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Retention of phytohemagglutinin with carboxyterminal tetrapeptide KDEL in the nuclear envelope and the endoplasmic reticulum

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Abstract. Soluble proteins that reside in the lumen of the endoplasmic reticulum are known to have at their carboxyterminus the tetrapeptides KDEL or HDEL. In yeast and mammalian cells, these tetrapeptides function as endoplasmic reticulum (ER)-retention signals. To determine the effect of an artificially-introduced KDEL sequence at the exact carboxyterminus of a plant secretory protein, we modified the gene of the vacuolar protein phytohemagglutinin-L (PHA) so that the amino-acid sequence would end in *LNKDEL* rather than *LNKIL*, and expressed the modified gene in transgenic tobacco with a seed-specific promoter. Analysis of the glycans of PHA showed that most of the control PHA had one endoglycosidase H-sensitive and one endoglycosidase H-resistant glycan, indicating that it had been processed in the Golgi complex. On the other hand, a substantial portion of the PHA-KDEL (about 75% at mid-maturation and 50% in mature seeds) had two endoglycosidase H-sensitive glycans. Phytohemagglutinin with two endoglycosidase H-sensitive glycans is normally found in the ER. Using immunocytochemistry we found that a substantial portion of the PHA-KDEL was present in the ER or accumulated in the nuclear envelope while the remainder was found in the protein storage vacuoles (protein bodies). We interpret these data to indicate that carboxyterminal KDEL functions as an ER retention-retardation signal and causes protein to accumulate in the nuclear envelope as well as in the ER. The incomplete ER retention of this protein which is modified at the exact carboxyterminus may indicate that structural features other than carboxyterminal KDEL are important if complete ER retention is to be achieved.

Key words: Endoplasmic reticulum – *Nicotiana* (transgenic) – Nuclear envelope – Phytohemagglutinin – Protein targeting

Introduction

The formation and maintenance of the numerous organelles present in eukaryotic cells requires the delivery of newly-synthesized proteins to their correct cellular locations. The synthesis of nearly all proteins starts in the cytosol and proteins enter each kind of organelle in a unique way. Understanding this intracellular protein traffic is one of the central issues in cell biology (Burgess and Kelly 1987; Klausner 1989; Pelham 1989). Targeting of protein to specific organelles depends on the interaction of targeting signals or domains of the protein with receptors and the identification of both the domain and the receptors is the subject of much recent research. The endoplasmic reticulum (ER) serves as the port of entry for luminal and membrane proteins that may be secreted or targeted to the ER, Golgi complex, endosomes, vacuoles or the plasma membrane. Most proteins that enter the ER do so because they have a signal peptide or a specific membrane spanning domain, spend only a short time there; some proteins, the ER resident proteins, are retained in the lumen of the ER or remain associated with its membranes. Thus, mechanisms must exist that allow resident ER proteins to be sorted from secretory proteins and proteins that will be targeted to other organelles.

Evidence from a number of laboratories shows that proteins that reside in the lumen of the ER of mammalian, yeast and plant cells have a carboxyterminal domain consisting of the four amino acids KDEL (lysine-aspartic acid-glutamic acid-leucine) or HDEL (histidine-aspartic acid-glutamic acid-leucine) (Pelham 1989). Furthermore, it has now been shown for both mammalian and yeast cells that these tetrapeptides are both neces-

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Abbreviations: endoH = endoglycosidase H; ER = endoplasmic reticulum; Mr = relative molecular mass; PHA = phytohemagglutinin; SDS = sodium dodecyl sulfate; PAGE = polyacrylamide gel electrophoresis; TBST = Tris-buffered saline containing Tween 20

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 A   N   L   V   L   N   K   I       L   *           Wild-type PHA-L
.CC AAC TTG GTC CTC AAC AAA ATC     CTC TAG ACT C
 GG TTG AAC CAG GAG TTG TTT CTA CTT GAG ATC TGA G-5' 36mer primer
.CC AAC TTG GTC CTC AAC AAA GAT GAA CTC TAG ACT C
 A   N   L   V   L   N   K   D   E   L   *           PHA-KDEL
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Fig. 1. Oligonucleotides at the 5'-end of PHA used in the polymerase chain reaction. *Upper line* shows the amino-acid sequence of PHA at its carboxyterminus, and the *second line* indicates the mRNA nucleotide sequence that corresponds to it. The *middle line* (marked 36 mer primer) shows the nucleotide sequence used in the polymerase chain reaction. The *fourth* and *fifth line* show the mRNA and the amino-acid sequence, respectively, of the mutant PHA-KDEL

sary and sufficient for the retention of proteins in the ER (Munro and Pelham 1987; Pelham 1988). Proteins that end in HDEL or KDEL are not simply held in the ER, but are continuously retrieved from a post-ER compartment, either the *cis*-Golgi or a salvage compartment before the *cis*-Golgi (Ceriotti and Colman 1988; Pelham 1988, 1989). Recently, Inohara et al. (1989) obtained the derived amino-acid sequence of the ER-associated auxin-binding protein of maize and found that this protein has a carboxyterminal KDEL sequence.

To test the possibility that carboxyterminal KDEL on a plant secretory protein can function as an ER retention signal in plant cells, we modified the complimentary DNA (cDNA) of phytohemagglutinin-L (PHA) at the 3' end of the coding sequence. Phytohemagglutinin, the seed lectin of the common bean, *Phaseolus vulgaris* L., is a vacuolar glycoprotein that is synthesized on the ER, and its transport to the vacuole is mediated by the Golgi apparatus (Chrispeels 1983). To obtain a change exactly at the carboxyterminus of PHA, we changed and slightly elongated the nucleotide sequence so that the amino-acid sequence would end in LNKDEL instead of LNKIL, the normal carboxyterminus of PHA-L. The genes for normal PHA-L and the mutant PHA-KDEL were introduced into tobacco with seed-specific promoters, and we examined the subcellular location and post-translational modification of the mutant protein. We showed earlier that the introduction of the PHA-L gene in tobacco results in the seed-specific expression of the protein which is correctly processed and targeted to protein storage vacuoles (protein bodies) (Sturm et al. 1988). Newly-synthesized PHA-L (in the ER) has two high-mannose glycans that are attached to Asn12 and Asn60. Transport through the Golgi complex results in the conversion of the glycan attached to Asn60 into a complex (endoglycosidase H-resistant) glycan (Vitale et al. 1984b; Sturm and Chrispeels 1986). The specificity of this conversion is the same in tobacco as in bean (Sturm et al. 1988). The presence of a complex glycan on PHA can normally be taken as evidence that the protein has left the ER and the *cis*-Golgi compartment and is now in or, more likely, beyond the *trans*-Golgi cisternae (Kornfeld 1987; Sturm et al. 1987). The results from the present experiments show that a large proportion of the PHA-KDEL in tobacco seeds has two high-mannose glycans, indicating that it has not been modified in the Golgi complex. Immunocytochemistry shows that a substantial proportion of PHA-KDEL is in the ER and the nuclear envelope; however, retention in the ER was not complete and a substantial portion of the PHA-KDEL progressed to the protein storage vacuoles.

Material and methods

Chimeric PHA genes. The control PHA gene chimera consists of the 5' upstream sequence of β-phaseolin, the coding sequence of PHA-L, and the 3' sequence of PHA-L as described in Voelker et al. (1989). The mutant PHA with carboxyterminal KDEL was made by polymerase chain reaction (PCR) mutagenesis at the 3'-end using the two oligonucleotide shown in Fig. 1. The oligonucleotide at the 5'-end used for the polymerase chain reaction had the sequence 5'-CTTCCCGAGTGGGTGAGCGTT-3' and covered amino acids 199 to 205 of PHA-L. As a template the pSPLec2 plasmid (Hoffman and Donaldson 1987) containing the PHA-L coding sequence. The protocol for PCR synthesis of a modified 3' end PHA-L gene fragment is described in Hoffman and Hundt (1988). The resulting plasmid pSPKDEL contains the KDEL C-terminal coding mutation as shown by nucleotide-sequence analysis. The plasmid was cut with HindIII, treated with mung bean nuclease (Epicentre Technologies, Madison, Wis., USA), and recut with SacI. The lectin gene fragment was isolated from a polyacrylamide gel and ligated with pPPZ60AB (Hoffman et al. 1987), partially digested with ScaI and completely digested with SacI. The resulting construct pPPKDEL has 850 bp (base pairs) of the 5' upstream sequence of β-phaseolin, 19 bp of phaseolin untranslated coding sequence, the PHA-KDEL coding sequence, the PHA-L poly(A) addition signal, the β-phaseolin poly(A) addition signal, and 3' flanking region. The chimeric gene can be removed from pPPKDEL as a 3 kb BamH1 fragment for insertion into an *Agrobacterium* vector. All restriction endonucleases were from Promega (Madison, Wis., USA) and New England Biolabs (Beverly, Mass., USA).

Plant transformation. The chimeric genes were inserted into the vector Bin19 (Bevan 1984) and after a triparental mating transconjugant (*Agrobacterium tumefaciens* (strain LBA4404) carrying the recombinant Bin19 plasmid were selected. Leaf discs of *Nicotiana tabacum* cv. Xanthi were incubated with transconjugant *A. tumefaciens* for 2 d (Horsch et al. 1985). After two to three weeks callus formed at the edges of the leaf discs and shoots appeared. These kanamycin-resistant shoots were transplanted and roots were induced as described by Voelker et al. (1987). Kanamycin-resistant tobacco plants were transferred to soil and grown to maturity in a greenhouse. Seeds were harvested at different stages of maturity and analyzed. All results shown, except those in Fig. 5, are for the high-expressing plant KDEL-4.

Protein, protein extraction, endoglycosidase-H digestion, and immunoblotting. Purified PHA-L protein was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). For the protein extraction of tobacco seeds, fresh material was ground at 0° C with a buffer containing 50 mM/Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol), pH 8, 30 mM NaCl, 0.1% Triton X-100 (octyl phenoxy polyethoxyethanol), and 1% β-mercaptoethanol. The supernatant of the subsequent centrifugation (14000·g for 5 min) was the extract. For immunoblotting, appropriate quantities of protein (determined according to Lowry et al. 1951) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the proteins were transferred to nitrocellulose, and PHA detected using a rabbit anti-PHA-L antibody generated as described in Voelker et al. (1987). We used goat antirabbit horseradish peroxidase cou-

pled immunoglobulins G (IgGs) (Bio-Rad Laboratories, Richmond, Cal., USA) as secondary antibody and 4-chloro-1-naphtol as a peroxidase substrate. Endoglycosidase-H (endoH) digestions of extracts were carried out according to Trimble and Maley (1984).

Immunocytochemistry. Immunocytochemical localization was accomplished as detailed in Herman et al. (1989). Briefly, embryos and endosperm of mid- to late maturation transgenic tobacco seeds fixed in 4% formaldehyde, 2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, were excised. The embryos and endosperms were then dehydrated in a graded ethanol series and the tissues embedded in LR White resin (Ted Pella Co., Tustin Calif., USA). Thin sections were mounted on nickel grids, were blocked with 10% fetal bovine serum in Tris-buffered saline containing Tween-20 (polyoxyethylene sorbitan monolaureate) (TBST: 20 mM Tris-HCl, pH 7.4, 0.15 M NACl, 0.5%, v/v, Tween-20) for 10 min at room temperature. The grids were sequentially labeled with 10 µg/ml affinity-purified anti-PHA IgG in TBST for 30 min, washed twice with TBST, and then indirectly labeled with 10 nm colloidal gold-goat anti-rabbit IgG (Jannsen, Antwerpen, Belgium) for 5 min at room temperature. The grids were then washed twice in TBST, once in distilled water, and then stained with 5% (w/v) aqueous uranyl acetate for 30 min at room temperature. In parallel control samples labeled with nonimmune rabbit IgG incubated in the same conditions were used. The labeled grids were examined with Hitachi (Tokyo, Japan) 300 and 500 electron microscopes.

Results

The glycosylation status of PHA. Analysis by immunoblot of PHA in mid-mature seeds of control plants containing the normal gene for PHA-L shows the presence of a doublet of full-length polypeptides and several processing products of the protein (see Sturm et al. 1989, and Fig. 2, lane 2). The upper band of the doublet is PHA with two high-mannose glycans that has not yet reached the protein-storage vacuoles, and the lower band is PHA with one high-mannose and one complex glycan that has arrived in the vacuoles. The polypeptide-processing products are also found in the vacuoles. At seed maturity (Fig. 2, lane 3), there is less PHA overall (intact polypeptide and processing products), consistent with a slow breakdown of PHA in tobacco seeds. The polypeptide with two high-mannose glycans has largely disappeared at seed maturity. In the seeds of plants transformed with PHA-KDEL, there is at mid-maturity a triplet of full-length polypeptides: a large amount of the upper band (arrow) as well as two slightly smaller polypeptides, the middle one corresponding to mature PHA in control seeds. At seed maturity (Fig. 2, lane 5), there is still a triplet of PHA-KDEL with approximately equal proportions of the upper and middle polypeptides.

To check the glycosylation status of the PHA and PHA-KDEL polypeptides, we digested them with endo-H prior to the immunoblot analysis. The results for standard PHA-L (Fig. 3, lanes 1 and 2) show a change in Mr of about 2 kDA as is to be expected when one high-mannose glyan is removed (Vitale et al. 1984a, b). The PHA from plants transformed with the normal PHA-L gene moves as a doublet, and there is an inversion of the polypeptides upon treatment with endo-H (Fig. 3, lanes 3 and 4) as judged by the relative intensities of

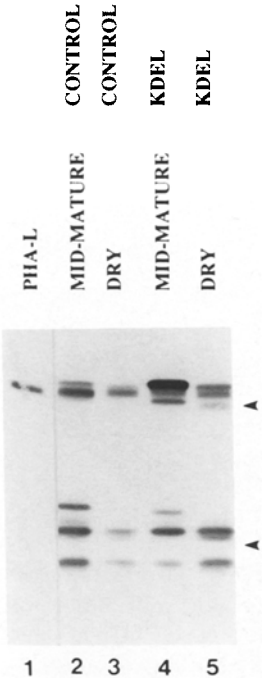

Fig. 2. Immunoblot analysis of PHA and PHA-KDEL in the seeds of transgenic tobacco. Seed extracts were prepared at seed maturity (*lanes 3* and *5*) or 20 days after pollination (*lanes 2* and *4*). *Lane 1* contains standard PHA-L. Note the doublet of full-length PHA molecules in *lane 2* and the great excees (*arrow*) of the upper member of the doublet in *lane 4*. The *arrowheads* on the *right* indicate the positions of molecular-weight markers of Mr 18000 and 26000

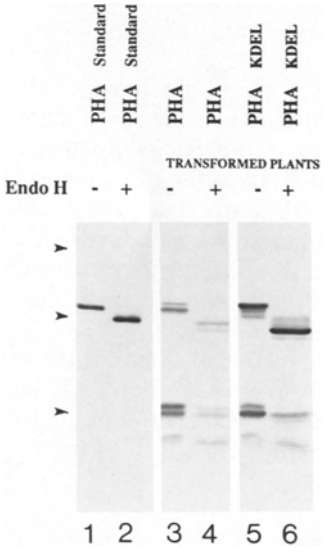

Fig. 3. Immunoblot analysis of PHA and PHA-KDEL after removal of high-mannose glycans with endoglycosidase H. Standard PHA-L in *lanes 1–2*; PHA and PHA-KDEL from transformed tobacco seeds in *lanes 3–4* and *5–6*, respectively. Removal of one high-mannose glycan results in a shift of 2000 Da (compare *lanes 1* and *2*) and of two high-mannose glycans in a shift of 4000 Da (compare most abundant polypeptide in *lanes 5* and *6*). Molecular weight markers of Mr 18000, 26000 and 43000 on the *left*

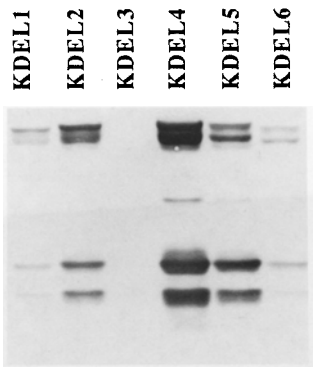

Fig. 4. Immunoblot analysis of PHA-KDEL in different transformants showing varying levels of expression

the band. The Mr of the most abundant polypeptide (upper band in lane 3) changes its Mr by 4 kDa (lower band in lane 4). The Mr of the other polypeptide changes by 2 kDa upon treatment with endo-H. This is in agreement with our earlier work (Sturm et al. 1988) that seeds of tobacco plants transformed with the PHA-L gene contain two forms of PHA-L: one with two endo-H-sensitive chains located mostly in the ER, and mature PHA-L, located mostly in the protein storage vacuoles. The two endo-H-sensitive chains are probably of the high mannose type, but we cannot rule out the possibility that they are hybrid oligosaccharides. Such oligosaccharides are also sensitive to endo-H. In the PHA-KDEL sample, the upper, abundant polypeptide changes its Mr after endo-H digestion by 4 kDa consistent with the interpretation that it has two high-mannose glycans. This means that in the PHA-KDEL seeds a large proportion of the polypeptide that is produced does not undergo Golgi-processing and polypeptides with two high-mannose chains persist in mature seeds of transgenic tobacco in seeds of plants transformed with normal PHA.

To determine if the ratio of the upper band of the doublet (two high-mannose glycans) to the lower band of the doublet (one high-mannose and one complex glycan) is related to the level of PHA-KDEL expression, we checked a number of transformants that gave differing levels of PHA-KDEL accumulation in mature seeds. These different levels of expression are probably caused by so-called position effects of the β-phaseolin promoter (Sengupta-Gopalan et al. 1985; Voelker et al. 1989). The results (Fig. 4) show that seeds from all plants had an approximately equal distribution between the two polypeptides in the doublet. This eliminates the possibility that examining low or high expressers will bias the results.

Phytohemagglutinin-KDEL binds to thyroglobulin. To make sure that the lack of glycan processing was not caused by improper folding of PHA, we checked the binding of PHA to porcine thyroglobulin linked to Sepharose beads. Phytohemagglutinin is a lectin that binds to the carbohydrate sidechains of thyroglobulin. Such binding requires that PHA be in a native configuration. The immunoblot shown in Fig. 5 indicates that both

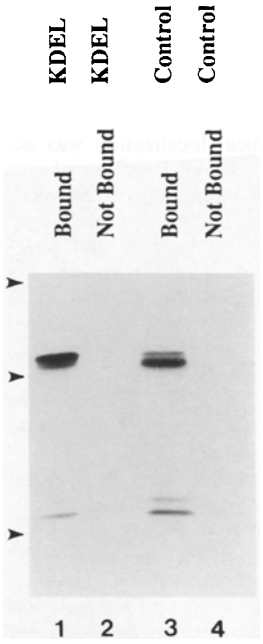

Fig. 5. Immunoblot analysis of PHA and PHA-KDEL after binding to thyroglobulin-Sepharose. The bound fractions are shown in *lanes 1* and *3*, the unbound fractions in *lanes 2* and *4*

normal PHA and PHA-KDEL bind equally well to thyroglobulin-Sepharose. We conclude, therefore, that the folding of PHA-KDEL is not grossly perturbed and that the carbohydrate-binding site is undisturbed.

Subcellular location of PHA-KDEL. The subcellular distribution of PHA with carboxyterminal KDEL expressed in mid- to late-maturation embryos and endosperms was examined by electron microscopic immunocytochemistry. Affinity purified antibodies diluted into a high concentration of fetal bovine serum resulted in specific labeling with a very low nonspecific background. The cellular distribution of PHA-KDEL in an embryo storage parenchyma cell is shown in Fig. 6. The matrix of the protein storage vacuoles in the storage parenchyma tissue was labeled with a high density of gold particles (Fig. 6). The central crystalloid region of the protein storage vacuole was devoid of label except for a very low level of nonspecifically bound gold particles. The presence of PHA-KDEL in the protein-storage-vacuole matrix and its absence from the crystalloid is an identical localization pattern to that observed in transgenic tobacco seed cells that express normal PHA (Sturm et al. 1988). Cells in the provascular region of the embryo contain protein storage vacuoles which are typically devoid of the crystalloid structure and contain a matrix of lower electron density. The protein storage vauoles in the provascular cells do not accumulate high levels of normal PHA or PHA-KDEL (data not shown). Observations on maturing jackbean cotyledons with electron-microscopic immunocytochemistry also showed little accumulation of the lectin concanavalin A in the provascular cell protein storage vacuoles (Herman and Shannon 1984). Dense gold label was also observed on

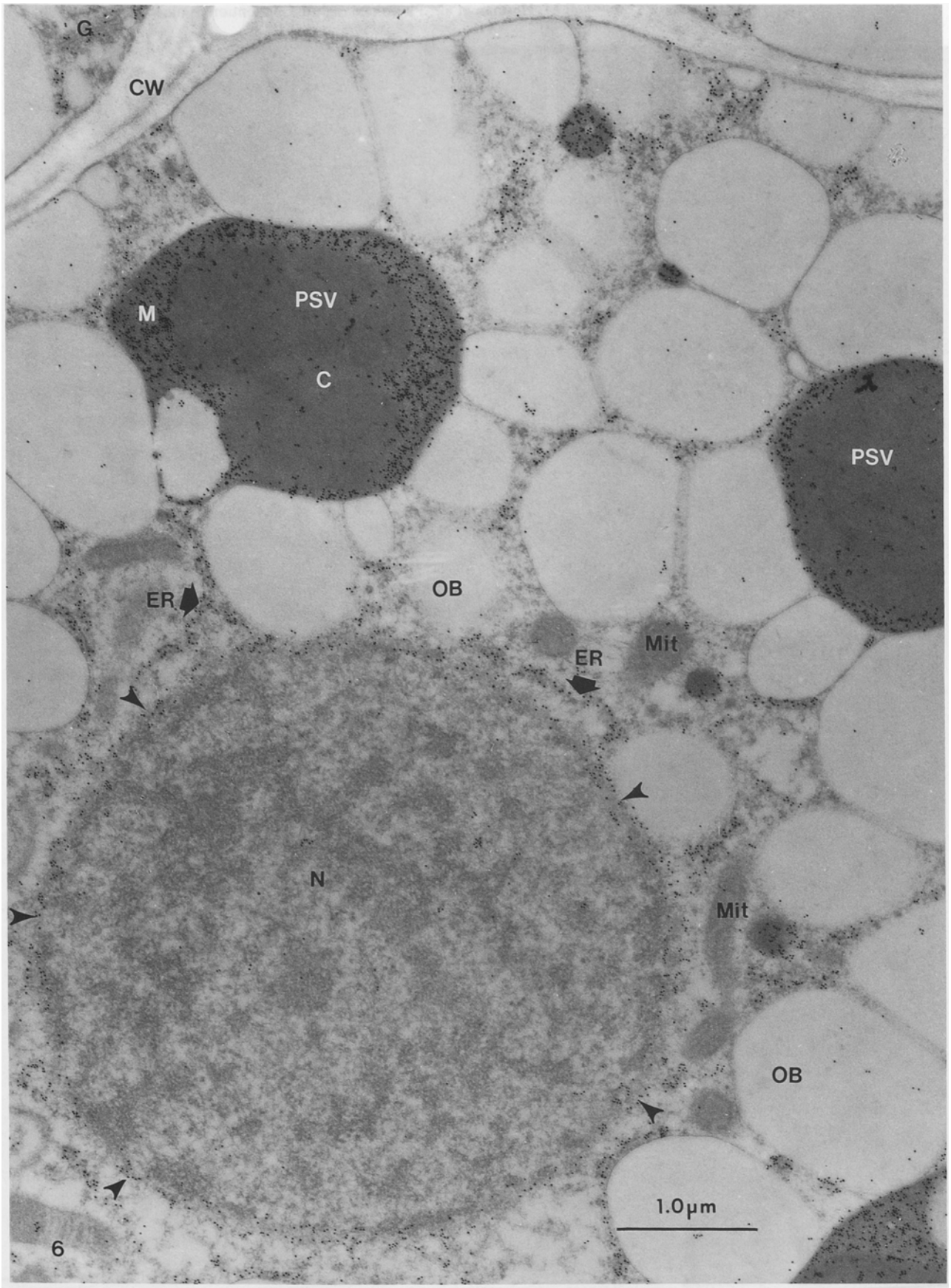

Fig. 6. The localization of PHA-KDEL in a storage parenchyma cell of a transgenic tobacco embryo is shown. A high density of gold particles label the entire intracellular endomembrane system including the endoplasmic reticulum (*ER, arrows*), the entire nuclear envelope (*arrowheads*), Golgi apparatus (*G*) and the matrix region (*M*) of the protein storage vacuole (*PSV*). In contrast the crystalloid region (*C*) of the protein storage vacuole exhibits very little gold particle label. Very little gold label is associated with the plasma membrane and extracellular space and cell wall (*CW*), indicating PHA-KDEL is not secreted from the cell. Other seed cell organelles including the oil bodies (*OB*) and mitochondria (*Mit*) exhibit only very low nonspecific labeling. × 25300

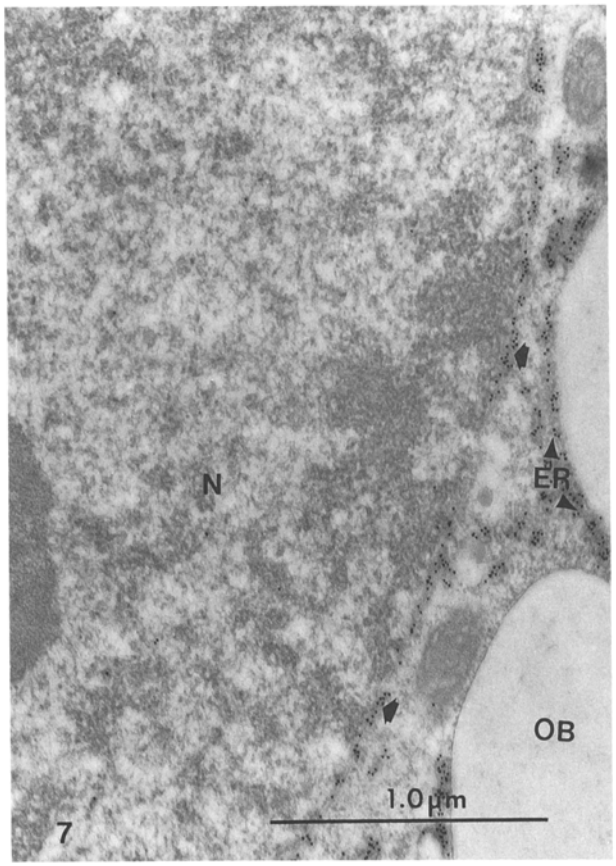

Fig. 7. The accumulation of PHA-KDEL in the nuclear envelope is shown. About one quarter of the nucleus is shown here which is labeled with a high density of gold particles around the entire periphery (*arrowheads*). Specific label is absent on the interior of the nucleus (*N*). The endoplasmic reticulum (*ER*) adjacent to the nucleus is labeled with gold particles at about the same density as the nuclear envelope. Specific gold label is absent on the oil bodies (*OB*). ×37000

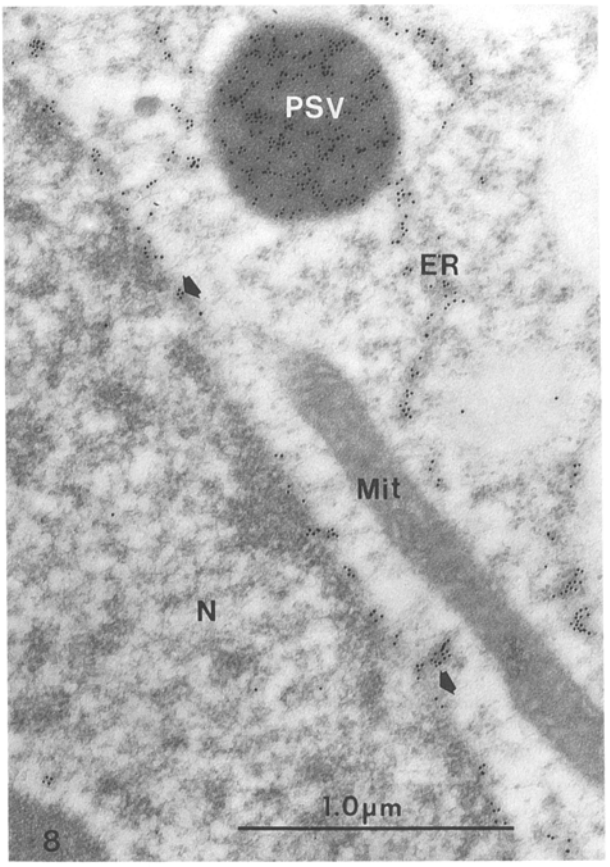

Fig. 8. The accumulation of PHA-KDEL in the endomembrane system components, the nuclear envelope (*arrowheads*), protein storage vacuole (*PSV*) and endoplasmic reticulum (*ER*) are shown. Specific labeled gold particles are almost completely absent on the cytoplasm surrounding the endomembrane system and on the oil bodies (*OB*) and mitochondria (*Mit*). ×37000

segments of the ER and the nuclear envelope (Figs. 6, 7, 8). The entire periphery of the nucleus was labeled with the average label density observed to be similar to that observed on the ER (Fig. 6). The immunocytochemistry assay does not have sufficient resolution to permit us to determine whether the label is on one or both nuclear envelope membranes or in the lumen between them. Little labeling was observed in the nuclear periphery of provascular cells (data not shown), indicating that there is a direct correlation between the label density on the protein storage vacuole and nucleus. Gold particles were also observed on the cisternae of the Golgi apparatus (Fig. 6), which has been previously been shown to mediate deposition of lectins in the protein storage vacuole (Chrispeels 1983; Herman and Shannon 1984). The PHA-KDEL label is restricted to the intracellular components of the secretory system. No label above background was found to be associated with the cell wall and plasma membrane, indicating that PHA-KDEL is not secreted (Fig. 6). Organelles that are not part of the secretory system, mitochondria and oil bodies, were devoid of label except for an occasional nonspecific gold particle (Figs. 6, 8).

Discussion

Our results show that the presence of carboxyterminal KDEL on a secretory protein (the vacuolar protein PHA-L) results in a partial retention of the protein in the secretory system. Although we obtained extensive gold-labeling of the ER, there was also abundant gold-labeling over the protein storage vacuoles. One way to explain these results is to postulate that the high expression of this transgene results in poor retrieval and recycling of PHA-KDEL from the post-ER compartment back to the ER. The β-phaseolin promoter driving PHA-L expression in tobacco is quite strong, giving rise to the accumulation of 1% of the total seed protein (Dorel et al. 1988; Voelker et al. 1989). Another explanation is indicated by the observation that the ratio of the two glycosylated forms of PHA-KDEL (polypeptides with two high-mannose glycans and polypeptides with one high-mannose and one complex glycan) is constant in mature seeds, regardless of the level of expression and accumulation. A third explanation of partial retention is that the carboxyterminal KDEL tetrapeptide is not optimally displayed at the surface of the protein to be

readily recognized by the putative receptor. Less than optimal display would cause poor recognition and inefficient recycling of PHA-KDEL back to the ER. This means that it is not sufficient to have carboxyterminal KDEL, but that the tetrapeptide must also be positioned in such a way that it can readily be recognized by its receptor (Vaux et al. 1989).

Zagouras and Rose (1990) modified two secretory proteins (rat growth hormone and the α-subunit of human chorionic gonadotrophin) in such a way that the sequence SEKDEL was added to the exact carboxytermini of the proteins. This modification resulted in a reduction in the rate of transport of these proteins out of monkey COS cells, but did not prevent their secretion. The half-time for secretion was increased sixfold for both proteins. These experiments and our results lead to the conclusion that features of protein structure other than carboxyterminal KDEL must be important for absolute retention in the ER.

Our results also show that a much larger proportion of PHA-KDEL than of control PHA has two high-mannose glycans as opposed to a high-mannose glycan and a complex glycan. This is true for both mid-mature and mature seeds. The presence of complex glycans on a glycoprotein can be taken as evidence that the protein is in, or has passed through the Golgi complex, and the absence of complex glycans normally means that the protein is still in the ER or *cis*-Golgi. We have shown previously that the upper polypeptide of the PHA doublet in seeds from plants transformed with the PHA-L gene is in the cytoplasm (presumably in the ER), while the lower polypeptide of the doublet is in the protein storage vauoles (Sturm et al. 1988). By this criterion, approximately half the PHA-KDEL remains in the ER (and nuclear envelope) at seed maturity. In the seeds that contain PHA-KDEL there is a triplet of full-length PHA molecules. We do not know what the lowest band of this triplet represents and whether it corresponds to a proteolytic or a glycolytic processing product of normal PHA. Unfortunately, we have not yet devised a suitable method to isolate ER from the oil-rich tobacco seeds or to recover protein storage vacuoles quantitatively. We cannot exclude the possibility that the presence of KDEL at the carboxyterminus inhibits the processing of the glycan at Asn 60 by somehow preventing one of the processing enzymes in the Golgi to carry out its normal modification reaction.

Particularly noteworthy is the presence of PHA-KDEL in the nuclear envelope and to our knowledge this is the first report of such a localization for a protein that is modified at the carboxyterminus with the purpose of causing ER-retention. It is important to note that normal PHA (Sturm et al. 1988) and PHA modified by removal of the glycosylation sites (Voelker et al. 1989) expressed in transgenic tobacco cells are not found in association with the nuclear envelope. No nuclear labelling has been reported in any of the other electron-microscopic immunocytochemistry studies of vacuolar storage proteins and lectins (see Herman 1988 for review). This indicates that the KDEL modification of PHA induces a redirection of part of the total PHA-KDEL to the nuclear envelope. The similar density of gold labeling on both the ER and nuclear envelope suggests that KDEL-proteins sequestered within the lumen of these two organelles are in concentration equilibrium. Ultrastructural observations have shown that the nuclear envelope is continuous with the ER in many types of cells, and it is therefore not unexpected that proteins that are in the lumen of the ER can be found in the nuclear envelope, especially if transport out of the ER is prevented. This is exactly what Doms et al. (1989) observed when transport of proteins out of the ER was inhibited with brefeldin A. Rose et al. (1989) also observed a perinuclear staining in yeast of the KAR2 protein, a yeast homolog of the mammalian ER protein BiP/GRP 78. Hardwick et al. (1990) have shown that yeast cells labeled with anti-HDEL antibodies and examined by immunofluorescence light microscopy resulted in staining of strands of presumptive ER, material close to the plasma membrane, and the perinuclear region. Whether this perinuclear staining means a location in the lumen of the nuclear envelope remains to be determined. They concluded that anti-HDEL antibodies recognize a small family proteins which are normally associated with the ER.

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Erratum

Maltose excretion by the symbiotic *Chlorella* of the heliozoan *Acanthocystis turfacea*

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The inscriptions on the left and right side of Fig. 1 were misplaced. The inscription on the left side should have read "^{14}C in cells (cpm $\times 10^{-3}$)" and that on the right side "^{14}C in the medium (cpm $\times 10^{-3}$)".