Roles for the Sarco-/Endoplasmic Reticulum in Cardiac Myocyte Contraction, Protein Synthesis, and Protein Quality Control

Although the function of the sarcoplasmic/endoplasmic reticulum (SR/ER) in cardiac contractile calcium handling is well established, its roles in protein synthesis, folding, and quality control in cardiac myocytes are not as clear. This review explores evidence suggesting that, in cardiac myocytes, protein synthesis and contractile calcium handling may be physically and functionally integrated.

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The essence of a healthy heart lies in the proper and coordinate function of the chambers of the heart, which comprise the cardiac atria and ventricles. These chambers are each composed of four main cell types: endothelial cells, smooth muscle cells, fibroblasts, and cardiac myocytes. Most of the mass of the heart is cardiac myocytes, which are responsible for the life-supporting, rhythmic contractions that underlie cardiac pump function. Although each chamber serves important functions, since it constitutes the largest muscle mass of the heart and is responsible for pumping oxygenated blood to most tissues, the left ventricle is often considered the powerhouse chamber of the heart. Thus, in terms of many aspects of heart health and pathology, it is the cardiac myocytes comprising the left ventricle that have been the focus of many studies. These studies have shown that efficient regulation of the cytosolic calcium that drives contraction is critical for proper heart function.

The regulation of cytosolic calcium levels in cardiac myocytes involves an elaborate intracellular membrane system called the sarcoplasmic reticulum (SR) (5, 63). A great deal is known about SR structure and function in terms of cardiac contraction (43, 44). In adult cardiac myocytes, the SR surrounds myofilaments and is well known for its roles in regulating the storage, release, and re-uptake of calcium, also called contractile calcium handling (58, 64). Proper myocyte contraction requires a precise balance of the expression and function of the myriad SR-luminal and membrane proteins involved in contractile calcium handling. An imbalance in the activities and/or levels of these proteins can impair cardiac contractility and lead to pathological dysfunction (22, 24). For example, in the failing heart, the levels of the ATPase responsible for pumping calcium back into the SR lumen are reduced; this reduction is believed to be a major contributor to the disease phenotype (24). Thus the synthesis of the proteins responsible for contractile calcium handling and the routing to their proper destinations are essential for proper cardiac myocyte function. However, the location and mechanisms of these processes in cardiac myocytes are not well understood.

Since most calcium handling proteins reside in either the lumen of the SR or in the SR membrane, it is reasonable to posit that contractile calcium handling proteins in cardiac myocytes are synthesized the same way as ER-luminal and transmembrane proteins in noncardiac myocytes. As shown in pioneering experiments by George Palade and others, ER luminal and transmembrane proteins, which account for ~35% of all proteins, are synthesized on ribosomes bound to the endoplasmic reticulum (ER) or rough ER (rER) (49, 54). The remaining proteins are made on free cytosolic ribosomes. Proteins synthesized on the rER include nearly all secreted, ER-luminal, and trans-membrane proteins, herein grouped together as secreted/membrane proteins. Following their synthesis on the rER, secreted/membrane proteins are folded, posttranslationally modified, and transported to their final destinations by what is called the classical, or conventional, secretory pathway (14, 23, 30, 48). At the foundation of the pathway is an elaborate rER-associated, protein synthesis, folding, and quality control system (41, 60).

The locations of the rER and secreted/membrane protein synthesis in cardiac myocytes are not well characterized. Some evidence suggests that secreted/membrane protein synthesis machinery resides in non-SR locations, such as the nuclear envelope and a diffuse perinuclear location, whereas other evidence suggests that it is near to, or part of, the SR. This evidence suggests that either SR proteins are synthesized elsewhere and then transported to the SR and/or that they are synthesized in the SR. Both mechanisms have important functional implications in terms of maintaining optimal myocardial contraction and thus...
averting pathology. For example, if SR proteins are synthesized elsewhere, then efficient synthesis, folding, and transport to their final destinations are required to maintain effective SR contractile calcium handling. Alternatively, if SR proteins are synthesized in the SR, then the protein synthesis, folding, and quality control machinery must also be located in the SR, which implicates a previously unappreciated function for the SR, as well as the possibility that there exists important functional and physical integration between protein synthesis and SR calcium handling machinery. In addressing these topics, this review will examine the cell biology of secreted/membrane protein synthesis, contractile calcium handling in cardiac myocytes, studies that address the location of secreted/membrane protein synthesis in cardiac myocytes, and interactions between the secreted/membrane protein synthesis and the calcium handling machinery that could have important functional implications in cardiac myocytes.

Cell Biology of Secretory/Membrane Protein Synthesis

The ER, discovered in 1945 (53), is the most expansive organelle, occupying a large amount of space in most cells and accounting for at least 50% of the cellular membrane (60). In 1952, electron microscopy was used to delineate two types of ER, the smooth ER (sER) and rER (54). Soon thereafter, it was shown that the rER differed from the sER in that the cytosolic leaflet of the rER is decorated with ribosomes (FIGURE 1, A AND B) (13, 49). Today, it is recognized that there are numerous structurally and functionally distinct domains of the ER, including the rER, nuclear envelope (NE) (FIGURE 1C), transitional

![Diagram of smooth ER, rough ER and nuclear envelope](http://physiologyonline.physiology.org/)
ER, and sER, the latter of which can be further divided into peripheral or cortical ER, also known as plasma membrane-associated membrane (PAM), as well as the mitochondrial-associated ER, also called mitochondrial-associated membrane (MAM) (60). One function attributed to every form of ER is the storage, regulated release, and reuptake of calcium (3). For example, depending on the cell type, neurohormonal receptor activation and/or membrane depolarization can stimulate the release of calcium from the ER into the cytosol, where it can regulate an array of processes, such as cardiac myocyte contraction (FIGURE 1D). However, it is the nature of most processes activated by cytosolic calcium that the activation must be relatively short-lived, which means increases in cytosolic calcium must be transient. The transient nature of changes in cytosolic calcium levels is accomplished by the coordinated release and reuptake of calcium back into the ER soon after its release (FIGURE 1E).

Although the nuclear envelope (NE) was originally identified as a membranous structure that surrounds and, therefore, defines the nucleus and was not considered part of the ER network (71), it was later shown that the outer leaflet of the nuclear envelope is decorated with ribosomes (39) and that the NE performs many of the functions initially ascribed solely to the rER, including calcium storage and regulated release (FIGURE 1, F AND G), as well as protein synthesis (FIGURE 1H) (15). It is also thought that some proteins bound for the classical secretory pathway, which includes secretory/membrane proteins, are synthesized in the NE.

Although our understanding of SR function in contractile calcium handling is deep, in some ways, our knowledge of other functions of the SR is relatively shallow and compares to our understanding of the functions of the NE some 40 years ago. A particular deficit in our understanding of SR function in the heart concerns its potential roles in protein synthesis and folding. In fact, very few studies have examined the location and mechanism of secretory/membrane protein synthesis in cardiac myocytes. This is surprising, considering the important roles served by proteins that are secreted from cardiac myocytes, such as hormones (e.g., atrial natriuretic factor), growth factors (e.g., cardiotrophin), stem cell homing factors (e.g., stromal cell derived factor-1), and cytokines (e.g., TGF-β), as well as membrane proteins, such as G-protein-coupled receptors (e.g., α- and β-adrenergic receptors) and calcium-handling proteins, such as SR/ER calcium ATPase (SERCA), phospholamban (PLN), L-type voltage-operated calcium channels (CaV1.2), the ryanodine receptor (RyR), and the inositol trisphosphate receptor (IP₃R). Thus, although the functions of such proteins have been studied for decades, the critical issue of how and where they are made is not well understood (31, 40).

By analogy to the NE, which was not originally thought to function in protein synthesis, it can be posited that, in addition to calcium handling, the SR may also participate in some aspects of secreted/membrane protein synthesis and folding in cardiac myocytes.

Although not well studied in cardiac myocytes, the mechanism of secreted/membrane protein synthesis has been delineated in other cell types. Secreted/membrane proteins synthesized by rER-associated ribosomes are translocated across the rER membrane by co-translational translocation (FIGURES 1, H AND I, AND 2A), upon which they adopt either an ER luminal (FIGURE 1J) or ER transmembrane (FIGURE 1K) configuration. In addition to ribosomes, there exists an elaborate molecular machinery associated with the rER that is required for secreted/membrane protein synthesis, folding, modification, and trafficking. For example, there are at least 20 polypeptides that constitute the channel, or translocon, through which nascent secreted/membrane proteins pass (12, 42, 55). As nascent proteins are translocated through the ER membrane translocon, chaperones and oxidoreductases, many of which require calcium as well as glycosyltransferases, most of which reside in the rER lumen or on the luminal side of the rER membrane (FIGURE 2B), facilitate nascent protein folding, disulfide bond formation, and N-linked glycosylation in the ER (75). All of these modifications are required for the synthesis of properly folded, functional secreted/membrane proteins. Once in the ER, correctly folded proteins that are destined to become permanent residents of the ER are retained in this location via several major mechanisms, including 1) active exclusion from vesicles that exit the ER and 2) retrieval from a post-ER compartment via retrograde transport back into the ER. An example of the second mechanism is that some ER residents have a COOH-terminal KDEL or a KDEL-like sequence. This motif fosters their binding to the KDEL-receptor in the Golgi, followed by their retrieval back to the ER (10, 52). Another example is that some transmembrane ER residents have an NH₂-terminal di-arginine motif or a COOH-terminal di-lysine motif, both of which foster retrieval from the Golgi back to the ER (28, 38). Alternatively, other proteins are retained in the ER through their interaction with other permanent ER residents (21). Luminal proteins that are not permanent residents of the ER proceed to the Golgi (FIGURE 2C) where they are sorted to their final destinations. For example, proteins bound for secretion are transported to secretory vesicles and eventually released from cells.
(FIGURE 2D), whereas membrane proteins, which are inserted into the ER membrane during their synthesis, are sorted to final destinations, such as the SR and sarcolemma (FIGURE 2E) (23).

Efficient secreted/membrane protein synthesis and folding require a relatively high level of calcium in the ER lumen, as well as the proper redox environment (8, 33, 72). Accordingly, pathological insults, such as ischemia, which can decrease ER calcium and perturb redox status, can impair ER protein folding (14, 50, 66). In most cells, ER calcium is released primarily through the IP₃ receptor and is returned back into the ER by sarco-/endoplasmic calcium ATPase (SERCA2b), which is associated with several other proteins that fine-tune its activity in response to sympathetic tone, such as phospholamban (PLN) (2, 3) (FIGURE 2F). The return of calcium from the cytosol back into the ER is critical for optimal function of the secreted/membrane protein synthesis and folding machinery, as

**FIGURE 2.** Calcium-induced calcium release, SR/ER protein synthesis, folding, and quality control in cardiac myocytes

I. ER Protein Synthesis, Folding and Quality Control

- ER protein synthesis, folding, and quality control. Shown is a diagram of the rough ER with attached ribosomes and mRNA engaged in translation (A), ER-luminal secreted/membrane protein synthesis machinery (B), transport of secreted and membrane proteins to the Golgi (C), trafficking of secreted proteins to secretory vesicles (D), and trafficking membrane proteins to final membrane destinations (E). In cardiac myocytes, the rough ER is considered to be mostly perinuclear, and thus contiguous with, if not the same as, the nuclear envelope. The reuptake of calcium from the cytosol into the ER lumen by SERCA2b and the associated regulatory protein phospholamban (F) is shown, as is the ER stress generated upon protein misfolding, which leads to the unfolded protein response (F) and to the degradation of potentially toxic terminally misfolded proteins by the ER-associated degradation (ERAD) pathway of protein quality control (G).

II. SR Ca²⁺-induced Ca²⁺ Release

- SR calcium-induced calcium release. Shown is a diagram of the junctional SR (j-SR) and longitudinal or free SR (f-SR) (H), as well as the t-tubule (I), the ryanodine receptor (RyR) (J), the L-type voltage-operated calcium channel (CaV; M), the release of calcium from the SR into the cytosol required to initiate cardiac myocyte contraction (K), and the reuptake of calcium back into the SR by SERCA2a and its associated regulatory protein phospholamban (L). Although not as abundant as the RyR, the IP3 receptor is localized to the cardiac myocyte SR, where it may play a role in NFAT-mediated hypertrophic growth (47, 57).

III. SR Protein Synthesis, Folding and Quality Control

- SR protein synthesis, folding, and quality control. Shown in the SR is the secreted/membrane protein biosynthetic and folding machinery (N), the ER-associated degradation protein quality control system (O), ER stress, and the unfolded protein response. This depiction is not meant to establish the location of these processes in the f-SR but to represent the possibility that they may be associated with the SR. It is formally possible that secreted/membrane protein biosynthesis, as well as the UPR and ERAD machinery, may be located anywhere in the SR and/or in a different membranous organelle that has not been defined but is near the SR.
evidenced by the potent effects of SERCA2 inhibitors, such as thapsigargin, on protein folding in the ER (35).

The impaired folding of proteins in the rER can cause ER stress, which results in the activation of a complex signaling program emanating from the ER, called the unfolded protein response (UPR).

In part, the UPR results in activation of a gene program that increases the expression of numerous proteins, many of which are targeted to the ER and designed to restore the ER folding environment (19, 20, 32, 44, 45). Among the genes that are upregulated in response to ER stress are those that encode ER-resident chaperones [e.g., glucose regulated protein 78 (GRP78)], disulfide isomerases (e.g., PDIA), and oxidoreductases (e.g., Ero-1α/β), most of which require calcium to perform their protein folding functions. The increased expression of such proteins promotes cell survival during stress and is therefore considered to be adaptive.

Upon ER stress, the accumulation of terminally misfolded proteins can be proteotoxic. Accordingly, in addition to its roles in protein synthesis, the rER is also the site for a protein quality control system, wherein terminally misfolded proteins in the ER are recognized by a collection of ER-luminal chaperones, primarily GRP78, and escorted back out of the ER in a retrograde manner (11, 62). En route across the rER membrane, terminally misfolded proteins are ubiquitinated by ER-associated ubiquitin ligases, which are oriented with their active sites on the cytosolic side of the ER. Following ubiquitination, terminally misfolded ER proteins are degraded by proteasomes, also associated with the cytosolic face of the rER (FIGURE 2G).

The protein quality control system responsible for degrading terminally misfolded proteins in the ER is called ER-associated degradation, or ERAD.

Since it contributes to the restoration of the ER protein-folding environment and promotes survival, ERAD is adaptive. However, if the adaptive UPR and ERAD are not sufficient to restore ER protein folding capacity and the cell cannot withstand the stress, maladaptive aspects of the ER stress response direct the cell to apoptosis.

It has long been believed that secretory and membrane proteins synthesized on the rER constitute up to 35% of cellular protein, thus emphasizing the essential nature of this organelle in protein synthesis. However, recent studies suggest that this is a considerable underestimate. For example, ribosomal profiling has shown that ribosomes associated with the rER are responsible for translating mRNAs that encode not only secretory and transmembrane proteins but, surprisingly, also proteins bound for other cellular locations (56). It is believed that the rER environment is actually more conducive to translation than the cytosol and that ribosomes on the rER likely contribute to the synthesis of proteins in addition to those that are secreted or membrane proteins.

Thus the rER is now recognized as the major site of protein synthesis in cells, which emphasizes the critical function of this organelle.

**Contractile Calcium Handling in Cardiac Myocytes**

The SR is a highly developed intracellular membrane system in striated muscle cells that is responsible for storage, release, and reuptake of the calcium required for contraction, i.e., contractile calcium handling. In cardiac myocytes, the SR surrounds the myofibrillar contractile machinery and is divided into junctional SR (j-SR) and longitudinal or free SR (f-SR) (FIGURE 2H). The j-SR is located near the t-tubules, the latter of which are invaginations of the plasma membrane or sarcolemma (FIGURE 2I) over the z-lines of sarcomeres (16). The release of contractile calcium from the SR takes place in response to an action potential, during which deploration of the sarcolemma is propagated from the cell surface into the inner regions of the cell via the t-tubules. Upon t-tubule depolarization, a small amount of calcium passes from outside to inside the cell through voltage-operated L-type calcium channels (CaV1.2) located in the t-tubules. Calcium entering via CaV1.2 travels a short distance, then binds to the RyR, a structurally complex calcium-activated channel located in the j-SR membrane (FIGURE 2J). Upon calcium binding, the RyR opens, thus allowing the very rapid eflux out of the SR of larger amounts of calcium that initiates contraction (FIGURE 2K) (4, 6, 36).

The process by which calcium influx through CaV1.2 activates SR calcium release via the RyR is calcium-induced calcium release (CICR).

The proper positioning of the CaV1.2 and RyR is critical for the coordinated release of SR calcium throughout each cardiac myocyte, which is required for a maximally productive contractile event (4). During the relaxation phase, most of the cytosolic calcium is pumped back into the SR by SERCA2a, which is tightly associated with a variety of proteins that can regulate the calcium transport activity of SERCA2a, such as PLN (FIGURE 2L) (51, 67). The relatively small amounts of cytosolic calcium that are not pumped back into the SR are extruded out of the cell, mostly by the sodium/calcium exchanger (NCX) located in the sarcolemma (4).

The process by which excitation of the sarcolemma leads to cardiac myocyte contraction is excitation-contraction coupling (ECC).

The f-SR lumen is contiguous with the lumen of the j-SR, such that calcium can diffuse between the
two structures (7). However, the proteins comprising the j- and f-SR differ. For example, SERCA2a and PLN are more abundant in the f-SR, whereas the RyR and its myriad accessory proteins, such as triadin, junctin, and calsequestrin, are located in very discrete regions of the j-SR, juxtaposed to the CaV1.2 in the t-tubule membrane (FIGURE 2M) (58). It is thought that the regional distribution of the proteins in j-SR and f-SR underlies region-specific functions.

Calcium-binding proteins are among many proteins that exhibit regional differences in their levels in the j-SR and f-SR. Like most calcium elsewhere in the ER lumen, calcium in the SR lumen is bound to a variety of these calcium-binding proteins, including calreticulin and calsequestrin. Calsequestrin is the most abundant SR luminal calcium-binding protein, and it is located primarily in the j-SR, where at least a portion of it resides in a complex with the RyR (9). Interestingly, during the contractile cycle, only ~50% of the calcium in the SR is released, which leaves a considerable amount of calcium in the SR (59). Although this residual SR calcium may be required to ensure a rapid rate of contractile calcium release from the SR, it is also possible that it has additional functions, such as participation in secreted/membrane protein synthesis.

Location of Secreted/Membrane Protein Synthesis in Cardiac Myocytes

Although a clear picture of the compartments in cardiac myocytes responsible for secreted/membrane protein synthesis has not yet emerged, in attempts to shed some light on this issue, several morphological studies have sought to locate the machinery necessary for this process, such as membrane-bound ribosomes or rER. Many studies have used electron microscopy to examine the ultrastructure of cardiac myocytes; although this technique may be useful for assessing some aspects of myocyte biology, such as myofibril and mitochondrial structure, as well as visualizing the the rER in some cell types such as professional secretory cells. However, due to the similar size of ribosomes and glycogen particles, the latter of which commonly are found to be associated with membranes in cardiac myocytes, the location and extent of the rER in cardiac myocytes has been difficult to determine and is not even mentioned in most electron microscopy studies (18). Nevertheless, there have been several electron microscopy studies that showed ribosomes to be associated with membranes in the perinuclear region, as well as near or contiguous with the f- and j-SR in cardiac myocytes (46, 61).

Other studies have used confocal fluorescence microscopy to identify specific components involved in secreted/membrane protein synthesis. For example, several studies showed that GRP78, which is best known for its roles in the folding of nascent secreted/membrane proteins, as well as ERAD, was located in the SR of skeletal muscle cells (70), as was protein disulfide isomerase (29). These findings are consistent with another study showing that, in isolated adult rat cardiac myocytes and in mouse heart sections, GRP78 was located in or near the SR as well as in a perinuclear region of the cell (1, 65). It was also shown that, in cardiac myocytes, a portion of translocon proteins colocalized with two SR proteins, SERCA2 and calsequestrin (29). In isolated adult mouse cardiac myocytes, TRAP, a member of the translocon, and S6 ribosomal protein, both of which are involved in secretory/membrane protein synthesis, were found in a perinuclear and peripheral staining pattern, the latter of which may represent localization to the SR (40). Moreover, in adult mouse cardiac myocytes, an antibody to KDEL, which has been used to locate rER resident proteins in other cell types, stained in a diffuse perinuclear pattern, as well as in regions of the cell occupied by the SR (68). In fact, this staining pattern was almost identical to that observed for GRP78 in cardiac myocytes in sections of adult mouse hearts (1). Thus there is evidence that elements of the secreted/membrane protein synthesis machinery are located in the nuclear envelope and perinuclear region, as well as in more peripheral areas that are either coincident with or very near the SR cardiac myocytes (FIGURE 2). However, significant challenges in this area remain, since the physical location and characteristics of secreted/membrane protein synthesis in adult cardiac myocytes during maturation and pathological re-modeling remain unknown. Moreover, although there is emerging evidence supporting the existence of discrete calcium pools in cardiac myocytes that support either contractility or gene regulation (26, 37, 69, 74), it remains unknown whether such discrete pools of calcium are involved in secreted/membrane protein synthesis and folding in the SR.

Interactions between Components of Secretory/Membrane Protein Synthesis and Calcium Handling Systems

Although there is no evidence of a direct interaction between protein synthesis and folding machinery and SR calcium handling proteins in the heart, several studies have reported physical and functional interactions between proteins involved in secreted/membrane protein biosynthesis and calcium handling proteins in other cell types, suggesting that protein synthesis and calcium handling in the SR/ER
might be integrated. For example, when associated with calcium, the ER luminal protein calretilicin binds to and reduces the activity of SERCA2 in a calcium-dependent manner (34). This is believed to represent a feedback loop wherein SERCA2-mediated refilling of the ER with calcium could be modulated when luminal calcium levels are sufficiently high. It is possible that this may be a role for the calretilicin that is known to be located in the F-SR of cardiac myocytes, where it is also known to bind to SERCA2a under conditions of oxidative stress (27). In another study, it was shown that, when ER calcium is low, the ER-luminal protein disulfide isomerase ERP44, which is involved with secreted/membrane protein synthesis, binds to the luminal domain of the IP$_3$R and decreases the efflux of calcium out of the ER into the cytosol (25).

An additional nexus between ER protein synthesis and calcium handling machinery involves ERAD and the IP$_3$R. The IP$_3$R is located in and is responsible for calcium release from the ER and is located in the SR and facilitates some calcium release from the SR, depending on the conditions. Several studies over the last few years have shown that the level of the IP$_3$-activated IP$_3$R is tightly regulated. In these studies, it was shown that soon after binding of IP$_3$, activated IP$_3$Rs are targeted to degradation by ERAD (73). Evidently, the same conformational changes that cause IP$_3$R channel opening lead to recognition of the IP$_3$R as a misfolded protein by ERAD components and ultimately results in IP$_3$R degradation. This complex regulatory system evidently reduces the sensitivity of ER calcium store depletion to IP$_3$, thus providing a regulatory mechanism by which cells can guard against the effects of ER calcium depletion. The tight regulation of the number of IP$_3$R is potentially important in the heart, where IP$_3$Rs, located in the NE as well as in the SR (17, 47), are believed to be responsible for the release of calcium during pathological hypertrophy (47, 74).

Conclusions

The location and mechanism of the synthesis, folding, and protein quality control for secreted/membrane proteins in cardiac myocytes are not well understood. Studies in other cell types have shown that the cellular machinery necessary for these processes resides in the rough ER and includes numerous calcium-requiring chaperones, oxidoreductases, and protein disulfide isomerases. Many components of this machinery are also localized to the nuclear envelope and perinuclear regions of cardiac myocytes, as well as to more peripheral areas of myocytes that are occupied by the SR. Thus, it is possible that, in addition to its roles in contraction, the SR may have important functions in secreted/membrane protein synthesis and folding. Moreover, certain components of the SR-associated protein synthesis and folding machinery may interact with, and perhaps regulate, SR-associated calcium-handling proteins, such as SERCA2a and the IP$_3$R. Thus the SR may serve as a hub for protein synthesis as well as contractile calcium handling.

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