Calumenin is a multiple EF-hand Ca\textsuperscript{2+}-binding protein located in the endo/sarcoplasmic reticulum of mammalian hearts. Calumenin belongs to the CREC family of Ca\textsuperscript{2+}-binding proteins having multiple EF-hands. Ca\textsuperscript{2+} homeostasis in the sarcoplasmic reticulum (SR) of mammalian hearts is maintained by RyR2, SERCA2 and other associated SR resident proteins. Evidence suggests that calumenin interacts with RyR2 and SERCA2, and therefore changes in the expression of calumenin could alter Ca\textsuperscript{2+} cycling in mouse heart. In this review, current knowledge of the biochemical and functional roles of calumenin in mouse heart is described. [BMB reports 2010; 43(3): 158-163]

INTRODUCTION

Calcium is an universal second messenger that plays important roles in various cellular processes such as gene expression, signal transduction, exocytosis and muscle contraction (1). In striated muscle cells, muscle contraction and relaxation cycles are governed by Ca\textsuperscript{2+} release and uptake occurring in the SR (2). RyR and SERCA are the two main proteins in the SR responsible for Ca\textsuperscript{2+} release and uptake, respectively. The SR contains also contains various resident proteins in the lumen that are involved in Ca\textsuperscript{2+} buffering. Fluctuation in the luminal Ca\textsuperscript{2+} concentration could modulate the quality and efficiency of protein folding and stability, as well as muscle contraction (3). Calsequestrin (CSQ) (4), calreticulin (CRT) (5), sarcalumenin (6), histidine-rich Ca\textsuperscript{2+}-binding protein (HRC) (7) and calumenin (8, 9) have all been identified as important SR luminal proteins that could regulate Ca\textsuperscript{2+} homeostasis in muscle cells.

Among SR resident proteins, calumenin was recently characterized to be 315 aa in length and has a calculated molecular weight of 37 kDa (10, 11). The N-terminal region of the calumenin protein contains a 19 aa signal sequence, whereas the C-terminal region has a unique 4 aa ER/SR retention signal (10). Calumenin is ubiquitously expressed in different tissues with a higher expression level found in the heart (10). Recent evidence suggests that calumenin is functionally associated with the release and uptake of Ca\textsuperscript{2+} in the SR during the Ca\textsuperscript{2+} cycling process (8, 9, 12).

Additionally, calumenin belongs to the CREC family of Ca\textsuperscript{2+}-binding proteins. The CREC family in mammalian cells is composed of a number of EF-hand proteins. The acronym CREC stands for Ca\textsuperscript{2+}-binding proteins of 45 kDa (Cab45), reticulocalbin, ER Ca\textsuperscript{2+}-binding protein of 55 kDa (ERC-55) and calumenin (13). Recently a number of other proteins also have been included in this protein family, and their physiological and biochemical functions have been under investigation. Regarding gene expression, the CREC family of proteins are encoded by five genes: RCN1, RCN2, RCN3, SDF4 and CALU (13).

This review attempts to summarize the recent advances contributing to the overall knowledge of the biochemical and physiological properties of calumenin in striated muscle, especially focusing on biochemical and functional properties in mouse heart.

Genomic organization of calumenin

Mouse calumenin gene was first cloned and characterized from mouse heart using the signal sequence trap method (10). Subsequent investigations have revealed that the calumenin gene has two alternatively spliced variants of equal length, named calumenin-1 and calumenin-2. Calumenin-2 was previously known as crotoxin-binding protein of 50 kDa (CBP50) or crocalbin (14, 15). In comparing mouse calumenin-1 and -2, the 19 aa signal sequence and 4 aa ER/SR retention sequences are identical (16). Calumenin protein contains one in vivo glycosylated N-glycosylation site at the 131st aa position. The aa sequences of mouse calumenin-1 and -2 have 92% identity and 95% homology (16), with the difference lying in EF-hands-1 and -2. Despite this, the conserved amino acids closely resemble the consensus EF-hand sequence (17).

Although the calumenin gene was initially mapped to the proximal portion of mouse chromosome 7, a recent investigation suggests its presence in chromosome 6 of the mouse genome (16). Localization of calumenin to mouse chromosome 6 as well as that to human chromosome 7 are in conserved synteny with each other. Furthermore, the mouse cal-
umenin gene contains six exons and five introns (16). Exon 2 is different between calumenin-1 and -2; exon 2 of calumenin-2 is 72 bp before that of calumenin-1 in the 5' to 3' direction (16).

Other CREC family members

Among other CREC family member proteins, the RCN1 gene encodes reticulocalbin protein (331 aa) containing a signal sequence, six EF-hands and the ER retention signal HDEL (18). Although previously reported to be localized only to the ER, reticulocalbin has recently been found to be localized at the surface of bone endothelial cells and prostate cancer cells (19). The translocation protein Sec63p located in the ER apparently interacts with reticulocalbin (20). Similarly, the RCN2 gene encodes the ER Ca2+-binding protein 55 kDa (ERC-55), which is 317 aa long and contains a signal sequence, six EF-hands and the HDEL ER retention signal in its C-terminus. This protein interacts with proPACE4. The SDF4 gene encodes Cab45, a 362 aa protein containing a signal sequence, six EF-hands and the C-terminal ER retention signal HDEF (23, 24). Cab45-C interacts with Munc18a and Munc 18b and is involved in the secretion process.

Calumenin polymorphisms

For the calumenin gene, at least 23 single nucleotide polymorphisms (one repeat or one insertion/deletion polymorphism) have been identified in the general population (25). The effect of calumenin polymorphism on the anticoagulant response was also reported (26). The polymorphism is localized to the 3'-untranslated region of the CALU gene.

Ca2+-binding properties of calumenin

Calumenin protein contains six EF-hands, each of which consists of typical helix-loop-helix motifs. The EF-hands undergo possible conformational changes upon Ca2+ binding. Specifically, the binding of Ca2+ to the individual EF-hands of calumenin was estimated to occur with a Kd of ~600 μM (11). To date, there is no clear structural explanation for the relatively low affinities of the EF-hands of calumenin when compared to the EF-hands found in other Ca2+-binding proteins (13).

Expression and localization of calumenin in striated muscle

Mouse and human calumenins are ubiquitously expressed in multiple tissues (10, 27). Both calumenin isoforms in mouse are abundant in muscle, and transcription levels are higher in cardiac muscle than in skeletal muscle. The mRNA expression level of calumenin is decreased in the adult mouse heart compared to embryonic stages (10). Likewise, calumenin protein expression during developmental stages was significantly decreased in adult mouse heart compared to embryonic and neonatal hearts (9). The level of calumenin protein steadily decreased in mouse heart until reaching a steady state level that was maintained throughout adulthood. This indicates that the pattern of calumenin expression is similar to that of other ER chaperone proteins such as CRT, glucose regulated protein 78, glucose regulated protein 94, protein disulfide isomerase and ER protein 57 (ERP57) (28).

Analysis of rabbit skeletal muscle showed that calumenin is abundant in the junctional fraction of rabbit skeletal SR where RyR1 is enriched, suggesting a possible interaction between calumenin and RyR1 (12). In ventricular myocytes and HL-1 cells, calumenin staining displayed clear localization along the Z-line and longitudinal axis of cardiomyocytes. This results suggest that calumenin is co-localized to areas of cardiomyocytes where SERCA2 and RyR2 are enriched (9).

Calumenin in EC coupling

The muscle sarcotubular system, consisting of the SR and transverse tubules (TT), regulates Ca2+ homeostasis within muscle cells and thereby muscle contraction and relaxation (2). Muscle contraction and relaxation along with other physiological parameters are dependent on the composition of the components of the Ca2+ handling apparatus. In skeletal muscle, the voltage sensor signal is transmitted to RyR via protein/protein interaction with the H-III loop of the dihydropyridine receptor (DHPR). In contrast, the coupling process in cardiac muscle depends on Ca2+ entering the fiber through the DHPR channel and initiating Ca2+-activated Ca2+ release through RyR. SERCA proteins are responsible for causing relaxation by pumping Ca2+ from the cytoplasm into the lumen of the SR. They are activated by an increase in cytoplasmic Ca2+ and inhibited as the luminal Ca2+ concentration increases towards maximum levels. The pump is most active during EC coupling when cytoplasmic Ca2+ is highest and stored Ca2+ is at its lowest. A number of proteins interact with SERCA proteins, thereby regulating Ca2+ homeostasis in the SR. Proteins such as CSQ, CRT, calumenin, sarcalumenin, and HRC are known to promote Ca2+ buffering in striated muscle (4-9).

Evidence shows that calumenin regulates Ca2+ release from the SR. For example, over-expression of calumenin in C2C12 cells significantly increased the storage capacity of Ca2+ in the SR, but at the same time decreased depolarization-induced Ca2+ release (12). The increased storage of SR Ca2+ can be attributed to increased Ca2+ buffering power inside the SR lumen due possibly to an increased level of calumenin protein. Although the physiological role of calumenin was not ex-