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Review article

Phospholamban interactome in cardiac contractility and survival: A new vision of an old friend

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ABSTRACT

Depressed sarcoplasmic reticulum (SR) calcium cycling, reflecting impaired SR Ca-transport and Ca-release, is a key and universal characteristic of human and experimental heart failure. These SR processes are regulated by multimeric protein complexes, including protein kinases and phosphatases as well as their anchoring and regulatory subunits that fine-tune Ca-handling in specific SR sub-compartments. SR Ca-transport is mediated by the SR Ca-ATPase (SERCA2a) and its regulatory phosphoprotein, phospholamban (PLN). Dephosphorylated PLN is an inhibitor of SERCA2a and phosphorylation by protein kinase A (PKA) or calcium–calmodulin-dependent protein kinases (CAMKII) relieves these inhibitory effects. Recent studies identified additional regulatory proteins, associated with PLN, that control SR Ca-transport. These include the inhibitor-1 (I-1) of protein phosphatase 1 (PP1), the small heat shock protein 20 (Hsp20) and the HS-1 associated protein X-1 (HAX1). In addition, the intraluminal histidine-rich calcium binding protein (HRC) has been shown to interact with both SERCA2a and triadin. Notably, there is physical and direct interaction between these protein players, mediating a fine-cross talk between SR Ca-uptake, storage and release. Importantly, regulation of SR Ca-cycling by the PLN/SERCA interactome does not only impact cardiomyocyte contractility, but also survival and remodeling. Indeed, naturally occurring variants in these Ca-cycling genes modulate their activity and interactions with other protein partners, resulting in depressed contractility and accelerated remodeling. These genetic variants may serve as potential prognostic or diagnostic markers in cardiac pathophysiology.

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1. Introduction

Cardiovascular disease is the leading cause of morbidity and mortality worldwide, with heart failure representing the fastest growing subcategory over the past decades. Aberrant Ca handling is a hallmark of heart failure, which is partially attributed to alterations in the function of the sarcoplasmic reticulum (SR). In cardiomyocytes, coordinated

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mobilization of cytosolic Ca by the SR is principally responsible for each cycle of cardiac contraction and relaxation. Ca is also an integral signaling molecule for numerous other cellular processes including survival and cell death. As such, the dysregulation of Ca handling observed in heart failure is linked to impaired cardiac muscle performance as well as tissue viability.

The level of intracellular Ca is regulated by a balance between the release of Ca into the cytosol and the removal of Ca by the combined action of several proteins in the cell. Cytosolic Ca is sequestered into the SR lumen by the sarcoplasmic reticulum Ca-ATPase (SERCA2a), whose activity is reversibly regulated by phospholamban (PLN), a 52 amino acid phosphoprotein [1]. Dephosphorylated PLN interacts with SERCA2a and inhibits the pumping activity, whereas phosphorylation of PLN by PKA and CAMKII during β-adrenergic stimulation relieves the inhibitory effects and augments the contractile parameters. Restoration of contractility to basal levels is mediated by protein phosphatase 1 (PP1), which dephosphorylates PLN [2–4]. Interestingly, PP1 is regulated by two PKA phosphoproteins, inhibitor-1 (I-1) and the small heat shock protein 20, Hsp20. Phosphorylation of inhibitor-1 or Hsp20 during β-adrenergic stimulation results in increases in their inhibitory activity for PP1, allowing for amplification of the stimulatory effects of PKA-phosphorylation in cardiomyocytes [1].

Recently, two other regulators of SR Ca-transport were identified. One of them is the small anti-apoptotic HS-1 associated protein X-1 (HAX1), which interacts with PLN and regulates SR Ca-cycling and contractility [5]. The other one is the histidine-rich calcium binding protein, HRC, which interacts with SERCA2a as well as the ryanodine receptor Ca release complex [6], mediating regulation of both SR Ca-uptake and release [7].

Thus, there is a multimeric SR Ca-transport ensemble composed of the regulatory partners: inhibitor-1/PP1/Hsp20, which are anchored to PLN through the regulatory subunit (RGL) of PP1 [8] and the transport complex of HAX/PLN/SERCA/HRC (Fig. 1).

2. SR calcium cycling in cardiac contractility and survival

2.1. Sarcoplasmic reticulum Ca-ATPase (SERCA)

SERCA is a 110 kD transmembrane protein, that belongs to a family of highly conserved proteins. SERCA2a is primarily expressed in the heart and is the mediator of calcium uptake by the SR, initiating relaxation. In human and experimental heart failure, the expression levels and enzymatic activity of SERCA2a are significantly decreased and these may underlie the depressed SR Ca-cycling [1,9]. The functional significance of alterations in SERCA2a levels has been examined using mouse models with overexpression or ablation of SERCA2a. Transgenic overexpression of SERCA2a resulted in significantly enhanced contractile parameters under baseline condition, which remained preserved under pressure overload without affecting mortality [10]. On the other hand, SERCA2a gene knock-out resulted in early embryonic lethality, while heterozygous mice exhibiting depressed function, survived without signs of heart disease [11].

Since early lethality of the targeted ablation of SERCA2a did not allow investigation of cardiac function, an inducible model with cardiac-specific deletion of SERCA2a was generated in order to gain insight into the mechanisms of SERCA2a deficiency [12]. Surprisingly, 4 weeks after inducible SERCA2a ablation in adult mice only moderately impaired cardiac function was observed with a relatively small

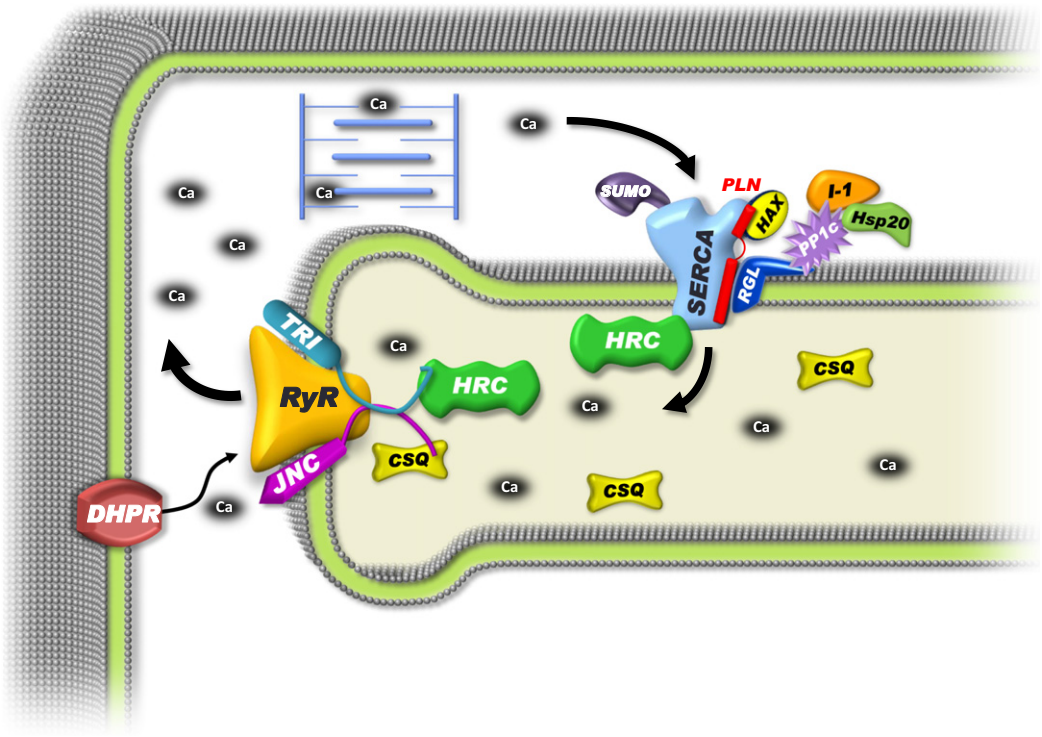

Fig. 1. Schematic representation of the PLN/SERCA interactome. In the classical view of EC-coupling, membrane depolarization activates sarcolemmal DHPR, causing an influx of calcium that activates the RyR complex, releasing calcium from the SR store. This calcium triggers myofilament contraction and is then transported back into the SR by SERCA2a, which is regulated by its binding partner phospholamban (PLN). However, this model is expanding with the identification of novel interacting and regulatory partners generating a much larger "interactome." At the center of this scheme is the classical PLN/SERCA-transport complex. HAX-1 binds to PLN and stabilizes its inhibitory interaction with SERCA2a. PLN also interacts with the RGL, the regulatory subunit of protein phosphatase 1 (PP1c), which anchors this enzyme along with its regulators inhibitor-1 (I-1) and Hsp20 to PLN/SERCA, thereby modulating SERCA2a function through PLN phosphorylation. SERCA2a is also regulated post-transcriptionally by the small ubiquitin-related modifier (SUMO-1), improving SERCA2a protein stability and activity. The histidine rich calcium binding protein (HRC) is a multi-functional protein that binds and inhibits SERCA2a as well as modulates RyR function through its interaction with triadin (TRI). The SR calcium release complex is comprised of RyR, TRI, junctin (JNC) and calsequestrin (CSQ).