EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 36, No. 3, 193-203, June 2004

Calcium cycling proteins in heart failure, cardiomyopathy and arrhythmias

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Accepted 11 June 2004

Abbreviations: ARVD, arrhythmogenic right ventricular cardiomyopathy; CPVT, catecholaminergic polymorphic ventricular tachycardia; CSQ2, cardiac calsequestrin; EC, excitation-contraction; FKBP, a FK506 binding protein; iRNA, Inhibitory RNA; ICa, the Ca^{2+} currents; JP2, junctophilin type 2; LCC, L-type Ca^{2+} channels; mAKAP, PKA anchoring protein; PKA, protein kinase A; PLN, phospholamban; RyR2, the cardiac ryanodine receptor; SAR, sarcalumenin; SERCA2a, the SR calcium ATPase 2a; SLN, sarcolipin; SR, sarcoplasmic reticulum

Abstract

A growing body of evidence, including studies using genetically engineered mouse models, has shown that Ca^{2+} cycling and Ca^{2+}-dependent signaling pathways play a pivotal role in cardiac hypertrophy and heart failure. In addition, recent studies identified that mutations of the genes encoding sarcoplasmic reticulum (SR) proteins cause human cardiomyopathies and lethal ventricular arrhythmias. The regulation of Ca^{2+} homeostasis *via* the SR proteins may have potential therapeutic value for heart diseases such as cardiomyopathy, heart failure and arrhythmias.

Keywords: calcium ATPase; calcium homeostasis; cardiomyopathy; heart failure; phospholamban; ryanodine receptor; sarcoplasmic reticulum

Introduction

Calcium is not only indispensable for normal muscle contraction and relaxation but also important as a second messenger of various signaling pathways in the heart. A growing body of evidence has shown that Ca^{2+} homeostasis and Ca^{2+}-dependent signaling pathways play a pivotal role in the development of cardiac hypertrophy, heart failure and arrhythmias. In this regard, two issues regarding the role of Ca^{2+} in the heart are attracting considerable attention. One is the discovery of Ca^{2+}/calmodulin-dependent calcineurin signaling pathway in cardiac hypertrophy (Olson and Williams, 2000; Wilkins and Molkentin, 2002) and the other is to identify the critical role of cardiac Ca^{2+} cycling in cardiomyopathy, heart failure and arrhythmias (Chien, 2000; Houser *et al.*, 2000; Scoote and Williams, 2002). In this review, we focus on the latter topic, especially on Ca^{2+} cycling proteins in the sarcoplasmic reticulum (SR).

The regulation of Ca^{2+} release and uptake *via* the cardiac sarcoplasmic reticulum

Periodic changes in Ca^{2+} concentration in cardiomyocytes are essential for cardiac contraction and relaxation, and the intracellular Ca^{2+} concentration is integrally regulated by proteins associated with the SR, an extensive intracellular membrane system. The SR consists of lipid bilayer organelle that surrounds each myofibril. In response to membrane depolarization, a small amount of extracellular Ca^{2+} enters the cardiomyocyte through the L-type Ca^{2+} channels. The Ca^{2+} influx triggers the release of Ca^{2+} from the SR into the cytosol through the cardiac ryanodine receptor (RyR2), initiating cardiac contraction. This event is known as Ca^{2+} induced- Ca^{2+} release. The relaxation is predominantly mediated by Ca^{2+} sequestration from the cytosol into the SR lumen via the SR calcium ATPase 2a (SERCA2a). The activity of the RyR2 and SERCA2a are known to be under fine-tuning by their intrinsic regulatory domains and associated SR proteins.

The RyR2 forms homotetramers consisting of 4 monomeric subunits, each of about 565-kDa to produce a bona fide ion channel. The subunit contains a high-conductance Ca^{2+}-selective pore, Ca^{2+} activation and inactivation sites, several phosphoryla-

tion sites, and multiple binding sites for a myriad of endogenous regulators including ATP, Mg^{2+}, and calmodulin. The RyR2 also forms a macromolecular complex by protein-protein interactions, including protein kinase A (PKA) and its anchoring protein (mAKAP), the protein phosphatases PP1 and PP2A, sorcin, calmodulin, a FK506 binding protein (FKBP12.6), and other proteins in the cytosol (Marks *et al.,* 2002a). In the luminal region of junctional SR membrane, the RyR2 is also associated with cardiac calsequestrin (CSQ), triadin, junctin, and junctate, which are all required for appropriate regulation of the Ca^{2+} release from the RyR2 (Muller *et al.,* 2002). Although cAMP-dependent PKA signal pathway plays the most important role in the regulation of the RyR-mediated Ca^{2+} release, the function of the RyR2 is regulated by many other factors, including several regulatory domains and protein-protein interactions with many molecules.

CSQ is a 55-kDa high capacity, moderate affinity Ca^{2+}-binding protein localized to the lumen of the junctional SR in cardiac muscle. CSQ forms a dense matrix in the SR lumen, where the protein appears to be physically connected to the RyR2 by anchoring strands. Biochemical evidence suggests that CSQ actively participates in muscle contraction by regulating the amount of luminal Ca^{2+} store (Sitsapesan and Williams, 1997). This regulatory effect of CSQ may be mediated by CSQ-anchoring proteins such as triadin and junctin (Zhang *et al.,* 1997). Triadin was first identified as a 95-kDa protein in skeletal muscle junctional SR membrane (Caswell *et al.,* 1991) and subsequently three cardiac triadin isoforms (triadin 1, 2 and 3) were cloned. Junctin was originally identified

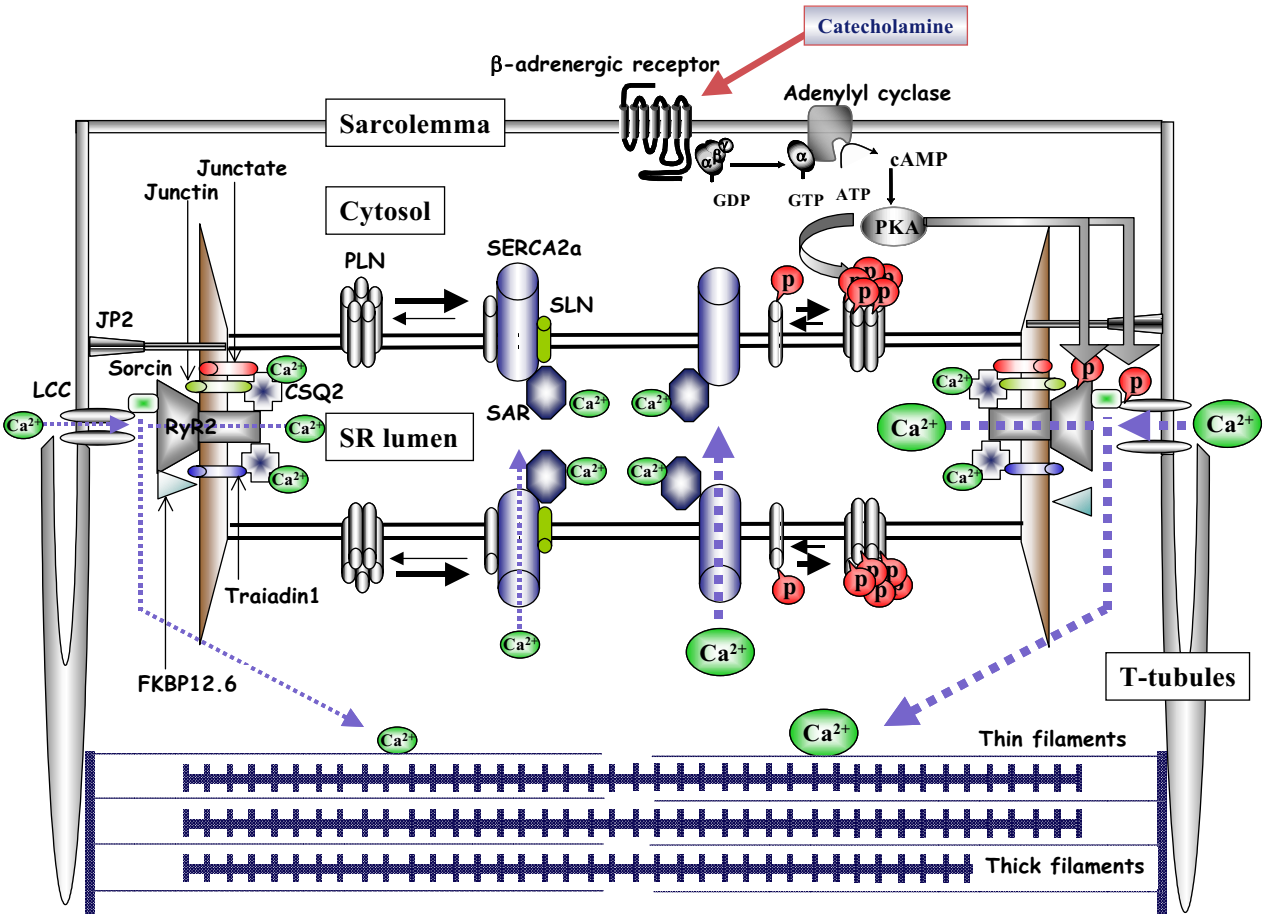

Figure 1. Major cardiac SR proteins involved in Ca^{2+} cycling in cardiomyocytes. The Ca^{2+} movement was demonstrated by blue broken lines. The right-sided panel shows the enhanced Ca^{2+} release and uptake by β-adrenergic stimulation via cAMP-dependent PKA signal pathway. PKA phosphorylates L-type Ca^{2+} channels (LCC), the cardiac ryanodine receptor (RyR2) and phospholamban (PLN). The phosphorylated RyR2 is dissociated from FKBP12.6, resulting in pronounced channel open probability. Phosphorylated PLN relieves its inhibition on SERCA2a activity, resulting in an increase in Ca^{2+} uptake into the SR. Junctophilin type 2 (JP2) is a membrane spanning protein between the sarcolemmal membrane and the SR. Sorcin is a penta-EF hand Ca^{2+}-binding protein that binds directly to both RyR2 and the LCC. The RyR2 is also associated with cardiac calsequestrin (CSQ2), triadin, junctin, and junctate. CSQ2 and sarcalumenin (SAR) are Ca^{2+}-binding proteins localized in the junctional and longitudinal SR, respectively. Sarcolipin (SLN) that is expressed predominantly in the atria may inhibit SERCA2a activity.

as a 26-kDa major CSQ-binding protein in cardiac and skeletal muscle junctional SR membranes (Jones *et al.,* 1995). Although triadin and junctin are the products of different genes, they exhibit intriguing structural and amino acid sequence similarities and play an important role in the regulation of Ca^{2+} release from the RyR2. Junctate, an alternative splicing form of the same gene generating junctin and aspartyl β-hydroxylase, is a newly identified 33-kDa Ca^{2+} binding protein in the integral SR membrane and three cardiac isoforms (junctate 1, 2 and 3) were cloned from the mouse heart (Treves *et al.,* 2000; Hong *et al.,* 2001).

Crosstalk between L-type Ca^{2+} channels on the sarcolemmal membrane and the RyR2 on the SR is a fundamental feature of excitation-contraction (EC) coupling in the heart. Junctophilin type 2 (JP2), a cardiac isoform of JP family, has been recently identified as a membrane spanning protein that contributes to the formation of the junctional membrane complexes between the sarcolemmal membrane and the SR (Takeshima *et al.,* 2000). JP2 is an essential component for the stabilization of the junctional membrane complexes and may play an important role in maintaining normal functional coupling of Ca^{2+} induced-Ca^{2+} release.

SERCA2a, a cardiac and slow-twitch skeletal muscle isoform of SERCA family that belongs to P-type ATPases, is the primary regulator of the rate of Ca^{2+} re-uptake during relaxation in the heart (Periasamy and Huke, 2001). The activity of SERCA2a is mainly regulated by its endogenous inhibitor, phospholamban (PLN). PLN, a 52 amino acid SR transmembrane phosphoprotein, inhibits Ca^{2+} uptake to interact with SERCA2a at their cytoplasmic and transmembrane domains. Phosphorylated PLN relieves its inhibition on SERCA2a activity, resulting in an increase in Ca^{2+} uptake into the SR (Frank and Kranias, 2000).

Sarcolipin has been identified as a counterpart of PLN in skeletal muscle (Odermatt *et al.,* 1998). The expression of mouse sarcolipin mRNA, however, was most abundant in the atria and was undetectable in the ventricles, indicating an atrial chamber-specific expression pattern in the heart (Minamisawa *et al.,* 2003b). Atrial chamber-specific expression of sarcolipin mRNA was increased during development and was down-regulated in the atria of hypertrophic heart. In human, sarcolipin mRNA was also expressed in the atria, but not detected in the ventricles. Therefore, sarcolipin is likely to be an atrial chamber-specific regulator of Ca^{2+} cycling in the heart. It is intriguing to know whether sarcolipin plays an important role in the atrium-specific cardiac disorders such as atrial fibrillation.

Sarcalumenin is a Ca^{2+}-binding protein localized in the longitudinal SR. Sarcalumenin isoforms are generated as 160- and 53-kDa glycoproteins by the alternative splicing of the primary transcript derived from its gene, and are specifically expressed in skeletal and cardiac muscle cells (Leberer *et al.,* 1990). The amino-terminal half of the 160-kDa isoform is characterized by the juxtapositions of negatively charged residues resembling to that of CSQ. Although several studies have suggested that sarcalumenin works as a Ca^{2+}-buffering protein like CSQ and regulates SERCA activity (Martin, 1990), the physiological role of sarcalumenin remains largely unknown at the present.

The functional relevance between β-adrenergic sysytem/cAMP-dependent PKA signal pathway and Ca^{2+} cycling in the heart has been warranted (Figure 1). cAMP-dependent PKA signal pathway plays a pivotal role in the regulation of Ca^{2+} release and uptake in the SR. Marx *et al.* demonstrated that the phosphorylated RyR2 by PKA is dissociated from FKBP12.6, resulting in pronounced channel open probability. Furthermore, the hyperphosphorylated RyR2 increased SR Ca^{2+} leak from the SR in patients with heart failure (Marx *et al.,* 2000). Yano *et al.* also found that the binding of FKBP12.6 to the RyR2 was decreased in pacing-induced heart failure when compared with normal hearts (Yano *et al.,* 2000). As to Ca^{2+} uptake into the SR, phosphorylation at PLN serine-16 by PKA is the predominant event leading to a proportional increase in the rate of Ca^{2+} uptake and accelerates ventricular relaxation (Colyer, 1998). PLN is considered to be mostly responsible for the effects of β-adrenergic stimulation on cardiac contractility and relaxation, since PLN deficient mice display the maximal contraction without β-adrenergic stimulation (Luo *et al.,* 1994). A decrease in PLN phosphorylation has been demonstrated in patients or animal models of heart failure, resulting in a decrease in Ca^{2+} uptake into the SR (Schmidt *et al.,* 1999; Sande *et al.,* 2002). Therefore, abnormal β-adrenergic/cAMP-dependent PKA signal pathway induces the imbalance of Ca^{2+} release and uptake in the SR, resulting in the reduced Ca^{2+} content in the SR. This is a central physiological hallmark of a number of animal models of heart failure, as well as in human failing hearts.

Genetically engineered animal models

Genetically engineered mice, such as transgenic and knockout mice, have proved to be extremely useful for understanding the regulation of many molecules involved in EC coupling and Ca^{2+} cycling in the heart, and have given strong insights for pathophysiological roles of the SR proteins (Kadambi and Kranias, 1998; Dillmann, 1999).

Table 1. Genetically engineered animal model.

Transgene	Cardiac phenotypes	References
RyR-2 KO	Embryonic lethal with morphological abnormalities in the heart tube Large vacuolate SR and structurally abnormal mitochondria	Takeshima, 1998 EMBO J
FKBP12 KO	Embryonic lethal with severe dilated cardiomyopathy and ventricular septal defects Noncompaction of left ventricular myocardium	Shou,1998 Nature
FKBP12.6 KO	Cardiac hypertrophy in the male but not in the female Loss of myofibril organization Exercise-induced vetricular tachycardia	Xin,2002 Nature Wehrens, 2003 Cell
Sorcin TG	Impaired contraction and relaxation without overt cardiac hypertrophy	Meyers, 2003 J Biol Chem
Junctin TG	Generalized cardiomegaly with systolic dysfunction Bradycardia and atrial fibrillation Increased fibrosis	Zhang, 2001 J Mol Cell Cardiol Hong, 2002 FASEB J Kirchhefer, 2003 Cardiovas Res
Triadin 1 TG	Cardiac hypertrophy with impaired contractility and relaxation	Kirchhefer, 2001 J Biol Chem
Junctate 1 TG	Dilated cardiomyopathy with severe systolic dysfunction Bradycardia with various arrhythmias	Cho, 2003 J Am Coll Cardiol
CSQ2 TG	Cardiac hypertrophy with systolic dysfunction	Sato,1998 J Biol Chem Cho, 1999 J Biol Chem
SERCA2 KO	Embryonic lethal in homozygous mice Impaired contraction and relaxation in heterozygous mice	Periasamy, 1999 J Biol Chem Ji, 2000 J Biol Chem
SERCA2a KO	High incidence of perineonatal mortality and cardiac malformations Mild cardiac hypertrophy with impaired cardiac contractility and relaxation	Ver Heyen, 2001 Cir Res
SERCA2a TG	Enhanced cardiac contractility and relaxation	He, 1997 J Clin Invest Baker, 1998 Cir Res
SERCA1 TG	Enhanced cardiac contractility and relaxation	Loukianov, 1998 Cir Res
SERCA2b TG	Enhanced cardiac contractility and relaxation	Greene, 2000 J Biol Chem
mutant SERCA2a TG	K397/400E, lack of a functional association with PLN Enhanced cardiac contractility and relaxation	Nakayama, 2003 FASEB J
PLN KO	Enhanced cardiac contractility and relaxation	Luo, 1994 Cir Res
PLN TG	Impaired cardiac contractility and relaxation without overt cardiac remodeling (2-fold overexpression) Heart failure with aging (4-fold overexpression)	Kadambi, 1996 J Clin Invest Dash, 2001 Circulation
mutant PLN TG		
C41F	Monomeric form of PLN; Less pronounced inhibitory effect when compared with wild-type PLN	Chu, 1998 Cir Res
S16A, T17A	Non-phosphorylatable form of PLN; Maximal inhibition of SERCA2a activity	Brittsan, 2000 J Biol Chem
L37A, I40A	Monomeric, dominant-acting, superinhibitory PLN; Impaied contractility with cardiac hypertrophy	Zvaritch, 2000 J Biol Chem
V49G	A superinhibitor of SERCA2a affinity for Ca2+; Impaired cardiac function and heart failure	Haghighi, 2001 J Biol Chem
N27A	A PLN hinge domain mutant; Impaired cardiac function and heart failure	Schmidt, 2002 Cardiovasc Res
S16E	A pseudophosphorylated PLN; Enhanced cardiac contractility and relaxation	Hoshijima, 2002 Nat Med
R9C	Blockade of PKA-mediated PLN phosphorylation; Impaired cardiac function and heart failure	Schmidt, 2003 Science

Knockout mice lacking RyR2 die at approximately embryonic day 10 with morphological abnormalities in the heart tube. Prior to embryonic death, large vacuolate SR and structurally abnormal mitochondria began to develop in the knockout cardiomyocytes, and the vacuolate SR appeared to contain high concentrations of Ca^{2+}. This result suggests that RyR2 is absolutely required for cellular Ca^{2+} homeostasis most probably as a major Ca^{2+} release channel to maintain the developing SR (Takeshima *et al.,* 1998). Alterations in RyR2 accessory proteins are also associated with pathogenesis in the heart. Ablation of FKBP12.6 gene caused cardiac hypertrophy in male mice, but not in female, despite the similar dysregulation of Ca^{2+} release in male and female knockout mice (Xin *et al.,* 2002). FKBP12.6-null mice consistently exhibited exercise-induced cardiac ventricular arrhythmias that cause sudden cardiac death. (Wehrens *et al.,* 2003). Since FKBP 12.6 stabilizes RyR2 channel activity and prevents aberrant activation of RyR2 during the resting phase of the cardiac cycle, this data suggests that unstable RyR2 channel can induce life-threatening arrhythmias. Although FKBP12 is predominantly associated with skeletal RyR (RyR1), FKBP12-deficient mice displayed severe dilated cardiomyopathy and ventricular septal defects without abnormality in skeletal muscle (Shou *et al.,* 1998).

Sorcin is a penta-EF hand Ca^{2+}-binding protein that binds directly to both RyR2 and the L-type Ca^{2+} channel. The transgenic mice exhibited no cardiac hypertrophy and no change in expression of other calcium regulatory proteins. However, significant reductions in global indices of contraction and relaxation were observed in the transgenic hearts. In addition, Ca^{2+} transient amplitudes were significantly depressed and the Ca^{2+} currents (I_{Ca}) inactivation rate of the L-type Ca^{2+} channel was significantly accelerated in transgenic myocytes (Meyers *et al.,* 2003).

Overexpression of the junctin under the control of α-myosin heavy chain promoter caused bi-atrial and bi-ventricular enlargements, impaired LV systolic function, bradycardia, atrial fibrillation, and increased fibrosis (Zhang *et al.,* 2001; Hong *et al.,* 2002; Kirchhefer *et al.,* 2003). Transgenic mice overexpressing triadin 1 in atria and ventricles demonstrated cardiac hypertrophy and impaired contractility and relaxation (Kirchhefer *et al.,* 2001). Cardiac-specific overexpression of junctate 1 resulted in dilated cardiomyopathy with severely depressed LV systolic function and various arrhythmias such as atrial fibrillation, ventricular premature beats and sinus pause. The reduced SR Ca^{2+} content, enhanced L-type Ca^{2+} current density, and the prolonged action potential duration may account for the bradycardia in the junctate 1 transgenic heart (Cho *et al.,* 2003). Thus, the transgenic mice with cardiac-specific overexpression of triadin 1, junctin and junctate 1 show distinct cardiac phenotypes, suggesting that these junctional SR transmembrane proteins are of functional relevance for the regulation of the SR Ca^{2+} release in the heart.

To elucidate the physiological significance of cardiac CSQ (CSQ2) in the cardiac excitation-contraction coupling, two independent lines of transgenic mice overexpressing CSQ2 in the heart have been generated (Jones *et al.,* 1998; Sato *et al.,* 1998). Although cardiac-targeted overexpression of CSQ2 results in a marked increase in SR Ca^{2+} storage in the both models, SR Ca^{2+} release was impaired upon depolarization, leading to depressed contractile parameters and cardiac hypertrophy. Transition from concentric LV hypertrophy to overt heart failure was clearly demonstrated and defective β-adrenergic receptor signaling preceded the development of dilated cardiomyopathy (Cho *et al.,* 1999). These findings suggest that chronic suppression of Ca^{2+} release caused by overexpression of CSQ2 or the excess amount of CSQ2 *per se* also initiate a cascade of molecular events that activates the program of cardiac hypertrophy and/or heart failure. In contrast, no phenotypic feature of heart failure was observed up to 17 months of age in ventricles of the model of Sato et al., which expresses higher amount of CSQ2 in the myocardium, suggesting that endogenous traits of the mouse strains also influence the outcomes (Sato *et al.,* 2003).

The genetic ablation of JP2 in mice caused wider gap size of the junctional membrane complexes and deficient $[Ca^{2+}]_i$ transients in cardiomyocytes, resulting in embryonic lethality (Takeshima *et al.,* 2000). These findings indicate that JP2 is essential to form normal junctional membrane complexes and efficient Ca^{2+} induced-Ca^{2+} release in the heart. The expression of JP2 was up-regulated during normal development and was down-regulated in a hypertrophic or dilated cardiomyopathic mouse model (Minamisawa *et al.,* unpublished data). JP type 1 (JP1) is a skeletal muscle type of JP isoforms. JP1 knockout mice died shortly after birth and exhibited deficiency of triad junctions and contraction in skeletal muscles (Ito *et al.,* 2001). Transgenic mice overexpressing JP1 exibted abnormal junctional membranes, in which the T-tubules were rolled up with the SR membranes. However, authentic triad formation was not induced by JP1 overexpression in cardiac myocytes, suggesting that ectopic JP1 expression cannot convert the diad to the triad in cardiac myocytes (Komazaki *et al.,* 2003).

Two independent groups generated transgenic mice overexpressing SERCA2a in the heart. The increase in SERCA2a expression resulted in myocardial contractility and relaxation by increasing SR Ca^{2+} transport (He *et al.,* 1997; Baker *et al.,* 1998). Transgenic

mice overexpressing a high calcium affinity SERCA2a mutant (K397/400E), lacking a functional association with PLN, were also generated. The transgenic mouse hearts showed increased contraction and relaxation, with increases in the amplitude of Ca^{2+} transient and rapid Ca^{2+} decay (Nakayama *et al.,* 2003). Moreover, transgenic mice overexpressing the fast-twitch skeletal muscle type of SERCA (SERCA1a) (Loukianov *et al.,* 1998) or SERCA2b (Greene *et al.,* 2000) in a heart-specific manner were also generated. Both mice demonstrated enhanced myocardial contractility and increased Ca^{2+} transport function, indicting that both SERCA isoforms can substitute for SERCA2a *in vivo*.

Complete ablation of SERCA2 (both SERCA2a and 2b) resulted in embryonic lethality (Periasamy *et al.,* 1999). Heterozygous mutant hearts that expressed 65% of the protein levels of SERCA2 compared with wild-types showed impaired cardiac contractility and relaxation (Ji *et al.,* 2000). When SERCA2a gene was specifically ablated, homozygous mutant hearts expressed only SERCA2b of which the protein levels were reduced to 40% of total SERCA2 in wild-type mice. SERCA2a deficiency led to increased incidence of perineonatal mortality and cardiac structural malformations as well as mild cardiac hypertrophy with impaired cardiac contractility and relaxation (Ver Heyen *et al.,* 2001). This data indicates that SERCA2a is essential for normal cardiac development and function.

Transgenic mice expressed two-fold higher levels of PLN in the heart displayed impaired cardiac contractility and relaxation without any phenotypic alterations including heart-to-body mass ratio, cardiomyocyte size and morphology (Kadambi *et al.,* 1996). However, transgenic mice overexpressing PLN at 4-fold normal levels exhibited the development of overt heart failure and a premature mortality with aging (Dash *et al.,* 2001). To elucidate whether the site-specific mutations of PLN alter cardiac contractility and relaxation, more than 10 transgenic mice overexpressing various types of mutant PLN have been also generated (Chu *et al.,* 1998; Brittsan *et al.,* 2000; Zvaritch *et al.,* 2000; Haghighi *et al.,* 2001; Hoshijima *et al.,* 2002; Schmidt *et al.,* 2002; Schmitt *et al.,* 2003). Among them, an increase in cardiac contractility and relaxation is detected only in the transgenic mice overexpressing a S16E peudo-phosphorylated PLN mutant (Hoshijima *et al.,* 2002). Several transgenic mice exhibited impaired cardiac contractility and relaxation, resulting in cardiac remodeling such as cardiac hypertrophy (Zvaritch *et al.,* 2000) and heart failure (Haghighi *et al.,* 2001; Schmidt *et al.,* 2002; Schmitt *et al.,* 2003).

Mice heterozygous and homozygous for the PLN-ablated gene have been extensively evaluated by Dr. Kranias and her colleagues (Kadambi and Kranias, 1998). Reduction or ablation of PLN resulted in "supernormal" cardiac contraction and relaxation without any phenotypic alterations at the gross morphology or ultrastructural levels in mice (Luo *et al.,* 1994).

Human cardiomyopathy caused by genetic mutations in Ca^{2+} cycling molecules

Cardiomyopathy is defined as a disease of the myocardium associated with cardiac dysfunction by either intrinsic/genetic disorders of myocardium, or extrinsic specific events like ischemia, pressure and volume overloads, abnormal metabolism, inflammation or toxic agents. Primary cardiomyopathy is a group of intrinsic disorders of the myocardium. To date, more than 20 genes have been identified as being responsible for cardiomyopathy. Despite the diverse etiologies, Ca^{2+} cycling defect is a physiological hallmark of all forms of cardiomyopathy. Therefore, the genes involved in Ca^{2+} cycling have been considered responsible for cardiomyopathy, and candidate gene approach has been employed in many laboratories.

In this regard, mutations in the RyR2 have been identified as the cause of arrhythmogenic right ventricular dysplasia (ARVD), a specific type of cardiomyopathy (Tiso *et al.,* 2001). This is the first SR gene which causes cardiomyopathy. Mutations in the RyR2 also cause cathecholamine-induced polymorphic ventricular tachycardias (Marks *et al.,* 2002b). These data indicate that RyR2 is responsible not only for cardiomyopathy and heart failure, but also for life-threatening arrhythmias.

Two independent groups have recently found that mutations of the PLN gene cause human dilated cardiomyopathy. One is a missense mutation at residue 9 (Arg⟶Cys) which is proposed to block PKA-mediated phosphorylation of PLN (Schmitt *et al.,* 2003). The other is a nonsense mutation at residue 39 (Leu⟶stop) (Haghighi *et al.,* 2003). In the former study, they generated Arg9Cys mutant PLN transgenic mice, in which PKA-mediated phosphorylation of PLN at serine 16 was blocked. The decay of Ca^{2+} transient was delayed in mutant myocytes, indicating impaired Ca^{2+} uptake. The mice recapitulated human heart failure. In the latter study, the heterozygous individuals with Leu39stop mutation exhibited hypertrophy without decreased contractile performance, and individuals homozygous displayed the diminished expression of PLN in the SR (null PLN) and dilated cardiomyopathy. The authors claimed that PLN is

essential to maintain normal cardiac function in human, in contrast to mice in which PLN deficiency enhances cardiac contractility and relaxation without any adverse effects. These opposite results from different species have to be verified in detail, since, as we will discuss later, PLN is thought to be one of the prime targets for novel therapeutic invention of heart failure.

In addition, a single nucleotide transition, -77A⟶G, in the PLN promoter region was found in a patient with late-onset type of hypertrophic cardiomyopathy (Minamisawa *et al.,* 2003a). The mutation was found to increase PLN promoter activity using neonatal rat myocytes, suggesting that -77A⟶G mutation in the PLN promoter region increases the PLN expression in the heart. The mutation has not been found more than normal 300 individuals so far. Therefore, the mutation in the promoter region may play an important role in the development of hypertrophic cardiomyopathy in human. Since different mutations in several sarcomeric proteins such as β-myosin heavy chain and troponin T cause dilated and hypertrophic cardiomyopathies (Seidman and Seidman, 2001), PLN is also likely to be associated with both dilated and hypertrophic cardiomyopathies. Here, the genes involved in Ca^{2+} cycling have become the real candidates responsible for cardiomyopathy. It should be intriguing to investigate mutations of other SR genes in patients with cardiomyopathy.

Life-threatening ventricular arrhythmias caused by genetic mutations in Ca^{2+} cycling molecules

A growing body of evidence indicates that mutations of RyR2 and CSQ2 cause catecholaminergic polymorphic ventricular tachycardia (CPVT) and ARVD2 (Lahat *et al.,* 2001; Laitinen *et al.,* 2001; Bauce *et al.,* 2002; Postma *et al.,* 2002; Priori *et al.,* 2002; Lahat *et al.,* 2003). Although the molecular mechanisms underlining the relation between genotypes and phenotypes remain unclear, one may assume that the greater amount of Ca^{2+} release from the mutant hearts may induce an elevated diastolic cytoplasmic Ca^{2+} during exercise- or catecholamine-induced stress, resulting in diastolic afterdepolarizations that can initiate fatal ventricular tachyarrhythmias. This increased Ca^{2+} release can be mediated by FKBP12.6. Recently, Wehrens et al demonstrated that a derivative of 1,4-benzothiazepine (JTV519) increased the affinity of FKBP12.6 for RyR2, which stabilized the closed state of RyR2 and prevented the Ca^{2+} leak that triggers arrhythmias (Wehrens *et al.,* 2004). Thus, further investigation of mutations in other SR genes related to Ca^{2+} release such as FKBP12.6, triadin 1 and junctin will be warranted.

The modulation of the SR proteins is potential therapeutic strategy for cardiomyopathy, heart failure, and arrhythmias

Our current therapy for cardiomyopathy and heart failure is primarily palliative and is not biologically targeted because of poor understanding in stress pathways leading to the progression of cardiac muscle dysfunction. The lessons from animal studies and human genetics revealed, however, that a decrease in Ca^{2+} uptake due to reduced SERCA2a activity and an increase in Ca^{2+} leak from the SR due to instability of the RyR2 play an important role in the development of cardiomyopathy, heart failure and arrhythmias. Therefore, the restore of the SR function may be novel therapeutic strategy for these abnormalities.

Genetic approaches and pharmacological interventions, designed to increase SERCA2a activity or inhibit PLN function, may prove valuable in preventing or reversing the adverse physiological impairment in cardiomyopathy and heart failure. It may be a fundamental approach to increase SERCA2a activity by simply increasing the expression level of SERCA2a. This can be achieved through SERCA2a gene transfer. Dr. Hajjar's group demonstrated that muscle contractility and relaxation were restored by adenovirus-mediated SERCA2a gene transfer in an animal model of pressure overload (Miyamoto *et al.,* 2000) as well as in cardiomyocytes from patients with heart failure (del Monte *et al.,* 2001). They recently demonstrated that improving intracellular Ca^{2+} cycling by overexpression of SERCA2a restored contractile function and reduced ventricular arrhythmias during cardiac ischemia-reperfusion (del Monte *et al.,* 2004).

Inhibition of PLN function or disruption of the interaction between PLN and SERCA2a is an alternative way to increase SERCA2a activity. Reduction of PLN expression by decreasing PLN transcription, or disrupting PLN mRNA stability seems to have promising value for pharmaceutical interventions to improve cardiac performance. Adenovirus-mediated antisense expression of PLN coding region resulted in the successful depression of PLN mRNA and protein and increased Ca^{2+} uptake in neonatal rat myocytes (He *et al.,* 1999; Eizema *et al.,* 2000). Inhibitory RNA (iRNA) for PLN mRNA can be an alternate to decrease PLN protein in the heart. Recent studies demonstrated that certain PLN mutants increased contractility and relaxation of normal and pathological hearts when they were transferred into

myocytes and animal hearts using adenovirus or adeno-associated vector (Minamisawa *et al.,* 1999; Hoshijima *et al.,* 2002; Iwanaga *et al.,* 2004). Therefore, selective disruption of the interaction between SERCA2a and PLN is effective to prevent or reverse cardiac performance in dilated cardiomyopathy and heart failure. So far augmented SERCA2a activity has beneficial effects on the cardiac function in rodent models of heart failure. This can be a novel inotropic therapy for cardiac dysfunction, at least for a short period. However, it should be carefully evaluated whether augmented SERCA2a activity affects morbidity and mortality in human for a long period, since a null PLN mutation causes dilated cardiomyopathy in human described above (Haghighi *et al.,* 2003).

In addition to reduced SR Ca^{2+} uptake, increased Ca^{2+} leak through RyRs is a significant component of altered EC coupling in heart failure. A leak of Ca^{2+} from the SR decreases SR Ca^{2+} content and release of systolic Ca^{2+}, and it may be a trigger for arrhythmias. Moreover, altered cytosolic Ca^{2+} by increased Ca^{2+} leak may contribute to altered gene expression and myocardial remodeling. FKBPs are a good candidate to stabilize the RyR to reduce the leak of Ca^{2+} through RyRs. In this regard, Prestle et al. demonstrated that Ca^{2+} leak through RyRs was reduced in adenoviral mediated FKBP12.6 overexpressed cardiomyocytes (Prestle *et al.,* 2001). Yao et al. recently reported that the prevention of Ca^{2+} leak through RyRs improved ventricular function and prevention of heart failure in a dog model, using the agent JTV519, which restores the FKBP12.6-mediated stabilization of RyR (Yano *et al.,* 2003). JTV519 also prevented fetal ventricular arrhythmias caused by the Ca^{2+} leak from the RyR2 (Wehrens *et al.,* 2004). Therefore, reducing SR leak is a promising approach to improve Ca^{2+} cycling of the failing heart as well as arrhythmias.

More than 100 years after Ringer's discovery indicating that Ca^{2+} is essential for normal muscle contraction, we have been realizing that the modulation of intracellular Ca^{2+} concentration via manipulating the SR proteins is promising tactics to fight a monster syndrome, "heart failure".

Acknowledgment

This work was supported in part by the Ministry of Education, Science, Sports and Culture of Japan (to S.Minamisawa), the Organization for Pharmaceutical Safety and Research Grant MF-16 (to Y. Sato), the Japan Health Sciences Foundation Grant KH23106 (to Y. Sato), and the Research Center for Bioresource and Health (RCBH) and KOSEF (to MC Cho).

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