Unique Defense Strategy by the Endoplasmic Reticulum Body in Plants

Kenji Yamada1,2, Ikuko Hara-Nishimura3 and Mikio Nishimura1,2,*

1Department of Cell Biology, National Institute for Basic Biology, Nishigo-naka 38, Okazaki 444-8585, Aichi, Japan
2School of Life Science, Graduate University for Advanced Studies (Sokendai), Okazaki 444-8585, Aichi, Japan
3Department of Botany, Graduate School of Science, Kyoto University, Kitashirakawa-oiwake, Kyoto 606-8502, Kyoto, Japan

*Corresponding author: E-mail, mikosome@nibb.ac.jp; Fax, +81-564-53-7400.

(Received September 8, 2011; Accepted November 1, 2011)

The endoplasmic reticulum (ER) is a site for the production of secretory proteins. Plants have developed ER subdomains for protein storage. The ER body is one such structure, which is observed in Brassicaceae plants. ER bodies accumulate in seedlings and roots or in wounded leaves in Arabidopsis. ER bodies contain high amounts of the β-glucosidases PYK10/BGLU23 in seedlings and roots or BGLU18 in wounded tissues. These results suggest that ER bodies are involved in the metabolism of glycoside molecules, presumably to produce repellents against pests and fungi. When Arabidopsis root is homogenized, PYK10 formed large protein aggregates that include other β-glucosidases (BGLU21 and BGLU22), GDSL lipase-like proteins (GLL22) and cytosolic jacalin-related lectins (PBP1/JAL30, JAL31, JAL33, JAL34 and JAL35). Glucosidase activity increases by the aggregate formation. NAI1, a basic helix–loop–helix transcription factor, regulates the expression of the ER body proteins PYK10 and NAI2. Reduced expression of NAI2, PYK10 and BGLU21 resulted in abnormal ER body formation, indicating that these components regulate ER body formation. PYK10, BGLU21 and BGLU22 possess hydrolytic activity for scopolin, a coumaroyl thioglucoside glucosidase; the pyk10 mutants are more susceptible to the symbiotic fungus Piriformospora indica. Therefore, it appears that the ER body is a unique organelle of Brassicaceae plants that is important for defense against pests and fungi.

Keywords: Arabidopsis thaliana • Brassicales • Endoplasmic reticulum • ER body • β-Glucosidase • Pathogen.

Abbreviations: AM, arbuscular mycorrhizae; BGLU, β-glucosidase; bHLH, basic helix–loop–helix; CBB, Coomassie brilliant blue; COI, coronatin insensitive; COP, coat protein complex; DIMBOA, 2,4-dihydroxy-7-methoxy-1, 4-benzoazin-3-one; ER, endoplasmic reticulum; GFP, green fluorescent protein; GH, glycosyl hydrolase; GLL, GDSL lipase-like; JA, jasmonic acid; JAL, jacalin-related lectin; JAZ, jasmonate ZIM-domain; KVs, KDEL-tailed protease-accumulating vesicles; LEB, long ER body; MelA, methyl jasmonate; 4-MUF, 4-methylumbelliferyl-β-D-glucoside; 4-MUG, 4-methylumbelliferyl-β-D-glucoside; PAC, precursor-accumulating; PB, protein body; PBP, PYK10-binding protein; PI, P. indica insensitive; rER, rough endoplasmic reticulum; sER, smooth endoplasmic reticulum; SP, signal peptide; TGG, thioglucoside glucosidase; TSA, Tonsoku-associating protein; VSP, vegetative storage protein.

Introduction

Because plants are sessile; they cannot escape from a rapidly changing environment. Plants are often exposed to various environmental stresses such as extreme temperatures, drought and pest attack. Recent results revealed that dynamic changes in organelles underlie the tolerance of plants to environmental stress. For example, morphological changes in vacuoles and small vesicular structures occur during salt and zinc ionic stress (Hamaji et al. 2009, Kawachi et al. 2009), vacuolar morphology in the endodermis is important for the shoot gravitropic response (Niihama et al. 2009) and changes in chloroplast positioning are important for maintaining efficient photosynthesis and evading photodamage (Kadota et al. 2009, Yamada et al. 2009a, Suetsugu et al. 2010). Therefore, focusing on organelle differentiation may reveal new insights into plant survival strategies (Hayashi and Nishimura 2009, Homi et al. 2009, Kamigaki et al. 2009, Mano et al. 2009, Nagano et al. 2009, Niihama et al. 2009, Sakamoto et al. 2009).

The endoplasmic reticulum (ER) is a network-like structure that is bound by a single membrane. The ER is a dynamic organelle composed of various functional domains (Staehelin 1996). It includes the rough ER (rER), smooth ER (sER) and nuclear envelope. The rER is a well-understood organelle that is coated by ribosomes and is responsible for the synthesis of secretory proteins. The sER has no ribosomes but instead accumulates a series of lipid biosynthesis enzymes. In addition, the ER accumulates specific types of seed storage proteins, such as prolamin or zein, to produce protein bodies (PBs) in...
the endosperm of maize (*Zea mays*) and rice (*Oryza sativa*) (Herman 2008, Yasuda et al. 2009, Kumamaru et al. 2010, Satoh-Cruz et al. 2010, Nagamine et al. 2011). KDEL-tailed protease-accumulating vesicles (KVs) and ricinosomes are ER-derived structures that accumulate specific proteins with ER retention signals such as the papain-type proteases in the dying tissues of mungbean (*Vigna mungo*) and castor bean (*Ricinus communis*) seedlings (Toyooka et al. 2000, Schmid et al. 2001). Most ER-derived transport vesicles are coat protein complex (COP) II vesicles that are responsible for the transport of proteins passing through the Golgi apparatus (Alberts et al. 2002). On the other hand, the maturing cotyledons of pumpkin (*Cucurbita maxima*) produce precursor-accumulating (PAC) vesicles that are ~200 nm in diameter and involved in the bulk transport of seed protein precursors (Hara-Nishimura et al. 1998, Hara-Nishimura et al. 2004). Similar structures occur in the seeds of *maigo* mutants that have a defect in the transport of seed storage proteins between the ER and Golgi in *Arabidopsis thaliana* (Li et al. 2006, Shimada et al. 2006, Takahashi et al. 2010). PAC vesicles are larger than COP II vesicles, are species specific and appear during specific stages in the plant life cycle. In Arabidopsis, several research groups have identified another type of ER structure that has been designated as the ER body/fusiform body (Gunning 1998, Ridge et al. 1999, Hayashi et al. 2001). The size and shape of the ER body is different from other ER-derived structures. The ER body is ~10 μm long and 1 μm wide, and is the largest ER structure in plants. It is of great interest to understand how and why plants form this unique organelle. Recent studies have revealed the mechanisms of ER body formation and its possible function. This review focuses on these topics.

**The ER body is a specific domain of the endoplasmic reticulum**

The ER body was first observed in the epidermis of Arabidopsis seedlings expressing SP–GFP–HDEL (Mitsuhashi et al. 2000), which is a green fluorescent protein (GFP) fused to a signal peptide (SP) at its N-terminus and His-Asp-Glu-Leu (HDEL), an ER retention signal, at its C-termina (Haseloff et al. 1997, Ridge et al. 1999, Hayashi et al. 2001). In these plants, GFP fluorescence was observed in the ER network, as expected. However, in addition to the ER network, novel rod-shaped structures were observed in the cotyledons (Fig. 1A). In support of this observation, ultrastructural analysis by electron microscopy showed that there are rod-shaped structures surrounded by a single membrane with attached ribosomes in Arabidopsis (Fig. 1B) (Gunning 1998, Hayashi et al. 2001). A connection between the structures and the ER was observed, confirming that they were subdomains of the ER (Gunning 1998). Immunoelectron microscopy using an anti-GFP antibody revealed that the structures accumulated GFP in transgenic plants expressing SP–GFP–HDEL, demonstrating that the rod-shaped structures observed by GFP fluorescence and the rod-shaped structures observed by electron microscopy were the same structures (Hayashi et al. 2001). These structures were termed ER bodies (Hayashi et al. 2001) or fusiform bodies (Hawes et al. 2001, Nelson et al. 2004) since they are fusiform structures derived from the ER. ER bodies are filled with condensed materials (Fig. 1B), suggesting that they function in the storage of proteins (Hayashi et al. 2001). Consistent with this, the specific gravity of ER bodies is higher than that of the ER and they are easily precipitated during low-speed centrifugation at 1,000×g (Hayashi et al. 2001). ER bodies sometimes move very quickly in the cell along the longitudinal axis. A movie of ER body movement is available in the Plant Organelles Database 2 (PODB2, http://podb.nibb.ac.jp/ Organelome/) (Mano et al. 2011). Fluorescence recovery after photobleaching analysis revealed that GFP fluorescence in the ER body was easily recovered after GFP was photo-bleached (K. Tamura, personal communication), suggesting that GFP flows from the ER to the ER body. Although ER bodies can move very quickly, most seem to be connected to the ER, which can be observed in electron micrographs (Gunning 1998).
ER bodies are observed in Brassicaceae plants

ER bodies were first identified in Arabidopsis; however, similar structures had already been investigated in electron micrographs between 1965 and 1978 (Bonnett and Newcomb 1965, Iversen 1970a, Iversen 1970b, Esau 1975, Hoefert 1975, Jørgensen et al. 1977, Behnke and Eschlbeck 1978). These structures were long and rod shaped and surrounded by ribosomes. In these reports, the structures were referred to as dilated cisternae. These structures frequently included substantial internal tubular structures that ran longitudinally through the ER bodies (Hoefert 1975, Jørgensen et al. 1977, Behnke and Eschlbeck 1978). ER bodies in Arabidopsis sometimes also include indistinct fibrillar structures (Hayashi et al. 2001, Matsushima et al. 2002, Matsushima et al. 2003a). These observations suggested that components of the ER bodies form fibrillar aggregated structures. Based on these observations, ER bodies were determined to exist exclusively in Brassicaceae plants such as radish (Raphanus sativus), white mustard (Sinapis alba), caner (Capparis spinosa), cleome (Cleome spinosa) and field penny-cress (Thlaspi arvense) (Iversen 1970a, Iversen 1970b, Behnke and Eschlbeck 1978). The order Brassicales includes the family Brassicaceae, Resedaceae and Tovariaceae (The Angiosperm Phylogeny Group 2003). ER body-like protein aggregates were observed in the vacuoles of Tovaria pendula, a species of Tovariaceae (Behnke and Eschlbeck 1978). However, no ER body has been identified in Resedaceae (Iversen 1970b, Behnke and Eschlbeck 1978). Interestingly, Brassicaceae plants specifically accumulate glucosinolates (β-thioglucoside-N-hydroxysulfates) that are essential for the defense strategy (Rodman et al. 1998, Fahey et al. 2001, Beekwilder et al. 2008, Bednarek et al. 2009, Clay et al. 2009). Therefore, the relationship between glucosinolate metabolism and ER bodies was addressed (Behnke and Eschlbeck 1978), and myrosinase (β-thioglucosidase) activity was detected in ER bodies by cytochemical electron microscopy in white mustard (Iversen 1970a). ER body-like structures have also been observed in non-Brassicaceae plants, such as the California poppy (Eschscholtzia douglasii) (Iversen 1970b), tobacco (Nicotiana tabacum) (Hawes et al. 2001) and humble plant (Mimosa pudica) (Esau 1975), although it is possible that the structures seen in these plants are KVs or ricinosome-related structures, which accumulate proteases and are observed in a wide range of species (Schmid et al. 1999, Okamoto et al. 2003, Senatore et al. 2009).

There are two types of ER bodies in Arabidopsis: seedling and root ER bodies and wound-inducible ER bodies

Based on accumulation patterns, we can categorize ER bodies into two types in Arabidopsis: seedling and root ER bodies, and wound-inducible ER bodies. Arabidopsis seedlings accumulate ER bodies in the epidermis (Hayashi et al. 2001). Dry seeds have no ER bodies, but 4 d of imbibition induces ER body accumulation in the cotyledon (Matsushima et al. 2003a), suggesting that ER bodies are synthesized de novo after seed germination. The ER bodies in cotyledons disappear slowly with the progression of senescence (Matsushima et al. 2002). Root tissues in Arabidopsis always accumulate ER bodies (Matsushima et al. 2002). This is not specific for Arabidopsis because ER bodies are observed in the roots of various Brassicaceae plants and they do not appear to be restricted to the epidermal tissue (Bonnett and Newcomb 1965, Iversen 1970b).

There are few ER bodies in rosette leaves under normal conditions but, when plants are wounded, the accumulation of ER bodies increases (Matsushima et al. 2002, Matsushima et al. 2003a). The accumulation of ER bodies is relatively slow and takes 48–66 h (Matsushima et al. 2002). Jasmonic acid (JA) is a well-known hormone that mediates wound response, induces resistance against insect/pathogen attack (McConnell et al. 1997, Vijayan et al. 1998, Li et al. 2002, Chini et al. 2007, Sato et al. 2011) and regulates the expression of wound-inducible genes, such as the vegetative storage protein (VSP) genes (León et al. 2001, Lorenzo et al. 2004). Methyl jasmonate (MeJA) treatment of rosette leaves induces ER body formation 37 h after treatment, along with the accumulation of VSP proteins (Matsushima et al. 2002). These responses were abolished in the coi1 mutant that has a defect in the JA receptor (Matsushima et al. 2002). Ethylene is another plant hormone that mediates plant defense responses against herbivores (O’Donnell et al. 1996, Stotz et al. 2000, Onkokesung et al. 2010). Ethylene reduced the effect of JA on ER body formation (Matsushima et al. 2002), suggesting that JA and ethylene exert antagonistic effects on ER body formation. These observations suggested that ER bodies are involved in resistance against insects or pathogens.

β-Glucosidase is the main component of the ER body

We have isolated an Arabidopsis mutant lacking ER bodies in seedlings and roots, termed the nai1 mutant. Comparison of the nai1 mutant and wild-type plants by proteomic analysis revealed that ER bodies accumulate PYK10/BGLU23, a β-glucosidase with an ER retention signal (Hara-Nishimura and Matsushima 2003, Matsushima et al. 2003a, Matsushima et al. 2003b). This protein was clearly evident when total proteins in seedlings or roots were separated by electrophoresis and stained with Coomassie brilliant blue (CBB), suggesting that PYK10 is highly accumulated in the ER bodies of seedlings and roots. PYK10 is a member of the GH1 family, which is composed of 47 members in Arabidopsis (Xu et al. 2004). Among these β-glucosidases, there are eight PYK10-type β-glucosidases (BGLU18, BGLU19, BGLU20, BGLU21, BGLU22, PYK10/BGLU23, BGLU24 and BGLU25) that have SPs at the N-terminus and putative ER retention signals at the C-terminus. Of these, only BGLU18 is strongly induced by
wounding (Ogasawara et al. 2009). BGLU18 proteins accumulate in cotyledons after wounding, and immunoelectron microscopy analysis shows that BGLU18 also localizes in the ER body after wounding in the nai1 mutant (Ogasawara et al. 2009). These observations demonstrate that BGLU18 is a component of wound-inducible ER bodies. Microarray data support these observations (Table 1), which show that the expression of PYK10 is high in seedlings and BGLU18 is induced by wounding and MeJA treatment (Stotz et al. 2000). The expression of BGLU21 and/or BGLU22 is high in the roots of MeJA-treated plants (Table 1), suggesting that BGLU21 and/or BGLU22 proteins are the components of ER bodies in roots and MeJA-treated leaves.

β-Glucosidase activity in Arabidopsis seedlings can be measured with the artificial substrates 4-methylumbelliferyl-β-D-glucoside (4-MUG) and 4-methylumbelliferyl-β-D-fucoside (4-MUF) (Matsushima et al. 2004, Nagano et al. 2005, Nagano et al. 2008). Cleavage of 4-MUG and 4-MUF was reduced in the nai1 mutant, which does not accumulate PYK10 (Matsushima et al. 2004), demonstrating that ER body β-glucosidases have both glucosidase and fucosidase activity.

Plants accumulate various glycoside molecules that are derived from secondary metabolism (Gachon et al. 2005, Ketudat 2010). Several glycosides that are involved in defense mechanisms, such as cyanogenic glucosides, saponins, glucosinolates or DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoazoin-3-one) glycoside (Mattiacci et al. 1995, Konno et al. 1999, Tattersall et al. 2001, Carpinella et al. 2005, Beekwilder et al. 2008, Morant et al. 2008). Most of these compounds are stored in the inactive state and become activated by the removal of glycone. β-Glucosidases hydrolyze these molecules into glyccone and aglycone. Thus, they are essential for the production of toxic compounds that mediate plant defense against insects or fungi (Mattiacci et al. 1995, Tattersall et al. 2001, Ketudat 2010). These β-glucosidases are substrate specific; for example, maize β-glucosidase Glu1 hydrolyzes DIMBOA glucoside but not the cyanogenic glucoside dhurrin (Czjzek et al. 2000). To understand the function of the ER body, it was very important to identify the endogenous substrate of ER body β-glucosidases from the numerous glucosides in Arabidopsis. Recent research revealed that BGLU21, BGLU22 and PYK10 hydrolyze scopolin and esculin (Ahn et al. 2010). Scopolin accumulates to high levels in Arabidopsis roots (Kai et al. 2006, Kai et al. 2008), which are the primary tissues for ER body localization, suggesting that scopolin may be an endogenous substrate of ER body β-glucosidases.

Glucosinolates are specific to the Brassicales and are composed of β-thioglucoside and N-hydroxyminosulfate groups. Arabidopsis accumulates three types of glucosinolates: aliphatic, benzyl and indolyl glucosinolates (Brown et al. 2003). Myrosinase is a β-thioglucosidase that specifically hydrolyzes glucosinolates and constitutes the ‘mustard oil bomb’ involved in plant defense against insects (Burmeister et al. 1997). Arabidopsis has typical myrosinases (TGG1/BGLU38, TGG2/BGLU37) that hydrolyze aliphatic glucosinolates (Andersson et al. 2009, Watanabe-Sugimoto et al. 2009), but neither TGG1 nor TGG2 was identified in a proteome analysis of the ER body. PYK10 has high homology to PEN2/BGLU26 that is an atypical myrosinase hydrolyzing two indolyl glucosinolates, indol-3-ylmethylglucosinolate (13G) and 4-methoxyindol-3-ylmethylglucosinolate (4M13G) (Bednarek et al. 2009). However, BGLU21, BGLU22 and PYK10 failed to hydrolyze sinigrin, an aliphatic glucosinolate (Ahn et al. 2010), suggesting that ER body β-glucosidases are not involved in the defense strategy using aliphatic glucosinolates in Arabidopsis.

### Table 1 Expression levels of genes that encode β-glucosidases with putative ER retention signals in Arabidopsis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cotyledon a</th>
<th>Hypocotyl a</th>
<th>Root b</th>
<th>Root c</th>
<th>Wounding 0 h c</th>
<th>Wounding 24 h c</th>
<th>Hormone control d</th>
<th>Hormone MeJA d</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGLU18</td>
<td>1,334.4</td>
<td>1,301.9</td>
<td>24.8</td>
<td>149.1</td>
<td>567.3</td>
<td>2,779.8</td>
<td>336.8</td>
<td>2,551.0</td>
</tr>
<tr>
<td>BGLU19</td>
<td>1.6</td>
<td>6.1</td>
<td>2.7</td>
<td>1.8</td>
<td>8.6</td>
<td>4.4</td>
<td>1.5</td>
<td>11.3</td>
</tr>
<tr>
<td>BGLU20</td>
<td>4.0</td>
<td>0.9</td>
<td>1.1</td>
<td>1.2</td>
<td>0.6</td>
<td>0.8</td>
<td>6.8</td>
<td>5.1</td>
</tr>
<tr>
<td>BGLU21/BGLU22</td>
<td>2.9</td>
<td>110.4</td>
<td>1,934.4</td>
<td>1,817.2</td>
<td>58.7</td>
<td>22.2</td>
<td>625.7</td>
<td>1,902.5</td>
</tr>
<tr>
<td>BGLU23(PYK10)</td>
<td>272.2</td>
<td>2,982.8</td>
<td>2,982.3</td>
<td>2,398.9</td>
<td>1,406.7</td>
<td>1,569.9</td>
<td>3,154.7</td>
<td>2,834.1</td>
</tr>
<tr>
<td>BGLU24</td>
<td>0.8</td>
<td>6.3</td>
<td>12.4</td>
<td>7.1</td>
<td>4.7</td>
<td>7.1</td>
<td>13.2</td>
<td>44.9</td>
</tr>
<tr>
<td>BGLU25</td>
<td>58.6</td>
<td>89.6</td>
<td>182.6</td>
<td>136.4</td>
<td>62.1</td>
<td>53.7</td>
<td>362.5</td>
<td>306.4</td>
</tr>
</tbody>
</table>

The expression of these β-glucosidases is extracted from microarray data that can be found in the online database (http://www.bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi) (Winter et al. 2007). Because of their sequence homology, it is difficult to distinguish between the expression of BGLU21 and BGLU22.

Tissue damage activates ER body β-glucosidases; PYK10 forms a large enzyme complex to increase activity

Proteomic and transcriptomic analysis of the nai1 mutant has enabled identification of the bulk of the proteins/genes involved in PYK10 function (Matsushima et al. 2004, Nagano et al. 2005, Nagano et al. 2008). Among these, the PYK10-binding protein 1 (PBP1), a member of the jacalin-related
lectin (JAL) family of proteins, was investigated in detail (Nagano et al. 2005). Unlike PYK10, PBP1 does not have an SP, and immunocytochemical analysis showed that PBP1 is not localized within the ER body (Nagano et al. 2005). Expression of the PBP1 gene and the accumulation of PBP1 protein were reduced in the nai1 mutant (Nagano et al. 2005). These results suggest that PBP1 is a cytosolic protein whose expression is regulated by NAI1. Interestingly, when Arabidopsis seedlings were homogenized, PYK10 bound to PBP1 and became insoluble (Nagano et al. 2005). The glucosidase activity of PYK10 toward 4-MUG is increased after the incubation of homogenate, especially in the insoluble fraction (Nagano et al. 2005). When equal protein amounts of PYK10 in the soluble or insoluble fractions were analyzed for glucosidase activity, PYK10 in the insoluble fraction had more activity than PYK10 in the soluble fraction (Nagano et al. 2005). These results suggest that PYK10 forms a large aggregate when cells are damaged, and the PYK10 aggregate has higher enzyme activity. In the pbp1 knockout mutant, the formation of the PYK10 aggregate was not reduced but the glucosidase activity of PYK10 was decreased (Nagano et al. 2005). This raises the possibility that cytosolic PBP1 increases PYK10 activity when plants are damaged by insect chewing or pathogen intrusion (Fig. 2). Proteome analysis of the PYK10 aggregate purified from incubated samples revealed that numerous proteins were included in the aggregate. These proteins were separated into three main categories: PYK10-type β-glucosidases (BGLU21, BGLU22 and PYK10/BGLU23), JALs (PBP1/JAL30, JAL31, JAL33, JAL34 and JAL35) and GDSL lipase-like (GLL) protein (GLL22) (Nagano et al. 2008). These proteins interacted with each other to form a large aggregate after a 24 h incubation of seedling extract (Nagano et al. 2008). Based on the finding that the glucosidase activity of the PYK10 aggregate is increased, it is reasonable to assume that these proteins may regulate the activity of PYK10 in the aggregate. Analysis of knockout mutants revealed that JALs and GLLs regulate the size of the PYK10 aggregate (Nagano et al. 2008). The size of the PYK10 aggregate is reduced in jal23 and jal31 mutants and increased in pbp1 and jal22 mutants (Nagano et al. 2008), indicating that JALs regulate the size of the PYK10 aggregate in an antagonistic manner. These observations are very similar to those made with regard to other β-glucosidases that associate with modifier proteins to alter their glucosidase activity, including JAL and GLL proteins (Lambrix et al. 2001, Kittur et al. 2007, Kissen and Bones 2009).

### Mechanism of ER body formation

The specific accumulation of PYK10 in ER bodies implies that special mechanisms exist for the formation of ER bodies...
A forward genetics approach was taken to understand this mechanism. Arabidopsis expressing SP–GFP–HDEL was mutagenized and observed with fluorescence microscopy to isolate mutants that exhibited defective organization of the ER (Matsushima et al. 2003b, Yamada et al. 2008, Nagano et al. 2009, Nakano et al. 2009). The mechanism of ER body formation was uncovered by analyzing mutants with defects in ER body formation, such as nai1, nai2 and long ER body (leb) mutants (Matsushima et al. 2004, Yamada et al. 2008, Nagano et al. 2009, Yamada et al. 2009b).

In the nai1 mutant, there was no ER body accumulation in the seedlings and roots, and the formation of abnormal ER bodies was induced by jasmonate treatment (Matsushima et al. 2004). The NAI1 gene encodes a basic helix–loop–helix (bHLH)-type transcription factor (Matsushima et al. 2004), and transcriptomic analysis showed that the NAI1 gene regulates the expression of various genes, including PYK10 and PBP1. This suggests that NAI1 regulates other components required for ER body formation. In addition to the nai1 mutant, the nai2 mutant also lacks ER bodies in the seedlings and roots (Yamada et al. 2008). The NAI2 gene encodes a unique protein that has an SP, and immunocytochemical analysis revealed that the NAI2 protein accumulates in ER bodies (Yamada et al. 2009a).

Therefore, NAI2 is an ER body component that regulates the formation of ER bodies in seedlings and roots (Fig. 3). NAI2 gene expression is reduced in the nai1 mutant (Yamada et al. 2008), suggesting that NAI1 regulates ER body formation through the expression of NAI2. Interestingly, NAI2 has a specific domain that is present in Brassicales plants but not in plants of other orders, suggesting that NAI2 evolved in Brassicales. In the nai2 mutant, the accumulation of PYK10 was reduced compared with the wild type, indicating that ER body formation by NAI2 is responsible for the accumulation of PYK10 in Arabidopsis (Yamada et al. 2008). There are two other NAI2 homologs in Arabidopsis: one is TSA1 (Tonsoku-associated protein 1) and the other is At3g15960 (Suzuki et al. 2005). Expression of the TSA1 gene is induced by JA treatment or wounding (data not shown) and was strongly correlated with the expression of BGLU19 in a search of online databases, such as ATTED II (http://atted.jp) (Obayashi et al. 2011). This suggests that TSA1 is involved in the formation of induced ER bodies in wounded leaves.

Recently, PYK10 and BGLU21 were shown to be responsible for the ER body formation (Nagano et al. 2009). A mutant with elongated ER bodies (−25 μm diameter) was isolated and termed the leb mutant (Nagano et al. 2009). The number of ER bodies was reduced in the leb mutant (Nagano et al. 2009), indicating that the leb gene regulates both the number and size of ER bodies in seedlings. Unexpectedly, the gene responsible for the leb mutation was PYK10 (Nagano et al. 2009). PYK10 forms a disulfide bond to create an ~170 kDa multimer (Nagano et al. 2009). A point mutation at Cys29 in PYK10 inhibits the formation of the multimer, resulting in a reduction of PYK10 protein levels in the leb mutant (Nagano et al. 2009). This result implies that the reduction of ER body components induces the alteration of ER body morphology. Analysis of the T-DNA insertion mutants of PYK10 and BGLU21 further validated this hypothesis. The BGLU21 gene encodes the putative second major ER body β-glucosidase expressed in seedlings and roots (Table 1). pyk10 bglu21 and leb bglu21 double knockout mutants exhibited significantly longer ER bodies (~50 μm), suggesting that these ER body components are required to maintain the structure of the ER body. Based on these observations, NAI2 and ER body β-glucosidases appear to control ER body formation, while NAI2 appears to facilitate β-glucosidase aggregation to form the ER body in the ER (Fig. 3).

ER body formation accompanying PYK10 expression is tissue and developmental stage dependent and requires transcription factor NAI1. On the other hand, the wound-induced hormone JA is involved in the formation of inducible ER bodies (Matsushima et al. 2002). JA signaling is transduced by the JA-Ile receptor COI1, which degrades JAZ proteins to activate the bHLH-type transcription factor AtMyc2 (Chin et al. 2007, Thines et al. 2007). ER body formation was not observed in the coi1 mutant (Matsushima et al. 2002), suggesting that COI1 plays a crucial role in wound-induced ER body formation.

Several lines of evidence suggest that ER bodies are involved in defense mechanisms in plants. Wounding or application of the wound hormone JA induces the formation of ER bodies (Matsushima et al. 2002, Hara-Nishimura and Matsushima 2003), as does the damage induced by pest chewing. Direct evidence that ER bodies are involved in plant defense unexpectedly came from an experiment using the mutualistic fungus Piriformospora indica (Sherameti et al. 2008). Brassicaceae plants are resistant to symbiotic arbuscular
mycorrhizal (AM) fungi, but some fungi of non-AM groups, such as *P. indica*, are able to infect Brassicaceae plants and enhance host plant growth (Kumari et al. 2003). Therefore, it became a model system for understanding plant–fungus mutualistic interactions in Arabidopsis (Serrinberg et al. 2007, Sherameti et al. 2008, Schäfer et al. 2009, Vadassery et al. 2009). *Piriformospora indica*-insensitive (*pii*) mutants that did not promote growth after infection with *P. indica* were isolated (Sherameti et al. 2008). An 8bp deletion in the promoter region of NA11 was identified, and expression of the NA11 gene was strongly reduced in the *pii-4* mutant, suggesting that the NA11 gene is responsible for *pii* (Sherameti et al. 2008). NA11 regulates the expression of PYK10. PYK10 protein accumulated in the roots of *P. indica*-infected plants compared with non-infected plants (Peškan-Berghofer et al. 2004), and the *pyk10* mutant showed dissolution of mutualism (Sherameti et al. 2008). This suggests that NA11 and PYK10 are involved in the mutualistic interaction between Arabidopsis and *P. indica*. Importantly, increased expression of the fungal transcription factor 1 (*cPitef1*) was detected in the *pyk10* and *na11* mutants, indicating that the growth of *P. indica* was high in these mutants compared with wild-type plants (Sherameti et al. 2008). These results suggest that the phenotype observed in these mutants was caused by the reduction of plant vigor due to hyperinfection of the fungus. PYK10 in Arabidopsis roots may be important for restricting fungal infection to a level that maintains a mutualistic interaction. Scopoletin, the hydrolyzed product of scopolin, inhibits the plant pathogen *Fusarium verticillioides*, although its activity is low (Carpinella et al. 2005). This implies that the hydrolysis of scopolin by ER body β-glucosidases is required for resistance against pathogens.

The β-glucosidases and glucosides are stored in separate compartments in cells and tissues to prevent constitutive catalytic reactions that produce toxic compounds and would damage plants. Brassicaceae plants have a glucosinolate–myrosinase defense system in which glucosinolate accumulates in sulfur-rich S-cells and myrosinase produces myrosin cells in the stems and leaves (Wittstock and Gershenzon 2002, Ueda et al. 2006). Both myrosinase and glucosinolate accumulate in vacuoles, but they are stored in different plant tissues. In oat (*Avena sativa*), enzyme and substrate are stored in different parts of the cell. A β-glucosidase, avenocidase, accumulates in chloroplasts, while the substrate avenosic acid accumulates in vacuoles. Avenosic acid, an antifungal saponin, is not hydrolyzed by avenocidase until the cell collapses (Gus-Mayer et al. 1994, Nisius 1988). In the roots of Arabidopsis, the ER body acts as a depository site for β-glucosidases, whereas scopolin accumulates in vacuoles (Taguchi et al. 2000). This ensures that scopolin will not be hydrolyzed until the cell collapses in response to pathogen or herbivore attack. The roots of Brassicaceae plants possess another β-glucosidase system that is also based on the ER body, and the compartmentalization of enzymes and substrates is also separate in this system.

Conclusions and future perspectives

ER bodies were discovered during the study of organelle differentiation using GFP imaging of live cells and ultrastructural analysis with electron microscopy (Gunning 1998, Ridge et al. 1999, Hayashi et al. 2001). These techniques allowed for the isolation of Arabidopsis mutants in order to analyze ER body formation and function. Based on these studies, a clearer understanding of the unique mechanisms of ER body formation and ER body function has emerged. The transcription factor NA11 regulates the expression of ER body-related genes, including ER body components PYK10, NA12 and cytosolic PBP1 (Matsushima et al. 2004, Nagano et al. 2005, Nagano et al. 2008, Yamada et al. 2008, Nagano et al. 2009). PYK10 and NA12 are important for ER body formation (Fig. 3) (Yamada et al. 2008, Nagano et al. 2009). Following cell damage caused by pest or pathogen attack, PYK10 aggregates and forms a large complex with PBP1, JALs and GLLs, which increases its glucosidase activity (Fig. 2) (Nagano et al. 2005, Nagano et al. 2008). This PYK10 activity is responsible for resistance against hyperinfection by symbiotic fungi by activating toxic compounds such as scopolin (or indole glucosinolates) (Sherameti et al. 2008, Ahn et al. 2010). Importantly, ER bodies are abundant in seedlings and roots, which contact the soil. This suggests that ER bodies in these tissues may be crucial for resistance to various soil fungi.

The theory of ER bodies and plant defense presented thus far is attractive, but many questions remain to be answered. For example, glucosinolates are attractive candidates for the substrate of PYK10, but PYK10 did not hydrolyze sinigrin, an aliphatic glucosinolate (Ahn et al. 2010). The only identified endogenous substrate of PYK10-type β-glucosidases is scopolin. However, the function of scopolin during infection by fungi such as *P. indica* is still elusive. Further, it is unknown whether the wound-inducible ER body is responsible for defense against pests such as aphids or thrips. Finally, the defense system proposed for ER bodies in Brassicaceae plants has yet to be identified in non-Brassicaceae plants. These questions remain to be answered in future studies.

Funding

This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT) [Grants-in-Aid for Scientific Research to K.Y. (Nos. 21770057 and 21113527), I.H.-N. (Nos. 22700014 and 22247006) and M.N. (No. 22120007)].

Acknowledgments

We thank Kentaro Tamura, Kyoto University, for the data obtained by FRAP analysis of ER bodies.
References


Ogasawara, K., Yamada, K., Christaller, J.T., Kondo, M., Hatsugai, N., Hara-Nishimura, I. et al. (2009) Constitutive and inducible ER bodies...


