Plant Cell Monogr (4)
D.G. Robinson: The Plant Endoplasmic Reticulum
DOI 10.1007/7089_055/Published online: 24 February 2006


# Transport of Proteases to the Vacuole: ER Export Bypassing Golgi?

Takashi Okamoto

Department of Biological Sciences, Tokyo Metropolitan University, Minami-osawa 1-1, Hachioji, 192-0397 Tokyo, Japan
*okamoto-takashi@c.metro-u.ac.jp*

**Abstract** Most vacuolar proteases are transported from the endoplasmic reticulum (ER) to vacuoles via the Golgi apparatus. However, higher plants possess a unique papain-type protease, termed KDEL-tailed protease. This protease has a Lys – Asp – Glu – Leu (KDEL) sequence at its C-terminus, which is known as a retention signal of soluble proteins to the ER, although the protease localizes and functions in vacuoles. Investigations on the intracellular trafficking pathway of this unique enzyme have suggested that the protease is transported from the ER to vacuoles by bypassing the Golgi apparatus. In this review, Golgi-dependent vacuolar trafficking of proteases is first explained, then the Golgi-independent vacuolar transport pathway of the KDEL-tailed protease is described.

## 1 Vacuolar Proteases and Their Trafficking Pathways Along the Endomembrane System

A major function of plant vacuoles is protein degradation and processing, and many vacuolar proteases have been identified as mediators for these proteolytic events. Recent proteomic analysis of vacuoles isolated from Arabidopsis rosette leaves indicated that at least 23 kinds of proteases, including cysteine, serine and aspartic proteases, exist in vacuoles (Carter et al. 2004).

Papain-type proteases (EC3.4.22), which possess a cysteine residue at the active site forming a catalytic triad and generally show broad substrate specificity, are thought to be the most abundant proteases in plant vacuoles (Rawlings and Barrett 1994). Cysteine proteases are initially synthesized as larger precursor proteins with *N*-terminal prodomains of approximately 120 amino acid residues. The prodomain functions as an inhibitor of the protease by occluding the active site and is essential for correct folding of the protein (Vernet et al. 1995; Carmona et al. 1996). Papain superfamily proteases are widely distributed among eukaryotes (Rawlings and Barrett 1994; Betri and Storer 1995) and are localized in lytic compartments (vacuoles or lysosomes) or secreted.

In plant cells, it is known that vacuolar transport signals of soluble proteins can be divided into three categories: sequence specific vacuolar sorting signals (ssVSS), *C*-terminal signals (csVSS), and physical structural signals

(psVSS; Matsuoka and Neuhaus 1999; Vitale and Raikhel 1999). The typical tetra sequence of ssVSS is an Asn – Pro – Ile – Arg (NPIR) motif on the *N*-terminal prosequence of vacuolar proteins. Vacuolar targeting via the NPIR motif was first identified on the polypeptide of sporamin, a storage protein of sweet potato (Matsuoka and Nakamura 1991), and papain-type proteases also contain the NPIR motif on the *N*-terminal prosequence (Matsuoka and Neuhaus 1999). Barley aleurain is the best characterized papain-type protease in terms of the molecular mechanism of vacuolar trafficking. Holwerda et al. (1992) revealed that the NPIR sequence on the *N*-terminal prosequence of aleurain functions as a vacuolar targeting signal, and a putative receptor, termed BP-80, for the vacuolar sorting signals was isolated from maturing Pea cotyledons (Paris et al. 1997).

Homologues of BP-80 have been isolated in pumpkin (PV72; Shimada et al. 1997), Arabidopsis (AtELP; Ahmed et al. 1997) and mung bean (VmVSR; Tsuru-Furuno et al. 2001), and they are called vacuolar sorting receptors (VSRs). VSRs are type I integral membrane proteins consisting of three epidermal growth factor motifs and a protease-associated region at the luminal domains (Mahon and Bateman 2000). It has been shown that BP-80 and AtELP bind in vitro to aleurain and AtALEU, an Arabidopsis aleurain, respectively, via the NPIR sequence (Paris et al. 1997; Ahmed et al. 2000), and that they localize in the Golgi apparatus and prevacuolar compartment (Ahmed et al. 2000). Recently, the effects of heterologous expression of the luminal region of a PV72 tagged with an ER-retention signal, HDEL, on the intracellular transport of AtALUE were observed (Watanabe et al. 2004). The heterologous expression of PV72-HDEL in Arabidopsis resulted in accumulation of the proform of AtALUE in leaf cells of the transgenic plants, suggesting that the VSR mislocalized in ER trapped the proform of AtALUE, and that VSR functions as a receptor for the protease in vivo as well as in vitro. Through these investigations, it has been suggested that papain-type protease possessing ssVSS is transported from the ER to vacuoles via the Golgi apparatus, and that the NPIR sequence recognized by VSR at the Golgi apparatus is a determinant for the vacuolar traffic.

Aspartic proteases (EC3.4.23) are also major proteases found in vacuoles (Elpidina et al. 1990; Runeberg-Roos et al. 1994; Hiraiwa et al. 1997; Ramalho-Santos et al. 1997; Mutlu et al. 1999), and the majority of plant aspartic proteases belong to the A1 family (Rawlings and Barret 1999; Simoes and Faro 2004). It has been revealed that aspartic proteases are widely distributed among vertebrates, plants, yeast, nematodes, fungi and virus (Davies 1990), and that plant enzymes show sequence similarities to their animal counterparts (Runeberg-Roos et al. 1991; Codeiro et al. 1994; Hiraiwa et al. 1997). However, it is noteworthy that plant aspartic proteases possess an inserted sequence of approximately 100 amino acid residues between the *N*- and *C*-terminal regions of the mature enzyme, and that this inserted sequence is not found in animal counterparts. The plant specific insert (PSI) is highly

similar to that of saposins, which are known as lysosomal sphingolipid-activating proteins that appear to be required for hydrolysis of sphingolipids by specific lysosomal hydrolases (O'Brien and Kishimoto 1991; Weiler et al. 1995). Analysis of the crystal structure of the proform of phytepsin, a barley aspartic protease, indicated that the PSI is expected to form an external loop on the surface of the molecules and to have a putative membrane binding region, suggesting the possibility that it plays a role in vacuolar transport via membrane-associated receptors (Kervinen et al. 1999). Direct evidence of the involvement of PSI in vacuolar trafficking was obtained from observations of intracellular localization of phytepsin and PSI-deleted phytepsin heterologously expressed in tobacco cells. Tormakangas et al. (2001) reported that deletion of PSI results in secretion of mutant protease, whereas intact phytepsin continued to localize in vacuoles in the tobacco cells. In addition, phytepsin is known to be glycosylated and acquires complex oligosaccharides, which occurs in the Golgi apparatus (Costa et al. 1997). From these various studies, it has been suggested that aspartic proteases are transported to the vacuoles via the Golgi-complex using PSI as a transport signal, although the possible receptor for PSI has yet to be identified.

## 2
## Golgi-Independent Vacuolar Transport of a KDEL-Tailed Protease

### 2.1
### KDEL-Tailed Proteases

Eukaryotic cells are divided into distinct subcellular compartments or organelles enclosed by one or more membranes. Because protein synthesis occurs mainly in the cytosol, proteins of subcellular compartments have intracellular localization signals that determine their final destinations. A transient signal peptide allows co-translational entry into the lumen of the ER. The ER is the starting compartment for vesicular trafficking to the Golgi apparatus, vacuoles and cell surface along the secretory pathway. Because secretion following a route mediated by the Golgi apparatus is the default destination for proteins introduced into the ER, proteins localizing in the ER, Golgi apparatus or vacuoles must have additional signals. Most soluble ER residents have a permanent *C*-terminal KDEL or HDEL tetrapeptide sequence, which constitutes an ER retention signal (Munro and Pelham 1987; Pelham 1989). The tetrapeptide is recognized by an ERD2-KDEL receptor in the Golgi apparatus, resulting in retrieval of H/KDEL proteins from this compartment back into the ER. The H/KDEL system is conserved through mammals, plants and yeasts (Denecke et al. 1992; Napier et al. 1992; Lee et al. 1993). Besides ER residents found in other eukaryotes, higher plants have unique papain-type proteases that possess KDEL-tails at the *C*-terminus, termed KDEL-tailed proteases (Akasofu et al.

**Table 1** KDEL-tailed proteases and their expression sites

| Enzyme | Plants | Organs showing expression | *C*-terminal sequences | Ref./accession no. |
|---|---|---|---|---|
| SH-EP | Mung bean | Germinated seeds | KDEL | Akasofu et al. (1989) |
| CysEP | Castor bean | Germinated seeds (endosperm) | KDEL | Schmid et al. (1998) |
| Proteinase A | Vetch bean | Germinated seeds | KDEL | Becker et al. (1997) |
| EP-C1 | French bean | Senescing leaves and pods | KDEL | Tanaka et al. (1993) |
| TPE4A | Pea | Immature ovules | KDEL | Cercos et al. (1999) |
| SEM102 | Day lily | Senescing leaves and flowers | RDEL | Valpuesta et al. (1995) |
| SEN11 | Day lily | Senescing leaves and flowers | KDEL | Guerrero et al. (1998) |
| O141 | Orchid | Mature ovules | RDEL | Nadeau et al. (1996) |
| REP-A | Rice | Germinated seeds | KDEM* | Shintani et al. (1997) |
| CysP1 | Soybean | Germinated seeds | KDEL | Ling et al. (2003) |
| CysP2 | Soybean | Germinated seeds | KDEL | Ling et al. (2003) |
| EP-A | Barley | Germinated seeds | TDEL* | Z97023 |
| PRT5 | Sandersonia | Senescing leaves | KDEL | AF133839 |
| AtCEP1 | Arabidopsis | —** | KDEL | At5g50260 |
| AtCEP2 | Arabidopsis | —** | KDEL | At3g48530 |
| AtCEP3 | Arabidopsis | —** | KDEL | At3g48540 |

* It is unknown whether the *C*-terminal of KDEM or TDEL sequence functions as an ER retention signal.
** The expression site of Arabidopsis KDEL-tailed proteases is unknown.

1989; Tanaka et al. 1993; Valpuesta et al. 1995; Becker et al. 1997; Guerrero et al. 1998; Schmid et al. 1998; Cercos et al. 1999).

In spite of the universal distribution of papain-type proteases, KDEL-tailed proteases appear to be restricted to the plant kingdom. KDEL-tailed proteases have been identified in at least 12 plant species, and most are expressed in senescing organs such as germinated cotyledons, senescing leaves and pods (Table 1). One such KDEL-tailed protease, designated SH-EP, was first isolated from cotyledons of mung bean seedlings as the enzyme responsible for degradation of storage proteins accumulated in protein storage vacuoles of cotyledon cells (Mitsuhashi et al. 1986). SH-EP is synthesized on membrane-bound ribosomes as a 43-kDa precursor through co-translational cleavage of the signal peptide, and the precursor is processed into a 33-kDa mature enzyme via 39- and 36-kDa intermediates during or after transport to the vacuoles (Mitsuhashi and Minamikawa 1989; Okamoto and Minamikawa 1998). Interestingly, regardless of the presence of the KDEL-tail on the polypeptide, it has been shown that mature SH-EP localizes in vacuoles (Okamoto et al. 1994). In the next section, the vacuolar transport pathway of this unique protease is explained.

## 2.2
## Packing of KDEL-Tailed Protease into KDEL-Vesicles at the ER

Immunogold-labeling of cotyledon cells from mung bean seedlings with an antibody against mature SH-EP revealed that a large amount of SH-EP is localized in a vesicle with a diameter of 200–500 nm (Fig. 1A; Toyooka et al. 2000). Here, the large vesicle containing KDEL-tailed protease is called the KDEL-vesicle. Accumulation of a large amount of SH-EP was observed at the edge or middle region of the ER, and KDEL-vesicles were frequently observed close to the ER (Fig. 1A,B). In addition, the ER often terminated in a small vesicle surrounded by a ribosome-attached membrane (Fig. 1C). This data suggested that SH-EP synthesized in the ER is packed into KDEL-vesicles formed at the edge or middle area of the ER, and that budding of the vesicle from the ER is the first step of SH-EP transport from the ER to vacuoles. Questions regarding whether SH-EP exists in KDEL-vesicles as proforms or mature enzyme, and whether the KDEL-tail of SH-EP is removed when the enzyme is packed into the KDEL-vesicles from ER thus arise. Dense labeling of KDEL-vesicles with antibodies against the *N*-terminal propeptide of SH-EP and *C*-terminal KDEL sequence suggested that SH-EP accumulates in the vesicles as enzymatically inactive proenzyme, and that the KDEL-tail of SH-EP is not removed during transport of proSH-EP from the ER to KDEL-vesicles.

KDEL-vesicles, termed ricinosomes, have been detected in endosperm cells of castor bean seedlings and in cells of senescing petals from day lily (Schmid et al. 1999). Moreover, Schmid et al. (2001) successfully isolated KDEL-vesicles from endosperms of castor bean seedlings, and further ana-

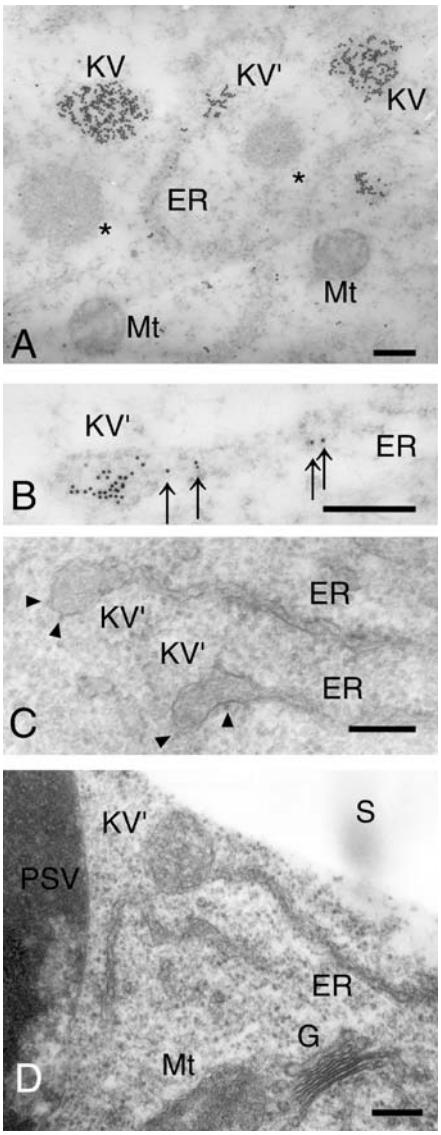


**Fig. 1** Electron photographs showing the budding of KDEL-vesicles from ER. **A** Accumulation of SH-EP at the edge of ER (KV′). The area where SH-EP localized was swollen. Two KDEL-vesicles existed close to the ER. **B** Magnified image of the accumulation of SH-EP at the edge of the ER (KV′). SH-EP in the lumen of the ER (*arrows*) seemed to be moving toward the edge of the ER where a large amount of SH-EP was accumulated (KV′). **C** Ultrastructure of cotyledon cells, showing ER terminates in a small vesicle (KV′). *Arrowheads* indicate ribosomes. **D** Ultrastructure of cotyledon cells, showing the existence of vesicles similar to KDEL-vesicles (KV′) adjacent to the ER. ER, endoplasmic reticulum; G, Golgi apparatus; KV, KDEL-vesicle; PSV, protein storage vacuole; S, starch granule; Mt, mitochondrion; Asterisk, unidentified cell compartment, *Bars*, 200 nm

lyzed the composition of matrix proteins in the vesicles by SDS-PAGE and subsequent protein sequencing of major proteins in the gel. Surprisingly, more than 90% of the proteins in the KDEL-vesicles were occupied by the proform of castor bean KDEL-tailed protease, designated CysEP. This indicates that KDEL-tailed proteases are highly concentrated in KDEL-vesicles, and that the vesicles might function as a "battery" of proteases in plant cells.

### 2.3
### KDEL-Vesicles Bypass the Golgi Apparatus

Involvement of the Golgi apparatus in vacuolar transport of KDEL-tailed proteases has been observed in detail, since vacuolar proteins are generally transported along the secretory pathway via the Golgi apparatus, from which vacuolar and secretory proteins are separately sorted. Despite the immunogold-labeling of ER and KDEL-vesicles in cotyledon cells of mung bean seedlings with anti-SH-EP antibody, the Golgi apparatus was never labeled with this antibody (Fig. 2A,B). On the other hand, when an antibody to another vacuolar protease, an asparaginyl endopeptidase, was employed for immunogold labeling, the Golgi apparatus and vacuoles were labeled, but the KDEL-vesicle was not (Fig. 2C). This selective labeling of KDEL-vesicles and Golgi apparatus with two kinds of vacuolar proteases indicates that cotyledon cells use two sorting pathways to transport proteolytic enzymes from the ER to vacuoles, a Golgi-mediated route for asparaginyl endopeptidase and a KDEL-vesicle mediated route for SH-EP. Moreover, a complex glycan antibody did not label KDEL-vesicles, but it did label the Golgi apparatus (Fig. 2D), indicating that proteins from the Golgi apparatus do not contribute to the content or formation of the vesicles. These immuno-cytochemical studies have shown that KDEL-vesicles bypass the Golgi apparatus and directly fuse with protein storage vacuoles (Fig. 2E,F). Bypassing the Golgi apparatus by KDEL-vesicles will probably be consistent even when organelle/vesicle sizes are compared, since the diameter of KDEL-vesicles (200–500 nm) is probably too large for discharge of their content into the Golgi apparatus without extreme structural consequences to this organelle.

### 2.4
### Involvement of the *C*-Terminal KDEL Sequence in Formation of KDEL-Vesicles and in Efficient Vacuolar Transport

Although most plant papain-type proteases are transported to vacuoles or secreted along the general secretory pathway, KDEL-tailed proteases are packed into KDEL vesicles, which directly fuse with vacuoles through a Golgi apparatus-independent pathway. The most notable difference between normal papain-type protease and KDEL-tailed protease is the presence of a KDEL sequence at the *C*-terminus, suggesting that the KDEL-tail is involved in this

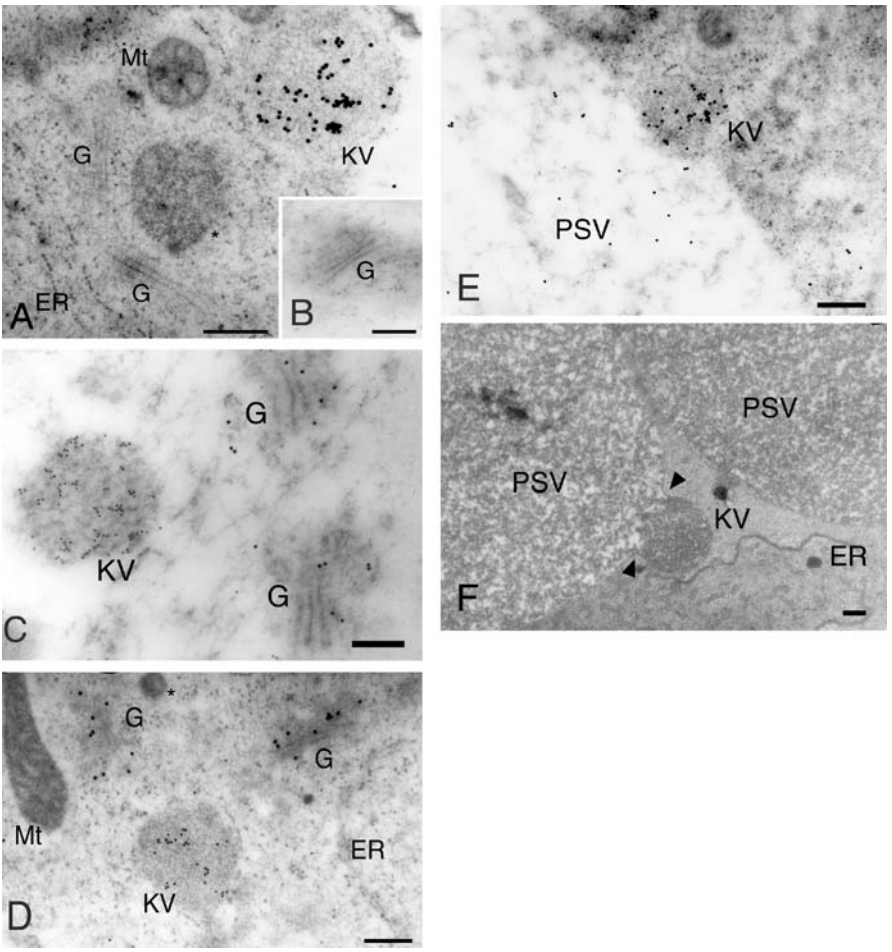


**Fig. 2** Electron photographs showing the transport of SH-EP via KDEL-vesicles by bypassing the Golgi apparatus. **A**,**B** The anti-SH-EP antibody did not immunogold-label the Golgi apparatus, although the KDEL-vesicle was labeled. **C** Antibodies to SH-EP (10-nm particles) and an asparaginyl endopeptidase (15-nm particles) selectively labeled the KDEL-vesicle and the Golgi apparatus, respectively. **D** The anti-complex glycan antibody (15-nm particles) and anti-SH-EP antibodies (10-nm particles) specifically immunogold-labeled the Golgi-apparatus and KDEL-vesicle, respectively. **E** KDEL-vesicle immunogold-labeled with the anti-SH-EP antibody fused with a protein storage vacuole (PSV). Gold particles localized in the PSV as well as the KDEL-vesicle. **F** Ultrastructural photograph showing that a vesicle similar to the KDEL-vesicle fuses to PSV. *Arrowheads* indicate the region where the KDEL-vesicle merges with the PSV membrane. ER, endoplasmic reticulum; G, Golgi apparatus; KV, KDEL-vesicle; Mt, mitochondrion; PSV, protein storage vacuole. *Bars*, 200 nm

unique vacuolar trafficking pathway. To address the possibility, SH-EP and its KDEL-deletion mutant (SH-EP$\Delta$KDEL) were heterologously expressed in Arabidopsis and intracellular localizations of these proteins were monitored. In cells from stems, cotyledons, rosette leaves and flowers of transformed plants expressing SH-EP, the enzyme accumulated in vesicles with diameters between 200–700 nm (Fig. 3A,B; Okamoto et al. 2003). These vesicles possibly correspond to KDEL-vesicles, since their size and the accumulation of KDEL-tailed protease are characteristic of KDEL-vesicles in cotyledons of mung bean seedlings. In addition, KDEL-vesicles in transgenic plants appeared to fuse with vacuoles (Fig. 3A). In contrast to heterologous expression of intact SH-

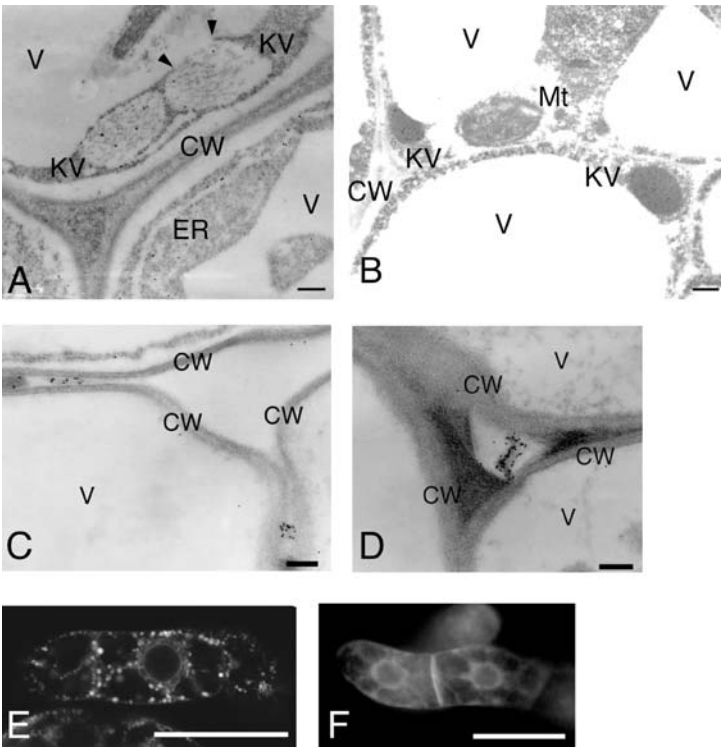


**Fig. 3** Heterologous expression of SH-EP (**A**,**B**,**E**) and SH-EP$\Delta$KDEL (**C**,**D**,**E**) in Arabidopsis (**A**–**D**) and tobacco BY2 cells (**E**,**F**). **A**,**B** Electron micrographs showing the development of KDEL-vesicles in stem (**C**) and cotyledon cells (**D**) of transgenic Arabidopsis expressing SH-EP. A KDEL-vesicle fused with a vacuole (*arrowheads* in panel **A**). **C**,**D** Electron photographs showing secretion of SH-EP$\Delta$KDEL from the cells of rosette leaves of transgenic Arabidopsis. Gold particles from the anti-SH-EP antibody were found at extracellular spaces and possible air spaces. **E**,**F** Intracellular localization of signal peptide (SP)-GFP-SHEP (**E**) or SP-GFP-SHEP$\Delta$KDEL (**F**) in tobacco BY-2 cells. Small foci derived from GFP fused with SH-EP were observed in the cells of panel **E**, however such signal was not detected in the cells of panel **F**. *Bars*, 200 nm (**A**–**D**) and 50 $\mu$m (**E**,**F**)

EP, formation of KDEL-vesicle was not detected in cells of transformed plants expressing SH-EPΔKDEL. Furthermore, SH-EPΔKDEL was mainly secreted and localized at the extracellular spaces and possible air spaces (Fig. 3C,D), indicating that the mutated protease was not packed into KDEL-vesicle but was secreted. The KDEL-tail of the protease will be essential for formation of KDEL-vesicles and subsequent vacuolar transport via the vesicles.

Additional confirmation of involvement of the KDEL-tail in formation of KDEL-vesicles was from GFP-labeling of the vesicles by heterologous expression of GFP-fused SH-EP or SH-EPΔKDEL in tobacco BY-2 cells. When signal peptide (SP)-GFP-SHEP was expressed, strong fluorescence was detected in small vesicles (Fig. 3E), and the diameter of the GFP-labeled small vesicles appeared to be similar to that of KDEL-vesicles, which were detected in cells of transformed Arabidopsis expressing SH-EP (Fig. 3A,B). In the case of SP-GFP-SHEPΔKDEL expression in tobacco cells, such a small vesicle was not observed, suggesting that deletion of the KDEL-tail from SH-EP resulted in loss of formation of the KDEL vesicle.

### 2.5
### Golgi-Independent Vacuolar Transport of Non-KDEL-Tailed Proteases

In addition to KDEL-tailed proteases, Arabidopsis RD21, a papain-type protease, and $\gamma$VPE, an asparaginyl endopeptidase, are known to be vacuolar proteases which are transported to vacuoles in a Golgi-independent manner. RD21 and $\gamma$VPE are localized in spindle-shaped vesicles, which are derived from ER ranging in size from 0.1–10 μm, and the vesicles, termed ER-bodies, are known to fuse with vacuoles via a Golgi apparatus-independent pathway as for the KDEL-vesicles (Hayashi et al. 2001; Hara-Nishimura et al. 2004). Matsushita et al. (2003) successfully identified a $\beta$-glucosidase possessing a KDEL-tail (PYK10) as the predominant protein component of ER-bodies (see also Hara-Nishimura in this volume). In addition, strong expression of PYK10 protein in cells appears to be needed for formation/induction of ER-bodies. Although involvement of the KDEL-tail of PYK10 in the formation of ER-bodies remains to be clarified, aggregation of PYK10 at the ER where ER-bodies are formed might occur via its KDEL-tail. RD21 and $\gamma$VPE are considered to be trapped in ER-bodies during aggregate formation of abundant PYK10 in the ER, resulting in the direct ER-vacuole transport of these non-KDEL-tailed proteases via ER-bodies (Hara-Nishimura et al. 2004).

## 3
## Conclusions and Prospects

The vacuolar trafficking pathways of the major plant vacuolar proteases, papain-type and aspartic proteases, are summarized in Fig. 4. Proforms of

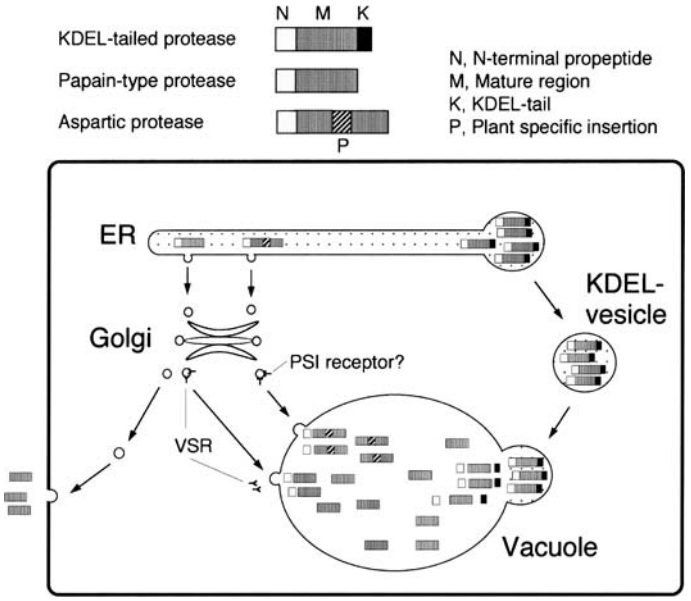


**Fig. 4** A model of the intracellular transport pathways for papain-type and aspartic proteases in plant cells. Proforms of non-KDEL papain-type proteases are transported from ER to vacuoles via ssVSS signal (NPIR motif), which is recognized by VSR existing on the Golgi apparatus. Proforms of aspartic proteases are also transported from ER to vacuoles through the Golgi apparatus, in which the proenzymes are recognized by putative receptor for PSI. Proforms of KDEL-tailed papain-type proteases synthesized in the lumen of ER are packed into KDEL-vesicles probably via the KDEL-tail. KDEL-vesicles filled with the proenzymes bud off from the ER, bypass the Golgi apparatus and fuse with vacuoles, resulting in the release of proenzymes into the inside of the vacuoles. When the *C*-terminal KDEL sequence was deleted from the KDEL-tailed protease, mutant protease is secreted probably along the endomembrane system. All vacuolar proteases described in the figures are activated by proteolytic processing after or during the transport of proenzymes to the vacuoles

normal papain-type (without KDEL-tail) and aspartic proteases are first transported from the ER to Golgi apparatus, and then they are recognized by VRS and putative receptor for PSI on the Golgi apparatus, respectively, resulting in transport of the proenzymes to vacuoles. After or during transport to the vacuoles, the proenzymes are converted to mature enzymes through proteolytic processing. In the case of KDEL-tailed proteases, proenzymes are accumulated at the ER and packed into KDEL-vesicles without removal of the KDEL-tail. These KDEL-vesicles, in which KDEL-tailed proteases are predominantly concentrated, bypass the Golgi apparatus and directly fuse with vacuoles. In the vacuoles, proenzymes are converted into active form by the removal of the *N*-terminal prosequence through possible autocatalysis of the enzyme. *C*-terminal propeptide containing the KDEL-tail will be removed by

other vacuolar proteases (Okamoto et al. 2001). With deletion of the KDEL-tail from the protease, mutant proteins are not packed into KDEL vesicles, but are mainly secreted possibly along the endomembrane system.

Why do plant cells utilize KDEL-vesicles for vacuolar transport of the protease? It has been reported that secretion of the KDEL-tailed protease (SHEP$\Delta$KDEL) results in growth defects of transformed plants through possible degradation of proteins existing in the extracellular space (Okamoto et al. 2003). In general, KDEL-tailed proteases are expressed at extremely high levels in senescing cells in which massive turnover and recapture of cellular materials occur. Since the vacuolar trafficking pathway via the Golgi apparatus appears to be saturable (Frigerio et al. 1998), plant cells may utilize the KDEL-tail for aggregation of highly expressed dangerous enzymes, such as papain-type proteases, at the ER. In addition to efficient and massive vacuolar transport of protease by large KDEL-vesicles, packing them into KDEL-vesicles may be a way to escape saturation of the vacuolar trafficking route along the endomembrane system and saturation-induced miss-sorting of the vacuolar protease. Induction/formation of KDEL-vesicles in cells of most tissues of transgenic plants by heterologous expression of KDEL-tailed proteases is an example of the enormous plasticity of the ER in plant cells.

## References

Akasofu H, Yamauchi D, Mitsuhashi W, Minamikawa T (1989) Nucleotide sequence of cDNA for sulfhydryl-endopeptidase (SH-EP) from cotyledons of germinating Vigna mungo seeds. Nucleic Acids Res 17:6733

Ahmed SU, Bar-Peled M, Raikhel NV (1997) Cloning and subcellular location of an Arabidopsis receptor-like protein that shares common features with protein-sorting receptors of eukaryotic cells. Plant Physiol 114:325–336

Ahmed U, Rojo E, Kovaleva V, Venkataraman S, Dombrowski JE, Matsuoka K, Raikhel NV (2000) The plant vacuolar sorting receptor AtELP is involved in transport of NH(2)-terminal propeptide-containing vacuolar proteins in Arabidopsis thaliana. J Cell Biol 149:1335–1344

Becker C, Senyuk VI, Shutov AD, Nong VH, Fischer J, Horstmann C, Muntz K (1997) Proteinase A, a storage-globulin-degrading endopeptidase of vetch (Vicia sativa L.) seeds, is not involved in early steps of storage-protein mobilization. Eur J Biochem 248:304–312

Berti PJ, Storer AC (1995) Alignment/phylogeny of the papain superfamily of cysteine proteases. J Mol Biol 246:273–283

Carmona E, Dufour E, Plouffe C, Takebe S, Mason P, Mort JS, Menard R (1996) Potency and selectivity of the cathepsin L propeptide as an inhibitor of cysteine proteases. Biochemistry 35:8149–8157

Carter C, Pan S, Zouhar J, Avila EL, Girke T, Raikhel NV (2004) The vegetative vacuole proteome of Arabidopsis thaliana reveals predicted and unexpected proteins. Plant Cell 16:3285–3303

Cercos M, Santamaria S, Carbonell J (1999) Cloning and characterization of TPE4A, a thiol-protease gene induced during ovary senescing and seed germination in pea. Plant Physiol 119:1341–1348

Cordeiro MC, Xue ZT, Pietrzak M, Pais MS, Brodelius PE (1994) Isolation and characterization of a cDNA from flowers of Cynara cardunculus encoding cyprosin (an aspartic proteinase) and its use to study the organ-specific expression of cyprosin. Plant Mol Biol 24:733–741

Costa J, Ashford DA, Nimtz M, Bento I, Frazao C, Esteves CL, Faro CJ, Kervinen J, Pires E, Verissimo P, Wlodawer A, Carrondo MA (1997) The glycosylation of the aspartic proteinases from barley (Hordeum vulgare L.) and cardoon (Cynara cardunculus L.). Eur J Biochem 243:695–700

Davies DR (1990) The structure and function of the aspartic proteinases. Annu Rev Biophys Biophys Chem 19:189–215

Denecke J, De Rycke R, Botterman J (1992) Plant and mammaliam sorting signals for protein retention in the endoplasmic reticulum contain a conserved epitope. EMBO J 11:2345–2355

Elpidina EN, Dunaevsky YE, Belozersky MA (1990) Protein bodies from Buckwheat seed coyledons – Isolation and characterization. J Exp Bot 51:969–977

Frigerio L, de Virgilio M, Prada A, Faoro F, Vitale A (1998) Sorting of phaseolin to the vacuole is saturable and requires a short *C*-terminal peptide. Plant Cell 10:1031–1042

Guerrero C, Calle M, Reid MS, Valpuesta V (1998) Analysis of the expression of two thiolprotease genes from daylily (Hemerocallis spp.) during flower senescence. Plant Mol Biol 36:565–571

Hara-Nishimura I, Matsushima R, Shimada T, Nishimura M (2004) Diversity and formation of endoplasmic reticulum-derived compartments in plants. Are these compartments specific to plant cells? Plant Physiol 136:3435–3439

Hayashi Y, Yamada K, Shimada T, Matsushima R, Nishizawa NK, Nishimura M, Hara-Nishimura I (2001) A proteinase-storing body that prepares for cell death or stresses in the epidermal cells of Arabidopsis. Plant Cell Physiol 42:894–899

Hiraiwa N, Kondo M, Nishimura M, Hara-Nishimura I (1997) An aspartic endopeptidase is involved in the breakdown of propeptides of storage proteins in protein-storage vacuoles of plants. Eur J Biochem 246:133–141

Holwerda BC, Padgett HS, Rogers JC (1992) Proaleurain vacuolar targeting is mediated by short contiguous peptide interactions. Plant Cell 4:307–318

Kervinen J, Tobin GJ, Costa J, Waugh DS, Wlodawer A, Zdanov A (1999) Crystal structure of plant aspartic proteinase prophytepsin: inactivation and vacuolar targeting. EMBO J 18:3947–3955

Lee HI, Gal S, Newman TC, Raikhel NV (1993) The Arabidopsis endoplasmic reticulum retention receptor functions in yeast. Proc Natl Acad Sci USA 90:11433–11437

Ling JQ, Kojima T, Shiraiwa M, Takahara H (2003) Cloning of two cysteine proteinase genes, CysP1 and CysP2, from soybean cotyledons by cDNA representational difference analysis. Biochim Biophys Acta 1627:129–139

Mahon P, Bateman A (2000) The PA domain: a protease-associated domain. Protein Sci 9:1930–1934

Matsuoka K, Nakamura K (1991) Propeptide of a precursor to a plant vacuolar protein required for vacuolar targeting. Proc Natl Acad Sci USA 88:834–838

Matsuoka K, Neuhaus J-M (1999) Cis-elements of protein transport to the plant vacuoles. J Exp Bot 50:165–174

Matsushima R, Kondo M, Nishimura M, Hara-Nishimura I (2003) A novel ER-derived compartment, the ER body, selectively accumulates a beta-glucosidase with an ER-retention signal in Arabidopsis. Plant J 33:493–502

Mitsuhashi W, Koshiba T, Minamikawa T (1986) Separation and characterization of two endopeptidases from cotyledons of germinating Vigna mungo seeds. Plant Physiol 80:628–634

Mitsuhashi W, Minamikawa T (1989) Synthesis and posttranslational activation of sulfhydryl-endopeptidase in cotyledons of germinating Vigna mungo seeds. Plant Physiol 89:274–279

Munro S, Pelham HBR (1987) A *C*-terminal signal prevents secretion of luminal ER proteins. Cell 48:899–907

Mutlu A, Chen X, Redy SM, Gal S (1999) The aspartic proteinase is expressed in Arabidopsis thaliana seeds and localized in the protein bodies. Seed Sci Res 9:75–84

Napier RM, Fowke LC, Hawes CR, Lewis M, Pelham HBR (1992) Immunological evidence that plants use both HDEL and KDEL for targeting proteins to the endoplasmic reticulum. J Cell Sci 102:261–271

Nadeau JA, Zhang XS, Li J, O'Neill SD (1996) Ovule development: identification of stage-specific and tissue-specific cDNAs. Plant Cell 8:213–239

O'Brien JS, Kishimoto Y (1991) Saposin proteins: structure, function, and role in human lysosomal storage disorders. FASEB J 5:301–308

Okamoto T, Nakayama H, Seta K, Isobe T, Minamikawa T (1994) Posttranslational processing of a carboxy-terminal propeptide containing a KDEL sequence of plant vacuolar cysteine endopeptidase (SH-EP). FEBS Lett 351:31–34

Okamoto T, Minamikawa T (1998) A vacuolar cysteine endopeptidase (SH-EP) that digests seed storage globulin: Characterization, regulation of gene expression, and posttranslational processing. J Plant Physiol 152:675–682

Okamoto T, Shimada T, Hara-Nishimura I, Nishimura M, Minamikawa T (2003) *C*-terminal KDEL sequence of a KDEL-tailed cysteine proteinase (sulfhydryl-endopeptidase) is involved in formation of KDEL vesicle and in efficient vacuolar transport of sulfhydryl-endopeptidase. Plant Physiol 132:1892–1900

Okamoto T, Toyooka K, Minamikawa T (2001) Identification of a membrane-associated cysteine protease (MCP) with possible dual roles in the endoplasmic reticulum and protein storage vacuole. J Biol Chem 276:742–751

Paris N, Rogers SW, Jiang L, Kirsch T, Beevers L, Phillips TE, Rogers JC (1997) Molecular cloning and further characterization of a probable plant vacuolar sorting receptor. Plant Physiol 115:29–39

Pelham HBR (1989) Control of protein exit from the endoplasmic reticulum. Annu Rev Cell Biol 5:1–23

Ramalho-Santos M, Pissarra J, Verissimo P, Pereira S, Salema R, Pires E, Faro CJ (1997) Cardosin A, an abundant aspartic proteinase, accumulates in protein storage vacuoles in the stigmatic papillae of Cynara cardunculus L. Planta 203:204–212

Rawlings ND, Barrett AJ (1994) Families of cysteine peptidases. Methods Enzymol 244:461–486

Rawlings ND, Barrett AJ (1999) MEROPS: the peptidase database. Nucleic Acids Res 27:325–331

Runeberg-Roos P, Kervinen J, Kovaleva V, Raikhel NV, Gal S (1994) The aspartic proteinase of barley is a vacuolar enzyme that processes probarley lectin in vitro. Plant Physiol 105:321–329

Runeberg-Roos P, Tormakangas K, Ostman A (1991) Primary structure of a barley-grain aspartic proteinase. A plant aspartic proteinase resembling mammalian cathepsin D. Eur J Biochem 202:1021–1027

Schmid M, Simpson D, Kalousek F, Gietl C (1998) A cysteine endopeptidase with a *C*-terminal KDEL motif isolated from castor bean endosperm is a marker enzyme for the ricinosome, a putative lytic compartment. Planta 206:466–475

Schmid M, Simpson D, Gietl C (1999) Programmed cell death in castor bean endosperm is associated with the accumulation and release of a cysteine endopeptidase from ricinosomes. Proc Natl Acad Sci USA 96:14159–14164

Schmid M, Simpson DJ, Sarioglu H, Lottspeich F, Gietl C (2001) The ricinosomes of senescing plant tissue bud from the endoplasmic reticulum. Proc Natl Acad Sci USA 98:5353–8535

Shimada T, Kuroyanagi M, Nishimura M, Hara-Nishimura I (1997) A pumpkin 72-kDa membrane protein of precursor-accumulating vesicles has characteristics of a vacuolar sorting receptor. Plant Cell Physiol 38:1414–1420

Simoes I, Faro C (2004) Structure and function of plant aspartic proteinases. Eur J Biochem 271:2067–2075

Shintani A, Kato H, Minamikawa T (1997) Hormonal regulation of expression of two cysteine endopeptidase genes in rice seedlings. Plant Cell Physiol 38:1242–1248

Tanaka T, Minamikawa T, Yamauchi D, Ogushi Y (1993) Expression of an endopeptidase (EP-C1) in Phaseolus vulgalis plants. Plant Physiol 101:421–428

Tormakangas K, Hadlington JL, Pimpl P, Hillmer S, Brandizzi F, Teeri TH, Denecke J (2001) A vacuolar sorting domain may also influence the way in which proteins leave the endoplasmic reticulum. Plant Cell 13:2021–2032

Toyooka K, Okamoto T, Minamikawa T (2000) Mass transport of a proform of a KDEL-tailed cysteine proteinase (SH-EP) to protein storage vacuoles by endoplasmic reticulum-derived vesicle is involved in protein mobilization in germinating seeds. J Cell Biol 148:453–563

Tsuru-Furuno A, Okamoto T, Minamikawa T (2001) Isolation of a putative receptor for KDEL-tailed cysteine proteinase (SH-EP) from cotyledons of Vigna mungo seedlings. Plant Cell Physiol 42:1062–1070

Valpuesta V, Lange NE, Guerrero C, Reid MS (1995) Up-regulation of a cysteine protease accompanies the ethylene-insensive senescence of daylily (Hemerocallis spp.) floweres. Plant Mol Biol 28:575–582

Vernet T, Berti PJ, de Montigny C, Musil R, Tessier DC, Menard R, Magny MC, Storer AC, Thomas DY (1995) Processing of the papain precursor. The ionization state of a conserved amino acid motif within the Pro region participates in the regulation of intramolecular processing. J Biol Chem 270:10838–10846

Vital A, Raikhel NV (1999) What do proteins need to reach different vacuoles? Trends Plant Sci 4:149–155

Watanabe E, Shimada T, Tamura K, Matsushima R, Koumoto Y, Nishimura M, Hara-Nishimura I (2004) An ER-localized form of PV72, a seed-specific vacuolar sorting receptor, interferes the transport of an NPIR-containing proteinase in Arabidopsis leaves. Plant Cell Physiol 45:9–17

Weiler S, Kishimoto Y, O'Brien JS, Barranger JA, Tomich JM (1995) Identification of the binding and activating sites of the sphingolipid activator protein, saposin C, with glucocerebrosidase. Protein Sci 4:756–764