ER-Derived Compartments Are Formed by Highly Regulated Processes and Have Special Functions in Plants

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The endoplasmic reticulum (ER) of plants is a part of the endomembrane system, a highly conserved system that functions in many lower and higher eukaryotes. Yet, as opposed to the ER of animal cells, the ER of plants also serves naturally as an origin for the generation of multiple ER-derived compartments having multiple functions in plant development and response to the environment. The major functions of ER-derived compartments are to store reserve compounds, such as storage proteins, storage oils, and rubber, as well as to accumulate massive amounts of hydrolytic enzymes in response to pathogen attacks. In addition, ER-derived compartments also transiently accumulate specific proteases that are subsequently internalized into the vacuoles and degrade seed storage proteins during germination (Hara-Nishimura et al., 2004). The diversity, biogenesis, final destination, and multiple functions of ER-derived compartments in plants are the subject of this Focus issue on ER-derived compartments and is discussed in a number of joint *Updates*. In addition, this Focus issue includes two research papers that are related to this topic.

HOW DO NASCENT ER-DERIVED BODIES INITIATE, AND HOW DO THEY MATURE AND BUD FROM THE CISTERNAL ER?

The formation of ER-derived protein bodies appears to be a complex process involving a number of factors. As discussed in three of the *Updates* of this Focus issue (Crofts et al., 2004; Hara-Nishimura et al., 2004; Vitale and Ceriotti, 2004), ER-derived protein bodies' formation includes specific targeting of mRNAs encoding the cargo proteins to the ER-derived protein bodies, interactions of the cargo proteins of these compartments among themselves and with ER-resident molecular chaperones, and also receptor-based mechanisms for the selection of the cargo proteins. It is also likely that the combination of these mechanisms enables efficient accumulation of the cargo proteins in these compartments. However, the mechanism by which the ER-derived protein bodies initiate is still unknown. Does this process involve differentiation of specific regions in the cisternal ER, or does it initiate upon sequestration of the cargo proteins into the cisternal ER and their formation of core elements inside it? Is the targeting of mRNAs important for the initiation of the nascent ER-derived protein bodies? If mRNA targeting is a prerequisite for the initiation of the ER-derived protein bodies, then the cisternal ER should contain specific docking regions for these mRNAs before the nascent ER-derived protein bodies initiate. The regulatory role of mRNA targeting in the formation of ER-derived protein bodies, as discussed by Okita and associates (Crofts et al., 2004), is also of special interest because mRNA targeting has not been studied extensively in plants. Therefore, the *Update* discussing mRNA targeting (Crofts et al., 2004) also provides important basic knowledge on the general machinery of mRNA targeting.

Several lines of evidence suggest that the cargo proteins of ER-derived compartments play a major role in their biogenesis. Arabidopsis (*Arabidopsis thaliana*) possess wound-inducible ER-derived bodies that accumulate a β-glycosidase containing a C-terminal ER-retention K/HDEL signal, but the Arabidopsis *nai1* mutant that lacks these bodies also does not accumulate this β-glycosidase (Hara-Nishimura et al., 2004). Notably, the *NAI1* gene encodes a transcription factor (Hara-Nishimura et al., 2004), and it will be interesting to see whether this transcription factor controls the expression of the β-glycosidase gene or the production of other elements related to the machinery of formation of the ER bodies containing this enzyme. Additional evidence for the importance of the cargo protein in the biogenesis of ER-derived compartments comes from analysis of Cys proteases that are formed in germinating seedlings. Some of these proteases, which transiently accumulate in ER-derived compartments, contain the ER-retention K/HDEL signal (Hara-Nishimura et al., 2004) whose deletion causes their exit to the Golgi rather than their transient accumulation in ER-derived compartments (Okamoto et al., 2003). This implies that the K/HDEL sequence is essential for their aggregation in the ER-derived compartments. Does the K/HDEL function by concentrating the proteins within the ER upon their efficient retrieval from the Golgi, or does its presence change the structural confirmation of these nascent proteins? The answers to these questions await further studies, but nevertheless, the participation of the

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K/HDEL signal in this process is a novel function of this sequence in the biogenesis of ER-derived compartments. Notably, the membranes surrounding the ER-derived protein bodies also contain receptors, which likely function in the selection and concentration of the cargo proteins within these compartments (Hara-Nishimura et al., 2004).

As discussed by Vitale and Ceriotti (2004), the maturation of the resident proteins of the ER-derived protein bodies within the ER involves special interactions with ER-resident molecular chaperones. This maturation process generally resembles the maturation of mal-folded proteins, which are destined for degradation by the ER-associated degradation pathway. Both maturation processes experience long-lasting interactions of the nascent protein with ER-resident molecular chaperones. Yet, the ER chaperone machinery can distinguish between the two classes of proteins, one class being recognized as mal-folded and targeted for degradation, while the other class is apparently recognized as correctly folded and therefore stably accumulates. It might be possible therefore that the two classes are differentially recognized by the ER chaperones already at their initial state of folding when the proteins are still at the monomer states. While the mal-folded proteins are likely to be partially insoluble already as monomers, the nascent proteins destined to ER-derived compartments may initially fold as soluble monomers, some of which are also able to escape the aggregation within the ER and exit the ER on route to the Golgi (Vitale and Ceriotti, 2004). It is also likely that specific interactions between the soluble monomers represent important prerequisites for their aggregation within the ER, as was observed for maize zein storage proteins (Kim et al., 2002).

Most ER-derived protein bodies disconnect and bud out from the cisternal ER but remain surrounded by ER membranes that either contain or lack attached ribosomes. The process of this budding is still entirely unknown, and it remains to be seen whether it is controlled by specific proteins analogous to the proteins controlling the Coat Protein II vesicles that bud from the ER on route to the Golgi (Nebenfuhr, 2002).

Not only proteins but also oils accumulate in ER-derived compartments. As discussed in the *Update* by Hsieh and Huang (2004), the biogenesis of ER-derived oil bodies and their final structure occur by complex processes that depend largely on the oil and a special group of proteins, mainly oleosins, which surround the oil in the oil bodies. The oleosins have special structural characteristics that are apparently important both to the biogenesis of the oil bodies and to their stability. Yet, the process of budding of the oil bodies from the ER and whether the oleosins regulate this process too has still to be elucidated.

INTERNALIZATION OF ER-DERIVED COMPARTMENTS INTO THE VACUOLES

ER-derived compartments can either reside in the cytosol or internalize into the vacuoles. The final destination of these compartments apparently depends on their cargo content and on the plant species and cell types that they are formed. Potential mechanisms of internalization of ER-derived compartments into the vacuoles and the significance of this process are discussed by Herman and Schmidt (2004), who names this process ERVT, analogous to the CVT pathway that delivers proteins from the cytosol to the vacuole in yeast (*Saccharomyces cerevisiae*; Noda et al., 2002). Microscopically, this process appears similar to autophagy, but the mechanism by which it operates is still unknown. Plants contain homologs of many yeast genes associated with autophagy (Doelling et al., 2002; Hanaoka et al., 2002), but whether these proteins control the internalization of the ER-derived compartments into the vacuoles still remains to be elucidated. Herman also discusses the possibility that similar mechanisms may operate in plant cells to dispose and turnover ER membrane within the vacuoles and that ER-derived compartments may take a ride on such mechanisms to be internalized into the vacuoles. Are ER-derived compartments specifically recognized for internalization into the vacuole, as occurs in the yeast CVT pathway, or do they take a ride on a more bulky process of internalization into the vacuoles as occurs by the classical stress-associated autophagy processes? Another interesting, still open question is whether plants, being sessile organisms that need to adapt to their environment quite frequently, operate autophagy processes under regular growth conditions to enable frequent turnover processes and remodeling of their cells.

WHY DO PLANTS FORM ER-DERIVED PROTEIN BODIES?

The answer to this question is not simple, but since plants contain multiple ER-derived compartments, it is likely that they serve functions that are especially important to the life cycles of plants. One of the major functions of ER-derived compartments is to store storage proteins in developing seeds. Plant seed storage proteins have two special characteristics. They accumulate in massive amounts and also have special aggregative characteristics, which apparently enable their efficient desiccation during seed maturation and rehydration during seed germination. Notably, plant seeds generally synthesize two distinct classes of storage proteins, namely globulins and prolamins, and deposit them in protein bodies by two distinct mechanisms. The globulins, which are generally more abundant in the embryos, are transported as soluble proteins from the ER via the Golgi to the vacuoles where they finally aggregate. The prolamins, which are generally more abundant in the endosperm tissues, already aggregate in the ER and are generally deposited in ER-derived protein bodies. Since the endosperm tissue dies at the end of seed maturation, it is possible that this tissue has adapted a massive synthesis of prolamins and their accumulation in ER-derived protein bodies because the

endomembrane system of the endosperm, including its vacuoles, progressively disintegrate with the maturation process. In addition, massive deposition of prolamins in ER-derived protein bodies may also enable an efficient utilization of the limiting space of the endosperm cells, which also accumulate other storage reserves, particularly starch. The stress-induced accumulation of massive amounts of hydrolytic enzymes in ER-derived compartments (Hara-Nishimura et al., 2004) may also enable an efficient utilization of the limiting intracellular space of plant cells and perhaps also a rapid discharge of these hydrolytic enzymes in response to pathogen attacks.

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