Published in final edited form as:

Sci Signal. ; 7(350): re11. doi:10.1126/scisignal.2005671.

MicroRNAs Meet Calcium: Joint Venture in ER Proteostasis

Fabian Finger and Thorsten Hoppe*

Institute for Genetics and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Joseph Stelzmann Str. 26, 50931 Cologne, Germany

Abstract

The endoplasmic reticulum (ER) is a cellular compartment that possesses a key function in protein translation and folding. Maintaining its integrity is of fundamental importance for organism's physiology and viability. The dynamic regulation of intraluminal ER Ca^{2+} concentration directly influences the activity of ER-resident chaperones and stress response pathways that balance protein load and folding capacity. Here, we review the emerging evidence that microRNAs play important roles in adjusting these processes to frequently changing intracellular and environmental conditions to modify ER Ca^{2+} handling and storage and maintain ER homeostasis.

Introduction

A fundamental challenge during organism's lifespan is to maintain a functional proteome that can adapt in response to physiological and environmental stresses. The complex coordination of diverse pathways, collectively termed the protein homeostasis (proteostasis) network, provides protein quality control 1–4. Proteome maintenance starts at the ribosome, continues by correct folding of the nascent polypeptide chain 5,6, and ends with the degradation of damaged and dispensable proteins. Proteostasis mechanisms are interconnected and tightly regulated, consisting of transcription, translation, protein folding, and degradation pathways, such as autophagy and the ubiquitin-proteasome-system (UPS) 1–4. Misregulation of proteostasis is detrimental for the physiology and lifespan of organisms and contributes to various age-related diseases, including neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases 7.

An adaptive stress response known as the unfolded protein response (UPR) prevents the overload of misfolded or aggregation-prone proteins in the lumen of the endoplasmic reticulum (ER) and is an important mechanism of proteostasis. The UPR^{ER} consists of three different branches represented by the sensor proteins inositol-requiring enzyme 1 α (IRE1 α), activating transcription factor 6 (ATF6), and double-stranded RNA-activated protein kinase-like ER kinase (PERK) 8,9. IRE1 α is a type 1 transmembrane protein that oligomerizes in response to increased abundance of misfolded proteins in the ER lumen (known as ER stress). Oligomerization of IRE1 α leads to trans-autophosphorylation through its

*Correspondence should be addressed to T.H. thorsten.hoppe@uni-koeln.de.

Competing Interest

The authors declare that they have no competing financial or personal interests.

cytoplasmic kinase domain. In this activated state, IRE1 α shows site-specific endonucleolytic activity, which promotes unconventional splicing of the mRNA encoding X-box binding protein 1 [(XBP1), also known as homologous to ATF/CREB1 (HAC1) in yeast]. XBP1 functions as transcription factor promoting the expression of UPR genes 10. ATF6, in its inactive form, is a transmembrane protein. In ER stress conditions, ATF6 is transported from the ER to the Golgi apparatus and cleaved by Golgi-resident proteases. The cytosolic part of ATF6 moves to the nucleus and activates the transcription of UPR genes 11. Similar to IRE1 α , PERK is a type 1 transmembrane protein with a cytosolic kinase domain. Increased abundance of unfolded proteins in the ER lumen triggers PERK oligomerization, trans-autophosphorylation, and phosphorylation of the α -subunit of eukaryotic translation initiation factor-2 (eIF2 α). Phosphorylation by PERK inactivates eIF2 α , reducing protein synthesis and the protein load in the ER 12. A cell's ability to handle proteotoxic stress and misfolded proteins in the ER declines with age, which is partly due to decreased activity of these proteostasis mechanisms 13–16.

The lumen of the ER is of particular importance for proteostasis as it harbors key components for folding, trafficking, and posttranslational modifications of proteins destined for secretion or incorporation into membranes 17. Chaperone proteins located in the lumen of the ER, including calreticulin (CALR), immunoglobulin binding protein [(BIP), also known as glucose-regulated protein 78 (GRP78) or heat shock 70kDa protein 5 (HSPA5)], and protein disulfide isomerases (PDIs), are part of an elaborate molecular network that facilitates correct folding, quality control, and alleviation of proteotoxic stress 18,19. CALR is a soluble ER chaperone that supports protein folding and prevents protein aggregation in the ER lumen. CALR also delays degradation of polypeptides until they are accurately folded 20–22. The Hsp70 family member BIP is described as a master regulator of the ER because it binds and modulates many ER processes and functions, such as the UPR sensor proteins IRE1 α , ATF6, and PERK 23. In non-stressed conditions, BIP binds to the luminal domains of all three UPR sensor proteins and thereby, maintains them in an inactive state. In the context of ER stress, BIP binds to misfolded proteins to prevent aggregation, and no longer binds to the UPR sensor proteins, which activates the UPR^{ER} 24,25. In addition to UPR sensor proteins, BIP also binds to translocon (TLC), a protein complex that build an ER channel necessary for the translocation of proteins during translation and the retrotranslocation of proteins destined for degradation by the 26S proteasome [a process called ER-associated degradation (ERAD)] 26. Through binding of TLC BIP seals the translocation pore to prevent ER Ca²⁺ leakage and to maintain ER homeostasis 27,28, and thus, TLC is also termed ER Ca²⁺ leak channel 29. PDIs, a class of oxidoreductases in the ER, catalyze disulfide bond formation, isomerization, and reduction of nascent proteins, and bind hydrophobic peptides to assist in correct folding 30,31. Thus, PDIs are foldase enzymes with chaperone activity 32. CALR, BIP, and PDIs directly bind Ca²⁺, which enables their interaction with polypeptides and other chaperones, and thereby, influences their ability to promote folding of nascent polypeptides 33. Consequently, these chaperones are highly sensitive to the concentration of ER Ca²⁺ and alterations of ER luminal Ca²⁺ increases the abundance of misfolded proteins and activates the UPR^{ER} 34,35. These ER chaperones, among others, are important Ca²⁺ binding proteins that contribute to optimal ER Ca²⁺

storage 33,36. Thus, maintenance of ER Ca^{2+} homeostasis is crucial for proteostasis and needs to be tightly regulated.

Ca^{2+} flux across the ER membrane, and thus, the luminal Ca^{2+} concentration, relies on the activity of specialized transporters and receptors located in the membrane. Ryanodine receptors (RYRs) and inositol 1,4,5-trisphosphate (IP3) receptors (IP3Rs) are the major regulators of Ca^{2+} release from the ER to the cytosol 33,37. In nonexcitable cells, such as endothelia, blood cells, and hepatocytes, Ca^{2+} release is predominantly mediated by IP3Rs. Activation and opening of the Ca^{2+} channels of IP3Rs are dependent on IP3, a second messenger derived from cleavage of phosphatidylinositol 4,5-bisphosphate (PIP2) by phospholipase C (PLC). PLC is activated either by G protein-coupled receptors or receptor tyrosine kinases in the plasma membrane 38. Ligands that trigger Ca^{2+} transport include neurotransmitters, hormones, and growth factors. The release of Ca^{2+} from ER stores modulates different cellular processes, such as muscle contraction and neuronal stimulation 38. RYRs are primarily present in neurons; as well as skeletal, cardiac, and smooth muscle cells, where they are particularly important for excitation-contraction coupling processes mediated by Ca^{2+} 39–42. Release of Ca^{2+} from the ER leaves the ER in a state of Ca^{2+} depletion, which activates Ca^{2+} entry across the plasma membrane, a process called store-operated Ca^{2+} entry (SOCE) 43. Molecular components of this mechanism are the stromal interaction molecules (STIMs), ER membrane proteins, and the ORAI subunits, which physically interact at junctions between the ER and the plasma membrane. These proteins form a Ca^{2+} release-activated Ca^{2+} (CRAC) channel complex that replenishes ER Ca^{2+} concentration 44. The activity of sarco(endo)plasmic reticulum Ca^{2+} ATPases (SERCAs) facilitate uptake of Ca^{2+} into the ER lumen. SERCAs are transmembrane proteins that pump two Ca^{2+} ions for each hydrolyzed ATP, thereby maintaining high ER luminal and low cytosolic Ca^{2+} concentration 45–47. Ca^{2+} uptake by SERCAs is modulated by ER-resident chaperones, such as CALR 48,49.

microRNAs – a class of endogenous short (~21-23 nucleotides long) non-coding RNAs – act posttranscriptionally to decrease the abundance of target proteins and thereby, govern diverse cellular processes from development to death 50. The interaction of microRNAs with target mRNAs results in translational repression, mRNA degradation, or a combination of both 51. Individual microRNAs presumably bind to various different mRNAs, resulting in complex posttranscriptional regulation of a diverse set of genes encoding proteins that often lie within specific pathways or cell processes. Thus, miRNAs may have wide ranging physiological consequences 52. microRNAs that target components of the UPR^{ER} or those that are regulated by genes linked to the UPR^{ER} are extensively reviewed. This literature presents an overview of microRNAs that directly connect to the three branches of the UPR^{ER} and focus on downstream pathways, such as the induction of apoptosis 53–56. Here, we highlight the role of microRNAs in the regulation of proteins that are important for ER Ca^{2+} homeostasis and maintaining luminal Ca^{2+} concentration including Ca^{2+} transporters and channels, IP3R and SERCA, and ER-resident, Ca^{2+} -binding chaperones, CALR, BIP, and PDIs. ER Ca^{2+} homeostasis directly influences protein folding and adaptive stress responses and thus, ER proteostasis 36,57. Increasing evidence supports the idea that microRNAs are critical regulators of the proteostasis network by fine-tuning ER Ca^{2+} homeostasis.

Ca²⁺ flux across the ER membrane

Ca²⁺ uptake in the ER

ER Ca²⁺ homeostasis relies on the activity of different transmembrane channels and receptors. Among those, SERCA mediates the uptake of Ca²⁺ from the cytosol into the ER, reestablishes optimal ER Ca²⁺ concentration after triggered Ca²⁺ release, and counteracts Ca²⁺ leakage from the ER. In mammals, the SERCA protein family is encoded by three genes (*SERCA1-3*). Each gene gives rise to different splice isoforms, which are expressed in a tissue-specific manner throughout development and in adult organisms. The protein encoded by *SERCA2b* is found almost ubiquitously throughout tissues and localizes to the ER. In contrast, the protein encoded by *SERCA2a* is exclusively found in skeletal and heart muscle and functions in the SR 58. Decreased abundance of SERCA2a and decreased SERCA2a-dependent Ca²⁺ pumping are associated with pathological conditions of the heart and heart failure 59.

The abundance of SERCA2a is regulated by at least two different microRNAs. miR-328 was initially described in studies on atrial fibrillation (AF) 60. AF is a common form of cardiac arrhythmia that is associated with a higher risk of ischemic stroke and can progress into heart failure 61. The abundance of miR-328 is decreased in a mouse model of AF 60. Transgenic mice stably expressing miR-328 in cardiac tissue display hypertrophic changes, such as enlarged size of the heart, increased appearance of ventricular tissue, and the elevated expression of hypertrophic markers like ANP, BNP and β -MHC, in addition to phenotypes associated with the maladaptive responses to AF 62. Cardiac hypertrophy occurs in response to damage, aging, or hypertension and initially starts with the enlargement of individual cardiomyocytes and progressive fibrosis, and eventually leads to cell death and progression into heart failure 63. miR-328 directly targets *SERCA2a* mRNA and thereby reduces the abundance of SERCA2a in mouse cardiomyocytes in vivo and in cultured neonatal rat ventricular myocytes (NRVMs). Stable expression of miR-328 and consequent low abundance of SERCA2a disrupt reuptake of Ca²⁺ in isolated mice cardiomyocytes stimulated with caffeine to induce release of ER Ca²⁺. This prolongs the transient increase of Ca²⁺ in the cytosol, which enhances the activation of downstream signaling molecules, such as the phosphatase calcineurin and its target, the transcription factor NFAT 62,64. Activation of calcineurin and the associated induction of nuclear localization of NFAT stimulates the expression of genes that promote cardiac hypertrophy 65. The inhibition of miR-328 in mice counteracts the activation of calcineurin and the nuclear localization of NFAT and attenuates cardiac hypertrophy 62.

miR-25 also directly targets *SERCA2a* mRNA, and reduces the abundance of SERCA2a in human cardiomyocytes 66. SERCA2a is important for cardiac contractility and is involved in cardiac hypertrophy. SERCA2a determines both the relaxation and contraction of cardiomyocytes by reducing cytosolic Ca²⁺ and controlling SR Ca²⁺ load 59. Stable expression of miR-25 in HL-1 cardiomyocytes, which contract spontaneously 67, delayed Ca²⁺ reuptake in the SR after contraction 66. The abundance of miR-25 is increased in human cardiomyocytes from patients with severe heart failure. Intravenous injection of single strand oligonucleotides that antagonize miR-25 function in mice with induced heart

failure increases the abundance of SERCA2a to normal physiological amounts and improves cardiac function 66. Thus, miR-25 and miR-328, and perhaps other microRNAs, play a crucial role in Ca^{2+} homeostasis by targeting the key regulator SERCA2a (Figure 1) and thus, may represent targets for therapeutic intervention against cardiac hypertrophy.

Neuronatin (NNAT) is an ER membrane protein 68 and a potential inhibitor of SERCA 69. NNAT regulates intracellular Ca^{2+} during adipogenesis of 3T3-L1 cells⁷⁰ and neurogenesis of embryonic stem cells⁷¹. miR-708 directly targets *NNAT* mRNA in metastatic breast cancer cells. The abundance of miR-708 is decreased in metastatic compared to nonmetastatic breast cancer cells and inversely correlates with NNAT abundance. Stable overexpression of miR-708 in metastatic breast cancer cells decreases NNAT and promotes aberrant Ca^{2+} reuptake in the ER after ATP-induced release, resulting in transiently increased cytosolic Ca^{2+} concentration⁷². Cell migration, including that of metastatic tumor cells, relies on intracellular Ca^{2+} ⁷³. Thus, miR-708 can regulate Ca^{2+} homeostasis in breast cancer by suppressing NNAT abundance⁷² (Figure 1), which could be relevant to metastatic cell migration. NNAT has structural similarities to phospholamban and sarcolipin, which are transmembrane proteins that inhibit SERCA^{68,74}, suggesting that NNAT may also antagonize SERCA activity. Likewise, phospholamban and sarcolipin might also be regulated by microRNAs providing an additional layer of regulation of Ca^{2+} homeostasis. Therefore, knowledge of how microRNAs can directly and indirectly regulate SERCA-dependent Ca^{2+} homeostasis will be important for understanding normal physiology, cardiac hypertrophy, cancer metastasis, and potentially several other pathological conditions.

Ca^{2+} release from the ER

Maintenance of ER homeostasis requires the regulated release of Ca^{2+} from the ER by specialized channels and receptors. IP3Rs are important for the controlled transport of Ca^{2+} out of the ER in response to IP3 signaling³⁸. miR-786 targets *ELO-2* mRNA, which encodes a fatty acid elongase involved in defecation in *Caenorhabditis elegans*^{75,76}. ELO-2 modulates the fatty acid composition of cellular membranes, mainly through addition of palmitate⁷⁶, which could change the activity of membrane proteins, including IP3Rs. miR-786 acts upstream of IP3Rs in worms and miR-786-deficient worms have defects in the coordinated calcium oscillations in intestinal cells⁷⁵, which are required for defecation and are caused by oscillatory IP3R-dependent Ca^{2+} release from the ER^{77,78}. Thus, miR-786 may be a common and conserved mechanism of regulation of IP3R.

miR-133a also plays a role in IP3R-dependent Ca^{2+} release. In rats, pressure overload induced by aortic banding causes hypertrophy of cardiomyocytes and increases the abundance of IP3R II in these cells⁷⁹. IP3R II is an isoform of IP3R that is the predominant form found in cardiac cells⁸⁰. miR-133a reduces the abundance of IP3R II in multiple cell types, including NRVMs and adult rat ventricular myocytes (ARVMs) in cell culture as well as mouse cardiomyocytes in vivo⁸¹. Notably, miR-133a directly binds the mRNA encoding IP3R II in HEK293 cells⁸¹. Thus, both miR-786 and miR-133a regulate the abundance of IP3R isoforms and thereby, control Ca^{2+} release from the ER.

In addition to IP3Rs, RYRs are important for the regulated release of Ca^{2+} ⁴¹. In ARVMs the muscle-specific microRNA miR-1 regulates RYR2, a cardiac-specific isoform of the

RyR family, indirectly by targeting the B56 α regulatory subunit of the protein phosphatase PPA2 82. PPA2 is a heterotrimeric protein that consists of a structural A domain, a regulatory B domain, and a catalytic C domain. The B regulatory subunits facilitate substrate specificity and subcellular localization of the phosphatase and are differentially expressed in different cell types 83. Overexpression of miR-1 in cardiomyocytes causes hyperphosphorylation and activation of RyR2 by calmodulin-dependent protein kinase (CaMKII), which results in increased Ca²⁺ release from the SR in response to caffeine 82. Moreover, increased cytosolic Ca²⁺ triggers Ca²⁺ release from the SR 84 and is essential for the activation of muscle contractions 41. Thus, activation of RyR2 is implicated in cardiac arrhythmia 85. Overexpression of miR-1 results in decreased abundance of B56 α , inhibition of PP2A and increased activity of RyR2 in ARVMs 82.

Ca²⁺ dependent ER chaperones

CALR

In addition to the role of Ca²⁺ pumps and channels, Ca²⁺ binding chaperones present in the ER lumen also regulate ER Ca²⁺ homeostasis 33. Moreover, depletion of ER Ca²⁺ inhibits chaperone function and results in the accumulation of misfolded proteins and the induction of the UPR^{ER} 33,86. Pathological conditions of the heart, such as ischemia, are associated with loss of cardiac function due to oxidative stress and Ca²⁺ dysregulation 87,88 and activation of ATF6 in cardiomyocytes 89. Transgenic expression of constitutively active ATF6 in the heart of mice increases the abundance of UPR-associated genes including *CALR* 88. *CALR* mRNA contains miR-455 binding sites and microRNA sequencing identified miR-455 to be downregulated in the heart of mice with constitutively active ATF6 90. Exposing NRVMs to tunicamycin to induce the UPR^{ER} or expressing constitutively active ATF6 in these cells decreases the abundance of miR-455 and increases the expression of *CALR*. Overexpression of pre-miR-455 decreases the abundance of CALR, whereas transfection with oligonucleotides that antagonizes miR-455 increases abundance of CALR 90. Thus, ER stress increases CALR, at least in part, by decreasing miR-455 (Figure 2). In embryonic stem cells, both, loss of CALR and depletion of ER Ca²⁺ stores, impairs the secretion of the Wnt- β -catenin signaling ligand WNT3A 91 and decreases the abundance of miR-302 92. The miR-302 family was shown to promote cell cycle progression and maintenance of pluripotency in embryonic stem cells 93. Thus, these data suggest that ER stress influences the abundance of multiple microRNAs that feed back to maintain ER Ca²⁺ homeostasis and proteostasis..

PDIs

PDIs facilitate disulfide bond formation and isomerization of nascent proteins in the ER lumen 94 and are regulated by microRNAs. When ER Ca²⁺ stores are empty, CALR binds and inhibits the activity of PDIs 95 (Figure 2). In addition, Ca²⁺ binding directly promotes the activity of PDIs 96. The expression of the gene encoding PDI-associated 6 (PDIA6) is increased in NIH-3T3 cells in response to various ER stress inducing agents, including thapsigargin, tunicamycin, and brefeldin A. PDIA6 binds to IRE1 α and knockdown of PDIA6 decreases XBP1 splicing induced by thapsigargin, indicative of decreased IRE1 α activity. *PDIA6* mRNA and PDIA6 protein abundance are decreased by miR-322,

implicating a direct interaction. The abundance of miR-322 is decreased in NIH-3T3 cells in response to depletion of ER Ca^{2+} stores 97. Thus, miR-322 likely supports ER Ca^{2+} homeostasis by regulating the UPR^{ER} sensor IRE1 α through PDIA6. A disulfide bond at Cys¹⁴⁸ in the luminal domain of IRE1 α promotes its activity. PDIA6 directly binds to Cys¹⁴⁸, reducing the disulfide bond and activity of IRE1 α 98. Thus, PDIA6 limits the UPR^{ER} and maintains it in a physiological state, and this mechanism is likely modulated by miR-322. However, understanding whether miR-322 contributes to resistance to ER stress will require further investigation.

BIP

The ER chaperone BIP is a master regulator of the UPR^{ER} that is important for ER Ca^{2+} storage, and thus, regulation of BIP is crucial for ER Ca^{2+} homeostasis and protein folding 23. Members of the miR-30 family (miR-30a, b, c, d, and e) directly target BIP in rat cardiac muscle and vascular smooth muscle cells. ER stress induced by H_2O_2 decreases the abundance of miR-30 and increases the abundance of BIP in these cells. The increase in BIP due to ER stress is abrogated by transfection with synthetic miR-30 mimetic oligonucleotides. However, miR-30 transfection only causes a small decrease in the abundance of *BIP* mRNA, suggesting that repression of mRNA translation is responsible for decreased BIP in response to miR-30 binding. Transfection with oligonucleotides that antagonize miR-30 function increases the abundance of BIP in the absence of ER stress-inducing agents and causes ER stress in NRVMs and in rat aorta vascular smooth muscle cells (RAVSMCs). Transfection with miR-30 mimetics decreases the abundance of ER chaperones and increases the survival of NRVMs and RAVSMCs exposed to H_2O_2 suggesting that endogenous miR-30 may protect cells from ER stress 99.

BIP is involved in cardiovascular disease. The abundance of BIP is increased in cardiovascular diseases, including heart failure, ischemia, and stroke 100–102. miR-181a is one of many microRNAs that changes expression in response to ischemia or stroke in the brain 103,104. In a mouse stroke model, the expression of miR-181a is increased in the ischemic core and decreased in the surrounding tissue (penumbra). In contrast, BIP is decreased in the ischemic core and increased in the penumbra. Intracerebroventricular infusion of oligonucleotides that antagonize miR-181a reduces the size of the infarcted area in mice given strokes 105, suggesting that inhibition of cerebral miR-181a is neuroprotective.

Various cancers exhibit induced ER stress, accompanied with increased abundance of BIP, compared to the respective unaffected tissue. Here, BIP plays a critical role in tumor initiation, progression, and metastasis 106,107. In prostate, colon, and bladder cancer cell lines, three microRNAs - miR-30d, miR-181a, and miR-199a-5p cooperate to suppress the translation of BIP. The expression of these microRNAs is decreased and inversely correlates with the abundance of *BIP* in tumor samples from patients with these cancers. The cooperative binding of miR-30d, miR-181a, and miR-199a-5p to *BIP* mRNA decreases the abundance of BIP. On the contrary, binding of each of these microRNAs alone does not result in decreased BIP abundance. In C42B prostate cancer cells, ER stress induced by thapsigargin increases the abundance of BIP, and this effect is prevented by transfection with

precursor miR-30d, miR-181a, and miR-199a-5p. Stable expression of these microRNAs in HCT116 colon cancer cells inhibits growth when the cells were grown as subcutaneous xenografts in mice 108. These findings suggest that decreased abundance of miR-30d, miR-181a, and miR-199a-5p and the consequent increase in BIP and the UPR^{ER} may be a mechanism that protects tumor cells from ER stress-dependent damage and thereby enables tumor cell survival.

Modulation of the UPR^{ER} and future perspectives

The accumulation of misfolded proteins in the lumen of the ER causes induction of the UPR^{ER}, which supports ER folding capacity by reducing protein translation and regulating the abundance of chaperones and enzymes that reestablish proteostasis 8,9. MicroRNAs that regulate or are expressed in response to activation of the stress sensors IRE1 α , ATF6, and PERK are reviewed elsewhere 53,54,56, outlining their important role in proteostasis. Here, we focused on microRNAs that inhibit proteins necessary for ER Ca²⁺ homeostasis and thereby influence ER proteostasis 34–36 (Table 1). Given the clear role of ER Ca²⁺ for chaperone function and adaptive stress responses 34–36, we consider microRNAs that fine-tune ER Ca²⁺ homeostasis to be part of the proteostasis network.

Prolonged ER stress induces proteolysis through multiple mechanisms, including autophagy, which is modulated by microRNAs 109. For example, deletion of *miR-34* in *C. elegans* increases the expression of autophagy-related genes and extends lifespan 110. Moreover, in mammalian cells miR-34 directly binds the mRNA of autophagy-related gene 9 (Atg9). Atg9 is required for autophagy, and thus miR-34 reduces autophagic flux 110. The activation of autophagy requires the release of Ca²⁺ ER stores, mainly through IP3Rs, and from mitochondria 111. Thus, the microRNAs that modulate ER Ca²⁺ homeostasis, such as those described in this review, may also influence the activation of autophagy.

Chronic activation of the UPR^{ER} causes cells to undergo apoptosis 112. Different members of the B-cell lymphoma 2 (BCL-2) protein family provide either pro- or anti-apoptotic functions 113. For example, the BCL-2 family members BAK and BAX promote mitochondrial outer membrane permeabilization and release of cytochrome c and other apoptotic factors into the cytosol 114. In addition, BCL-2 and BCL-xL interact with IP3Rs to control Ca²⁺ flux from ER to mitochondria 115. Both processes promote formation of apoptosomes and activation of caspases, which induce apoptosis 116. The abundance of miR-29 is increased by ER stress in neuronal tissue of amyotrophic lateral sclerosis (ALS) mice 117 and targets several BCL-2 family proteins 113, suggesting that miR-29 links ER stress to apoptosis. Moreover, microRNAs that control the abundance of IP3Rs, thereby regulating Ca²⁺ release from the ER, such as miR-786 and miR-133a 75,81, may indirectly influence the initiation of apoptosis.

In addition to the roles of microRNAs in ER Ca²⁺ homeostasis (Table 1), the UPR^{ER}, autophagy, and apoptosis, microRNAs also modulate other proteostasis pathways, including mitophagy 118, the response to low temperatures 119, mTOR signaling 120, and energy metabolism 121. In contrast, there is little evidence for microRNAs that directly target key regulators of the UPS. Given the relevance of the UPS in many pathological and age-related

conditions including Alzheimer's and Parkinson's diseases, it may be particularly important to study whether and how microRNAs affect this process.

In multicellular organisms, the induction of specific proteostasis pathways is often regulated non cell-autonomously, indicating extensive physiological crosstalk between different tissues 122. For example, the heat shock response in *C. elegans* depends on specific thermosensory neurons and downstream signaling that affects non-neuronal tissues. Functional inhibition of these neurons abrogates the ability to sense ambient temperature and the associated behavioral responses and prevents the systemic heat shock response, resulting in decreased thermal tolerance 123,124. In addition, activation of IRE-1 and subsequent XBP-1 splicing in neuronal cells can induce the UPR^{ER} in the worm intestine. Expression of constitutively active XBP-1 in worm neurons inhibits age-related decline of the UPR^{ER} and increases resistance to ER stress, prolonging lifespan¹⁴. Thus, although the microRNAs discussed in this review regulate target mRNAs within diverse tissues, such as cardiac and smooth muscle 60,62,66,82, intestine 75, and neuronal cells 105, it is possible that these microRNAs also coordinate ER Ca²⁺ homeostasis and proteostasis among organs and thus, contribute to systemic stress responses.

Concluding remarks

In this review we highlight microRNAs that regulate key components in ER Ca²⁺ homeostasis (Table 1), including transmembrane channels and receptors that control Ca²⁺ flux across the ER membrane and Ca²⁺-binding, ER-resident chaperones important for ER Ca²⁺ storage. These microRNAs and their targets were mainly characterized in pathological conditions and future work is needed to elucidate whether these relationships have a more general physiological relevance. Furthermore, this evidence suggests that some of these microRNAs may be putative therapeutic targets in disease prevention or treatment, especially with regard to heart failure. In conclusion, it is clear that microRNAs act at different levels to regulate ER Ca²⁺ homeostasis and maintain optimal luminal Ca²⁺ conditions and thus fine-tune ER proteostasis mechanisms.

Acknowledgments

We are grateful for critical comments on the manuscript from Dr. André Franz, Paul Pirson and members of the Hoppe laboratory. T.H. is supported by the Deutsche Forschungsgemeinschaft (CECAD, FOR885, SFB635, KFO 286, and DIP8 grant 2014376) and the European Research Council (consolidator grant 616499). We apologize for not having cited valuable contributions due to size limitation.

References

1. Roth DM, Balch WE. Modeling general proteostasis: proteome balance in health and disease. *Curr Opin Cell Biol.* 2011; 23(2):126–34. DOI: 10.1016/j.ceb.2010.11.001 [PubMed: 21131189]
2. Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU. Molecular chaperone functions in protein folding and proteostasis. 2013; :323–55. DOI: 10.1146/annurev-biochem-060208-092442
3. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem.* 2009; 78:959–91. DOI: 10.1146/annurev.biochem.052308.114844 [PubMed: 19298183]
4. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science.* 2008; 319(5865):916–9. DOI: 10.1126/science.1141448 [PubMed: 18276881]

5. Drummond DA, Wilke CO. Mistranslation-induced protein misfolding as a dominant constraint on coding-sequence evolution. *Cell*. 2008; 134(2):341–52. DOI: 10.1016/j.cell.2008.05.042 [PubMed: 18662548]
6. Powers ET, Balch WE. Costly mistakes: translational infidelity and protein homeostasis. *Cell*. 2008; 134(2):204–6. DOI: 10.1016/j.cell.2008.07.005 [PubMed: 18662533]
7. Frost B, Diamond MI. Prion-like mechanisms in neurodegenerative diseases. *Nat Rev Neurosci*. 2010; 11(3):155–9. DOI: 10.1038/nrn2786 [PubMed: 20029438]
8. Ron D. Translational control in the endoplasmic reticulum stress response. *J Clin Invest*. 2002; 110:1383–1388. DOI: 10.1172/JCI0216784 [PubMed: 12438433]
9. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol*. 2007; 8(7):519–29. DOI: 10.1038/nrm2199 [PubMed: 17565364]
10. Sidrauski C, Walter P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell*. 1997; 90(6):1031–9. DOI: 10.1016/S0092-8674 [PubMed: 9323131]
11. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian Transcription Factor ATF6 Is Synthesized as a Transmembrane Protein and Activated by Proteolysis in Response to Endoplasmic Reticulum Stress. *Mol Biol Cell*. 1999; 10(11):3787–3799. DOI: 10.1091/mbc.10.11.3787 [PubMed: 10564271]
12. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature*. 1999; 397(6716):271–4. DOI: 10.1038/16729 [PubMed: 9930704]
13. Henis-Korenblit S, Zhang P, Hansen M, et al. Insulin/IGF-1 signaling mutants reprogram ER stress response regulators to promote longevity. *Proc Natl Acad Sci U S A*. 2010; 107(21):9730–5. DOI: 10.1073/pnas.1002575107 [PubMed: 20460307]
14. Taylor RC, Dillin A. XBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity. *Cell*. 2013; 153(7):1435–1447. DOI: 10.1016/j.cell.2013.05.042 [PubMed: 23791175]
15. Shemesh N, Shai N, Ben-Zvi A. Germline stem cell arrest inhibits the collapse of somatic proteostasis early in *Caenorhabditis elegans* adulthood. *Aging Cell*. 2013; 12(5):814–22. DOI: 10.1111/ace.12110 [PubMed: 23734734]
16. Ben-Zvi A, Miller Ea, Morimoto RI. Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc Natl Acad Sci U S A*. 2009; 106(35):14914–9. DOI: 10.1073/pnas.0902882106 [PubMed: 19706382]
17. Araki K, Nagata K. Protein folding and quality control in the ER. *Cold Spring Harb Perspect Biol*. 2011; 3(11):a007526.doi: 10.1101/cshperspect.a007526 [PubMed: 21875985]
18. Braakman I, Hebert DN. Protein folding in the endoplasmic reticulum. *Cold Spring Harb Perspect Biol*. 2013; 5(5):a013201.doi: 10.1101/cshperspect.a013201 [PubMed: 23637286]
19. Voisine C, Pedersen JS, Morimoto RI. Chaperone networks: tipping the balance in protein folding diseases. *Neurobiol Dis*. 2010; 40(1):12–20. DOI: 10.1016/j.nbd.2010.05.007 [PubMed: 20472062]
20. Hebert DN, Zhang JX, Chen W, Foellmer B, Helenius A. The number and location of glycans on influenza hemagglutinin determine folding and association with calnexin and calreticulin. *J Cell Biol*. 1997; 139(3):613–23. DOI: 10.1083/jcb.139.3.613 [PubMed: 9348279]
21. Hebert DN, Foellmer B, Helenius a. Calnexin and calreticulin promote folding, delay oligomerization and suppress degradation of influenza hemagglutinin in microsomes. *EMBO J*. 1996; 15(12):2961–8. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=450237&tool=pmcentrez&rendertype=abstract>. [PubMed: 8670797]
22. Peterson JR, Ora A, Van PN, Helenius A. Transient, lectin-like association of calreticulin with folding intermediates of cellular and viral glycoproteins. *Mol Biol Cell*. 1995; 6(9):1173–1184. DOI: 10.1091/mbc.6.9.1173 [PubMed: 8534914]
23. Hendershot LM. The ER function BiP is a master regulator of ER function. *Mt Sinai J Med*. 2004; 71(5):289–97. [Accessed August 7, 2014] [PubMed: 15543429]
24. Bertolotti, a, Zhang, Y., Hendershot, LM., Harding, HP., Ron, D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat Cell Biol*. 2000; 2(6):326–32. DOI: 10.1038/35014014 [PubMed: 10854322]

25. Morris, Ja, Dorner, aJ, Edwards, Ca, Hendershot, LM., Kaufman, RJ. Immunoglobulin Binding Protein (BiP) Function Is Required to Protect Cells from Endoplasmic Reticulum Stress but Is Not Required for the Secretion of Selective Proteins. *J Biol Chem.* 1997; 272(7):4327–4334. DOI: 10.1074/jbc.272.7.4327 [PubMed: 9020152]
26. Meusser B, Hirsch C, Jarosch E, Sommer T. ERAD: the long road to destruction. *Nat Cell Biol.* 2005; 7(8):766–72. DOI: 10.1038/ncb0805-766 [PubMed: 16056268]
27. Alder NN, Shen Y, Brodsky JL, Hendershot LM, Johnson AE. The molecular mechanisms underlying BiP-mediated gating of the Sec61 translocon of the endoplasmic reticulum. *J Cell Biol.* 2005; 168:389–399. DOI: 10.1083/jcb.200409174 [PubMed: 15684029]
28. Hammadi M, Oulidi A, Gackière F, et al. Modulation of ER stress and apoptosis by endoplasmic reticulum calcium leak via translocon during unfolded protein response: involvement of GRP78. *FASEB J.* 2013; 27(4):1600–9. DOI: 10.1096/fj.12-218875 [PubMed: 23322163]
29. Van Coppenolle F, Vanden Abeele F, Slomianny C, et al. Ribosome-translocon complex mediates calcium leakage from endoplasmic reticulum stores. *J Cell Sci.* 2004; 117:4135–4142. DOI: 10.1242/jcs.01274 [PubMed: 15280427]
30. Wallis AK, Freedman RB. Assisting oxidative protein folding: How do protein disulphide-isomerases couple conformational and chemical processes in protein folding? *Top Curr Chem.* 2013; 328:1–34. DOI: 10.1007/128-2011-171 [PubMed: 21630134]
31. Bulleid NJ. Disulfide bond formation in the mammalian endoplasmic reticulum. *Cold Spring Harb Perspect Biol.* 2012; 4:a013219.doi: 10.1101/cshperspect.a013219 [PubMed: 23125019]
32. Klappa P, Freedman RB, Zimmermann R. Protein Disulphide Isomerase and a Luminal Cyclophilin-Type Peptidyl Prolyl Cis-Trans Isomerase are in Transient Contact with Secretory Proteins During Late Stages of Translocation. *Eur J Biochem.* 2008; 232(3):755–764. DOI: 10.1111/j.1432-1033.1995.0755a.x
33. Burdakov D, Petersen OH, Verkhratsky A. Intraluminal calcium as a primary regulator of endoplasmic reticulum function. *Cell Calcium.* 2005; 38(3–4):303–10. DOI: 10.1016/j.ceca.2005.06.010 [PubMed: 16076486]
34. Groenendyk J, Agellon LB, Michalak M. Coping with endoplasmic reticulum stress in the cardiovascular system. *Annu Rev Physiol.* 2013; 75:49–67. DOI: 10.1146/annurev-physiol-030212-183707 [PubMed: 23020580]
35. Durose JB, Tam AB, Niwa M. Intrinsic Capacities of Molecular Sensors of the Unfolded Protein Response to Sense Alternate Forms of Endoplasmic Reticulum Stress. 2006 Jul.17:3095–3107. DOI: 10.1091/mbc.E06
36. Coe H, Michalak M. Calcium binding chaperones of the endoplasmic reticulum. *Gen Physiol Biophys.* 2009; 28(Spec No):F96–F103. [Accessed August 13, 2014] [PubMed: 20093733]
37. Berridge MJ. The endoplasmic reticulum: a multifunctional signaling organelle. 2002; 32:235–249. DOI: 10.1016/S0143-4160(02)00182-3
38. Berridge MJ. Inositol trisphosphate and calcium signalling. *Nature.* 1993; 361:315–325. DOI: 10.1038/361315a0 [PubMed: 8381210]
39. Clapham DE. Calcium signaling. *Cell.* 1995; 80:259–268. DOI: 10.1016/0092-8674(95)90408-5 [PubMed: 7834745]
40. Bootman M, Berridge M. The elemental principles of calcium signaling. *Cell.* 1995; 83:675–678. DOI: 10.1016/0092-8674(95)90179-5 [PubMed: 8521483]
41. Lanner JT, Georgiou DK, Joshi AD, Hamilton SL. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harb Perspect Biol.* 2010; 2(11):a003996.doi: 10.1101/cshperspect.a003996 [PubMed: 20961976]
42. Fill M, Copello Ja. Ryanodine receptor calcium release channels. *Physiol Rev.* 2002; 82(4):893–922. DOI: 10.1152/physrev.00013.2002 [PubMed: 12270947]
43. Smyth JT, Dehaven WI, Jones BF, et al. Emerging perspectives in store-operated Ca²⁺ entry: roles of Orai, Stim and TRP. *Biochim Biophys Acta.* 2006; 1763(11):1147–60. DOI: 10.1016/j.bbamcr.2006.08.050 [PubMed: 17034882]
44. Lewis RS. Store-operated calcium channels: new perspectives on mechanism and function. *Cold Spring Harb Perspect Biol.* 2011; 3(12)doi: 10.1101/cshperspect.a003970

45. Kaufman RJ, Malhotra JD. Calcium trafficking integrates endoplasmic reticulum function with mitochondrial bioenergetics. *Biochim Biophys Acta*. 2014; :1–7. DOI: 10.1016/j.bbamcr.2014.03.022
46. Olesen C, Picard M, Winther A-ML, et al. The structural basis of calcium transport by the calcium pump. *Nature*. 2007; 450(7172):1036–42. DOI: 10.1038/nature06418 [PubMed: 18075584]
47. Toyoshima C. How Ca^{2+} -ATPase pumps ions across the sarcoplasmic reticulum membrane. *Biochim Biophys Acta*. 2009; 1793(6):941–6. DOI: 10.1016/j.bbamcr.2008.10.008 [PubMed: 19010358]
48. John LM, Lechleiter JD, Camacho P. Differential modulation of SERCA2 isoforms by calreticulin. *J Cell Biol*. 1998; 142(4):963–73. DOI: 10.1083/jcb.142.4.963 [PubMed: 9722609]
49. Arnaudeau S, Frieden M, Nakamura K, Castelbou C, Michalak M, Demaurex N. Calreticulin differentially modulates calcium uptake and release in the endoplasmic reticulum and mitochondria. *J Biol Chem*. 2002; 277(48):46696–705. DOI: 10.1074/jbc.M202395200 [PubMed: 12324449]
50. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. *Development*. 2005; 132:4653–4662. DOI: 10.1242/dev.02073 [PubMed: 16224045]
51. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet*. 2008; 9:102–114. DOI: 10.1038/nrg2290 [PubMed: 18197166]
52. Lim LP, Lau NC, Garrett-Engele P, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*. 2005; 433:769–773. DOI: 10.1038/nature03315 [PubMed: 15685193]
53. Byrd AE, Brewer JW. Micro(RNA)managing endoplasmic reticulum stress. *IUBMB Life*. 2013; 65(5):373–81. DOI: 10.1002/iub.1151 [PubMed: 23554021]
54. Maurel M, Chevet E. Endoplasmic reticulum stress signaling: the microRNA connection. *Am J Physiol Cell Physiol*. 2013; 304:C1117–26. DOI: 10.1152/ajpcell.00061.2013 [PubMed: 23515532]
55. Urra H, Dufey E, Lisbona F, Rojas-Rivera D, Hetz C. When ER stress reaches a dead end. *Biochim Biophys Acta*. 2013; 1833(12):3507–17. DOI: 10.1016/j.bbamcr.2013.07.024 [PubMed: 23988738]
56. Chitnis N, Pytel D, Diehl JA. UPR-inducible miRNAs contribute to stressful situations. *Trends Biochem Sci*. 2013; 38(9):447–52. DOI: 10.1016/j.tibs.2013.06.012 [PubMed: 23906563]
57. Ashby MC, Tepikin AV. ER calcium and the functions of intracellular organelles. *Semin Cell Dev Biol*. 2001; 12(1):11–7. DOI: 10.1006/scdb.2000.0212 [PubMed: 11162742]
58. Vangheluwe P, Raeymaekers L, Dode L, Wuytack F. Modulating sarco(endo)plasmic reticulum Ca^{2+} ATPase 2 (SERCA2) activity: cell biological implications. *Cell Calcium*. 2005; 38(3–4): 291–302. DOI: 10.1016/j.ceca.2005.06.033 [PubMed: 16105684]
59. Meyer M, Schillinger W, Pieske B, et al. Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy. *Circulation*. 1995; 92:778–784. DOI: 10.1161/01.CIR.92.4.778 [PubMed: 7641356]
60. Lu Y, Zhang Y, Wang N, et al. MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. *Circulation*. 2010; 122(23):2378–87. DOI: 10.1161/CIRCULATIONAHA.110.958967 [PubMed: 21098446]
61. Nattel S. New ideas about atrial fibrillation 50 years on. *Nature*. 2002; 415(6868):219–26. DOI: 10.1038/415219a [PubMed: 11805846]
62. Li C, Li X, Gao X, et al. MicroRNA-328 as a regulator of cardiac hypertrophy. *Int J Cardiol*. 2014; 173(2):268–76. DOI: 10.1016/j.ijcard.2014.02.035 [PubMed: 24631114]
63. Dorn GW. The fuzzy logic of physiological cardiac hypertrophy. *Hypertension*. 2007; 49(5):962–70. DOI: 10.1161/HYPERTENSIONAHA.106.079426 [PubMed: 17389260]
64. Wilkins BJ, Molkentin JD. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem Biophys Res Commun*. 2004; 322(4):1178–91. DOI: 10.1016/j.bbrc.2004.07.121 [PubMed: 15336966]

65. Shimoyama M, Hayashi D, Takimoto E, et al. Calcineurin Plays a Critical Role in Pressure Overload-Induced Cardiac Hypertrophy. *Circulation*. 1999; 100(24):2449–2454. DOI: 10.1161/01.CIR.100.24.2449 [PubMed: 10595959]
66. Wahlquist C, Jeong D, Rojas-Muñoz A, et al. Inhibition of miR-25 improves cardiac contractility in the failing heart. *Nature*. 2014; 508(7497):531–5. DOI: 10.1038/nature13073 [PubMed: 24670661]
67. Claycomb WC, Lanson Na, Stallworth BS, et al. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proc Natl Acad Sci U S A*. 1998; 95(6):2979–84. [PubMed: 9501201]
68. Joseph R, Dou D, Tsang W. Molecular cloning of a novel mRNA (neuronatin) that is highly expressed in neonatal mammalian brain. *Biochem Biophys Res Commun*. 1994; 201:1227–1234. doi:S0006291X84718365 [pii]. [PubMed: 8024565]
69. Oyang EL, Davidson BC, Lee W, Poon MM. Functional characterization of the dendritically localized mRNA neuronatin in hippocampal neurons. *PLoS One*. 2011; 6(9):e24879.doi: 10.1371/journal.pone.0024879 [PubMed: 21935485]
70. Suh YH, Kim WH, Moon C, et al. Ectopic expression of Neuronatin potentiates adipogenesis through enhanced phosphorylation of cAMP-response element-binding protein in 3T3-L1 cells. *Biochem Biophys Res Commun*. 2005; 337(2):481–9. DOI: 10.1016/j.bbrc.2005.09.078 [PubMed: 16223607]
71. Lin H-H, Bell E, Uwanogho D, et al. Neuronatin promotes neural lineage in ESCs via Ca(2+) signaling. *Stem Cells*. 2010; 28(11):1950–60. DOI: 10.1002/stem.530 [PubMed: 20872847]
72. Ryu S, McDonnell K, Choi H, et al. Suppression of miRNA-708 by polycomb group promotes metastases by calcium-induced cell migration. *Cancer Cell*. 2013; 23(1):63–76. DOI: 10.1016/j.ccr.2012.11.019 [PubMed: 23328481]
73. Yang S, Zhang JJ, Huang XY. Orai1 and STIM1 Are Critical for Breast Tumor Cell Migration and Metastasis. *Cancer Cell*. 2009; 15:124–134. DOI: 10.1016/j.ccr.2008.12.019 [PubMed: 19185847]
74. Asahi M, Kurzydowski K, Tada M, MacLennan DH. Sarcolipin inhibits polymerization of phospholamban to induce superinhibition of sarco(endo)plasmic reticulum Ca²⁺-ATPases (SERCAs). *J Biol Chem*. 2002; 277(30):26725–8. DOI: 10.1074/jbc.C200269200 [PubMed: 12032137]
75. Kemp BJ, Allman E, Immerman L, et al. miR-786 regulation of a fatty-acid elongase contributes to rhythmic calcium-wave initiation in *C. elegans*. *Curr Biol*. 2012; 22(23):2213–20. DOI: 10.1016/j.cub.2012.09.047 [PubMed: 23141108]
76. Kniazeva M, Sieber M, McCauley S, Zhang K, Watts JL, Han M. Suppression of the ELO-2 FA elongation activity results in alterations of the fatty acid composition and multiple physiological defects, including abnormal ultradian rhythms, in *Caenorhabditis elegans*. *Genetics*. 2003; 163(1): 159–69. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1462428&tool=pmcentrez&rendertype=abstract>. [PubMed: 12586704]
77. Dal Santo P, Logan Ma, Chisholm AD, Jorgensen EM. The inositol trisphosphate receptor regulates a 50-second behavioral rhythm in *C. elegans*. *Cell*. 1999; 98(6):757–67. DOI: 10.1016/S0092-8674(00)81510-X [PubMed: 10499793]
78. Espelt MV, Estevez AY, Yin X, Strange K. Oscillatory Ca²⁺ signaling in the isolated *Caenorhabditis elegans* intestine: role of the inositol-1,4,5-trisphosphate receptor and phospholipases C beta and gamma. *J Gen Physiol*. 2005; 126(4):379–92. DOI: 10.1085/jgp.200509355 [PubMed: 16186564]
79. Carè A, Catalucci D, Felicetti F, et al. MicroRNA-133 controls cardiac hypertrophy. *Nat Med*. 2007; 13(5):613–8. DOI: 10.1038/nm1582 [PubMed: 17468766]
80. Harzheim D, Movassagh M, Foo RS-Y, et al. Increased InsP3Rs in the junctional sarcoplasmic reticulum augment Ca²⁺ transients and arrhythmias associated with cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2009; 106(27):11406–11. DOI: 10.1073/pnas.0905485106 [PubMed: 19549843]
81. Drawnel FM, Wachten D, Molkenin JD, et al. Mutual antagonism between IP(3)RII and miRNA-133a regulates calcium signals and cardiac hypertrophy. *J Cell Biol*. 2012; 199(5):783–98. DOI: 10.1083/jcb.201111095 [PubMed: 23166348]

82. Terentyev D, Belevych AE, Terentyeva R, et al. miR-1 overexpression enhances Ca(2+) release and promotes cardiac arrhythmogenesis by targeting PP2A regulatory subunit B56alpha and causing CaMKII-dependent hyperphosphorylation of RyR2. *Circ Res.* 2009; 104(4):514–21. DOI: 10.1161/CIRCRESAHA.108.181651 [PubMed: 19131648]
83. McCright B, Rivers AM, Audlin S, Virshup DM. The B56 family of protein phosphatase 2A (PP2A) regulatory subunits encodes differentiation-induced phosphoproteins that target PP2A to both nucleus and cytoplasm. *J Biol Chem.* 1996; 271:22081–22089. DOI: 10.1074/jbc.271.36.22081 [PubMed: 8703017]
84. Fabiato A. Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J Gen Physiol.* 1985; 85:247–289. DOI: 10.1085/jgp.85.2.247 [PubMed: 2580043]
85. Ai X, Curran JW, Shannon TR, Bers DM, Pogwizd SM. Ca²⁺/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca²⁺ leak in heart failure. *Circ Res.* 2005; 97:1314–1322. DOI: 10.1161/01.RES.0000194329.41863.89 [PubMed: 16269653]
86. Gidalevitz T, Prahlad V, Morimoto RI. The stress of protein misfolding: from single cells to multicellular organisms. *Cold Spring Harb Perspect Biol.* 2011; 3doi: 10.1101/cshperspect.a009704
87. Scarabelli TM, Gottlieb Ra. Functional and clinical repercussions of myocyte apoptosis in the multifaceted damage by ischemia/reperfusion injury: old and new concepts after 10 years of contributions. *Cell Death Differ.* 2004; 11(Suppl 2):S144–52. DOI: 10.1038/sj.cdd.4401544 [PubMed: 15608693]
88. Martindale JJ, Fernandez R, Thuerlauf D, et al. Endoplasmic reticulum stress gene induction and protection from ischemia/reperfusion injury in the hearts of transgenic mice with a tamoxifen-regulated form of ATF6. *Circ Res.* 2006; 98:1186–1193. DOI: 10.1161/01.RES.0000220643.65941.8d [PubMed: 16601230]
89. Doroudgar S, Thuerlauf DJ, Marcinko MC, Belmont PJ, Glembotski CC. Ischemia activates the ATF6 branch of the endoplasmic reticulum stress response. *J Biol Chem.* 2009; 284(43):29735–45. DOI: 10.1074/jbc.M109.018036 [PubMed: 19622751]
90. Belmont PJ, Chen WJ, Thuerlauf DJ, Glembotski CC. Regulation of microRNA expression in the heart by the ATF6 branch of the ER stress response. *J Mol Cell Cardiol.* 2012; 52(5):1176–82. DOI: 10.1016/j.jmcc.2012.01.017 [PubMed: 22326432]
91. Lai S-L, Chien AJ, Moon RT. Wnt/Fz signaling and the cytoskeleton: potential roles in tumorigenesis. *Cell Res.* 2009; 19(5):532–45. DOI: 10.1038/cr.2009.41 [PubMed: 19365405]
92. Groenendyk J, Michalak M. Disrupted WNT Signaling in Mouse Embryonic Stem Cells in the Absence of Calreticulin. *Stem Cell Rev.* 2014; 10(2):191–206. DOI: 10.1007/s12015-013-9488-6 [PubMed: 24415131]
93. Liao B, Bao X, Liu L, et al. MicroRNA cluster 302-367 enhances somatic cell reprogramming by accelerating a mesenchymal-to-epithelial transition. *J Biol Chem.* 2011; 286(19):17359–64. DOI: 10.1074/jbc.C111.235960 [PubMed: 21454525]
94. Ferrari DM, Söling HD. The protein disulphide-isomerase family: unravelling a string of folds. *Biochem J.* 1999; 339(Pt 1):1–10. DOI: 10.1042/0264-6021:3390001 [PubMed: 10085220]
95. Corbett EF, Michalak M. Calcium, a signaling molecule in the endoplasmic reticulum? *Trends Biochem Sci.* 2000; 25(7):307–11. DOI: 10.1016/S0968-0004(00)01588-7 [PubMed: 10871879]
96. Primm T, Walker K, Gilbert H. Facilitated protein aggregation. Effects of calcium on the chaperone and anti-chaperone activity of protein disulfide-isomerase. *J Biol Chem.* 1996; doi: 10.1074/jbc.271.52.33664
97. Groenendyk J, Peng Z, Dudek E, et al. Interplay Between the Oxidoreductase PDIA6 and microRNA-322 Controls the Response to Disrupted Endoplasmic Reticulum Calcium Homeostasis. *Sci Signal.* 2014; 7(329):ra54–ra54. DOI: 10.1126/scisignal.2004983 [PubMed: 24917591]
98. Eletto D, Eletto D, Dersh D, Gidalevitz T, Argon Y. Protein disulfide isomerase A6 controls the decay of IRE1α signaling via disulfide-dependent association. *Mol Cell.* 2014; 53(4):562–76. DOI: 10.1016/j.molcel.2014.01.004 [PubMed: 24508390]

99. Chen M, Ma G, Yue Y, et al. Downregulation of the miR-30 family microRNAs contributes to endoplasmic reticulum stress in cardiac muscle and vascular smooth muscle cells. *Int J Cardiol.* 2014; 173(1):65–73. DOI: 10.1016/j.ijcard.2014.02.007 [PubMed: 24612558]
100. Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. *J Cell Biol.* 2012; 197(7):857–67. DOI: 10.1083/jcb.201110131 [PubMed: 22733998]
101. Minamino T, Kitakaze M. ER stress in cardiovascular disease. *J Mol Cell Cardiol.* 2010; 48(6): 1105–10. DOI: 10.1016/j.yjmcc.2009.10.026 [PubMed: 19913545]
102. Nakka VP, Gusain A, Raghubir R. Endoplasmic reticulum stress plays critical role in brain damage after cerebral ischemia/reperfusion in rats. *Neurotox Res.* 2010; 17:189–202. DOI: 10.1007/s12640-009-9110-5 [PubMed: 19763736]
103. Dharap A, Bowen K, Place R, Li L-C, Vemuganti R. Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome. *J Cereb Blood Flow Metab.* 2009; 29(4):675–87. DOI: 10.1038/jcbfm.2008.157 [PubMed: 19142192]
104. Yuan Y, Wang JY, Xu LY, Cai R, Chen Z, Luo BY. MicroRNA expression changes in the hippocampi of rats subjected to global ischemia. *J Clin Neurosci.* 2010; 17(6):774–8. DOI: 10.1016/j.jocn.2009.10.009 [PubMed: 20080409]
105. Ouyang Y-B, Lu Y, Yue S, et al. miR-181 regulates GRP78 and influences outcome from cerebral ischemia in vitro and in vivo. *Neurobiol Dis.* 2012; 45(1):555–63. DOI: 10.1016/j.nbd.2011.09.012 [PubMed: 21983159]
106. Dong D, Stapleton C, Luo B, et al. A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. *Cancer Res.* 2011; 71(8):2848–57. DOI: 10.1158/0008-5472.CAN-10-3151 [PubMed: 21467168]
107. Luo B, Lee aS. The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. *Oncogene.* 2013; 32(7):805–18. DOI: 10.1038/nc.2012.130 [PubMed: 22508478]
108. Su S-F, Chang Y-W, Andreu-Vieyra C, et al. miR-30d, miR-181a and miR-199a-5p cooperatively suppress the endoplasmic reticulum chaperone and signaling regulator GRP78 in cancer. *Oncogene.* 2013; 32(39):4694–701. DOI: 10.1038/nc.2012.483 [PubMed: 23085757]
109. Titone R, Morani F, Follo C, Vidoni C, Mezzanzanica D, Isidoro C. Epigenetic Control of Autophagy by MicroRNAs in Ovarian Cancer. *Biomed Res Int.* 2014; 2014:343542.doi: 10.1155/2014/343542 [PubMed: 24877083]
110. Yang J, Chen D, He Y, et al. MiR-34 modulates *Caenorhabditis elegans* lifespan via repressing the autophagy gene *atg9*. *Age (Dordr).* 2013; 35(1):11–22. DOI: 10.1007/s11357-011-9324-3 [PubMed: 22081425]
111. Decuypere J-P, Bultynck G, Parys JB. A dual role for Ca(2+) in autophagy regulation. *Cell Calcium.* 2011; 50(3):242–50. DOI: 10.1016/j.ceca.2011.04.001 [PubMed: 21571367]
112. Ellis RE, Yuan JY, Horvitz HR. Mechanisms and functions of cell death. *Annu Rev Cell Biol.* 1991; 7:663–698. DOI: 10.1146/annurev.cellbio.7.1.663 [PubMed: 1809356]
113. Ouyang Y-B, Giffard RG. microRNAs affect BCL-2 family proteins in the setting of cerebral ischemia. *Neurochem Int.* 2013; doi: 10.1016/j.neuint.2013.12.006
114. Bender T, Martinou J-C. Where killers meet--permeabilization of the outer mitochondrial membrane during apoptosis. *Cold Spring Harb Perspect Biol.* 2013; 5:a011106.doi: 10.1101/cshperspect.a011106 [PubMed: 23284044]
115. Ouyang Y-B, Giffard RG. ER-Mitochondria Crosstalk during Cerebral Ischemia: Molecular Chaperones and ER-Mitochondrial Calcium Transfer. *Int J Cell Biol.* 2012; 2012:493934.doi: 10.1155/2012/493934 [PubMed: 22577383]
116. Giorgi C, Baldassari F, Bononi A, et al. Mitochondrial Ca(2+) and apoptosis. *Cell Calcium.* 2012; 52(1):36–43. DOI: 10.1016/j.ceca.2012.02.008 [PubMed: 22480931]
117. Nolan K, Mitchem MR, Jimenez-Mateos EM, Henshall DC, Concannon CG, Prehn JHM. Increased Expression of MicroRNA-29a in ALS Mice: Functional Analysis of Its Inhibition. *J Mol Neurosci.* 2014; doi: 10.1007/s12031-014-0290-y
118. Barde I, Rauwel B, Marin-Flore RM, et al. A KRAB/KAP1-miRNA cascade regulates erythropoiesis through stage-specific control of mitophagy. *Science.* 2013; 340(6130):350–3. DOI: 10.1126/science.1232398 [PubMed: 23493425]

119. Lyons PJ, Lang-Ouellette D, Morin P. CryomiRs: towards the identification of a cold-associated family of microRNAs. *Comp Biochem Physiol Part D Genomics Proteomics*. 2013; 8(4):358–64. DOI: 10.1016/j.cbd.2013.10.001 [PubMed: 24212287]
120. Totary-Jain H, Sanoudou D, Ben-Dov IZ, et al. Reprogramming of the microRNA transcriptome mediates resistance to rapamycin. *J Biol Chem*. 2013; 288(9):6034–44. DOI: 10.1074/jbc.M112.416446 [PubMed: 23300087]
121. El Azzouzi H, Leptidis S, Dirks E, et al. The hypoxia-inducible microRNA cluster miR-199a~214 targets myocardial PPAR δ and impairs mitochondrial fatty acid oxidation. *Cell Metab*. 2013; 18(3):341–54. DOI: 10.1016/j.cmet.2013.08.009 [PubMed: 24011070]
122. Taylor RC, Berendzen KM, Dillin A. Systemic stress signalling: understanding the cell non-autonomous control of proteostasis. *Nat Rev Mol Cell Biol*. 2014; 15:211–7. DOI: 10.1038/nrm3752 [PubMed: 24556842]
123. Prahlad V, Cornelius T, Morimoto RI. Regulation of the cellular heat shock response in *Caenorhabditis elegans* by thermosensory neurons. *Science*. 2008; 320(5877):811–4. DOI: 10.1126/science.1156093 [PubMed: 18467592]
124. Prahlad V, Morimoto RI. Neuronal circuitry regulates the response of *Caenorhabditis elegans* to misfolded proteins. *Proc Natl Acad Sci U S A*. 2011; 108(34):14204–9. DOI: 10.1073/pnas.1106557108 [PubMed: 21844355]

Overline

Cell Biology

One sentence summary

Multiple microRNAs converge to regulate calcium homeostasis in the endoplasmic reticulum.

Gloss

The endoplasmic reticulum (ER) is an intracellular compartment that governs maturation and folding of secretory and transmembrane proteins. Maintenance of ER integrity is tightly regulated to avoid protein overload and protein aggregation. Optimal ER luminal conditions and specialized ER-resident proteins called chaperones are central for this quality control. One important factor influencing ER protein homeostasis is the concentration of Ca^{2+} in the ER. Thus, mechanisms that regulate the Ca^{2+} flux across the ER membrane are carefully controlled. MicroRNAs, a class of short non-coding RNAs that act posttranscriptionally to reduce protein abundance, are known to modulate diverse cellular processes. In this review, consisting of two figures, one table, and 124 references, we focus on the role of microRNAs that coordinate ER Ca^{2+} homeostasis with proteostasis pathways.

Figure 1. MicroRNAs modulating Ca^{2+} flux across the ER membrane.

An overview of microRNAs that regulate the intraluminal ER Ca^{2+} concentration. Ca^{2+} uptake in the ER lumen depends on the pumping activity of SERCA, which is regulated by interaction with different effectors such as NNAT. Conversely, the release of Ca^{2+} from internal ER stores depends on the activity of IP3Rs and RYRs. Both import and export of Ca^{2+} is modulated by microRNAs. Direct inhibition by microRNAs through mRNA targeting is depicted with red bars.

Figure 2. MicroRNAs and Ca²⁺ dependent ER chaperones.

(A) The ER chaperones CALR and PDI interact in distinct Ca²⁺ concentrations. Low intraluminal Ca²⁺ level enables CALR to bind PDI and inhibit its activity. High Ca²⁺ concentration prevents this interaction, resulting in increased activity of both PDI and CALR. (B) Depletion of ER Ca²⁺ stores decreases the abundance of miR-455 and miR-322 and increases the abundance of CALR and PDI. PDI and IRE1α represent a negative feedback loop in ER stress regulation. The abundance of PDIs are increased by activation of the UPR^{ER} and, PDIs inhibit IRE1α activity through reduction of a disulfide bond required for activation and oligomerization. (C) BIP binds to the ER stress sensors IRE1α, ATF6, and PERK, thereby inhibiting their activation. BIP also binds TLC and seals the pore to prevent Ca²⁺ leakage. ER stress decreases the abundance of miR-30, miR-181a, and miR-199-5p, which increases BIP and enhances protein folding. To provide this chaperone function, BIP dissociates from ER stress sensors and TLC and induces UPR^{ER}.

Table 1

microRNAs affecting Ca²⁺ flux and ER chaperones

target	microRNA	cell type	species	reference
Ca²⁺ flux across ER membrane				
SERCA2a	miR-328	cardiac muscle	<i>M. musculus</i>	Li <i>et al.</i> , 2014
	miR-25	cardiac muscle	<i>H. sapiens</i>	Wahlquist <i>et al.</i> , 2014
NNAT	miR-708	breast cancer	<i>H. sapiens</i>	Ryu <i>et al.</i> , 2013
IP3R II	<i>mir-786</i>	intestinal	<i>C. elegans</i>	Kemp <i>et al.</i> , 2012
	miR-133a	cardiac muscle	<i>R. norvegicus</i>	Drawnel <i>et al.</i> , 2012
RYR2	miR-1	cardiac muscle	<i>R. norvegicus</i>	Terentyev <i>et al.</i> , 2009
Ca²⁺ dependent ER chaperones				
CALR	miR-455	cardiac muscle	<i>M. musculus</i>	Belmont <i>et al.</i> , 2012
	miR-302a, b, c, d	embryonic stem (ES)	<i>M. musculus</i>	Liao <i>et al.</i> , 2011
PDI/A6	miR-322	embryonic fibroblasts (NIH-3T3)	<i>M. musculus</i>	Groenendyk <i>et al.</i> , 2014
BIP	miR-30a, b, c, d, e	cardiac/vascular smooth muscle	<i>R. norvegicus</i>	Chen <i>et al.</i> , 2014
	miR-30d, miR-181a, miR-199-5p	various cancer	<i>H. sapiens</i>	Su <i>et al.</i> , 2013
	miR-181	neuronal	<i>M. musculus</i>	Ouyang <i>et al.</i> , 2011