899–907.
18. Matsuoka, K., Matsumoto, S., Hattori, T., Machida, Y. & Nakamura, K. (1990) *J. Biol. Chem.*, in press.
19. Faye, L. & Chrispeels, M. J. (1987) *Planta* **170**, 217–224.
20. Voelker, T., Herman, E. M. & Chrispeels, M. J. (1989) *Plant Cell* **1**, 95–104.
21. Sonnewald, U., von Schaewen, A. & Willmitzer, L. (1990) *Plant Cell* **2**, 345–355.
22. Maeshima, M., Sasaki, T. & Asahi, T. (1985) *Phytochemistry* **24**, 1899–1902.
23. Hattori, T., Yoshida, N. & Nakamura, K. (1989) *Plant Mol. Biol.* **13**, 563–572.
24. Hattori, T., Nakagawa, T., Maeshima, M., Nakamura, K. & Asahi, T. (1985) *Plant Mol. Biol.* **5**, 313–320.
25. Hattori, T., Ichihara, S. & Nakamura, K. (1987) *Eur. J. Biochem.* **166**, 535–538.
26. Kimura, T., Takeda, S., Asahi, T. & Nakamura, K. (1990) *J. Biol. Chem.* **265**, 6079–6085.
27. Kunkel, T., Roberts, J. D. & Zakour, R. A. (1987) *Methods Enzymol.* **154**, 367–382.
28. Kay, R., Chan, A., Dely, M. & McPherson, J. (1987) *Science* **236**, 1299–1302.
29. Hood, E. E., Halmer, G. L., Fraley, R. T. & Chilton, M.-D. (1986) *J. Bacteriol.* **168**, 1291–1301.
30. Ikeda, T., Matsumoto, T. & Noguchi, M. (1976) *Agric. Biol. Chem. (Tokyo)* **40**, 1765–1770.
31. An, G. (1987) *Methods. Enzymol.* **153**, 292–305.
32. Nagata, T., Okada, T., Takebe, I. & Matsui, C. (1981) *Mol. Gen. Genet.* **184**, 161–165.
33. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038.
34. Savarino, K. A., Stork, P., Ventimiglia, R., Mandel, G. & Goodman, R. H. (1989) *Cell* **57**, 11–19.
35. Zhu, X., Ohta, Y., Jordan, F. & Inouye, M. (1989) *Nature (London)* **339**, 483–484.
36. Valls, L. A., Winther, J. R. & Stevens, T. H. (1990) *J. Cell Biol.* **111**, 361–368.
37. Tague, B. W., Dickinson, C. D. & Chrispeels, M. J. (1990) *Plant Cell* **2**, 533–546.