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How does the stressed out ER find relief during virus infection?

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Abstract

The endoplasmic reticulum and Golgi network (ERGN) is vital to most cellular biosynthetic processes. Many positive strand RNA viruses depend upon the ERGN for replication, maturation, and egress. Viruses induce changes in ER architecture and stimulate fatty acid synthesis to create environments that can scaffold replication complexes, plant virus movement complexes, or virion maturation. Potato virus X (PVX) and Turnip mosaic virus (TuMV) each encode small membrane binding proteins that embed in the ERGN and activate the unfolded protein response (UPR). The UPR ensures ERGN homeostasis in the face of environmental assaults that could negatively impact the biosynthetic functions of the ERGN. This article explores the relationship between ER stress, the UPR, and membrane synthesis occurring during virus infection.

Highlights

- RNA viruses regulate ER membrane synthesis and the unfolded protein response
- Changing ER architecture increases the cell's ability to handle viral biosynthetic needs
- The unfolded protein response is central to managing virus induced ER stress

Introduction

The endoplasmic reticulum (ER) and Golgi apparatus comprise an extensive membrane network of tubules and vesicles that reaches most organelles in the cell, stretching from the nucleus through the plasmodesmata [1-4]. The ER has multiple complex functions including calcium storage, protein synthesis, protein folding, and membrane lipid synthesis. Protein chaperones reside in the ER lumen to facilitate protein folding, disulfide bond formation, and carbohydrate modifications. Distinct protein signaling complexes also reside at the ER and control stress responses to abiotic and biotic environmental assaults that cause alterations in ER morphogenesis, calcium homeostasis, or protein folding. Moreover, alterations of ER homeostasis can be caused by heat, drought or pathogen infection and are ultimately linked to the reduction in plant productivity and yields.

Positive strand RNA viruses and some reoviruses infecting mammals and plants are unique models for exploring ER membrane dynamics including ER stress signaling and its outcomes. First, the ER network has a large malleable surface area that is used as a physical scaffold by many positive strand RNA virus species to shape a protective membrane bound environment housing the viral replication machinery [5,6]. These 'viroplasm' compartments are centers for virus replication which concentrate virus and host factors needed to produce new genomes. Plant bromoviruses, dianthoviruses and tombusviruses induce invaginations or other compartments that remain tethered to the planar ER or organellar membrane [7-10]. Potyviruses and potexviruses are known to induce vesicles and larger membrane rearrangements that are identified to be significant for virus replication or intercellular transport [11-16]. It is generally accepted that such compartments occur to protect viral RNAs from degradation by cellular nucleases and host silencing machinery. Forming these replication centers involves virus induced membrane synthesis, changes in fatty acid metabolism, rearrangement of extant membranes, and viral recruitment of cellular factors to these new subcellular domains [10,17-21]. This raises questions about whether virus stimulated membrane synthesis is the cause of morphological changes in the ER; whether viruses provoke morphological changes to the ER cause pathological stress, or if membrane synthesis and morphological changes in the ER are components of a pro-survival response to virus-induced ER stress. In particular, the signaling machinery that controls ER membrane proliferation in response to plant virus infection is not yet uncovered, however there is new research in mammalian cells and yeast to try to understand the relationship of ER membrane expansion with ER stress and the unfolded protein response.

Potexviruses, potyviruses, and reovirus membrane binding proteins trigger ER stress.

In general, positive strand RNA viruses place an enormous biosynthetic burden on the ER to produce viral and cellular proteins. Such an increase load also increases the possibility for malformed proteins to accumulate. ER stress occurs when homeostasis is disrupted such as by the accumulation of malformed proteins, and a response system known as the unfolded protein response (UPR) is activated to provide relief [22,23]. The UPR serves to enhance the ER protein folding and degradation capabilities of the cell in order to re-establish ER homeostasis. In plants there are two major branches of the UPR that are activated by abiotic and biotic stressors (Figure 1). One pathway is mediated by inositol-requiring protein 1 (IRE-1) which is a

sensor of ER stress that catalyzes the unconventional splicing of bZIP60 transcripts. *Arabidopsis* has two copies: IRE1a and IRE1b which localize to the perinuclear ER [24]. A 23 nt intron is removed by IRE1 activity which results in a frameshift in the expression of downstream sequences. The unspliced bZIP60 has a transmembrane domain near the C-terminus which is removed by splicing and the truncated transcription factor localizes to the nucleus where it increases expression of key ER-resident chaperones including BiP. This pathway is reported to play a role in potyvirus and potexvirus infection, is activated by bacterial infection, and is also linked to salicylic acid mediated defense [24,25]. The second pathway, which is not activated by viral infections, involves bZIP17/bZIP28. These membrane tethered transcription factors, are retained in the ER through binding BiP. Heat, osmotic stress or chemical agents will activate bZIP17 or bZIP28 which are then released and move through the Golgi to their final destination in the nucleus where they similarly activate UPR gene expression [26,27].

Malformed proteins are exported from the ER for degradation, typically via the proteasome or autophagic machinery [27-30]. ER associated protein degradation (ERAD) is a machinery that actively prevents the toxic build-up of aberrant proteins in the ER which could otherwise lead to cell death. There are many examples of mammalian viruses that co-opt or subvert the ERAD machinery. For example Herpes virus U2 and US11 proteins ~~downregulate the class I major histocompatibility complex (MHC) molecules by facilitating their degradation via the~~ ~~are identify the major histocompatibility protein class I via the ERAD machinery for proteasomal degradation via the ERAD machinery.~~ A similar example is ~~t~~The HIV Vpu protein which mediates turnover of the CD4 proteins soon after translation via ERAD [31]. These viral proteins act as client adapter proteins that present the targets to the proteasome machinery for degradation. Noack et al (2015) described that RNA viruses such as the Japanese encephalitis flavivirus or certain members of the Nidovirales use "ER tuning vesicles" as centers for progeny production. ERAD tuning vesicles are a recently described pathway in yeast and mammals that participates in the delivery of ERAD regulators to the ER lumen to bind and dislocate malformed client proteins [32]. Until now the ER tuning pathway has not been described in plants, and therefore, we can only speculate about possible interactions involving plant RNA viruses. However there is preliminary data pointing toward the possible role for ERAD in potexvirus infection. Specifically, the ~~Potato virus X (PVX, a potexvirus)~~ PVX TGB3 protein activates expression of SKP1, a cofactor in the 26S proteasome needed for protein turnover. Second, PVX TGB2 protein has been shown to drive TGB3 to ER associated vesicles [15,33]. Attempts to uncover the nature of these TGB2-related vesicles have failed to identify any known markers that associate with ER to Golgi or endocytic transport (data not shown), but it is worth considering that if ERAD tuning vesicles exist in plants, these could be a scaffold for potexvirus complexes.

The ~~PVX Potato virus X (PVX, a potexvirus)~~ TGB3 movement protein is an ER localized factor that functions in coordination with other viral movement proteins to aid trafficking of viral RNA between neighboring cells [13]. Turnip mosaic virus (TuMV, a potyvirus) 6K2 protein is also a small membrane binding protein that anchors the viral RNA to the ER via the viral linked protein (termed VPg) at the 5' end of the genome. The viral replicase and select host proteins are then recruited to these centers [34]. Thus, both PVX and TuMV rely on the ER for replication, and there is data to suggest that ER tubules become condensed in perinuclear regions. It is not clear from these studies whether membrane rearrangement and biogenesis are linked [13,15,16,34-37].

Rice black-streaked dwarf virus (RBSDV) is a reovirus with a double-stranded, segmented RNA genome. The P10 protein is the outer capsid protein that has three predicted transmembrane domains and associates with the ER. Researchers speculate that the outer capsid protein resides in the ER to aid assembly of intermediate virus particles from the viroplasm [6].

In separate studies, the PVX TGB3, TuMV 6K2, and RBSDV P10 proteins were identified for their specific ability to stimulate the UPR via activation of the bZIP60 transcription factor. Both the TuMV 6K2 and PVX TGB3 were shown to enhance bZIP60 mRNA levels as well as expression of BiP [6,33,38-40]. The TuMV 6K2 protein was shown to stimulate splicing of the bZIP60 transcripts by IRE-1. In bZIP60-silenced *N. benthamiana* plants inoculated with PVX-GFP, systemic virus movement was delayed and virus accumulation was reduced. TuMV was inoculated to wild-type and bZIP60 defective *Arabidopsis* plants and the levels of viral RNA ~~was-were~~ reduced in the mutant plants. Necrotic lesions were seen following overexpression of PVX TGB3 in *N. benthamiana* leaves, and these could be alleviated by overexpression of BiP. These data pointed to the likely explanation that upregulation of bZIP60-mediated UPR was important for the cell to maintain ER homeostasis reducing toxic accumulation of viral proteins in the ER. However activation of a specific ER-dependent cell death pathway has not been well demonstrated experimentally. Thus, while bZIP60 appears to contribute to both PVX and TuMV infection, it is not clear whether: a) IRE1 or bZIP60 interact directly with the PVX TGB3 or TuMV 6K2 proteins, b) bZIP60 is responsible for transcription of critical factors contributing to virus replication or movement c) enhanced bZIP60 transcription is needed to create a homeostatic environment in the ER that enables virus infection.

Linking membrane proliferation and rearrangements to ER stress

Plant viruses notoriously cause expansion and rearrangement of ER membranes to create environments needed to house viral replication complexes. Such physical changes in the ER should lead to altered homeostasis. Since UPR is the primary machinery controlling ER homeostasis, it is not unreasonable to infer that membrane expansion and UPR are related. This raises the question of whether expansion, rearrangement and ER stress are linked, and controlled by the UPR machinery.

In mammalian cells, IRE1 is linked to unconventional splicing of the XBP1 RNA which may be orthologous to the plant bZIP60 factor. XBP1 is a transcription factor that upregulates UPR related genes and was reported to promote biogenesis of the ER and Golgi network [41]. Flaviviruses such as Japanese encephalitis virus, Dengue virus, Hepatitis C virus have enveloped virions and encode glycoproteins that accumulate along the ER-Golgi network. Following replication, genomes are encapsulated and virion maturation occurs by budding through these cellular membranes. Early in virus infection, flaviviruses trigger the IRE1/XBP1 arm of the UPR to reduce cytotoxicity by virus infection [42,43]. Dengue virus, Hepatitis C virus, and West Nile virus stimulate fatty acid synthesis as a requirement for virus replication and maturation [44-47]. The relatedness of these events has led researchers to hypothesize that viruses require the IRE1/XBP1 pathway of the UPR to either: a) increase the surface area of the ER to support the buildup of glycoproteins and enable virion budding or; b) regulate ER expansion in order to reduce ER stress that may result from the additional burden of supporting the process of virus maturation. Among plant infecting viruses, Brome mosaic virus (BMV; a bromovirus) replication proteins 1a and 2a interact with the ER. Mutations in the *OLE1* gene, encoding delta9 fatty acid desaturase, inhibits virus replication [48]. Delta9 fatty acid desaturase converts saturated fatty acids to unsaturated. The composition of the membrane

lipids in the ER membranes that surround the viral replicase are important for successful replication. The Tomato bushy stunt virus (TBSV; a tombusvirus) p33 protein is reported to stimulate phospholipid synthesis in yeast. Upregulation of three biosynthesis genes named INO1, OPI3 and CHO1 in TBSV infected cells is be important for virus replication [49]. Interestingly, the yeast PAH1 gene encodes Pah1p which is required for lipid synthesis. Downregulation of PAH1 leads to proliferation and enlargement of ER membranes and promotes TBSV replication in yeast [19]. ~~In~~ *Arabidopsis* mutations in ~~the~~ PAH1 and PAH2 ~~genes~~ causes ER expansion. Overexpression of AtPAh2p in *N. benthamiana* reduces TBSV, Cucumber necrosis virus, and Red clover necrotic mosaic virus replication [19,50]. In these examples, membrane expansion is important to create the appropriate lipid environment, or create a new subdomain of the ER that can function for virus replication without hindering other activities of the ER. Membrane expansion seems to be achieved through transcriptional regulation of genes involved in membrane biogenesis.

The relationship between ER stress, membrane synthesis, and ER rearrangement seems to be complex. There is data to indicate that membrane synthesis and altered ER morphology can be uncoupled, but there is less knowl~~edgen~~ about how either event relates to UPR or ER stress regulation. This is highlighted in studies of membrane dynamics in yeast using chemical inducers of ER stress. Massive ER expansion is reported after treated with DTT or tunicamycin and is driven by *Ino2* and *Ino4* transcription factors that are involved in phospholipid synthesis [50]. Yeast IRE1 or Hac1 (XBP1 orthologue) mutants showed impaired membrane expansion when treated with the same chemical inducers of ER stress. Experiments were able to uncouple *Ino2/4* driven membrane expansion from the increase in ER chaperone levels to demonstrate that membrane expansion can occur independent of UPR although expansion of the ER seems to alleviate ER stress [50]. Early events in certain virus infections in mammals include activation of IRE1/XBP1 pathway and proliferation of ER membranes. For Dengue virus, the NS3 protein interacts with the ER and causes changes in ER architecture which do not appear to require IRE1/XBP1-mediated fatty acid synthesis [51]. Similarly with brome mosaic virus, ER rearrangement is the resulting action of the viral replication associated proteins that intentionally insert into pre-existing ER membranes [17,52]. The physical interactions of viral proteins with ER membranes appears to cause changes in membrane morphology. Potyviruses encode 6K2 and potexviruses encode TGB2 and TGB3 small membrane binding proteins that are demonstrated to enhance the accumulation of vesicles budding from the ER. For these plant virus examples it is assumed that membrane expansion is separate process from structural rearrangement. However, it is not yet know if the physical interactions of these proteins with the ER brings them in contact with the ER stress sensor IRE1 and activate the IRE/bZIP60 pathway and if membrane synthesis is a factor in managing ER stress.

Conclusions

ER stress can result from metabolic inhibition of protein folding or glycosylation which can ultimately affect cell survival. Membrane expansion enables cell survival by expanding the surface area of the ER to accommodate normal cellular functions as well as viral processes. Changes in ER architecture could be with the purpose of ensuring proper functioning of the ER or to meet an independent need of creating special ER domains that sequester virus processes from host defenses. Positive strand RNA viruses act on the ER early in infection to affect membrane expansion, rearrangement and UPR. The IRE1/XBP1 pathway in mammalian cells

has~~ve~~ also be linked to membrane biogenesis but it is not known if the related IRE1/bZIP60 pathway plays the same role in plants. While there is sufficient data to indicate that viral proteins reshape membranes by their physical interactions, it is not clear if these physical interactions activate IRE1 related signaling. Further research is also needed to determine if changes in gene expression affecting membrane proliferation and fatty acid composition are somehow linked to the IRE1/XBP1 (bZIP60) pathway. While BMV and TBSV are established models for discovering the functions of critical genes involved in fatty acid metabolism, data obtained in studies of PVX and TuMV indicates that UPR related transcriptional changes is another dimension of plant viral-ER interactions that could be useful for designing strategies to create environments that are less favorable to virus infection. It is reasonable to consider that these studies involving unrelated viruses will uncover knowledge about the ER that could be combined to engineer better strategies for eliminating virus infection and identify new useful cellular targets for crop improvement.

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Figure legend

Figure 1. The endoplasmic reticulum and ER stress pathways regulate communication between the nucleus and the mitochondria. In response to virus infection, ER localized IRE1 activates splicing of the bZIP60 mRNA. The truncated bZIP60 transcription factor goes to the nucleus. Two additional ER stress related transcription factors, bZIP28 and bZIP17, respond to other ER stresses. Upon activation they move through the Golgi and then to the nucleus. These three transcription factors overlap in their ability to regulate genes involved in protein folding, cell death regulation, membrane synthesis. Research is ongoing to the categories of ER stress related genes that play a role in virus infection.

Figure