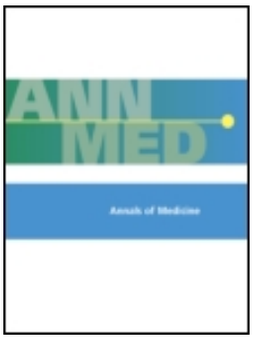

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◆ TRENDS IN MOLECULAR MEDICINE ◆

Phospholamban and cardiac contractility

Konrad Frank and Evangelia G Kranias

Over the last three decades, our knowledge and understanding of the role of phospholamban and its modulation of sarcoplasmic reticulum (SR) function has advanced significantly. Phospholamban is a key regulator of cardiac contractility and modulates SR Ca^{2+} sequestration by inhibiting the SR Ca^{2+}-ATPase (SERCA) in its dephosphorylated state. Upon phosphorylation, which is mediated through β-adrenergic stimulation, the inhibitory effect of phospholamban on the function of SERCA is relieved. This review summarizes recent advances that have been made towards understanding the modulation of SR Ca^{2+}-sequestration by phospholamban through the generation and characterization of genetically altered animal models. It also discusses the role of phospholamban in human heart failure and recent attempts to restore SR function in experimentally induced and human heart failure, which may be translated into future therapeutic approaches in the treatment of this disease.

Key words: cardiac contractility; phospholamban; relaxation; sarcoplasmic reticulum.

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Introduction

The sarcoplasmic reticulum (SR) is an intracellular membrane network in cardiac cells that plays an essential role in excitation–contraction coupling. Its main function is the storage and distribution of Ca^{2+} ions that initiate cardiac contraction and relaxation. During systole, the action potential facilitates a minor Ca^{2+} influx through sarcolemmal L-type Ca^{2+} channels. This, in turn, induces a major Ca^{2+} release from the SR stores through the Ca^{2+} release channel or the ryanodine receptor (1). Subsequently, Ca^{2+} binds to troponin C and initiates the cross-bridge movement of the myofilaments. The quick removal of Ca^{2+} either into the SR or the extracellular lumen is essential for cardiac relaxation. In human, rabbit, ferret and cat hearts, this Ca^{2+} sequestration during diastole is mainly facilitated by the SR Ca^{2+}-ATPase (SERCA) (70–80%) and to a lesser extent by the sarcolemmal Na^+/Ca^{2+} exchanger (20–30%) (2). Other systems, such as the slow Ca^{2+} removal systems, ie the sarcolemmal Ca^{2+}-ATPase and the mitochondrial Ca^{2+} uniporter, play only minor roles in intracellular Ca^{2+} extrusion during relaxation. In contrast, in both rat and mouse myocardium, SERCA plays a more predominant role with an approximately 90% contribution to the resequestration of Ca^{2+} (Na^+/Ca^{2+} exchanger: ~7% of Ca^{2+} resequestration) (2). Upon sarcoplasmic resequestration, Ca^{2+} binds mainly to the SR Ca^{2+} storage protein, calsequestrin (3) and to other Ca^{2+}-binding proteins, such as calreticulin (4) and the histidine-rich-binding protein (5).

The SERCA is under the regulatory control of the phosphoprotein phospholamban, which inhibits the apparent affinity of SERCA for Ca^{2+} in its dephosphorylated form. Upon phosphorylation of phospholamban through β-adrenergic stimulation and enhanced cyclic AMP-dependent protein kinase A activity, the inhibitory effect of phospholamban on SERCA is relieved. This leads to increased initial rates of SR Ca^{2+} uptake, accelerated relaxation and enhanced SR Ca^{2+} load, which is available for release through the ryanodine receptor resulting in enhanced relaxation.

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This review summarizes recent advances in our understanding of the modulation of SR Ca^{2+} sequestration by phospholamban focusing specifically on 1) the structure of phospholamban and its regulatory effects on SR function; 2) the physiological role of phospholamban as revealed by genetically altered models; 3) the expression and function of phospholamban in human heart failure; and 4) the potential future implications of phospholamban in the treatment of heart failure.

Structure of phospholamban and its regulatory effects on SR function

Phospholamban is an integral SR membrane protein that contains 52 amino acids. It is expressed mainly in cardiac muscle and, to a lesser extent, in slow-twitch skeletal, smooth muscles and endothelial cells (6–8). *In vitro* studies with purified SR membranes have demonstrated that phospholamban can be phosphorylated at three distinct sites: 1) serine-16 by cAMP-dependent protein kinase A; 2) threonine-17 by Ca^{2+} calmodulin-dependent protein kinase; and 3) serine-10 by Ca^{2+} phospholipid-dependent protein kinase (9–11). Phosphorylation by each protein kinase increases the apparent affinity of the SERCA for Ca^{2+} and, thereby, the rate of Ca^{2+} sequestration into the SR (Fig 1a) (12). The inhibitory effects of phospholamban are restored through dephosphorylation by an SR-associated phosphatase (13). During β-adrenergic stimulation, which represents the typical 'fight-or-flight' reaction and is one of the main regulatory mechanisms to increase low cardiac output, both serine-16 and threonine-17 become phosphorylated, but the cAMP-dependent phosphorylation site (serine-16) appears to be the most important mediator to enhance cardiac contractility (14, 15). This has also been shown in transgenic and gene-targeted mouse models (16, 17).

Phospholamban is a membrane-spanning protein. Its secondary and tertiary protein structure can be subdivided into two main domains: domain I is hydrophilic and contains the cytoplasmic region and the three phosphorylation sites (amino acids 1–30), whereas domain II includes the hydrophobic SR membrane-spanning part of the protein (amino acid residues 31–52) (18). Domain I contains two further motifs: domain Ia (residues 1–20) presumably forming an α-helical structure, and domain Ib (residues 21–30), which is likely to exist as a random coil. The transmembrane domain II has also been proposed to have an α-helical structure (19).

Until recently, little was known about the functional significance of phospholamban in the control of basal contractility; however, this was elucidated by genetic techniques that modified the expression levels of phospholamban and characterized the functional consequences *in vivo*.

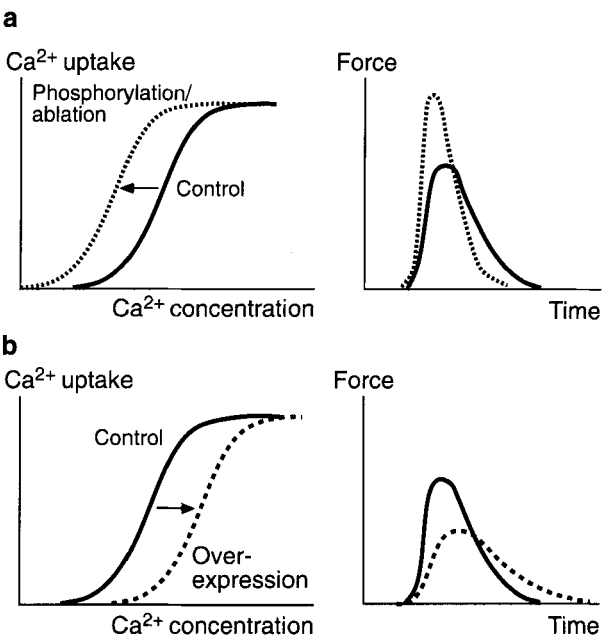

Figure 1. Effect of phospholamban phosphorylation and altered stoichiometric ratio of phospholamban to SERCA on SR Ca^{2+} uptake and cardiac contractility. (a) Phosphorylation or ablation of phospholamban in murine hearts results in an increased affinity of SERCA for Ca^{2+}, which is associated with enhanced peak force of contraction and accelerated relaxation. **(b)** Overexpression of phospholamban leads to decreased affinity of SERCA for Ca^{2+} accompanied by diminished peak force of contraction and prolonged relaxation. SR, sarcoplasmic reticulum; SERCA, SR Ca^{2+} -ATPase.

Physiological role of phospholamban: insights from transgenic and gene-targeted mouse models

The functional role of phospholamban has been elucidated by using transgenic and gene knock-out mouse models. The murine phospholamban gene was ablated by homologous recombination in embryonic stem cells, and phospholamban-deficient mice were generated (20). These mice were indistinguishable from their wild-type controls at the morphological and histological levels. Biochemical studies indicated that the affinity of the SERCA for Ca^{2+} was significantly increased in the hearts of phospholamban knock-out mice compared with their wild-type littermates. This finding was consistent with the previously described functional modulation of the SERCA upon phospholamban phosphorylation in native SR membranes (9) and in expression systems (21). Physiological measurements in isolated cardiac myocytes revealed enhanced myocyte mechanics and parallel

increases in amplitude and kinetics of rising and falling phases of Ca^{2+} transients (Fig 1a) (22, 23). Furthermore, phospholamban-deficient myocytes exhibited a higher SR Ca^{2+} load (24), and the L-type Ca^{2+} channel currents were significantly larger (25). The hyperdynamic contractile function, observed in phospholamban-deficient cardiomyocytes, was also apparent at the intact organ level when work-performing heart preparations under identical preload, afterload and heart rate (20) were used, as well as in intact animals in which left ventricular catheter techniques (26) and echocardiography were used (27). Thus, ablation of phospholamban was associated with significant increases in contractile parameters at the cellular, organ and intact animal levels.

Furthermore, isoproterenol stimulation resulted in an attenuated contractile response in isolated myocytes (22), hearts (20) or intact animals (27). Interestingly, the attenuation of β-agonist stimulation was not caused by alterations in the β-adrenergic signalling pathway or the degree of phosphorylation of other key cardiac regulatory phosphoproteins (28). In addition, no compensatory mechanisms were observed at the level of the SERCA, calsequestrin or the myofilaments, as revealed by the myosin ATPase activity and the Ca^{2+}-dependent force development in skinned fiber experiments (29, 30). However, the ryanodine receptor levels were decreased (~25%) in an attempt to regulate the release of the increased SR Ca^{2+} load (29). These results indicate that phospholamban is a key regulator of basal contractility and a major mediator of the β-agonist responses in the mammalian heart (31).

The phospholamban knock-out studies suggested that the relative stoichiometric ratio of phospholamban to SERCA is a critical determinant of the regulation of the cardiac contraction–relaxation cycle. Thus, in a second approach, the effects of over-expressing phospholamban through transgenesis in murine hearts was investigated by using the cardiac-specific α-myosin heavy-chain promoter (16). Utilizing this transgenic technique, phospholamban was over-expressed by 2-fold compared with hearts of wild-type mice. Similar to the phospholamban-deficient model, murine hearts from transgenic animals and their wild-type controls were analysed at the molecular, biochemical and physiological levels.

Transgenic mice over-expressing twice as high levels of phospholamban in the heart showed no morphological abnormalities or alterations in heart/body weight compared with their wild-type littermates. The affinity of the SERCA for Ca^{2+} was decreased in native SR preparations from transgenic hearts, and thus exhibited properties opposite to those of the phospholamban-deficient SR preparations (20). Compared with wild-type controls isolated cardiomyocytes from transgenic animals showed decreases in shortening fraction and rates of shortening and relengthening. The amplitude of the Ca^{2+} signal was also decreased, and the rate of decay of the Ca^{2+} transient in fura-2-loaded transgenic cardiac myocytes was significantly prolonged, which was consistent with the decreased Ca^{2+} affinity of SERCA (Fig 1b). These findings were consistent with parameters measuring depressed contractility at the intact organ and whole animal levels. However, the observed differences in contractile parameters, Ca^{2+} kinetics and depressed left ventricular function *in vivo* were abolished upon isoproterenol stimulation (16). These results suggest that in native SR, there is a fraction of SERCA molecules that is not subject to regulation by phospholamban.

A third approach studied effects of over-expression of a nonphosphorylatable form of phospholamban, in which the phosphorylation sites serine-16 and threonine-17 were mutated to alanine (32). Several transgenic lines were generated, which expressed phospholamban that was mutated 1.8-, 2.6-, 3.7- and 4.7-fold over wild-type levels. Cardiac SR Ca^{2+} uptake experiments of these lines revealed that increasing levels of phospholamban were accompanied by decreases in the apparent affinity for Ca^{2+}. Saturation of the phospholamban inhibitory effect on the affinity of the SERCA was achieved at a phospholamban to SERCA ratio of 2.6 to 1, suggesting that only 40% of the SERCA pumps are under the inhibitory regulation of phospholamban in wild-type murine hearts (32).

Based on these studies in genetically engineered phospholamban mouse models, increases in the apparent ratio of phospholamban to SERCA may be at least partially responsible for the depressed kinetics of Ca^{2+} transients and contractile parameters in the mammalian heart (33).

Expression levels and function of phospholamban in human heart failure

In human heart failure, several lines of evidence suggest that there are alterations in intracellular Ca^{2+} homeostasis, which may be related to an altered expression, function or regulation of Ca^{2+}-handling proteins. Along these lines, several groups have reported diminished peak and prolonged decay of Ca^{2+} transients in cardiomyocytes and papillary muscle strips that have been isolated from hearts of patients with end-stage heart failure (34, 35). These alterations in the rise and fall of the Ca^{2+} transient have important implications for excitation–contraction coupling and the development of increased diastolic tension in heart failure. Furthermore, they diminish frequency potentiation, which is one of the potent intrinsic mechanisms inducing cardiac inotropy (36–38).

Figure 2 summarizes alterations in SR function and protein levels as well as in phosphorylation levels of phospholamban in human congestive heart failure. Most studies in human heart failure patients have revealed that the expression of phospholamban remains unchanged compared with nonfailing myocardium (37–39), although one study reported a down-regulation (18%) of phospholamban (40). Based on transgenic and gene-targeted mouse model studies, the phospholamban to SERCA ratio has been shown to be a major regulator of cardiac contractility (32, 33). Thus, any alterations in the expression levels of phospholamban have to be examined in parallel with possible alterations in the expression levels of SERCA, as it is the ratio of phospholamban to SERCA that mainly regulates contractility. Along these lines, it remains controversial whether SERCA expression changes in human heart failure. Several groups have reported a down-regulation of the SERCA protein (36, 40), while others have not observed any significant changes in SERCA expression in end-stage failing human myocardium (37–39, 41). A decrease in the expression levels of SERCA may account for increased ratio of phospholamban to SERCA and, thus, increased inhibition of the affinity of SERCA and prolonged relaxation. In addition to possible changes in the protein levels of SERCA, the phosphorylation status of phospholamban at serine-16 or threonine-17 sites has been shown to decrease in human heart failure. Phosphorylation at the serine-16 site exhibited a 30% decrease (42), and similar changes were observed for the threonine-17 site (43). The decreases in serine-16 phosphorylation levels may reflect decreased expression of β-receptors (44), increased expression of G_i -protein levels (45) and increased β-AR-kinase-1 activity (46), which represent downstream alterations in the β-adrenergic signalling pathway. In light of these distinct changes in the expression and phosphorylation of SR Ca^{2+}-handling proteins, a disruption or alteration of the phospholamban–SERCA complex may be a target for therapeutic approaches.

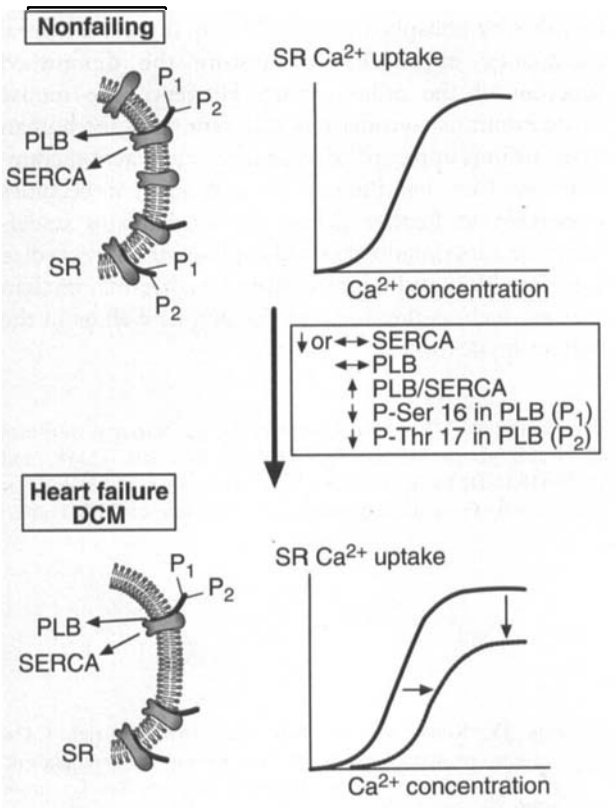

Figure 2. Schematic representation of alterations in SERCA and phospholamban (PLB) in human heart failure (dilated cardiomyopathy, DCM). Although expression levels of PLB are unchanged, the increased phospholamban to SERCA ratio and decreased phosphorylation at serine-16 (P-Ser-16) and threonine-17 (P-Thr-17) sites of phospholamban are associated with impairment of SR function in human heart failure. SERCA, sarcoplasmic reticulum Ca^{2+} -ATPase, SR, sarcoplasmic reticulum.

Potential implications of the phospholamban–SERCA complex in the treatment of heart failure

As outlined above, heart failure is associated with distinct changes in intracellular Ca^{2+} handling. Muscle relaxation and rapid removal of cytosolic Ca^{2+} by

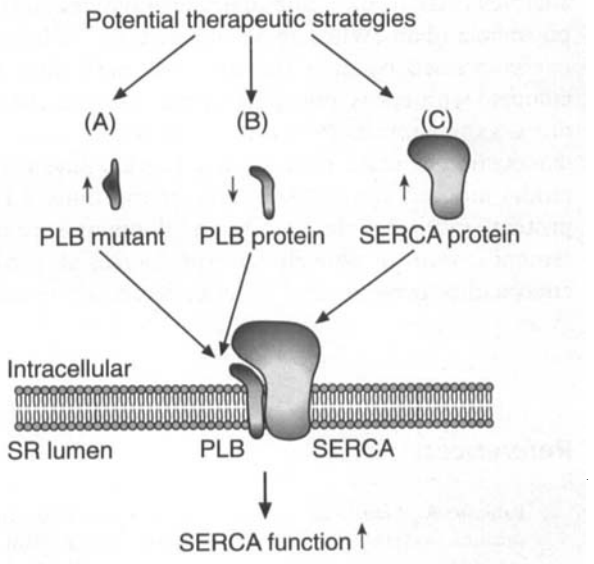

Figure 3. Schematic representation of different approaches to increase sarcoplasmic reticulum (SR) function through modulation of the activity of phospholamban (PLB) or the PLB to SERCA ratio. The function of the PLB–SERCA complex may be regulated either through: A) over-expression of a dominant-negative mutant of PLB, that replaces native PLB; B) down-regulation of PLB transcription/translation; or C) up-regulation of SERCA protein expression. SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase.

Based on the findings in genetically altered animal models and human heart failure, disruption or alteration of the phospholamban–SERCA complex may be a target for therapeutic approaches.

SERCA are impaired, resulting in increased diastolic calcium concentration. Consequently, the SR Ca^{2+} load decreases, and less Ca^{2+} is available for subsequent contractions. Thus, enhanced SR reuptake either through stimulation of the pump itself or dissociation of the phospholamban–SERCA complex may represent a potential therapeutic target to partially restore the perturbed intracellular Ca^{2+} handling in heart failure. Several approaches in animal models and human cardiomyocytes have followed this working hypothesis (Fig 3). Hajjar and co-workers used recombinant adenovirus-mediated gene transfer to increase the expression of SERCA in aortic-banded rats (49) or in isolated cardiomyocytes derived either from rat (47) or human failing hearts (48). These studies support the notion that an enhanced expression of SERCA restores disturbed intracellular Ca^{2+} handling by decreasing the relative ratio of phospholamban to SERCA. Other potential approaches to influence the phospholamban–SERCA complex may utilize dominant-negative mutants of phospholamban. When these mutants are sufficiently over-expressed by gene transfer techniques they can compete with native phospholamban. Derived from *in vitro* expression systems, this approach was first demonstrated in the context of a heart failure mouse model induced by gene ablation of the muscle LIM protein (50). In this study, a dominant-negative mutant form of phospholamban increased cardiac contractility even beyond baseline levels, observed in wild-type hearts. Recently, another approach to decrease the protein levels of phospholamban and thereby increase contractility was reported that used adenovirus-mediated gene transfer of phospholamban antisense cDNA in cultured neonatal rat cardiomyocytes (51). With this technology, a 70% reduction in phospholamban protein levels was accomplished in cultured neonatal rat cardiomyocytes after 72 h, and this was accompanied by increased rates of Ca^{2+} decay (51). Further studies will determine whether any of these approaches to modulate the phospholamban–SERCA complex may be included as a valuable therapeutic option in the treatment of heart failure.

Conclusion

Significant advances have been made in describing the functional role of phospholamban in cardiac contractility of the mammalian heart. This has been achieved through the generation and characterization of transgenic and gene knock-out mouse models with integrative approaches. The findings in the mouse may provide valuable insight into the mechanisms underlying the regulation of human cardiac contractility by phospholamban and may suggest potential therapeutic approaches to restore the diminished function of the failing heart. However, the mouse myocardium is considerably different from the human myocardium in regard to excitation–contraction coupling and Ca^{2+} handling. In that respect, it becomes important to further define the mechanisms underlying the functional role of phospholamban in cardiac Ca^{2+} handling and contractility in higher mammalian species, such as the dog and the pig, as well as in the human myocardium.

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References

1. **Fabiato A.** Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J Gen Physiol* 1985; 85: 291–320.
2. **Bers DM, Bassani JW, Bassani RA.** Na-Ca exchange and Ca fluxes during contraction and relaxation in mammalian ventricular muscle. *Ann N Y Acad Sci* 1996; 779: 430–42.
3. **MacLennan DH, Reithmeier RA.** Ion tamers. *Nat Struct Biol* 1998; 5: 409–11.
4. **Michalak M, Corbett EF, Mesaeli N, Nakamura K, Opas M.** Calreticulin: one protein, one gene, many functions. *Biochem J* 1999; 344: 281–92.
5. **Suk JY, Kim YS, Park WJ.** HRC (histidine-rich Ca2+ binding protein) resides in the lumen of sarcoplasmic reticulum as a multimer. *Biochem Biophys Res Commun* 1999; 263: 667–71.
6. **Ganim JR, Luo W, Ponniah S, Grupp I, Kim HW, Ferguson DG, et al.** Mouse phospholamban gene expression during development in vivo and in vitro. *Circ Res* 1992; 71: 1021–30.
7. **Lalli MJ, Shimizu S, Sutliff RL, Kranias EG, Paul RJ.** [Ca2+]i homeostasis and cyclic nucleotide relaxation in aorta of phospholamban-deficient mice. *Am J Physiol* 1999; 277: H963–70.

8. **Sutliff RL, Hoying JB, Kadambi VJ, Kranias EG, Paul RJ.** Phospholamban is present in endothelial cells and modulates endothelium-dependent relaxation. Evidence from phospholamban gene-ablated mice. *Circ Res* 1999; 84: 360–4.
9. **Kranias EG, Solaro RJ.** Phosphorylation of troponin I and phospholamban during catecholamine stimulation of rabbit heart. *Nature* 1982; 298: 182–4.
10. **Le Peuch CJ, Guilleux JC, Demaille JG.** Phospholamban phosphorylation in the perfused rat heart is not solely dependent on beta-adrenergic stimulation. *FEBS Lett* 1980; 114: 165–8.
11. **Simmerman HK, Collins JH, Theibert JL, Wegener AD, Jones LR.** Sequence analysis of phospholamban. Identification of phosphorylation sites and two major structural domains. *J Biol Chem* 1986; 261: 13333–41.
12. **Kadambi VJ, Kranias EG.** Phospholamban: a protein coming of age. *Biochem Biophys Res Commun* 1997; 239: 1–5.
13. **Kranias EG.** Regulation of calcium transport by protein phosphatase activity associated with cardiac sarcoplasmic reticulum. *J Biol Chem* 1985; 260: 11006–10.
14. **Mundina-Weilenmann C, Vittone L, Ortale M, de Cingolani GC, Mattiazzi A.** Immunodetection of phosphorylation sites gives new insights into the mechanisms underlying phospholamban phosphorylation in the intact heart. *J Biol Chem* 1996; 271: 33561–7.
15. **Kuschel M, Karczewski P, Hempel P, Schlegel WP, Krause EG, Bartel S.** Ser16 prevails over Thr17 phospholamban phosphorylation in the beta-adrenergic regulation of cardiac relaxation. *Am J Physiol* 1999; 276: H1625–33.
16. **Kadambi VJ, Ponniah S, Harrer JM, Hoit BD, Dorn GW 2nd, Walsh RA, et al.** Cardiac-specific overexpression of phospholamban alters calcium kinetics and resultant cardiomyocyte mechanics in transgenic mice. *J Clin Invest* 1996; 97: 533–9.
17. **Luo W, Chu G, Sato Y, Zhou Z, Kadambi VJ, Kranias EG.** Transgenic approaches to define the functional role of dual site phospholamban phosphorylation. *J Biol Chem* 1998; 273: 4734–9.
18. **MacLennan DH, Toyofuku T, Kimura Y.** Sites of regulatory interaction between calcium ATPases and phospholamban. *Basic Res Cardiol* 1997; 92: 11–5.
19. **Simmerman HK, Jones LR.** Phospholamban: protein structure, mechanism of action, and role in cardiac function. *Physiol Rev* 1998; 78: 921–47.
20. **Luo W, Grupp IL, Harrer J, Ponniah S, Grupp G, Duffy JJ, et al.** Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and loss of beta-agonist stimulation. *Circ Res* 1994; 75: 401–9.
21. **Jones LR, Simmerman HK, Wilson WW, Gurd FR, Wegener AD.** Purification and characterization of phospholamban from canine cardiac sarcoplasmic reticulum. *J Biol Chem* 1985; 260: 7721–30.
22. **Wolska BM, Stojanovic MO, Luo W, Kranias EG, Solaro RJ.** Effect of ablation of phospholamban on dynamics of cardiac myocyte contraction and intracellular Ca2+. *Am J Physiol* 1996; 271: C391–7.
23. **Li L, Chu G, Kranias EG, Bers DM.** Cardiac myocyte calcium transport in phospholamban knockout mouse: relaxation and endogenous CaMKII effects. *Am J Physiol* 1998; 274: H1335–47.
24. **Santana LF, Kranias EG, Lederer WJ.** Calcium sparks and excitation-contraction coupling in phospholamban-deficient mouse ventricular myocytes. *J Physiol (Lond)* 1997; 503: 21–9.
25. **Masaki H, Sato Y, Luo W, Kranias EG, Yatani A.** Phospholamban deficiency alters inactivation kinetics of L-type Ca2+ channels in mouse ventricular myocytes. *Am J Physiol* 1997; 272: H606–12.
26. **Lorenz JN, Kranias EG.** Regulatory effects of phospholamban on cardiac function in intact mice. *Am J Physiol* 1997; 273: H2826–31.
27. **Hoit BD, Khoury SF, Kranias EG, Ball N, Walsh RA.** In vivo echocardiographic detection of enhanced left ventricular function in gene-targeted mice with phospholamban deficiency. *Circ Res* 1995; 77: 632–7.
28. **Kiss E, Edes I, Sato Y, Luo W, Liggett SB, Kranias EG.** Beta-adrenergic regulation of cAMP and protein phosphorylation in phospholamban-knockout mouse hearts. *Am J Physiol* 1997; 272: H785–90.
29. **Chu G, Luo W, Slack JP, Tilgmann C, Sweet WE, Spindler M, et al.** Compensatory mechanisms associated with the hyperdynamic function of phospholamban-deficient mouse hearts. *Circ Res* 1996; 79: 1064–76.
30. **Schwinger RH, Brixius K, Savvidou-Zaroti P, Bolck B, Zobel C, Frank K, et al.** The enhanced contractility in phospholamban deficient mouse hearts is not associated with alterations in (Ca2+)-sensitivity or myosin ATPase-activity of the contractile proteins. *Basic Res Cardiol* 2000; 95: 12–8.
31. **Koss KL, Kranias EG.** Phospholamban: a prominent regulator of myocardial contractility. *Circ Res* 1996; 79: 1059–63.
32. **Brittsan AG, Carr AN, Schmidt AG, Kranias EG.** Maximal inhibition of SERCA2 Ca(2+) affinity by phospholamban in transgenic hearts overexpressing a non-phosphorylatable form of phospholamban. *J Biol Chem* 2000; 275: 12129–35.
33. **Koss KL, Grupp IL, Kranias EG.** The relative phospholamban and SERCA2 ratio: a critical determinant of myocardial contractility. *Basic Res Cardiol* 1997; 92: 17–24.
34. **Beuckelmann DJ, Nabauer M, Erdmann E.** Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circulation* 1992; 85: 1046–55.
35. **Dipla K, Mattiello JA, Margulies KB, Jeevanandam V, Houser SR.** The sarcoplasmic reticulum and the Na+/Ca2+ exchanger both contribute to the Ca2+ transient of failing human ventricular myocytes. *Circ Res* 1999; 84: 435–44.
36. **Hasenfuss G, Reinecke H, Studer R, Meyer M, Pieske B, Holtz J, et al.** Relation between myocardial function and expression of sarcoplasmic reticulum Ca(2+)-ATPase in failing and nonfailing human myocardium. *Circ Res* 1994; 75: 434–42.
37. **Schwinger RH, Bohm M, Schmidt U, Karczewski P, Bavendiek U, Flesch M, et al.** Unchanged protein levels of SERCA II and phospholamban but reduced Ca2+ uptake and Ca(2+)-ATPase activity of cardiac sarcoplasmic reticulum from dilated cardiomyopathy patients compared with patients with nonfailing hearts. *Circulation* 1995; 92: 3220–8.
38. **Frank K, Bolck B, Bavendiek U, Schwinger RH.** Frequency dependent force generation correlates with sarcoplasmic calcium ATPase activity in human myocardium. *Basic Res Cardiol* 1998; 93: 405–11.
39. **Movsesian MA, Karimi M, Green K, Jones LR.** Ca(2+)-transporting ATPase, phospholamban, and calsequestrin levels in nonfailing and failing human myocardium. *Circulation* 1994; 90: 653–7.
40. **Meyer M, Schillinger W, Pieske B, Holubarsch C, Heilmann C, Posival H, et al.** Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy. *Circulation* 1995; 92: 778–84.
41. **Linck B, Boknik P, Eschenhagen T, Muller FU, Neumann J, Nose M, et al.** Messenger RNA expression and immunological quantification of phospholamban and SR-Ca(2+)-ATPase in failing and nonfailing human hearts. *Cardiovasc Res* 1996; 31: 625–32.
42. **Schwinger RH, Munch G, Bolck B, Karczewski P, Krause**

EG, Erdmann E. Reduced Ca(2+)-sensitivity of SERCA 2a in failing human myocardium due to reduced serin-16 phospholamban phosphorylation. *J Mol Cell Cardiol* 1999; 31: 479–91.

43. **Dash R, Frank K, Moravec CS, Kranias EG.** Phospholamban phosphorylation and the apparent affinity of the sarcoplasmic Ca2+-ATPase for Ca2+ are depressed in failing human myocardium (abstract). *Circulation* 1999; 100: 2202.
44. **Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, et al.** Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N Engl J Med* 1982; 307: 205–11.
45. **Neumann J, Schmitz W, Scholz H, von Meyerinck L, Doring V, Kalmar P.** Increase in myocardial Gi-proteins in heart failure. *Lancet* 1988; 2: 936–7.
46. **Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ.** Altered expression of beta-adrenergic receptor kinase and beta 1- adrenergic receptors in the failing human heart. *Circulation* 1993; 87: 454–63.
47. **Hajjar RJ, Schmidt U, Kang JX, Matsui T, Rosenzweig A.** Adenoviral gene transfer of phospholamban in isolated rat cardiomyocytes. Rescue effects by concomitant gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase. *Circ Res* 1997; 81: 145–53.
48. **del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, et al.** Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation* 1999; 100: 2308–11.
49. **Miyamoto MI, del Monte F, Schmidt U, DiSalvo TS, Kang ZB, Matsui T, et al.** Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure. *Proc Natl Acad Sci U S A* 2000; 97: 793–8.
50. **Minamisawa S, Hoshijima M, Chu G, Ward CA, Frank K, Gu Y, et al.** Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* 1999; 99: 313–22.
51. **Eizema K, Fechner H, Bezstarosti K, Schneider-Rasp S, van Der Laarse A, Wang H, et al.** Adenovirus-based phospholamban antisense expression as a novel approach to improve cardiac contractile dysfunction: comparison of a constitutive viral versus an endothelin-1-responsive cardiac promoter. *Circulation* 2000;101: 2193–9.