

The Phospholamban Journey 4 Decades After Setting Out for Ithaka

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“As you set out for Ithaka, hope your voyage is a long one, full of adventure, full of discovery..... Keep Ithaka always in your mind. Arriving there is what you are destined for. But do not hurry the journey at all. Better if it lasts for years.....Ithaka gave you the marvelous journey. Without her you would not have set out.”

The quote above from the poem Ithaka by K. Kavafis is based on Homer’s Odyssey (8th century BC) and focuses on Odysseus’ journey to return to his kingdom in Ithaka (Greek: Ιθάκη, Ithaki) after the Troy war. Odysseus encounters numerous challenges, hardships, setbacks, but also beautiful new experiences, knowledge, and wisdom during this long voyage. Kavafis describes a journey that is both Odysseus’ and ours as we set out on a discovery voyage in our scientific careers. The poet urges us to live for the journey rather than the expected end point. It is the accumulated knowledge and wisdom through the years of scientific wandering and discovery that make our journey fun and enjoyable. Consistent with the poem’s theme, a journey to understand the functional role of phospholamban started over 40 years ago, on its identification in the heart. In this viewpoint, we present the lessons, challenges, shifting paradigms, controversies, and limitations of the phospholamban journey, which has been full of adventure and full of discovery!

Phospholamban Discovery

In the early 1970s, investigators were trying to determine whether the relaxation-promoting effects of catecholamines in the heart may also involve the sarcoplasmic reticulum (SR) function. Indeed, addition of cAMP (cyclic adenosine monophosphate) or PKA (protein kinase A) to crude microsomal vesicles increased calcium (Ca)-transport activity. Surprisingly, P³² labeling indicated that SERCA (sarcoplasmic reticulum Ca²⁺ ATPase 1) was not phosphorylated but another small protein of ≈22000 Da.¹ The identity of this phosphoprotein was not known, and discussions in Arnie Katz’s laboratory resulted in naming it phospholamban (PLN) from the Greek words: phosphorous and

lambano (receive). From that point on, several laboratories have been investigating the functional role of PLN and its regulatory effects on SERCA activity and heart function. We have learned a lot through the years, but the complexities of this simple interaction have been puzzling, as additional interacting partners have been discovered that link Ca-cycling through PLN/SERCA to both contractility and cell survival. Four decades later, we have a strong understanding of PLN, but there are still many unresolved questions, challenges, and controversies remaining.

PLN Function and Potential Therapeutic Promise

Detailed in vitro studies from several laboratories indicated that PLN regulates the rate-limiting steps in the SERCA2a activity. Initial studies focused on PLN phosphorylation by PKA at Ser16, whereas subsequent studies showed that PLN can also be phosphorylated by Ca-CAMKII (Ca²⁺-calmodulin-dependent protein kinase II) at Thr17. Phosphorylation of each site increases the Ca affinity of SERCA2a.² The first evidence on PLN phosphorylation in vivo was provided in 1982, using rabbit hearts perfused with [³²P]-orthophosphate and stimulated by isoproterenol. The ³²P-incorporation into phospholamban was associated with an increased rate of SR Ca uptake.³ Thus, PLN was called the stimulator of SERCA2a activity and cardiac function during β-adrenergic stimulation.

This notion on PLN’s role in the heart was challenged in the early 1990s. Genetically altered mouse models with reduced or ablated PLN expression (PLN-knock out) indicated that PLN is actually an inhibitor of the Ca affinity of SERCA under basal conditions, and inhibition is relieved on its phosphorylation during β-adrenergic stimulation.⁴ On the contrary, overexpression of PLN resulted in inhibition of SERCA2a and depressed contractility.⁵ Thus, there is a fraction of the SERCA pumps that is not functionally regulated by PLN, and this is ≈40% in mouse hearts. These lessons from mice were important in interpreting data from human and experimental heart failure (HF), which showed that SERCA levels diminish, whereas PLN levels do not get altered.⁶ Therefore, there is a greater fraction of SERCA pumps in the inhibited state by PLN in HF. In addition, the degree of PLN phosphorylation is decreased in HF, indicating an additional insult for the depressed function. Further characterization of transgenics with expression of PLN mutations confirmed its key role in cardiac function, and detailed structural/functional studies by several laboratories provided key insights into the mechanisms of SERCA2a regulation by PLN.^{7,8}

Test the PLN Promise: Good or Bad?

Several pharmaceutical companies launched efforts to identify a PLN inhibitor, but none of the identified molecules seemed specific for cardiac SERCA at efficacious doses. In parallel,

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academic laboratories tested the hypothesis that PLN ablation could rescue cardiac dysfunction and pathological remodeling by breeding strategies of various cardiomyopathy mouse models. Some studies clearly demonstrated the beneficial effects of improved SR Ca cycling through PLN ablation on cardiac function and remodeling, whereas others showed that normalization of myocyte Ca handling may not translate into improved cardiac function in vivo or into reversal of remodeling. This may be expected because rescue of the depressed SR function may represent only one of the multiple modulators in hypertrophic responses. In addition, genetic ablation of PLN is associated with several cellular adaptations to accommodate the enhanced Ca²⁺ cycling and energetic demand, which may further compound on the HF phenotype of genetic models. Nevertheless, these studies dampened the initial enthusiasm on PLN ablation as a therapeutic modality. Further support on the importance of PLN was provided by demonstrating rescue of a rat model of HF using RNA interference of PLN.⁹

The notion on targeting PLN in heart disease was further challenged by the identification of a human PLN-null mutation in HF patients. Interestingly, the first 2 human PLN mutations were simultaneously identified, but they were associated with opposite subcellular mechanisms or PLN actions. One of them, R9C, resulted in chronic inhibition of SERCA2a and early death in heterozygous carriers.¹⁰ However, the other mutation was associated with loss of PLN function (L39stop) and resulted in dilated cardiomyopathy (DCM) and premature death in the homozygous state.¹¹ This finding in human was puzzling as PLN ablation in mice resulted in hyperdynamic cardiac function that persisted through the aging process. Importantly, these studies clearly revealed the limitations in our understanding of the role of PLN in human heart and further challenged its potential role as a therapeutic target. One explanation is that the function of PLN is different between mouse and human hearts, and studies in mice have only partially contributed to the functional understanding of this protein in higher mammalian species. Alternatively, the PLN function may be similar between mice and human, but PLN mutations that are deleterious for humans are not necessarily so for mice. This may be because of the differences in cardiac reserve and regulation of Ca balance in the cardiomyocytes of the 2 species. Another explanation that is gaining acceptance is that the loss-of-function PLN mutations produce modified PLNs that traffic abnormally in the cell and cause damage by aberrant interactions with intracellular proteins. In addition, several differences underlie Ca-cycling regulation in human and mice.

Recently, more PLN mutations have been identified that are associated with an arrhythmogenic cardiomyopathy. The PLN R14del is characterized by a deletion of arginine 14 in the *PLN* gene. It was first discovered in a large Greek family with hereditary DCM. DCM patients heterozygous for the R14del mutation exhibit biventricular dilation, dysfunction, and ventricular arrhythmias.¹² A larger cohort has been identified in the Netherlands, where R14del carriers are at high risk for malignant ventricular arrhythmias and end-stage HF.¹³ A new mutation (R25C) in the human *PLN* gene has also been identified in a subset of DCM patients with ventricular arrhythmias and the need for implantable cardiac defibrillators. These known

phospholamban mutations are appearing more frequently in recent years, as genetic studies expand around the world. Despite their frequent appearance, the exact mechanisms by which the mutated PLN proteins cause cardiomyopathy or arrhythmias remain largely unknown. The abnormal relationship of phospholamban to SERCA2a in these disease states offers only a small part of the explanation. The mutant PLN protein exerts pathological effects by associating incorrectly and indiscriminately with other proteins in cardiomyocytes. However, it is not clear why some mutations are associated with contractile dysfunction whereas others with ventricular arrhythmias.

Complexities and Challenges: An Evolving PLN-Protein Interactome

Testing PLN as a potential therapeutic modality has proven to be far more complicated than originally thought because further investigation revealed that PLN does not act alone, but it forms a multimeric complex with several interacting partners¹⁴ (Table). PLN recruits the antiapoptotic protein HAX-1 (HCLS1-associated protein X-1), and this actually increases inhibition of SERCA. In addition, HAX-1 recruits the small Hsp90 (heat shock protein 90) from the endoplasmic reticulum to the PLN/SERCA2a ensemble, suggesting a functional coupling between endoplasmic reticulum stress signaling elements and calcium homeostasis. PLN also binds to Gm (muscle glycogen-targeting subunit of protein phosphatase 1), the anchoring subunit of protein phosphatase 1 (PP1), and AKAP (AKAP7γ/δ; AKAP15; AKAP18δ), the anchoring subunit of PKA, allowing for fine-tuning of the PLN phosphorylation status and, thus, SERCA activity. Moreover, PP1 interacts with the endogenous inhibitor-1 and the small Hsp20, and both of these are regulators of PP1 activity and Ca cycling (Table). Indeed, human genetic variants of inhibitor-1 (G109E) or Hsp20 (P20L) result in reduced binding and inhibition of PP1, suggesting aberrant enzymatic regulation of PLN activity in human carriers. Importantly, PKA phosphorylation of inhibitor-1 or Hsp20 increases PLN phosphorylation and contractility in cardiomyocytes. Interestingly, the levels of Hsp20 and its phosphorylation are significantly increased during ischemia/reperfusion and HF, which may represent a compensatory response to cardiac stress. The enhanced phosphorylation and activation of

Table. Functional Effects of PLN Partners

PLN Partners	Effects
SERCA2a	PLN inhibits SERCA2a and contractility
HAX-1	↑ PLN inhibition
HAX-1/Hsp90	↑↑ PLN inhibition
Gm/PP1	Dephosphorylates PLN; ↑ PLN inhibition
Gm/PP1/I-1/Hsp20	Inhibits PP1; ↓ PLN inhibition
AKAP/PKA	↑ P-PLN (S16); ↓ PLN inhibition
CAMKII	↑ P-PLN (T17); ↓ PLN inhibition

AKAP indicates protein kinase A anchoring protein (AKAP7δ/γ; AKAP15; AKAP18 δ); Gm, muscle glycogen-targeting subunit of PP1; I-1, inhibitor 1 of PP1; PKA, protein kinase A; PLN, phospholamban; PP1, protein phosphatase 1; and P-PLN, phosphorylated PLN.

Hsp20 concomitant with decreased phosphorylation and inactivation of inhibitor-1 in the same failing hearts reflect the complexities underlying PLN regulation in macromolecular complexes. Thus, any perturbations in either the levels or activity of PLN, postulated to hold therapeutic promise in HF, will impact altered regulation of Ca cycling and cell death through this fine-tuned protein network.

Challenges, Unresolved Questions, and Future Perspective

Although we have learnt a lot through the past 4 decades about PLN function in cardiac physiology and pathology, we are still facing several unresolved and key basic questions that hamper our in-depth understanding of this protein. For example, we do not currently know (1) the stoichiometry of SERCA to PLN in human heart; (2) the degree of PLN phosphorylation under nonstimulated conditions; (3) whether PLN phosphorylation is altered on a beat-to-beat basis; (4) whether PLN decreases are beneficial or detrimental in human hearts under physiological or pathological conditions; and (5) the role of PLN partners under stress or HF conditions.

In addition, it is now evident that PLN regulation involves a multimeric regulatome, and the consequences of targeting PLN in HF are not clear. Attempts to reduce PLN levels or activity may also eliminate part of the HAX-1 and Hsp90 benefits, as these PLN partners are cardioprotective and their recruitment to SR/endoplasmic reticulum may be especially important under stress conditions. In addition, targeting PLN may disrupt the Gm/PP1/inhibitor-1 and Hsp20 complex and diminish regulation of Ca cycling. More recently, micropeptides that regulate SERCA activity embedded within RNA transcripts (annotated as long noncoding RNAs) have been discovered. The relationship of these novel micropeptides to PLN and how they regulate SERCA is unknown and adds to the complexity of calcium regulation.

The ability to generate patient-specific human induced pluripotent stem cells (iPSCs) offers new opportunities to study the underlying mechanisms of PLN-associated pathologies. Recently, cardiomyocytes derived from iPSC of patients with PLN R14del displayed a strong arrhythmogenic phenotype, whereas engineered tissues using these cardiomyocytes demonstrated a significant decrease in force production.¹⁵ The iPSCs also offer the opportunity to correct the phenotype by genome editing. In addition, the possibility of inserting the disease genes in various iPSC lines may allow us to assess whether known genetic mutations or polymorphisms (introduced or embedded in these iPSC lines) amplify or suppress the impact of phospholamban mutations.

Journey to Ithaca Continues

Over 40 years of studies on PLN and the scientific quest for knowledge are nicely reflected in the poem Ithaca. Every single one of our discoveries brought us to a new harbor with surprising views and new knowledge, which prompted us to challenge and rethink our original plan for the journey but also excited us to continue the search for Ithaca. We have not arrived at Ithaca (understanding PLN function) yet, but the enjoyment of investigation and the marvelous experience of the journey are all that we can ask for.

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Disclosures

None.

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