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Membrane glycerolipid equilibrium under endoplasmic reticulum stress in *Arabidopsis thaliana*

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ABSTRACT

Endoplasmic reticulum (ER) is an indispensable organelle for secretory protein synthesis as well as metabolism of phospholipids and their derivatives in eukaryotic cells. Various external and internal factors may cause an accumulation of aberrant proteins in the ER, which causes ER stress and activates cellular ER stress responses to cope with the stress. In animal research, molecular mechanisms for protein quality control upon ER stress are well documented; however, how cells maintain lipid homeostasis under ER stress is an emerging issue. The ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE), two major phospholipid classes, is important under ER stress in animal cells. However, in seed plants, no study has reported on the changes in membrane lipid content under ER stress, although a number of physiologically important environmental stresses, such as heat and salinity, induce ER stress. Here, we investigated membrane glycerolipid metabolism under ER stress in Arabidopsis. ER stress transcriptionally affected PC and PE biosynthesis pathways differentially, with no significant changes in membrane glycerolipid content. Our results suggest that higher plants maintain membrane lipid equilibrium during active transcription of phospholipid biosynthetic genes under ER stress.

1. Introduction

In eukaryotic cells, the endoplasmic reticulum (ER) is an organelle, where secretory and membrane proteins are first translocated and go through a series of protein maturation steps including the addition of glycans and formation of disulfide bonds. To maintain ER homeostasis, a monitoring system collectively called ER quality control allows only properly folded proteins to leave the ER [1]. However, various external and internal factors may cause an accumulation of aberrant proteins in the ER, which potentially causes toxicity to cellular functions. To circumvent this stress (ER stress), multiple and evolutionally conserved cellular pathways termed the ER stress response are activated to alleviate the ER stress. Among these pathways, the unfolded protein response (UPR) has been most intensively studied in yeast and mammals as well as plants [2].

In higher plants, environmental stresses such as heat and high salinity are tightly linked with the ER stress response [3]. In the plant UPR, INOSITOL-REQUIRING TRANSMEMBRANE KINASE/ENDONUCLEASE 1 (IRE1) activates a transcriptional factor, bZIP60, by unconventional cytosolic splicing upon ER stress, then ER-stress response genes including IMMUNOGLOBULIN-HEAVY-CHAIN-BINDING PROTEINS (BiPs) are upregulated transcriptionally [4]. The second pathway of plant UPR is accomplished by sequential proteolytic reactions in the Golgi by site-1 and -2 proteases (S1P and S2P), which cleave bZIP17 and bZIP28 upon ER stress [5,6]. bZIP17 and bZIP28 are known as sensor proteins at the ER, similar to IRE1. They recognize aberrant proteins in the ER lumen upon ER stress and are translocated to the Golgi for the above-mentioned

Abbreviations: DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol.

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proteolytic reactions.

The ER is an indispensable organelle also for metabolism of phospholipids and their derivatives. In addition to protein homeostasis, recent works in yeast and mammalian cells have revealed that aberrant phospholipid metabolism leads to the ER stress and activates the UPR. For example, inhibition of phosphatidylcholine (PC) biosynthetic pathways induces ER stress for reduced PC biosynthesis and/or subsequent changes in phospholipid contents [7–9]. In particular, imbalanced ratio of PC to phosphatidylethanolamine (PE), two primary phospholipid classes in most eukaryotic cells, has been suggested as a cause of ER stress [10–12]. Contrary to the increasing interest in the mechanistic link between lipid functions and ER stress response in animals, little is known about the involvement of lipids in the ER stress response in seed plants. A pioneering work in maize showed that ER stress alters the enzyme activity of diacylglycerol kinase, cholinephosphate cytidylyltransferase, phosphatidylinositol (PI) 4-kinase, and PI 4-phosphate 5-kinase [13]; however, glycerolipid analysis of wild-type Arabidopsis seedlings has not been investigated. Given that ER is the major site of lipid biosynthesis, ER stress may be tightly associated with glycerolipid homeostasis also in seed plants.

To elucidate glycerolipid metabolism and function upon ER stress in seed plants, this work aimed to analyze glycerolipid profiles under ER stress in *Arabidopsis thaliana*. Glycerolipid contents of wild-type Arabidopsis seedlings maintained their composition upon ER stress treatment, although some changes were observed in the fatty acid composition. Because the ratio of PC and PE is a signature profile in ER-stressed yeast and mammalian cells, we analyzed the gene expression of enzymes involved in the biosynthesis of these phospholipid classes. The results showed dynamic patterns in the profile of gene expression during ER stress. The membrane glycerolipid profile may be maintained at the post-transcriptional level upon ER stress in *Arabidopsis thaliana*.

2. Materials and methods

2.1. Plant materials and culture conditions

Wild-type *Arabidopsis thaliana* (Columbia-0 ecotype) were grown under continuous light at 23 °C. For plate culture, Murashige and Skoog (MS) medium was used at half-strength condition [14]. Tunicamycin (Tm) treatment to 14-day-old seedlings was performed as previously described [15]. 1% dimethyl sulfoxide (DMSO) was used as mock treatment.

2.2. Quantitative reverse transcription-coupled polymerase chain reaction (qRT-PCR)

Whole seedlings of 14-day-old wild-type plants with 5 μg/ml Tm treatment at the times indicated (0, 5 or 24 h) were harvested for total RNA extraction by using the Qiagen RNeasy Plant Mini kit (Qiagen, Hilden, Germany) including DNase treatment. Subsequently, cDNA was synthesized by using the First Strand SuperScript III Reverse Transcription System (Life Technologies, Carlsbad, CA). qRT-PCR was performed with the 7500 Real Time PCR System (Applied Biosystems, Massachusetts, USA) as described [16] with the primers listed in Supplemental Table 1 [16–18]. Actin2 (At3g18780) was used as a control.

2.3. Lipid extraction and analysis

Total lipid was extracted from 14-day-old seedlings with or without Tm treatment by the method of Bligh and Dyer [19]. Analysis of glycerolipid classes was performed essentially as described [16,20]. Briefly, each lipid class was separated on a silica gel thin layer chromatography (TLC) plate by 2-dimensional TLC with the following solvent system (1st dimension, chloroform:methanol:ammonium solution = 120:80:8 [by vol.]; 2nd dimension, chloroform:methanol:acetic acid:water = 170:20:15:3 [by vol.]). Lipid spots were identified by primuline staining and scraped off from the TLC plate. Separated lipids were re-extracted from the silica gel and their acyl moieties were hydrolyzed and methylesterified to prepare fatty acid methyl esters (FAMEs) with HCl-methanol solution including 1 μM of pentadecanoic acid (15:0) as an internal standard. The resulting FAMEs were extracted with *n*-hexane and analyzed by gas chromatography (GC-2010; Shimadzu, Kyoto, Japan) with a FID detector and a ULBON HR-SS-10 column (Shinwa Chemical Industries, Japan). Data are mean ± SD from 9 biological replicates.

3. Results

3.1. Polar glycerolipid contents of Arabidopsis seedlings under ER stress

To investigate whether ER stress affects membrane lipid contents, we analyzed the lipid composition of 2-week-old wild-type seedlings of Arabidopsis treated with Tm for 24 h. Tm is a widely used chemical reagent to trigger the ER stress response by inhibition of *N*-linked protein glycosylation [21]. Levels of seven major polar glycerolipid classes analyzed (monogalactosyldiacylglycerol

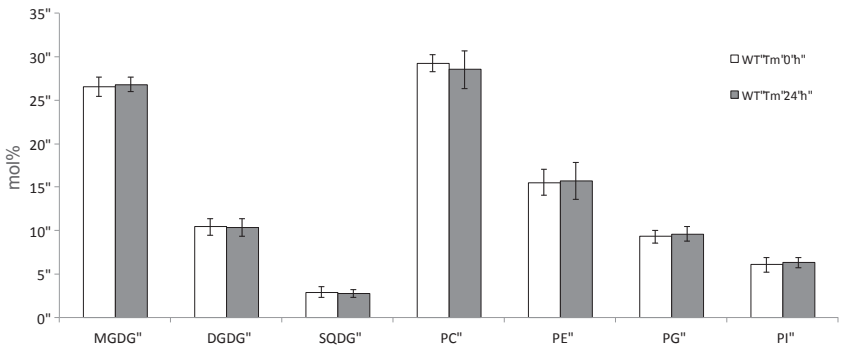

Fig. 1. Polar glycerolipid content of 14-day-old wild-type (WT) seedlings with or without tunicamycin (Tm) treatment. Fourteen-day-old seedlings were treated with 5 μg/ml Tm for 24 h. Data are mean ± SD with 9 biological replicates. Statistical significance was analyzed by Student's t-test. DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol.

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