SARCOLIPIN AND PHOSPHOLAMBAN AS REGULATORS OF CARDIAC SARCOPLASMIC RETICULUM CA2+ ATPASE

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Abstract

The cardiac sarcoplasmic reticulum calcium ATPase (SERCA2a) plays a critical role in maintaining the intracellular calcium homeostasis during cardiac contraction and relaxation. It has been well documented over the years that altered expression and activity of SERCA2a can lead to systolic and diastolic dysfunction. The activity of SERCA2a is regulated by two structurally similar proteins, phospholamban (PLB) and sarcolipin (SLN). Although, the relevance of PLB has been extensively studied over the years, the role SLN in cardiac physiology is an emerging field of study. This review focuses on the advances in the understanding of the regulation of SERCA2a by SLN and PLB. In particular, it highlights the similarities and differences between the two proteins and their roles in cardiac patho-physiology.

Keywords

SERCA2a; sarcolipin; phospholamban; calcium; regulation

Introduction

The cardiac sarco(endo)plasmic reticulum (SR) Ca^{2+} ATPase (SERCA2a) is a pivotal molecule for maintaining a balanced concentration of intracellular Ca^{2+} during the cardiac contraction-relaxation cycle[1,2]. SERCA2a promotes muscle relaxation by lowering the cytosolic Ca^{2+} concentration through active Ca^{2+} transport into the SR and, thereby, restores the intracellular Ca^{2+} needed for the next contraction cycle [1]. The expression and regulation of SERCA2a activity have been widely investigated, emphasizing its central role in the regulation of Ca^{2+} homeostasis during development and under a variety of patho-physiological conditions [3–6]. Studies from a variety of animal models of heart disease [7–9] and end stage human heart failure [10,11] suggest that defects in SR Ca^{2+} uptake function is one of the major contributing factors for the progression of heart failure. Several studies have demonstrated the role of SERCA2a interacting proteins in modulating pump activity and in normal and failing hearts. It is well established that in the heart SERCA2a activity is regulated by a small phosphoprotein, **phospholamban (PLB).** Recent studies have suggested that another small molecular weight protein, **sarcolipin (SLN)** is also involved in the regulation of SR Ca^{2+} ATPase activity. Current data suggest that these two proteins play important roles in regulating SERCA2a

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activity and cardiac physiology, however much remains to be understood. The focus of this review is to compare the functional significance of SLN and PLB, in particular their roles in SERCA2a regulation and cardiac contractility, and in cardiac patho-physiology.

Sarcolipin is structurally similar to PLB

Structural similarities between the PLB and SLN gene as well as the homology between their protein sequences (Fig 1), suggest that both PLB and SLN belong to the same family of proteins [12,13]. The 52 amino acids of PLB are organized into three domains. The cytoplasmic domain IA, consisting of residues 1–20, of which the first 16 are likely in an α-helical conformation, cytoplasmic domain IB consisting of residues 21–30 and domain II with residues 31–52 is the hydrophobic transmembrane domain which is probably in an α-helical conformation [13,27, 81]. On the other hand, SLN is a 31 amino acid SR membrane protein and shows a distribution pattern similar to SERCA2a and PLB [12,16]. Similar to PLB, the amino acids in SLN are organized into three domains; cytoplasmic domain, transmembrane and lumenal domains. The first 7 amino acids in SLN are hydrophilic and are cytoplasmic, the next 19 hydrophobic amino acids form a single transmembrane α-helix, and the last 5 hydrophilic amino acids are lumenal [12,17]. Amino acid sequence comparison and modeling studies have shown that the transmembrane helices of SLN and PLB share considerable homology [12,14,18,19]. In 19 transmembrane amino acids of SLN, 8 are identical, 7 share Val, Leu, or Ile, 3 share Thr, Trp, or Cys and one is Met for Ile substitution [12,19,20]. Amino acid conservation in the transmembrane domains of SLN and PLB suggests that both proteins interact in a similar way with SERCA [21,22]. There is substantial similarity between the N-terminal part of the transmembrane domain of SLN and domain Ib of PLB [19]. Domain Ib of PLB is proposed to be important for the dynamic protein-protein interaction which is regulated by the phosphorylation-dephosphorylation of Ser 16 and Thr 17, which modulates PLB function [19]. Although, the N-terminal cytoplasmic domain of SLN is not conserved among different species, Threonine 5 in the cytoplasmic domain of SLN is conserved, suggesting that it could serve as a potential phosphorylation site (discussed below) (Fig. 2). SLN has a unique C-terminal lumenal domain comprised of amino acids – RSYQY which is highly conserved among different species [12]. A recent study suggests that the lumenal domain could be involved in the retention of SLN in the ER. However, the same study shows that when co-expressed with SERCA, C-terminus of SLN is not needed for its retention in the ER suggesting that C-terminus may have a different function [23]. The flexible nature of the C-terminus also leaves Tyr-29 and Tyr-31 residues available for interactions with various aromatic residues in the transmembrane helices of SERCA and suggests that lumenal domain could be involved in the regulation of SERCA-SLN interaction [20,21,23].

SLN and PLB are differentially expressed during development and disease states

In the heart, PLB is expressed at higher levels in the ventricles, compared to the atria [24]. On the other hand, SLN levels are predominant in the atria than the ventricle [14–16,25]. PLB is also expressed at low levels in slow-twitch skeletal muscles [27,28]. The expression of SLN in the skeletal muscles varies among species. In smaller mammals, SLN mRNA is found at low levels in slow-twitch skeletal muscle, whereas, in larger mammals including human, SLN is expressed at higher levels in both slow- and fast-twitch skeletal muscles [12,14,15,26]. Thus there are chamber specific and species specific differences in the expression of PLB and SLN.

The expression of both PLB and SLN is regulated during development and modulated by the hormonal and patho-physiological state of the heart. The expression of SERCA2a [3], PLB [29–31], and SLN mRNA [25] increase several fold during heart development, displaying co-ordinate regulation and indicates the increase in SR function during heart development.

Thyroid hormone levels have been shown to modulate SR function by altering the expression of SERCA and its regulators. In hyperthyroidism, a decrease in both inhibitors PLB [6,32–34] and SLN [36] relative to SERCA2a is seen. In hypothyroidic hearts, SERCA levels are decreased, whereas PLB and SLN levels are unchanged [6,32,33,36]. These studies suggest that regulation of SERCA2a function can contribute to the altered contractile function in hypothyroid and hyperthyroid hearts.

In most forms of human and animal models of heart failure, alterations in the expression of SERCA and PLB are shown to be the major contributors for the altered calcium homeostasis. Studies showed that downregulation of PLB and SERCA proteins and decreased basal phosphorylation of PLB in end-stage heart failure correlated well with diastolic and systolic dysfunction [9,3537,38]. Similarly, the expression of SLN is also altered under a variety of pathological conditions. SLN mRNA is down-regulated in atrial myocardium of patients with chronic atrial fibrillation [39] and hypertrophic remodeling of atria [25]. A recent study also showed that SLN mRNA was up-regulated ~50 fold in the hypertrophied ventricles of Nkx2-5 null mice [40]. Studies on failing dog hearts induced by chronic pacing, showed that sarcolipin protein levels are up-regulated in the right atria [Babu et al unpublished]. However, the expression of SERCA and PLB are not altered in the above dog model suggesting a possible role for SLN in the altered Ca^{2+} homeostasis in the atria during heart failure. Whether altered expression of SLN contributes to abnormal atrial calcium homeostasis and plays a critical role in cardiac patho-physiology in human heart failure remains to be investigated.

Transgenic approaches to study the role of PLB and SLN in cardiac physiology

The mechanism of PLB action on SERCA and its relevance in cardiac muscle physiology has been studied extensively over the past several years. Studies using genetically altered mouse models have given important insight into the role of PLB in cardiac physiology. Using a PLB knockout mouse model [41], Dr. Kranias and colleagues provided the crucial evidence that PLB is an important regulator of the SERCA2a. They showed that absence of PLB enhanced SR calcium uptake and increased rates of contraction and relaxation. This was associated with an increase in SERCA2a affinity for calcium [41]. On the other hand, over-expression of PLB in the heart resulted in a decrease in SR Ca^{2+} uptake and depressed cardiac contractile performance *in vivo*. [42] These studies revealed that a shift in PLB: SERCA ratio leads to a corresponding shift in SERCA affinity for Ca^{2+}, so that an increase in the PLB: SERCA ratio leads to decreased Ca^{2+} affinity. Thus, an alteration in the PLB: SERCA ratio can affect SR Ca^{2+} transport. More recently, studies in human suggest that mutations in PLB [43,44] or the absence of PLB [45] can cause far more serious functional consequences, culminating in human heart failure. It is tempting to speculate that, in larger mammals PLB is essential for maintaining heart function, unlike in mouse. These studies underscore the importance of understanding species differences with regard to the role of PLB and SR Ca^{2+} transport, in general, between small and larger animals.

The physiological relevance of SLN in the heart was only recently identified with the help of two transgenic mouse models developed independently by the MacLennan lab and our lab. In the first case, Asahi et al [46] used rabbit cDNA to overexpress SLN in the mouse, by targeting a single copy of the α-MHC driven SLN construct into the *Hprt* locus of the X-chromosome. This resulted in heterogeneous expression of SLN in female mice due to X-chromosome inactivation. Therefore, only males were used in this study. Overexpression of SLN reduced the apparent Ca^{2+} affinity of the SERCA2a. *In vivo* measurements of cardiac function showed a significant decrease in +dP/dt and −dP/dt and led to ventricular hypertrophy. The inhibitory effect of SLN was reversed by treatment with the β-adrenergic agonist, isoproterenol, which

restored contractile function. They also reported that basal phosphorylation of PLB was decreased in the SLN transgenic hearts and in the presence of isoproterenol, PLB phosphorylation was restored to the level seen in wild-type controls. This was interpreted as an enhanced PLB phosphorylation, resulting in the dissociation of SLN from PLB and leading to the restoration of contractile function in the SLN transgenic hearts during β-adrenergic stimulation. By co-immunoprecipitation analysis using microsomes prepared from transgenic hearts, it was observed that SLN was bound to both SERCA2a and PLB, forming a ternary complex. These data suggested that SLN mediates its inhibitory effect on SERCA2a through stabilization of the SERCA2a-PLB complex and through the inhibition of PLB phosphorylation.

Our lab used the cardiac specific α-MHC promoter to overexpress mouse SLN in the atria and ventricles [47]. To study the role of SLN, the SLN: SERCA2a ratio was increased in the ventricle, where the level of SLN is naturally low. Overexpression of mouse SLN in the mouse ventricle did not lead to hypertrophy. The development of hypertrophy observed by Asahi et al. [46] is probably due to the overexpression of rabbit SLN in the mouse heart, which differs from mouse SLN at the N-terminus. SLN overexpression in the ventricle leads to decreased SERCA2a affinity for calcium, Ca^{2+} transient amplitude and shortening, and slowed relaxation. Consistent with Asahi et al [46] the +dP/dt and −dP/dt were significantly decreased, due to SLN overexpression. Similar results were found in myocytes and muscle preparations from mice overexpressing SLN, in comparison to the wild-type littermates. The inhibitory effect of SLN on SERCA2a was reversed upon β-adrenergic stimulation, suggesting that SLN is a reversible inhibitor of SERCA2a, similar to the role of PLB. In this study, we observed that an increase in SLN level does not affect PLB levels, PLB monomer to pentamer ratio and its phosphorylation status, and we concluded that the effect of SLN on SERCA2a is direct and is not mediated by a change in PLB monomer levels or its phosphorylation status. This was further confirmed by Gramolini et al. [48] by expressing SLN in the PLB null (−/−) background. This was achieved by mating the SLN transgenic mice, with cardiac specific overexpression of SLN, with the PLB KO mice. Overexpression of SLN in the absence of PLB led to a decrease in the affinity of SERCA2a for Ca^{2+}, impaired contractility, reduced calcium transient amplitude and slower decay kinetics, compared to PLB (−/−) animals. Further, in the SLN/PLB (−/−), mice isoproterenol restored the calcium dynamics to the levels seen in PLB (−/−) mice, suggesting that SLN could mediate the β-adrenergic response. The ventricular myocytes from PLB−/− mice did not show an increase in calcium handling in response to isoproterenol (ISO) which is consistent with the lack of PLB and its phosphorylation effects. Where as ventricular myocytes from SLN/PLB (−/−) showed an increased calcium transient amplitude as well as increased calcium decay kinetics, which suggests that SLN could be a mediator of β-adrenergic response and this response is independent of PLB. The lack of ISO -response in the PLB−/− ventricular myocytes, as well as other data showing very low levels of SLN suggests that SLN has little physiological role in the normal ventricle. But this does not rule out the possibility of its role in certain diseased conditions where the levels of SLN are altered. However, such conditions are yet to be reported to date. Further research in this area will determine the role of SLN in ventricular patho-physiology. These data suggest that SLN can mediate its inhibitory effect on SERCA2a independent of PLB, and could also be an important mediator of β-adrenergic response in the heart.

We recently developed a SLN knock out mouse model. Preliminary studies carried out using SLN null mice showed that loss of SLN leads to an increase in SR calcium uptake function and enhanced cardiac contractility. These studies further indicate that SLN is an important regulator of SERCA2a function and cardiac contractility [Babu et al unpublished data].

Based on the studies using genetically engineered mouse models it could be interpreted that an increase in the apparent ratio of either PLB or SLN, with respect to SERCA2a, may lead to depressed Ca^{2+} transport kinetics and contractile parameters in the mammalian heart.

SLN functionally differs from PLB

The available data suggests that SLN and PLB independently regulate SERCA2a activity [16,20,46–48]. Both PLB and SLN inhibit SERCA activity in the heart by lowering the apparent calcium affinity of the pump [42,46,47]. The inhibitory effect of SLN is relieved upon β-adrenergic stimulation, as observed for PLB [13,46–48]. Although, PLB and SLN inhibit SERCA2a activity, there might be subtle but important differences in their mechanism of regulation. For example, as illustrated in Figure 3, the inhibitory effect of PLB on SERCA2a is relieved at high calcium concentrations [42], whereas the inhibitory effect of SLN on SERCA2a is observed even at high calcium [46,47]. These functional differences could be attributed to the structural differences in the lumenal domains (C-terminus) of the two proteins. Although PLB and SLN are similar in their transmembrane domain, SLN has a longer C-terminal lumenal domain [12]. The lumenal amino acids -RSYQY in SLN are highly conserved between species and suggested to be interacting with various aromatic residues in the transmembrane helices of SERCA [12,20,21,23]. *Thus, the C-terminus of SLN could be involved in the Ca^{2+} independent inhibition of SERCA2a and could contribute for the inhibitory function of SLN at high calcium concentrations*.

It is well documented that PLB interacts with and inhibits SERCA2a activity in a reversible manner [14,49–51]. The inhibitory function of PLB is modulated by phosphorylation/ dephosphorylation and by an increase in intracellular Ca^{2+} concentration [52–54]. Phospholamban can be phosphorylated at two distinct sites; - serine 16 by cAMP-dependent protein kinase (PKA), and threonine 17 by Ca^{2+}-calmodulin–dependent protein kinase (CaMKII) during β-adrenergic stimulation [52,63,64]. Phosphorylation disrupts the physical interaction of PLB with SERCA2a and thus stimulates SR Ca^{2+} transport by increasing the affinity of the SERCA2a for Ca^{2+}, without a significant change in Vmax [51,64–69]. This, in turn, leads to an increase in the velocity of relaxation, SR Ca^{2+} load and, as a consequence, increased SR Ca^{2+} release and myocardial contractility [51,70] Whereas de-phosphorylation of PLB by type 1 phosphatase (pp1) leads to the inhibition of the SERCA [71].

Unlike PLB, the exact mechanism of SLN action on SERCA2a is not well understood. Data suggest that SLN can regulate SERCA2a activity by one of the following mechanisms: 1) the inhibitory function of SLN is mediated through PLB or 2) through the direct interaction of SLN with SERCA2a. The first evidence of the inhibitory function of SLN came from studies performed in HEK293 cells, where co-expression of SLN with either SERCA1a or SERCA2a resulted in a decreased pump affinity for Ca^{2+} [20,21]. These studies further suggested that when SLN is co-expressed with PLB, the inhibitory effect is enhanced. This super inhibitory effect on SERCA function is attributed to direct interaction of SLN with PLB leading to the destabilization of PLB pentamers. Accordingly, SLN forms a complex with PLB and thereby prevents polymerization of PLB to form pentamers. This leads to the formation of more monomers, the inhibitory form of PLB and super-inhibition of SERCA2a [14,73]. However, results from circular dichroism using chemically synthesized SLN and PLB, suggest that SLN in a hydrophobic environment is a highly stable protein, similar to the transmembrane region of PLB. These studies also suggest that, unlike PLB, SLN has only a weak ability to form oligomers and does not form heterodimers with PLB [14,74]. Therefore, it is unlikely that the inhibitory function of SLN on the SERCA2a is through polymerization. Further, this mechanism is feasible only when SLN and PLB are co-expressed. However, SLN is expressed at high levels in tissues which express either low amount of PLB (as in atria and slow-twitch skeletal muscle) or no PLB (as in fast twitch skeletal muscles). These observations suggest a

possible independent role for SLN, where SLN directly binds to SERCA2a and alters its affinity for Ca^{2+}. Co-immunoprecipitation studies have shown that SLN can interact with SERCA2a. SLN can bind SERCA either alone or in association with PLB [21]. Biochemical data and structural modeling using NMR suggest that both SLN and PLB bind SERCA in the same molecular groove and with similar mechanisms [19,20]. Mutagenesis studies by MacLennan and co-workers revealed that both SLN and PLB occupy the same interaction site in SERCA [20–22]. Results from a mouse model overexpressing SLN in PLB null background, further suggest that SLN is an effective inhibitor of SERCA2a and supports the hypothesis that SLN can regulate cardiac SERCA2a independent of PLB [48].

Further evidence from transgenic mouse models discussed earlier suggests that SLN could play a role in the β-adrenergic response in the heart. Gramolini et al. [48] recently provided evidence that SLN can be phosphorylated at the conserved Threonine 5 residue. Future studies in these directions are important towards understanding the regulation of SERCA2a in the atria and identify SLN as a potential therapeutic target to improve cardiac contractility.

β-adrenergic response in the atria could be mediated by SLN

It is well established that PLB is a major regulator of the β-adrenergic stimulatory effects in the heart [51,75,76]. Activation of β-adrenergic receptors in the sarcolemma, by increased levels of catecholamines, leads to the phosphorylation of PLB at Ser16 and Thr 17 sites via the cAMP-dependent kinase(PKA) and Ca^{2+}/calmodulin dependent kinase (CaMKII) signaling pathways, respectively [14,51]. It is important to note that Ser16 phosphorylation is a prerequisite for the phosphorylation of Thr 17. Further, there is evidence suggesting that Ser16 can be phosphorylated independently of Thr 17 *in vivo* and that phosphorylation of Ser16 by PKA is sufficient for mediating the maximal β-adrenergic response [77]. This indicates that PKA pathway is the major signaling pathway in response to β-adrenergic stimulation and that PLB is one of the main targets in the ventricle. An interesting observation in this regard is that some contractile response to β-adrenergic stimulation persists in animal models and cardiac myocytes totally devoid of PLB [78,80,82]. In particular, there is compelling data suggesting that the β-adrenergic response in the atria may depend on proteins other than PLB. Atrial SR has been shown to exhibit a four-fold lower level of PLB and a two-fold higher level of SERCA2a compared to ventricular SR [24,78,79]. However, a recent report by Kaasik et al [80] suggests that the decreased PLB levels in rat atria are not associated with a smaller response of SR Ca^{2+} uptake to β-adrenergic stimulation. On the contrary, Ca^{2+} uptake in the isoproterenol treated atria shows a much larger increase. In addition, rat atria respond to isoproterenol with much larger increases in developed tension, contractility and relaxation rates than ventricular muscle [80]. Thus, the observed increase in both Ca^{2+} uptake and contractile function could not be explained by decreased PLB levels and phosphorylation status. This suggests that the β-adrenergic response in the atria could be mediated by proteins other than PLB. SLN could be one such candidate. Results from the transgenic mouse model which overexpress SLN in PLB-null background [48] showed that the inhibitory effect of SLN can be relieved upon β adrenergic activation and suggested that SLN can act as a mediator of β-adrenergic response in the atria. These studies further identified Threonine -5 as a potential phosphorylation site and showed that a serine threonine protein kinase 16 (STK16) could phosphorylate SLN. The physiological relevance of STK16 and its role during β-adrenergic stimulation is yet to be investigated [14,48]. Preliminary i*n-vitro* data from our lab [Babu et al. unpublished results] suggest that SLN can be phosphorylated by CaMKII at the Threonine 5 and could also play a role in mediating beta adrenergic response in the atria[83]. Based on these studies and its abundant expression in the atria we are a proposing a model that shows SLN as a mediator of β-adrenergic response in the atria (Fig 4)..

Conclusion and Perspectives

PLB and SLN have emerged as two proteins which regulate the SERCA2a activity in the heart. Significant progress has been made towards understanding regulation of SERCA2a by PLB. The ratio of PLB to SERCA2a and its phosphorylation status are two key determinants of PLB action on SERCA2a. This concept has been unequivocally established using TG mouse models that express PLB at different levels or using a PLB null mouse model. On the other hand, the role of SLN in cardiac physiology is not as well understood. Studies from the MacLennan lab and our lab have established that SLN acts as a functional regulator of SERCA2a in the heart. When it comes to the mechanism of action of SLN there are still many unanswered questions. Most importantly what is the mechanism of SLN action on SERCA2a and how the inhibitory action of SLN on SERCA is relieved? One may speculate that phosphorylation of SLN is involved in regulating its mode of action. However, the kinases and phosphatases involved need to be identified. Most notably, SLN expression is absent in the ventricle, but present in significant amounts in the atria together with PLB. This indicates that SLN could play an important role in the regulation of SERCA activity in the atria much like the SERCA regulation by PLB in the ventricle. At this time it is also unclear if these two regulators are functionally similar as initially proposed. Is this a functional redundancy or do they play distinct roles in regulating SERCA2a? It is quite likely that the two proteins have evolved independently and adapted to unique functions in their cellular environment. It is also possible that the mode of action of SLN in the heart is different from the skeletal muscle due to entirely different neurohormonal environments. Future work should be directed towards the mechanism of SLN action on SERCA2a and determine if SLN is phosphorylated in response to beta adrenergic regulation in cardiac muscle.

Acknowledgments

Abbreviations

SERCA2a
Sarcoplasmic reticulum calcium ATPase

PLB
Phospholamban

SLN
Sarcolipin

SR
Sarcoplasmic Reticulum

Ca^{2+}
Calcium

Val
Valine

Leu
Leucine

Ile
Isoleucine

Thr

Threonine

Trp

Tryptophan

Cys

Cysteine

Met

Methionine

Ser

Serine

ER

Endoplasmic reticulum

MHC

Myosin heavy chain

PKA

Cyclic AMP dependent protein kinase

CaMKII

Calcium calmodulin dependent protein kinase

pp1

Protein phosphatase 1

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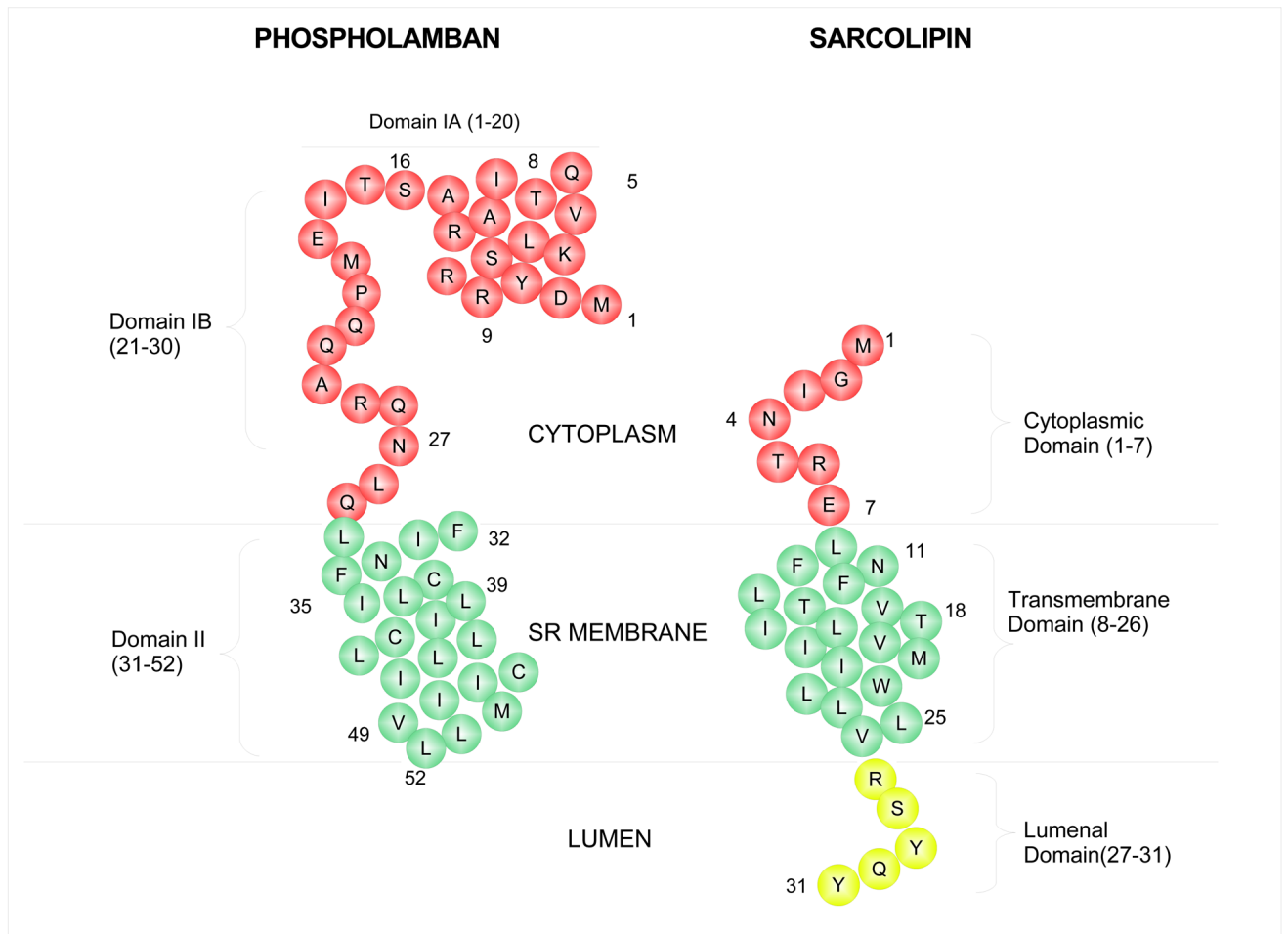
Fig 1. Schematic representation of homology between the PLB and SLN protein sequence
Horizontal lines denote the membrane boundaries and amino acids are shown in circles using their one letter code (see main text for detailed description)

SLN Amino acid sequence comparison

Mouse   MERST**Q**ELF**I**NFTVVLITV**L**LMWLLV<u>RSYQY</u>

Rat   MERST**Q**ELF**I**NFTVVLITV**L**LMWLLV<u>RSYQY</u>

Rabbit   MERSTREL**C**LNFTVVLITVIL**I**WLLV<u>RSYQY</u>

Pig   MERSTREL**C**LNFTVVLITVIL**I**WLLV<u>RSYQY</u>

Human   M**GIN**TRELFLNFT**I**VLITVILMWLLV<u>RSYQY</u>

Fig 2. Sequence comparison of SLN from different species [12,23]
The amino acids in red differ among the species. The conserved lumenal amino acids are underlined.
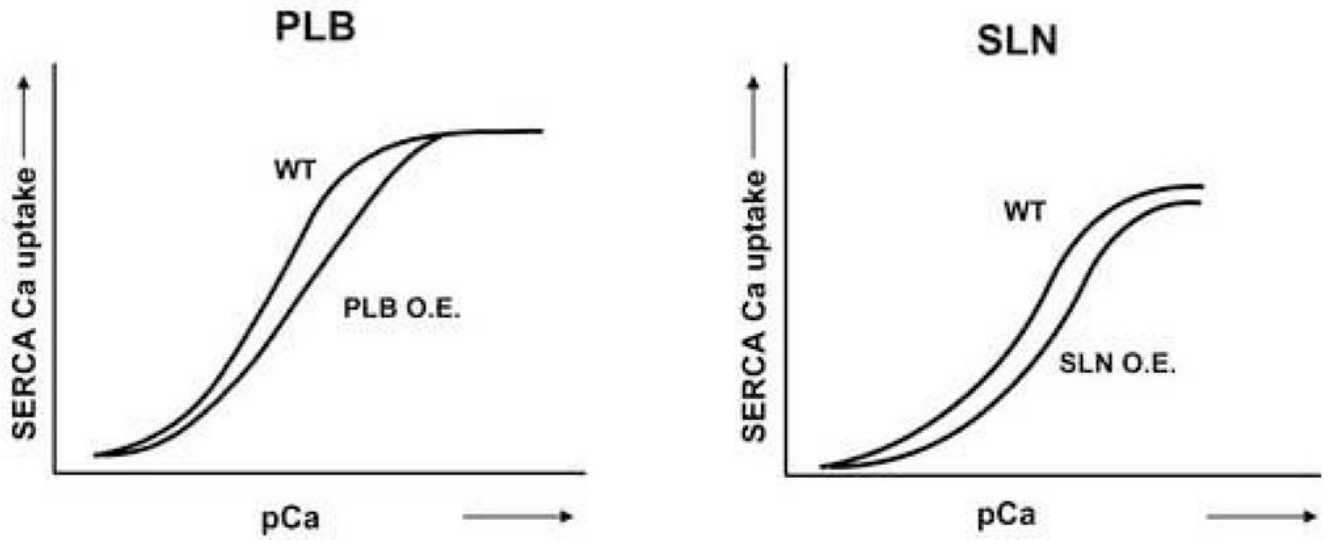
Fig 3. Illustration showing differences in the functional effect of PLB and SLN on SR Ca^{2+} uptake
Inhibitory effect of PLB overexpression (PLB O.E) on SERCA calcium uptake is relieved at high calcium concentrations [42] where as SLN overexpression (SLN O.E) is inhibitory even at high calcium compared to wild type (WT) [46,47] indicating subtle differences in the mechanism of action of the two regulators.
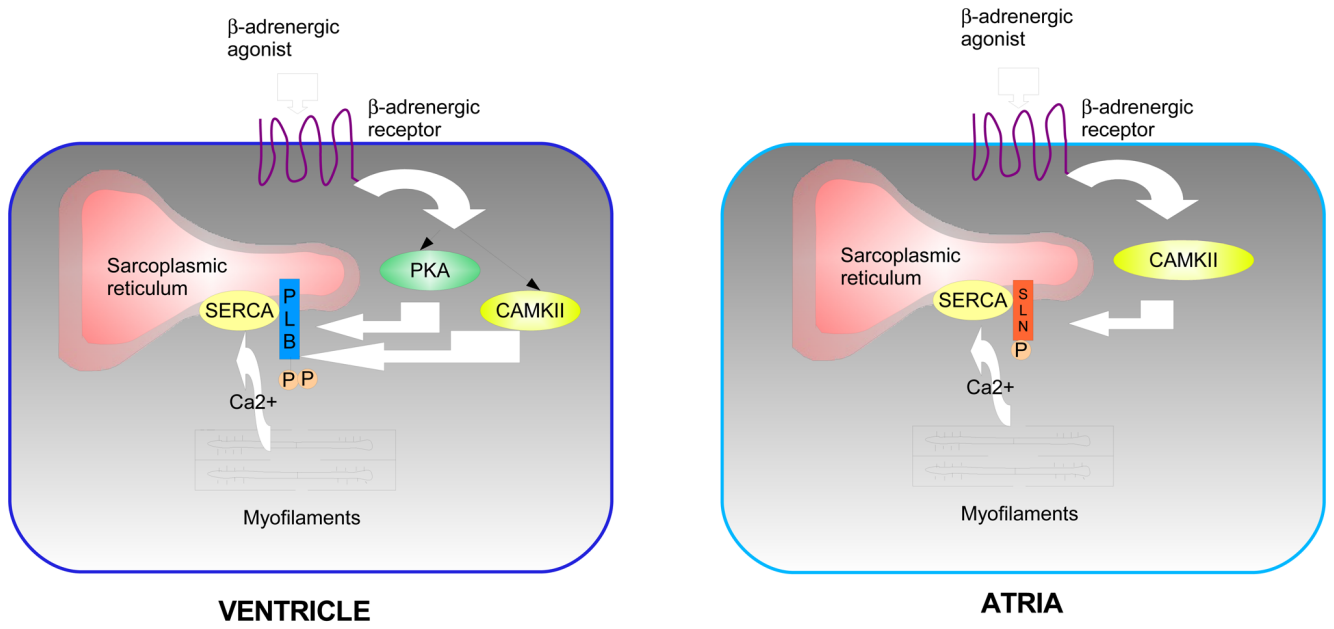
Fig 4. Proposed model depicting the role of PLB and SLN in mediating β-adrenergic response
Active calcium transport into the SR is facilitated by the SERCA2a pump and its activity is regulated by PLB and SLN .In the ventricle PLB is the principal mediator of the β-adrenergic response. In its dephosphorylated form PLB is an inhibitor of SERCA2a. Phosphorylation of PLB in response to β-adrenergic stimulation by protein kinase A (PKA) or Calcium calmodulin dependent kinase (CAMKII) relieves the inhibitory effect of PLB on SERCA2a. In the atria, SLN predominates and plays an important role in mediating the β-adrenergic response. Phosphorylation of SLN by CAMKII relieves the inhibitory effect of SLN on SERCA2a in the atria, activating SERCA2a leading the atrial muscle relaxation.