# Chapter 4
# The Endoplasmic Reticulum and the Cellular Reticular Network


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**Abstract** The endoplasmic reticulum and the other organelles of the eukaryotic cell are membrane-bound structures that carry out specialized functions. In this chapter, we discuss strategies that the cell has adopted to link and coordinate the different activities occurring within its various organelles as the cell carries out its physiological role.

**Keywords** Calcium signaling · Cell stress · Cellular reticular network · Homeostasis · Membrane contact sites

## Abbreviations

| | |
|---|---|
| ATF6 | activating transcription factor 6 |
| BiP | immunoglobulin binding protein |
| ER | endoplasmic reticulum |
| ERAD | ER-associated degradation |
| ERMAS | ER-mitochondria encounter structure |
| GRP | glucose regulated protein |
| HSP | heat shock protein |
| $InsP_3$ | inositol-1,4,5-trisphosphate |
| IRE | serine/threonine-protein kinase/endoribonuclease inositol-requiring enzyme |
| ORAI | $Ca^{2+}$ release-activated $Ca^{2+}$ channel |
| PERK | dsRNA-activated protein kinase-like ER kinase |
| PDI | protein disulfide isomerase |

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|---|---|
| RER | rough ER |
| SARAF | SOCE-associated regulatory factor |
| SCAP | SREBP cleavage activating protein |
| SER | smooth ER |
| SERCA | sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase |
| SOCE | store-operated $Ca^{2+}$ entry |
| SR | sarcoplasmic reticulum |
| SREBP | sterol-response element-binding protein |
| STIM | stromal-interacting molecule |
| RyR | ryanodine receptor |
| UPR | unfolded protein response |
| Xbp1 | X-box binding protein 1 |

## 4.1 Introduction

A defining feature of eukaryotic cells is the compartmentalization of various cellular functions specialized within organelles. These organelles are separated from each other by membranes and provide distinct protected environments where the cell can carry out various specialized functions at greater efficiencies by populating these structures with a unique set of proteins and lipids that can partition the required set of metabolites. On the other hand, segregation of functions poses a problem regarding substrate and metabolite exchange among membrane-bound compartments as well as intracellular communication that is essential for coordination of cellular metabolic activities. Recent developments have provided insights into the strategies employed by the cell to overcome the problem.

## 4.2 The ER

The ER is an extensive system of membranes arranged as a "net-like" network that in many cases occupies most of the interior of the cell (Fig. 4.1). This organelle houses a variety of critical ATP-requiring functions, such as maintenance of cellular homeostasis, synthesis of membrane-associated, luminal and secreted proteins, correct folding of proteins and glycoproteins, post-translational modification of proteins, lipid and steroid synthesis, $Ca^{2+}$ storage and signaling, to name a few [1–3]. In some cellular systems, the ER provides specialized functions such as support of excitation-contraction in muscle (sarcoplasmic reticulum, SR), detoxification as well as lipid synthesis (smooth ER, SER) such as in hepatic and intestinal cells, support of protein secretory functions such as in pancreatic and liver cells (rough ER, RER), and activation of cells of the immune and nervous systems ($Ca^{2+}$ signaling).

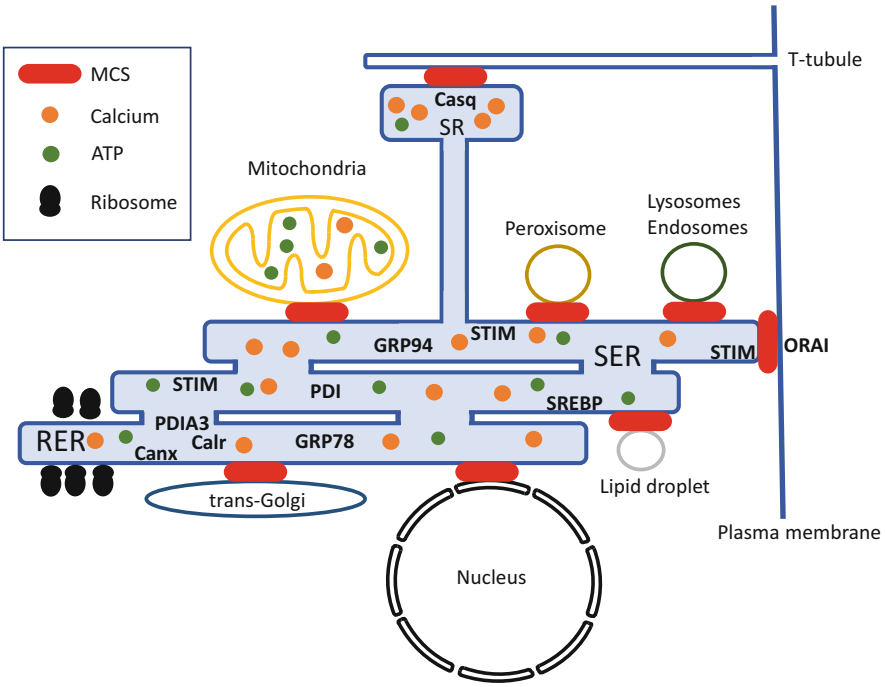


**Fig. 4.1** Cellular reticular network and interorganellar membrane contacts sites. The figure depicts the joining of ER membranes to cellular organelles to form the cellular reticular network from the plasma membrane to the nuclear envelope. The membrane contact sites enable rapid distribution and exchange of substrates, metabolites and signalling molecules to support proteostasis and lipidostasis. *Calr* calreticulin, *Casq* calsequestrin, *Canx* calnexin, *PDI* protein disulfide isomerase, *RER* rough endoplasmic reticulum, *SER* smooth endoplasmic reticulum

To perform these diverse functions, the ER engages a wide assortment of multifunctional integral membrane and luminal chaperones, folding enzymes and sensor molecules [4]. Chaperones are specialized proteins that assist in folding of polypeptides and in the assembly of multi-subunit proteins while folding enzymes accelerate folding process by supporting posttranslational modifications of newly synthesized proteins [5, 6]. Folding sensors play a key role in recognition of the properly folded or malfolded proteins [7]. These proteins are not only involved in ensuring the fidelity of protein folding, posttranslational modifications of newly synthesized proteins, but also contribute to storage of $Ca^{2+}$ ions, facilitation of $Ca^{2+}$ signaling, lipid and steroid synthesis and modification, and many other cellular roles beyond those occurring within the ER lumen [1, 8–11]. The ER also contains membrane-associated proteins that support cellular lipid and steroid synthesis [8, 9]. Importantly, ER resident chaperones and folding enzymes are major luminal ER $Ca^{2+}$ binding proteins, and together with the inositol-1,4,5-trisphosphate ($InsP_3$) receptor/$Ca^{2+}$ release channel, the ER $Ca^{2+}$-ATPase (SERCA) pump and

ER-associated $Ca^{2+}$ sensors stromal interacting molecule (STIM) proteins, are vital for $Ca^{2+}$-based intracellular communication [2, 12, 13]. The ER membrane also contains an assortment of integral membrane kinases, which together with the ER-resident chaperones and folding enzymes, regulate ER stress responses allowing cellular adaptation to many challenges originating from environmental, metabolic and intrinsic demands [14, 15]. Some organelles, such as mitochondria and nucleus, are also equipped with additional specialized stress coping mechanisms [16–20]. These mechanisms work in concert with ER stress coping strategies to preserve or regain cellular homeostasis and prevent cellular dysfunction.

## 4.3 ER and $Ca^{2+}$ Homeostasis

The ER is the main site of intracellular $Ca^{2+}$ storage and physiological source of $Ca^{2+}$ for intracellular signaling [2]. Disruption of $Ca^{2+}$ homeostasis within the ER as well as release of $Ca^{2+}$ from ER stores activate transcriptional and translational cascades that produce components involved in key pathways, such as the unfolded protein response (UPR), protein folding, ER-associated degradation (ERAD), expansion of the membrane systems as well as apoptosis [2, 12, 13]. Many of the ER proteins involved in $Ca^{2+}$ binding and signaling also participate in nearly all critical ER functions [4, 21–25]. These proteins collectively make up the luminal ER $Ca^{2+}$ stores, maintaining the total $Ca^{2+}$ concentration in the ER within the μM to mM range, which is critical for the preservation of the integrity and survival of the cell. Among these ER resident $Ca^{2+}$ binding proteins, calreticulin represents the major protein responsible for sequestering approximately 50% of luminal ER $Ca^{2+}$ [26]. Other ER chaperones, GRP94 and GRP78 (also known as immunoglobulin binding protein or BiP) have relatively low capacity for binding $Ca^{2+}$ and contribute approximately 30% of the total luminal ER $Ca^{2+}$ store by virtue of their abundance [27–29]. The remaining balance of the luminal ER $Ca^{2+}$ store is bound to proteins such as ER resident oxidoreductases, a PDI-like family of proteins [30–32]. In some cell types, such as muscle cells, calsequestrin is the major $Ca^{2+}$ binding protein in the lumen of SR [33, 34].

## 4.4 ER-Plasma Membrane Connection: Store-Operated Calcium Entry

After the release of $Ca^{2+}$ from the ER stores, the cell engages a recovery system to restore cellular $Ca^{2+}$ levels to the initial state and replenish luminal ER $Ca^{2+}$ stores *via* the action of the SERCA pump. Depletion of luminal ER $Ca^{2+}$ stores leads to activation of store-operated $Ca^{2+}$ entry (SOCE) mechanism [13, 35–37], a concept

initially proposed by Putney [38]. SOCE is an excellent example of coordination of $Ca^{2+}$ signaling between the ER lumen, the plasma membrane and the extracellular environment. This process involves the ER-resident $Ca^{2+}$ sensors STIM proteins and plasma membrane $Ca^{2+}$ channels known as Calcium Release-Activated Calcium Modulator (ORAI). STIM senses reductions in luminal ER $Ca^{2+}$ stores, resulting in oligomerization of STIM and complex formation with ORAI [37, 39, 40] bringing plasma membrane $Ca^{2+}$ channels close to ER membrane embedded $Ca^{2+}$ pumps. This ORAI-STIM interaction effectively causes the influx of $Ca^{2+}$ from the extracellular milieu, increasing cytoplasmic $Ca^{2+}$ levels and subsequently refilling of the luminal ER $Ca^{2+}$ stores.

In vertebrates, there are two isoforms of STIM proteins, STIM1 and STIM2 [13]. STIM1 is activated by receptor-mediated ER $Ca^{2+}$ release whereas STIM2 is involved in maintaining resting ER $Ca^{2+}$ concentration [41]. Both isoforms are ubiquitously expressed and share common structural features. STIM1 and STIM2 contain a single transmembrane domain and two EF-hands, a helix-loop-helix structural motif characteristic of $Ca^{2+}$-binding proteins [42], which are important for the $Ca^{2+}$ sensing activity. In addition to the N-terminal EF-hands, STIM proteins contain the sterile α motif (SAM) domain and three coiled-coil domains (CC1-3) at their C-terminal end, which are exposed to the cytoplasm and important for interaction with ORAI [13]. ORAIs are plasma membrane proteins with four transmembrane domains, which function as a $Ca^{2+}$ channel. The crystal structure of ORAI from *Drosophila melanogaster* revealed that the $Ca^{2+}$ channel is comprised of a hexameric assembly of ORAI subunits arranged around a central ion pore [43]. In mammalian cells, there are three isoforms of ORAI proteins, namely ORAI1, ORAI2 and ORA3. All the ORAI isoforms support functional SOCE [44]. ORAI1 and ORAI3 form multimeric $Ca^{2+}$ channels that are also regulated in a $Ca^{2+}$ store-independent way by lipid messengers arachidonic acid and leukotriene C4 [45–47]. No interaction between STIM and ORAI is apparent at resting ER $Ca^{2+}$ levels, but upon depletion of the luminal ER $Ca^{2+}$, STIM molecules oligomerize due to conformation changes at the CC1 domain. The STIM multimers then recruit and gate the ORAI channels at ER and plasma membranes junctions. The ER also contains a negative regulator of SOCE, known as SOCE-associated regulatory factor (SARAF), that associates with STIM to facilitate slow $Ca^{2+}$-dependent inactivation thus protecting cells from $Ca^{2+}$ overfilling. STIM-ORAI signaling requires additional associated proteins including CRACR2A, STIMATE, junctate, POST, and septins [13]. Luminal ER oxidoreductase PDIA3 can also control STIM function, and consequently STIM1-induced SOCE, by binding to two conserved cysteines in the luminal domain of STIM1 [48]. Additionally, PDIA3 can regulate SERCA2b, a non-muscle isoform of SERCA, enabling further dynamic control of ER $Ca^{2+}$ homeostasis [49]. The actions of PDIA3 illustrate how multiple ER activities, such as protein folding and posttranslational modification, are linked with $Ca^{2+}$ transport and maintenance of ER $Ca^{2+}$ homeostasis.

## 4.5 ER and Protein Quality Control

Considering that over 30% of proteins are synthesized and processed in the ER, it is not surprising that the ER has evolved a sophisticated protein quality control system to preserve proteostasis [1, 3, 10, 11, 50, 51]. Proteostasis (protein homeostasis) refers to optimal folding and function of proteome [51]. Proteostasis is accomplished by an elaborate mechanism that integrates key cellular processes, namely biosynthethic and degradative pathways as well as control of gene transcription. Disrupted proteostasis is detrimental for the survival of the cell and health of the organism. The cell has a wide repertoire of molecular chaperones, which include cytoplasmic heat shock proteins (HSPs) [52], a subset of glucose regulatory proteins (GRP78/BiP and GRP94), the lectin chaperones calreticulin, calnexin, and ER degradation-enhancing α-mannosidase-like protein [53, 54]. These chaperones are specialized with respect to their substrates. For example, calreticulin, calnexin and PDIA3 are the proteins that make up the core machinery responsible for ensuring quality control of newly synthesized glycoproteins [1, 3, 10, 11, 51]. The ER-associated oxidoreductases [protein disulphide isomerases (PDIs) and peptidyl prolyl isomerases (PPIs)], oligosaccharide transferases, glucosidases and mannosidases are responsible for protein modifications such as disulfide bond formation and N-linked glycosylation [1, 50, 55–57]. Depletion of luminal ER $Ca^{2+}$ inhibits chaperone function causing a global ER stress and disrupted proteostasis [22, 58, 59].

## 4.6 ER and Cellular Stress Coping Response Strategies

Loss of nutrients/energy homeostasis is a universal feature defining the induction of ER stress and impacts on all aspects of cellular function. $Ca^{2+}$ signaling may be the principal mechanism involved in recognizing, communicating the state of cellular reticular homeostasis, and coordinating the activities to multiple corrective strategies. Mitochondria and the nucleus also developed stress responses to prevent cellular dysfunction due to stress challenges, environmental and metabolic demands [16–20, 60]. Activation of the UPR leads to translational attenuation to prevent synthesis of new proteins in the ER, transcriptional induction of genes encoding chaperones, folding enzymes and other proteins involved in ERAD, controlled degradation of misfolded proteins, and activation of apoptosis if the cell is unable to re-establish ER homeostasis [14]. The UPR coping strategy in the ER is thought to be comprised of three separate pathways, each controlled by distinct ER-associated integral membrane sensor proteins: the ER kinase dsRNA-activated protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) embedded in the ER membrane and complexed with GRP78/BiP in the lumen of the ER [14]. Additionally, a controlled self-digestion and degradation process known as autophagy, is stimulated to help promote cell

survival by eliminating damaged cellular components [8, 61, 62]. Autophagy may also provide the cell with a short-term source of raw materials (such as amino acids and fatty acids) and energy substrates [63].

Luminal ER $Ca^{2+}$ binding proteins, such as GRP78/BiP, and the $Ca^{2+}$-dependent microRNA miR-322 have been identified as bonafide regulators of UPR [64, 65]. Calreticulin, a major ER $Ca^{2+}$ binding protein, associates with ATF6 in a carbohydrate-dependent manner, and together with GRP78/BiP (also an ER $Ca^{2+}$ binding protein), maintains ATF6 in its transcriptionally inactive membrane-bound precursor state [66]. PDIA5, under stress conditions, promotes ATF6α export from the ER and activation of its target genes [67]. PDIA6 is a regulator of IRE1 activity in response to depletion of luminal ER $Ca^{2+}$ stores [64, 68]. The binding of PDIA6 with the luminal domain of IRE1α in a cysteine-dependent manner has been shown to enhance IRE1α activity [64, 68]. This effect is specific for the IRE1α branch since PDIA6 has no influence on the activity of the PERK branch of the UPR pathway [64, 68]. Depletion of luminal ER $Ca^{2+}$ and activation of SOCE reduces the abundance of the microRNA miR-322, which in turn regulates PDIA6 mRNA stability and consequently IRE1α activity [64]. Other microRNAs have been shown to regulate components that maintain ER homeostasis, including channels that control $Ca^{2+}$ fluxes across the ER membrane [69]. The expression of these microRNAs is sensitive to changes in ER $Ca^{2+}$ homeostasis [69]. The luminal ER environment (amount of $Ca^{2+}$, composition of ER resident proteins) together with non-coding RNAs including microRNAs cooperate to control UPR status and to maintain homeostasis within the entire cellular reticular network [64, 69, 70].

## 4.7 ER and Lipid Metabolism

Cellular membranes are comprised of proteins as well as lipids, which include phospholipids, glycolipids and sterols [71]. The lipid constituents are not evenly distributed throughout the membrane systems of the cell. The plasma membrane and organellar membranes have characteristic lipid compositions which defines their identity. For instance, unesterified cholesterol is typically abundant in the mammalian plasma membrane and rare in ER membranes. Cardiolipin is enriched in mitochondrial membranes. Membrane leaflets may display lipid asymmetry as observed in the plasma membrane where phosphatidylserine is present in greater abundance in the inner leaflet whereas phosphatidylethanolamine is more highly represented in the outer leaflet. These lipids serve not only a structural role but also as a source of lipid metabolites that act as signaling molecules such as $InsP_3$, diacylglycerols and others [71]. Heterologous membrane systems do not readily mix or merge largely due to the physicochemical properties imparted by their distinct lipid compositions. It is thought that fusion of dissimilar membranes requires the action of specific proteins present at the sites of membrane interactions [71, 72]. The unique lipid composition of the plasma membrane and organellar membranes may also partly determine the characteristic proteome associated with these different

membrane systems by virtue of specific lipid-protein interactions that promote the retention of specific proteins in certain types of membrane environments.

Membrane lipid homeostasis, or lipidostasis, involves regulated synthesis of certain lipid species and lipid quality control to remove damaged lipids [51]. The ER is the major site of membrane lipid synthesis, although other organelles participate in producing biosynthetic intermediates, as in the case of phosphatidylserine synthesis (involves mitochondria) [73] and cholesterol synthesis (involves peroxisomes) [74, 75]. The enzymes responsible for these reactions are either embedded in membranes or located in the lumen of the organelles. Conversion of cholesterol into other bioactive compounds, such as steroid hormones, vitamin D metabolites and bile acids, also occurs in the ER in conjunction with other organelles [76, 77]. Vitamin D is known as a major regulator of systemic $Ca^{2+}$ homeostasis [78] and is also important in the maintenance of cell signaling pathways [12]. The secondary bile acid known as ursodeoxycholic acid is a potent proteostasis promoter [79]. Considering the intimate relationships between specific lipids and proteins that comprise the various membrane systems of the cell, we propose that the coordination of lipidostasis and proteostasis is a key aspect necessary for assembly of functional membrane units to preserve cellular function and viability [51]. A recent study established that specific amino acid residues within the transmembrane domain of SERCA make ordered contacts with membrane phosphatidylcholine residues that surround the transmembrane domain to facilitate $Ca^{2+}$ transport into the cell [80]. Disrupted lipidostasis is likely to curtail SERCA function and thus impair cellular $Ca^{2+}$ homeostasis.

The induction of the UPR has been observed to induce phosphatidylcholine synthesis. This was initially attributed to the activation of the IRE1 branch of the pathway since enforced expression of Xbp1s mRNA could recapitulate the effect of UPR induction [81]. The increase in the synthesis of phosphatidylcholine supports expansion of ER membranes and is likely a part of the response to resolve ER stress. Interestingly, there were no changes in the abundance of mRNA of genes that are known to participate in the synthesis of phosphatidylcholine, suggesting that induction of membrane lipid synthesis is accomplished via posttranscriptional mechanisms. ATF6 was also found capable of stimulating phosphatidylcholine synthesis in an Xbp1s-independent fashion [82]. It is not clear how these transcription factors augment phosphatidylcholine synthesis. Nevertheless, the findings support for the notion that lipidostasis is linked to proteostasis, considering that membrane assembly is a concerted process involving both lipids and proteins.

Deletion of calreticulin dramatically reduces luminal ER $Ca^{2+}$ stores, and causes the extreme elevation of blood lipids in mice and intracellular lipid accumulation in worms [83]. Sterol response element binding proteins (SREBPs) are master regulators of lipid homeostasis by regulating the expression of genes involved in cholesterol and triacylglycerol metabolism [9, 84, 85]. There are three SREBP isoforms known as SREBP-1a, SREBP-1c (both encoded by the SREBP1 gene) and SREBP-2 (encoded by the SREBP2 gene). These transcription factors are synthesized as precursor ER integral membrane proteins. Structurally, the SREBPs are composed of a transcription factor domain located in the N-terminal region of the protein, a

transmembrane domain, and a regulatory domain located in the C-terminal region of the protein that interacts with another ER membrane protein known as SREBP cleavage activating protein (SCAP). SREBP processing is triggered by the reduction of unesterified cholesterol concentration in the ER membrane [86]. When unesterified cholesterol is abundant in the ER membrane, the SREBP-SCAP complex is retained in ER. However, when the ER membrane is depleted of unesterified cholesterol, the SREBP-SCAP complex migrates to the Golgi apparatus where SREBP is sequentially processed by two Golgi resident proteases known as Site-1 and Site-2 proteases, respectively, to release its N-terminal fragment which is the active transcription factor. A recent discovery has added a twist in the complexity of this regulatory framework by elaborating the existence of a link between luminal ER $Ca^{2+}$ homeostasis and cholesterol metabolism [83]. The direct reduction of the luminal ER $Ca^{2+}$ caused the shrinkage of the unesterified cholesterol pool in the ER that triggers SREBP processing without altering the size of the total intracellular unesterified cholesterol pool [83]. This finding suggests that the size of the luminal ER $Ca^{2+}$ pool may be involved in determining the basal sensitivity setpoint of the cholesterol sensing mechanism inherent to the SREBP processing pathway, and thus highlight the importance of luminal ER $Ca^{2+}$ homeostasis in lipid metabolism.

## 4.8 ER and Membrane Contact Sites

The compartmentalization of specialized functions within discrete membrane bound organelles creates a challenge for efficient transport and exchange of molecules between compartments. Recent studies have documented the promiscuity of ER membranes for forming contacts with the plasma membrane and other organelles (mitochondria, peroxisomes, lysosomes, endosome, trans-Golgi, phagosomes, nuclear envelope) including lipid droplets [87–95]. These membrane contact sites occur between two heterologous membranes that are situated in very close proximity, approximately 10–30 nm, from each other and appear to be highly stable [36, 96, 97] except for STIM-ORAI (ER-plasma membrane) contacts which form transiently in the process of replenishing cellular $Ca^{2+}$ stores. Contacts between other organelles, not involving the ER, have not been observed. It is also noteworthy that membrane contact sites between ER membranes with ribosomes (i.e., RER) and other organellar membranes are observed, implying possible functional heterogeneity in the membranes that make up the ER [93]. The joining of ER membranes to organellar membranes forms the cellular reticular network [14] characterized by interconnected membranes, tubules, vesicles and cisternae spanning the plasma membrane to the nuclear envelope linked together by membrane contacts sites that form portals that facilitates rapid passage of transport of substrates and metabolites (such as nutrients, biosynthetic intermediates, ATP) and signaling molecules (such as lipid messengers, $Ca^{2+}$ ions) (Fig. 4.1) [14, 36, 94, 95, 98].

Recent studies have determined the identity of some of the molecules associated with membrane contacts sites [94, 99–101]. Proteins found at these structures

include $Ca^{2+}$ binding proteins (e.g., STIM, ORAI, SERCA, RyR), chaperones, transporters, and lipid binding and lipid transfer proteins. Membrane contact sites may represent highly specialized sites of function as suggested by studies on phospholipid synthesis [73, 102]. The existence of these structures may account for the rapid non-vesicular transport of various lipid metabolites between organelles, such as during biosynthesis of membrane lipids and cholesterol. Indeed, many of the proteins that have been characterized are lipid transfer proteins, whose substrates include fatty acids, phospholipids, sterols and their metabolites including those that serve as messengers [99, 103, 104]. In yeast, several specialized proteins associated with membrane contact sites between ER and mitochondria have been identified. These proteins form a functional unit referred to as ER-mitochondria encounter structure (ERMES) [96, 103, 105]. The equivalent functional unit in mammalian cells has not been described.

Furthermore, release of luminal ER $Ca^{2+}$ generates areas of $Ca^{2+}$ microdomains characterized by a high $Ca^{2+}$ concentration and occur at the contact region between the ER and plasma membrane where $Ca^{2+}$ channels on the plasma membrane open [37, 39, 40]. The formation of ER-membrane contacts and the complexity of $Ca^{2+}$ signaling proteins in these microdomains accounts for the specific characteristics of discrete $Ca^{2+}$ signals [89]. One of the best characterized membrane contacts are those formed between skeletal and cardiac muscle T (transverse)-tubules (extensions of the plasma membrane) and the SR [106–109]. These membrane contacts are the driving force to support excitation-contraction coupling in the muscle and the mechanisms behind the $Ca^{2+}$-induced $Ca^{2+}$ release in the cardiac muscle [110]. Membrane contacts between ER and mitochondria and $Ca^{2+}$ microdomains that form between $InsP_3R$ and $Ca^{2+}$ uniporters on the mitochondria support uptake of $Ca^{2+}$ by mitochondria to match energy supply by $Ca^{2+}$-dependent stimulation of oxidative phosphorylation [95, 111].

This versatile arrangement of contacts between ER and organellar membranes offers the cell an ability to influence and/or support highly specialized functions throughout the cellular reticular network. Impairment of the ER-organellar contact sites might be involved in the pathogenesis of diseases such as neurodegenerative diseases [51]. Understanding how the process involved in the formation, maintenance and function of ER-membrane contact sites are regulated will provide insight into the role of ER $Ca^{2+}$ in coordinating gene expression and cellular function.

## 4.9 Conclusions

The ER is an extensive network of membranes that occupies a major proportion of the cell interior. The ER and the other organelles are characterized by unique proteome and lipidome which together provide ideal environments that enable specialized functions within the cell. $Ca^{2+}$ homeostasis in general, and ER luminal $Ca^{2+}$ in particular, is essential for the function of the ER and reticular network. $Ca^{2+}$ is a key molecule involved in orchestrating the metabolic pathways occurring in

different compartments of the cell. Internal or external factors that result in the loss of nutrient and energy homeostasis impose stress on cellular functions. Coping response strategies operate to alleviate and eliminate stress in the ER and other organelles to maintain proteostasis and lipidostasis within the entire cellular reticular network. ER membranes can form resilient contacts with the membranes of other organelles forming a complex cellular reticular network. Membrane contact sites enable rapid distribution and exchange of substrates, metabolites and signalling molecules to ensure optimal cellular function and homeostasis.

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**Conflict of Interest** The authors declare no conflict of interest.

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