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Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3215; doi:10.3390/ijms19103215

Unfolded Protein Response-Dependent Communication and Contact among Endoplasmic Reticulum, Mitochondria and Plasma Membrane

Atsushi Saito[1]* and Kazunori Imaizumi[2]

[1] Department of Stress Protein Processing, Institute of Biomedical & Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan.

[2] Department of Biochemistry, Institute of Biomedical & Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan.

*Corresponding author: Atsushi Saito

Department of Stress Protein Processing, Institute of Biomedical & Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan.

Tel: +81-82-257-5131; Fax: +81-82-257-5134; E-mail: saitoa@hiroshima-u.ac.jp

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Abstract

The function of the endoplasmic reticulum (ER) can be impaired by the alternation of the extra- and intracellular environment such as disruption of calcium homeostasis, expression of mutated proteins and oxidative stress. In response to disruptions to ER homeostasis, eukaryotic cells activate canonical branches of signal transduction cascades, collectively termed the unfolded protein response (UPR). The UPR attempts to recover the protein folding capacity and avoid irreversible cellular damage. Additionally, the UPR has been shown to play unique physiological roles in the regulation of diverse cellular events, including cell differentiation and development and lipid biosynthesis. Recent studies have indicated that these important cellular events are also regulated by contact and communication among organelles. These reports suggest strong involvement among the UPR, organelle communication and regulation of cellular homeostasis. However, the precise mechanisms for the formation of contact sites and the regulation of its dynamics by UPR remain unresolved. In this review, we summarized the current understanding of how the UPR regulates morphological changes to the ER and the formation of contact sites between the ER and other organelles. We also review how UPR-dependent connections between the ER and other organelles affect cellular and physiological functions.

Keywords: Unfolded protein response; ER morphology; Mitochondria-associated ER membrane; ER-PM contact sites

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1. Introduction

The endoplasmic reticulum (ER) is the intracellular organelle responsible for the synthesis, folding, modification and assembly of secretory proteins. This organelle has a unique system, collectively known as the unfolded protein response (UPR), to maintain an optimal environment for protein quality control [1-2]. Accumulation of unfolded and/or misfolded proteins in the ER lumen leads to ER dysfunction and apoptotic cell death [3]. The UPR is activated to resolve protein misfolding events and thus ameliorate the ER environment and maintain homeostasis [4]. Additionally, the function of the UPR has been extended from maintenance of protein quality control to fine-tuning of cellular homeostasis and biological functions such as cell development, differentiation, glycogenesis and lipid metabolism [5-10]. Previous reports have indicated that regulation of these physiological processes is controlled by signal transduction events derived from the ER including the UPR [11-15]. Moreover, morphological changes and dynamics of the developed ER also manipulate cellular homeostasis through communication with other organelles [16-19]. Here, we discuss the current knowledge of the diverse functions and prospects of the UPR for regulating ER morphology and the formation of contact sites with mitochondria and the plasma membrane (PM).

2. ER stress transducers and key molecules of the UPR

The ER is a critical organelle for lipid synthesis, calcium storage, protein synthesis and post-translational modifications of many secretory and membrane proteins. An altered environment to the ER and/or cellular malfunction such as calcium depletion in the ER lumen, expression of mutated proteins, oxidative stress and ischemia cause accumulation of unfolded and/or misfolded proteins in the ER lumen, which perturbs ER

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functions. These abnormal conditions are collectively known as ER stress [1-3]. Three major canonical ER stress transducers, inositol-requiring kinase 1 (IRE1) [20], protein kinase R-like ER kinase (PERK) [21] and activating transcription factor 6 (ATF6) [22], are activated in response to ER stress. These three proteins initiate signaling events that induce the expression of chaperone molecules, attenuate protein translation and degrade unfolded proteins, and collectively these are referred to as the UPR [1-2, 4]. These ER stress transducers localize at the ER membrane. ER stress triggers IRE1 dimerization and trans-autophosphorylation [20]. Activated IRE1 processes a 26-nucleotide intron of the *x-box binding protein 1* (*Xbp1u*) mRNA (unspliced form of *Xbp1*) via its RNase activity. The splicing produces the mature *Xbp1s* mRNA (spliced form of *Xbp1*) [23-25], generating the transcription factor XBP1s. The target genes of XBP1s include molecular chaperones and ER-associated degradation (ERAD)-related genes [25]. PERK also oligomerizes and auto-phosphorylates in response to ER stress. Phosphorylated PERK directly phosphorylates the α subunit of eukaryotic initiation factor 2 (eIF2α). The phosphorylated eIF2α accelerates the disassembly of the 80S ribosome, and eventually this process suppresses global protein synthesis [21, 26-27]. Anomalistically, ATF4 escapes translational attenuation by phosphorylated eIF2α. The gene coding for ATF4 has open reading frames (ORFs) in its 5′-untranslated region. These upstream ORFs prevent translation of native ATF4 under normal conditions. The phosphorylation of eIF2α and the disassembly of the 80S ribosome circumvent these pseudo ORFs and promote the translation of native ATF4 [26, 28]. ATF4 drives the expression of several genes involved in amino acid metabolism [26, 29-30]. In turn, ATF4 induces the transcription of the transcription factor, CCAAT enhancer binding protein homologous protein (CHOP) [31-33]. Prolonged expression of CHOP induces cell death [34]. ATF6 translocates from the ER to the Golgi apparatus following ER

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stress. This protein undergoes subsequent processing by site-1 and site-2 proteases, respectively [35-36]. The cleaved N-terminal fragments move into the nucleus. The ATF6 N-terminal fragments act as transcription factors and induce the expression of ER molecular chaperones such as binding immunoglobulin protein (BiP) [22, 37]. Interestingly, recent studies have revealed that the UPR regulates biological functions and cellular homeostasis, as well as the removal of accumulated proteins [5]. These beneficial outcomes also regulate the ER morphology and the communication of the ER network with other organelles [16-18]. Thus, the UPR may orchestrate overall cellular homeostasis through ER dynamics and cooperative attachment among organelles. The perturbation of these systems can lead to the pathogenesis of various diseases including neurodegenerative diseases.

3. Morphological changes to the ER by the UPR

The regulation of ER biogenesis and expansion is controlled by the UPR. ER expansion is necessary to increase protein folding capacity to handle unfolded proteins that accumulate in the ER lumen [38-39]. XBP1, the downstream transcription factor of IRE1, is mainly responsible for ensuring sufficient regulation of ER biogenesis, including an increase in biosynthesis of ER proteins and lipid biogenesis [40-41]. When B cells become plasma cells, they undergo the acceleration of membrane biogenesis and the exponential expansion of ER that allow them to secrete large quantities of immunoglobulin [42-43]. The selective deletion of *Xbp1* in B cells is shown the similar amounts of phosphatidylethanolamine (PtdEtn), phosphatidylserine and phosphatidylglycerol to those of wild-type B cells [44]. By contrast, the significant decrease in the levels of phosphatidylcholine (PtdCho), sphingomyelin (SM) and phosphatidylinositol is observed

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in thse deficient cells. In addition, *Xbp1*-deficient B cells is inhibited the ER expansion following the lipopolysaccharide (LPS)-stimulated activation [40]. Thus, XBP1 drives morphological changes to the ER through the regulation of the amounts of membrane components including PtdCho. The morphological changes to the ER is most drastically affected by the biosynthesis of PtdCho, because PtdCho is the most abundant cellular phospholipid and a major component of ER membranes [45]. PtdCho is primarily produced from cytidine diphosphocholine (CDP-choline) [45]. Choline cytidylyltransferase (CCT) converts phosphocholine to CDP-choline in the presence of CTP [46]. The residual phosphocholine is transferred to diacylglycerol (DAG), yielding PtdCho [45]. Cholinephosphotransferase (CPT1) [47] or choline/ethanolaminephosphotransferase (CEPT1) [48] catalyzes this final step. The level and synthesis of CCT is upregulated in fibroblasts overexpressing a spliced form of XBP1 [49]. The increase in activity of CCT accelerates the production of PtdCho. Increasing the synthesis of PtdCho by transduction of CCT is sufficient for only a small expansion of rough ER. In contrast, the transduction of the spliced form of XBP1 yields a clear increase in PtdCho synthesis and promotes expression of abundant ER proteins, which leads to robust expansion of the ER. Therefore, XBP1 may orchestrate ER biogenesis by coordinating phospholipid biosynthesis and the expression of ER proteins. These ER expansions and morphological changes by UPR components may facilitate communication and contact between the ER and other organelles.

4. The mitochondria-associated ER membrane (MAM) and the UPR

The ER is physically and biologically connected to mitochondria. The specialized subdomain of the ER is called the mitochondria-associated ER membrane (MAM) [19].

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MAM is an intracellular lipid raft-like structure intimately involved in calcium homeostasis, lipid metabolism, apoptosis and mitochondrial functions [19, 50-51]. In mammalian cells, several types of connectors for MAM have been identified. Mitofusin 2 (MFN2) is a dynamin-related GTPase localized at the ER surface and mitochondria [52]. MFN2 contributes to the tethering between the ER and mitochondria by homologous interaction of ER-associated MFN2 with mitochondrial MFN2. MFN2 also forms a heterologous interaction with MFN1, a homologue protein only localized at the outer mitochondrial membrane [52]. Vesicle-associated membrane protein-associated protein B (VAPB), an ER protein, also forms a connection between the ER and mitochondria by interacting with protein tyrosine phosphatase-interacting protein 51 (PTPIP51) that is localized at mitochondria [53]. ER-resident glucose-regulated protein (GRP75) and mitochondrial voltage-dependent anion channel 1 (VDAC1) form a complex with subtype 3 of the 1, 4, 5-triphosphate receptor (IP3R3). This complex serves as a calcium exchange platform at MAM [54]. B-cell receptor-associated protein 31 (BAP31) localized at the ER interacts with mitochondrial fission 1 homolog (FIS1) and phosphofurin acidic cluster sorting protein-2 (PACS-2) as MAM connectors related to the induction of apoptosis [55-56]. Multiple molecules involved in protein quality control, autophagy, mitochondrial dynamics, lipid synthesis and the inflammasome, are recruited to MAM, suggesting that diverse signaling derived from MAM orchestrates these physiological events [57-60].

Dysfunctions of MAM may cause the pathogenesis of several diseases including metabolic diseases and neurodegenerative diseases [61-62]. The liver is highly involved in glucose homeostasis, and both metabolic inflexibility and insulin resistance predispose to the development of hepatic metabolic diseases. The functions of the developed ER and

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mitochondria, which play a central role in the regulation of hepatic metabolism, and their contact sites are also related to those events in the liver. A lot of recent studies have suggested that MAM is an important convergence for regulating hormonal and nutrient signaling in the liver [63-64]. In addition, several proteins in the insulin signaling pathway such as Akt and mammalian target of rapamycin (mTOR) complex 2 (mTORC2) are located in MAM, and manipulate insulin signaling [65]. The ER-mitochondria miscommunication leads to hepatic insulin resistance, that may trigger the pathogenesis of type 2 diabetes mellitus [66]. The dysfunctions of MAM is also involved in the development of neurodegenerative diseases including Parkinson's disease (PD) [67]. PD-related genes have been identified as a regulator for mitochondrial functions, and PD-associated mutations in these genes lead to mitochondrial dysfunction. α-synuclein (SNCA) is linked to familial as well as sporadic PD. This protein is shown to be enriched in the MAM fraction of the ER in mouse and human brain [68]. The other two PD-related proteins, Parkin and PTEN-induced kinase 1 (PINK1) are also involved in mitochondrial regulation. These proteins control mitochondrial degradation by mitophagy, a selective autophagy for degradation of old and damaged mitochondria [69]. MAM is known to constitute initiation sites for this process [70]. Parkin is enriched in the MAM fraction of neurons exposed to glutamate excitotoxicity [71]. PINK1 is also found in the MAM fraction during mitophagy [72]. Although the detailed involvement between MAM functions and PD is still unclear, future works will have to define how dysfunctions of MAM promote the pathogenesis of PD.

The induction of various MAM proteins correlates with ER stress. Of those proteins, Rab32 is a GTPase and localizes to the ER and mitochondria [73-74]. This protein regulates ER-mitochondria interactions and mitochondrial dynamics [75]. The expression of Rab32

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is upregulated upon brain inflammation in a mouse model and lesions of multiple sclerosis (MS) brain tissues [76-77]. The treatment of human neuroblastoma SH-SY5Y cells with ER stress inducers results in the transcriptional activation of Rab32, indicating that the expression of Rab32 may be under the control of ER stress-induced UPR. The induction of Rab32 *in vivo* parallels those of ER stress-related genes in active lesions of MS brain [77]. Rab32 is also known as a modulator for MAM properties [74]. The expressions of the other MAM regulatory proteins including MFN2, GRP75 and PACS-2 are upregulated in active lesions of MS brain, which is consistent with that observed for Rab32 expression [77]. Overexpression of Rab32 or expression of the dominant-active form of Rab32 in SH-SY5Y cells results in impaired mitochondrial dynamics and distribution, and the inhibition of neurite outgrowth. UPR-dependent Rab32 may manipulate neurite outgrowth via regulation of mitochondrial dynamics and the formation of MAM.

Sigma 1 receptor (Sig1R) is another MAM protein that is a chaperone protein highly expressed in spinal motor neurons and several peripheral organs including lung, kidney, liver pancreas, spleen, adrenal gland and heart [78-80]. This protein specifically localizes at MAM and forms a complex with ER chaperones under normal conditions. Calcium depletion in the ER lumen accelerates the dissociation of Sig1R from ER chaperones, followed by regulation of a variety of cellular functions such as the transfer of calcium signaling between the ER and mitochondria [79, 81]. A recent study has shown that Sig1R-deficiency attenuates ER-mitochondria crosstalk and triggers the degradation of moderate motor neurons in Sig1R-deficient mice, suggesting that Sig1R is a key factor for the integrity of MAM [82]. Sig1R-deficiency disrupts ER-mitochondria contacts, which impairs intracellular calcium signaling, and mitochondrial dynamics and transport. Interestingly, the expression of Sig1R is upregulated in response to ER stress [83]. Sig1R is

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transcriptionally upregulated by treatment with ER stress inducers. Many transcription factors including XBP1 (IRE1 pathway), ATF4 (PERK pathway) and ATF6 (ATF6 pathway) are activated downstream of the UPR. Knockdown experiments indicate that suppressing the expression of ATF4 decreases the level of Sig1R. Furthermore, strong binding of ATF4 to a 5′ upstream region of Sig1R was observed by a chromatin immunoprecipitation assay, suggesting that the expression of Sig1R is regulated by the PERK pathway of a UPR branch. These previous observations indicate that the UPR may fine-tune the functions of MAM through PERK pathway-dependent expression of Sig1R. Whereas, Sig1R can regulate UPR through the direct interaction with ER stress transducers. Sig1R binds to monomeric form of IRE1 at MAM under ER stress condition [84]. The interaction of Sig1R with IRE1 leads to correct the conformation of IRE1, followed by inducing the stabilization. Although these events transiently interfere with the dimerization and the autophosphorylation of IRE1, the stabilized IRE1 enables to exert a long-lasting activation. The knockdown of Sig1R causes the upset of IRE1-XBP1 signaling, resulting in the induction of apoptosis by ER stress. The report suggests that the stabilization of IRE1 by Sig1R at MAM serves the resistance against ER stress by ensuring the long-lasting activation of IRE1-XBP1 signaling.

Recent studies have reported that Sig1R may be involved in the etiology of neurodegenerative diseases. Alzheimer's disease (AD) is now accepted as being caused by amyloid β (Aβ) plaques and tau neurofibrillary tangles [85-86]. Aβ is generated at MAM and may affect the functions of the ER, mitochondria and MAM [87]. Knockdown of Sig1R in hippocampal neurons has been shown to cause neuronal degeneration. The uncontrolled expression of Sig1R leads to abnormal calcium shuttling from the ER to mitochondria [87]. Additionally, impaired expression of Sig1R is observed in the brain of

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$APP_{Swe/Lon}$ mice, the AD mouse model [Swedish (K670/M671) and London (V717I) mutations] [88], and postmortem cortical brain tissue of AD patients. The downregulation of Sig1R is also detected in putamen of PD and in the lumbar spinal cord of amyotrophic lateral sclerosis (ALS) patients [89-90]. Sig1R-knockdown cells exhibit vulnerability to dopamine toxicity, which is involved in the etiology of PD, resulting in the induction of apoptosis [91]. Sig1R-deficient mice show muscle weakness and loss of motor neurons [82]. The pathogenesis is similar to those of ALS. Sig1R-deficiency triggers impaired mitochondrial fission and transport in axons, leading to axonal degeneration. The pathogenesis of these neurodegenerative diseases involves the induction of ER stress and the UPR [92]. Thus, disturbance of the UPR-Sig1R axis may cause aberrant functions of MAM, resulting in the development of these diseases.

In contrast to the regulation of MAM by UPR-related molecules, several MAM connectors can modulate the UPR. The three branches (IRE1, PERK and ATF6 pathways) of the UPR induced by ER stress show excessive activation in MFN2-deficient mouse embryonic fibroblasts [93]. The over-activation of IRE1 and PERK pathways triggers the attenuation of ER stress-dependent apoptosis and autophagy, respectively, in these deficient cells. MFN2 interacts with PERK to suppress its activation under normal conditions. Loss of function of MFN2 causes an increase in reactive oxygen species (ROS) production, mitochondrial calcium overload and impaired mitochondrial morphology through the sustained activation of PERK. These data suggest that MAM connector MFN2 has unique roles in cooperatively orchestrating mitochondrial dynamics and the UPR. Additionally, PERK localized at MAM is also known as a MAM connector [94]. This connector promotes apoptosis following insults, requiring the transfer of ROS-mediated signals between the ER and mitochondria. Accelerated ROS production and

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the over-activation of PERK because of MFN2 deficiency, and an increase in ROS damage by PERK localized at MAM may synergistically accelerate ROS-based apoptosis. Consequently, the UPR and MAM may have a bidirectional communication that enables regulation of the ER and mitochondrial dynamics and cellular homeostasis (Figure 1).

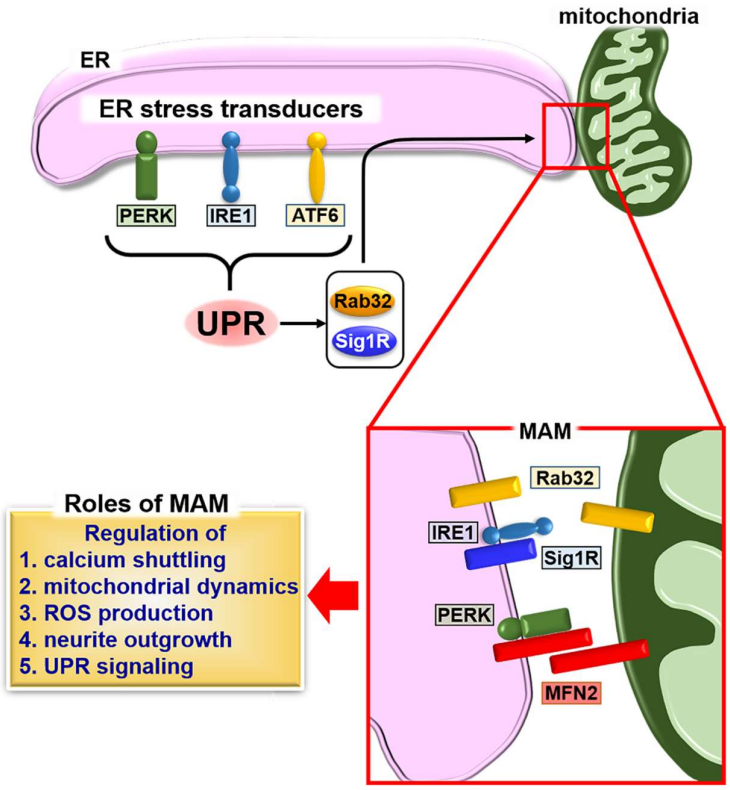

Figure 1. Schematic describing the formation of MAM and the UPR. The UPR induces the expression of MAM connectors Rab32 and Sig1R, followed by fine-tuning of calcium signaling, calcium shuttling, mitochondrial dynamics, ROS production and neurite outgrowth through the formation of MAM. Sig1R can bind to IRE1 to regulate its stabilization. The stabilized IRE1 causes the long-lasting activation, and promotes the cellular survival under ER stress condition. A second MAM connector, MFN2, interacts with PERK to inhibit its activity for regulating ROS production, calcium shuttling and mitochondrial morphology.

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5. ER-PM contact sites and the UPR

Regions of the ER closely apposed to the PM (the distance is typically within 10 to 30 nm) were first revealed by electron microscopic analysis [95]. ER-PM contact sites have emerged as key regulators of intracellular calcium dynamics [96-99]. Previous studies have shown that calcium depletion in the ER lumen triggers extracellular calcium influx through the PM at ER-PM contact sites to replenish the calcium concentration of the ER lumen [100-101]. Stromal-interacting molecule 1 (STIM1) is an integral ER protein that regulates the formation of ER-PM contact sites in response to calcium depletion in the luminal ER [102]. STIM1 senses a decrease in the intraluminal ER calcium level and undergoes conformational changes. The exposed domains following the conformational change to STIM1 preferentially target phosphatidylinositol 4,5-biphosphate (PI(4, 5)P2) enriched ER-PM contact sites [100, 103-104]. STIM1 directly recruits and forms a complex with Orai1, calcium channels localized at the PM, followed by replenishing calcium levels in the ER lumen [100, 105].

The tethering molecules are necessary to form ER-PM contact sites. Previos reports have identified at least four protein families as ER-PM tethering proteins in yeast and/or metazoans; extended-synaptotagmins (E-Syts) (Tricalbins in yeast), VAMP-associated proteins (VAPs) (Scs2/22 in yeast), transmembrane protein 16 (Tmem16) (Ist2 in yeast), and junctophilins (JPHs) [106-109]. Each of these protein families have been independently studied for their own cellular pathways, suggesting that ER-PM contact sites are area of a hub for numerous cellular signal transductions. Recent studies have indicated that the dysfunctions of the tethering may cause the disturbances of cellular functions. JPHs were identified by screening using monoclonal antibodies generated from mice immunized

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with membrane vesicles of rabbit skeletal muscles [109]. There are four junctophilins in mammals. These four JPHs have been mainly studied in the context of cell physiology in skeletal muscle. Four JPHs are selectively expressed in several tissues. JPH1 and JPH2 are preferentially expressed in both heart and skeletal muscle. On the other hand, JPH3 and JPH4 are highly expressed in brain and neuronal tissues [109-110]. Their C-terminal transmembrane domains are anchored in the sarcoplasmic reticulum (SR). Their N-termini contain basic membrane occupation and recognition nexus (MORN) domains. The MORN domain binds to phospholipids including phosphatidylserine and phosphatidylinositol (3,4,5)-trisphosphate [111]. In addition to those phospholipids, previous report has suggest that the purified junctophilins bind to electrostatically neutral lipids such as PtdCho and SM [111]. Defection of ER-PM contact sites formed by JPHs leads to the impaired calcium transients in cardiac myocytes. These abnormalities raise the possibility to induce weak heartbeats, cardiac arrest and eventual embryonic lethality in *Jph2*-deficient mice [109]. In addition, myotubes lacking JPH1 also exhibit a severe reduction in store-operated calcium entry (SOCE) occurring at ER-PM contact sites, low basal cytosolic calcium levels and low SR calcium store [112-113]. These studies indicate that the formation of ER-PM contact sites and SOCE mediated by ER-PM contact sites are important for regulation of calcium signaling in muscle cells.

The importance of ER-PM contact sites and SOCE has been also demonstrated in many other genetic studies. The heterozygous mice for the STIM1-null mutation normally grow to adulthood. By contrast, a majority (approximately 70%) of mice lacking STIM1 dies within a few hours after birth [114]. These mice show cyanosis before death caused by cardiopulmonary defect. Surviving *Stim1*-deficient mice display significant growth retardation. The weight of the deficient mice achieves approximately half of wild-type

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littermates at 3 and 7 week of age. Moreover, mice lacking functional STIM1 exhibit muscle fatigue and skeletal myopathy [115]. Loss of Orai1 in mice shows phenotypes similar to those in *Stim1*-deficient mice [116]. In addition to the functions of muscle cells, these deficient mice (*Stim1*- and *Orai1*-deficient mice) abolish the functions of immune cells, because the activation of immune cells is closely involved in SOCE. Mast cells lacking STIM1 exhibit the impaired activation of nuclear factor of activated T-cells (NFAT), which plays central roles in transcriptional regulation of cytokines [117], the suppressed degranulation and the reduction of cytokine production [118]. These abnormalities in *Stim1*-deficient mast cells are also observed in *Orai1*-deficient mast cells [119]. The reduction of SOCE and the inhibited cytokine production are shown not only in mast cells from *Stim1*- and *Orai1*-defecient mice but also in those of T cells [120-121]. Selective ablation of STIM1 in T cells shows a lymphoproliferative phenotype and a selective decrease in regulatory T cells [120]. These phenotypes in immune cells of *Stim1*- and *Orai1*-deficient mice are consistent with those of the severe combined immunodeficiency (SCID) symptoms in human patients who have mutations in *Stim1* or *Orai1* gene, resulting in the lack of normal STIM1 or Orai1 functions [116]. These genetic studies and clinical observations suggest the importance of the formation of ER-PM contact sites and SOCE via the contact sites in activation and maintenance of immune cells, as well as the functional regulation of muscle cells.

Morphological changes to the ER and regulation of ER dynamics are essential for the efficient formation of ER-PM contact sites. ER dynamics is regulated by microtubule- and actin-binding proteins [122-123]. Of those proteins, filamin A (FLNA) is known as a connector between the ER and actin cytoskeleton [122]. PERK has been identified as an interacting partner of FLNA at the ER membrane by a proximity-dependent biotin

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identification (BioID) assay [124]. PERK acts as a scaffold molecule for FLNA, enabling interlocking between F-actin and ER dynamics. The dimerization of PERK induced by calcium depletion in the ER lumen is necessary for the interaction between PERK and FLNA. Simultaneously, the decrease in calcium concentration in the ER lumen leads to activation of STIM1. The PERK-FLNA axis accelerates the remodeling and alters the polymerization dynamics of F-actin, followed by the relocalization of cortical ER containing STIM1 to the PM. These morphological changes to the ER are regulated by the PERK-FLNA connection and allow for the efficient formation of ER-PM contact sites and calcium influx to replenish the luminal calcium level. Thus, PERK manipulates morphological changes to the ER and the formation of ER-PM contact sites by its dimerization, but not via signal transduction.

The other ER stress transducer, IRE1, is also involved in cytoskeleton remodeling via interaction with FLNA. A yeast two-hybrid screen identified that FLNA binds to the cytosolic domain of IRE1 [125]. The dimerization of IRE1 is an essential step for the physiological interaction with FLNA. The IRE1 dimer acts as a scaffold molecule, and recruits FLNA and protein kinase C type α (PKCα). FLNA is phosphorylated by PKCα, followed by an increase in the remodeling of the actin cytoskeleton and cell migration. The interaction between IRE1 and FLNA implicates significant roles for ER functions and ER-derived signaling including the UPR at lamellipodia and filopodia, where active actin dynamics are observed. Additionally, the connection between IRE1 and FLNA implies the involvement of IRE1 in the formation of ER-PM contact sites, like those of PERK. As mentioned in Section 3, the IRE1 pathway is responsible for the regulation of ER biogenesis through manipulation of lipid biosynthesis. ER-PM contact sites are known to play roles in the supply of membrane lipids from the ER membrane to the PM

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[96]. Lipid transfer at ER-PM contact sites may facilitate extension of the PM and alter cellular morphology. The IRE1 pathway may bidirectionally regulate the formation of ER-PM contact sites via modulation of actin dynamics and the supply of membrane lipids at contact sites via fine-tuning of lipid biosynthesis. Collectively, these reports on PERK and IRE1 serve as reminders to the importance of defining the interactome of ER stress transducers, which may reveal unexpected mechanisms for cooperative regulation among the UPR, actin dynamics and ER-PM contact sites (Figure 2).

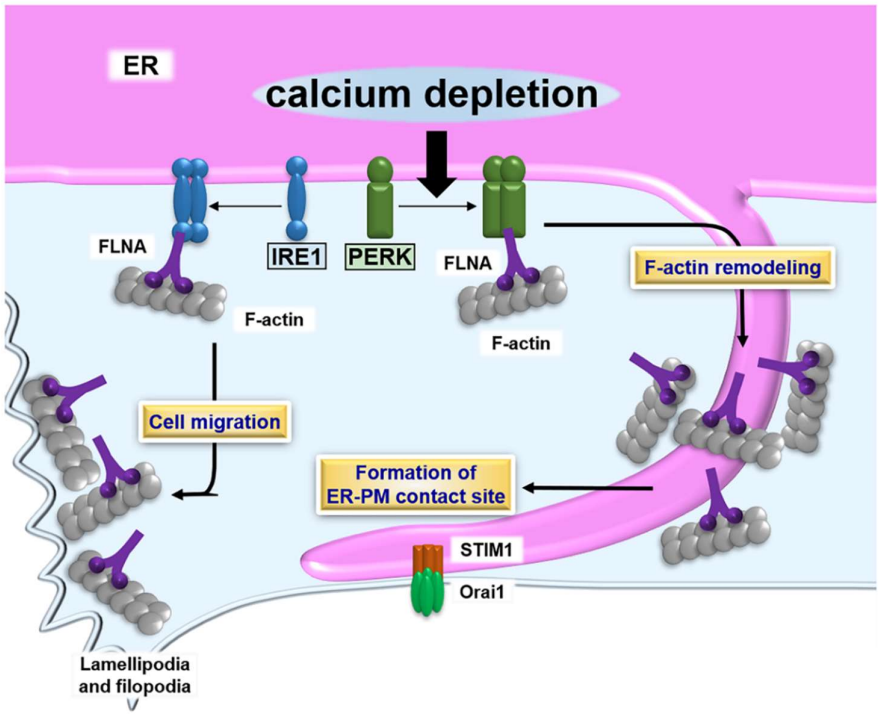

Figure 2. Schematic describing the formation of ER-PM contact sites and the UPR. PERK dimerizes in response to calcium depletion in the ER lumen. The PERK dimer binds to FLNA, which accelerates formation of ER-PM contact sites containing STIM1 and Orai1. The contact sites promote calcium influx, which returns calcium levels to normal values in the ER lumen. The IRE1 dimer also interacts with FLNA. Although the effect of the IRE1-FLNA interaction on the formation of ER-PM contact sites remains unclear, the binding of IRE1 to FLNA does modulate ER dynamics and cell migration.

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6. Concluding remarks

Understanding how ER morphology is regulated is essential for understanding how the ER communicates with other organelles. Moreover, characterizing this regulation is important for comprehending cellular homeostasis because a well-developed ER that spans the cytoplasm frequently changes its morphology and plays roles in bidirectional signal transmission through the formation of contact sites. These organelle contacts regulate the dynamics of each organelle and signal transduction, and ultimately orchestrate cellular homeostasis and biological functions. The UPR is a major signaling system found in the ER and is involved in regulating morphological changes to the ER. The mechanisms that regulate contact sites between the ER and other organelles are not fully understood. However, further studies will uncover new signaling cascades and concepts for organelle communication that is regulated by the UPR. Although not discussed in this review, research has also focused on characterizing contact sites between the ER and cellular components other than mitochondria and the PM (e.g., endosome and Golgi apparatus). Such studies may provide novel information that further elucidates the comprehensive regulation of cellular functions by the ER and UPR. These types of approaches may provide insight into new therapeutic targets for a variety of diseases including neurodegenerative diseases associated with the UPR and the organelle contact sites. The expansion of a broad spectrum of UPR signaling to regulation of morphological changes and contact sites should assist with deciphering the mechanisms for manipulating an intricate organelle network, which may lead to breakthroughs in therapeutic strategies that target various diseases.

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Acknowledgments

We thank the members of our laboratory, M. Kaneko, R. Asada, K. Matsuhisa and T. Okamoto for valuable discussions related to this work. This work was partly supported by grants from the Japan Society for the Promotion of Science KAKENHI (JP17H06416, JP17H01424), Mochida Memorial Foundation for Medical and Pharmaceutical Research and The Sumitomo Electric Industries Group Corporate Social Responsibility Foundation. We thank Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflicts of interest

We declare no competing financial interests.

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