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The Phospholamban Journey Four 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 ' journey to return to his kingdom in Ithaka (: 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, upon 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 function. Indeed, addition of cAMP or PKA to crude microsomal vesicles increased Ca-transport activity. Surprisingly, P^{32}-labeling indicated that SERCA was not phosphorylated but

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another small protein of ~ 22,000 Dalton[1]. The identity of this phosphoprotein was not known and discussions in Arnie Katz's lab 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 while subsequent studies showed that PLN can be also phosphorylated by Ca-CAMKII 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 [^{32}P]-orthophosphate and simulated by isoproterenol. The ^{32}P-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 B-adrenergic (B-AR) 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-KO) indicated that PLN is actually an inhibitor of the Ca-affinity of SERCA under basal conditions and inhibition is relieved upon its phosphorylation during B-AR stimulation[4]. On the other hand, 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 while 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 appeared specific for cardiac SERCA at efficacious doses. In parallel, academic labs tested the hypothesis that PLN ablation could rescue cardiac dysfunction and pathologic 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, while 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 since 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 a number of cellular adaptations to accommodate the enhanced Ca^{2+}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 two human PLN mutations were simultaneously identified but they were associated with opposite “sub-cellular 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 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 due to the differences in cardiac reserve and regulation of Ca-balance in the cardiomyocytes of the two 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 bi-ventricular 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 while 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 since further investigation revealed that PLN does not act alone but it forms a multimeric complex with several interacting partners[14] (Table 1). PLN recruits the anti-apoptotic protein HAX-1 and this actually increases inhibition of SERCA. In addition, HAX-1 recruits the small Hsp90 from the endoplasmic reticulum to the PLN/SERCA2a ensemble, suggesting a "functional coupling" between ER stress signaling elements and calcium homeostasis. PLN also binds to Gm, the anchoring subunit of protein phosphatase 1 (PP1), as well as 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 (I-1) and the small Hsp20 and both of these are regulators of PP1 activity and Ca-cycling (Table 1). 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 heart failure, which may represent a compensatory response to cardiac stress. The enhanced phosphorylation and activation of 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 heart failure, 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 four decades regarding 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: a) the stoichiometry of SERCA to PLN in human heart; b) the degree of PLN phosphorylation under non-stimulated conditions; c) whether PLN phosphorylation is altered on a beat to beat basis; d) whether PLN decreases are beneficial or detrimental in human hearts under physiological or pathological conditions; and e) the role of PLN partners under stress or heart failure 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/ER may be especially important under stress conditions. In addition, targeting PLN may disrupt the Gm/PP1/I-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 while 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.

The Journey to Ithaka Continues

Over forty years of studies on PLN and the scientific quest for knowledge are nicely reflected in the poem “ITHAKA”. 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 Ithaka. We have not arrived at Ithaka (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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Nonstandard abbreviations and acronyms

Gm	Muscle Glycogen-Targeting Subunit of Protein Phosphatase 1
DCM	dilated cardiomyopathy
HAX-1	HCLS1 Associated Protein X-1
Hsp	heat shock protein
iPSCs	induced pluripotent stem cells

References

1. Tada M, Kirchberger MA, Katz AM. Phosphorylation of a 22,000-dalton component of the cardiac sarcoplasmic reticulum by adenosine 3′:5′-monophosphate-dependent protein kinase. J Biol Chem. 1975; 250:2640–2647. [PubMed: 235523]
2. Simmerman HKB, Jones LR. Phospholamban: Protein Structure, Mechanism of Action, and Role in Cardiac Function. Physiol Rev. 1998; 78:921–947. [PubMed: 9790566]
3. Kranias EG, Solaro RJ. Phosphorylation of troponin I and phospholamban during catecholamine stimulation of rabbit heart. Nature. 1982; 298:182–184. [PubMed: 6211626]
4. Luo W, Grupp IL, Harrer J, Ponniah S, Grupp G, Duffy JJ, Doetschman T, Kranias EG. Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and loss of beta-agonist stimulation. Circ Res. 1994; 75:401–409. [PubMed: 8062415]

5. Kadambi VJ, Ponniah S, Harrer JM, Hoit BD, Dorn GW, Walsh RA, Kranias EG. Cardiac-specific overexpression of phospholamban alters calcium kinetics and resultant cardiomyocyte mechanics in transgenic mice. J Clin Invest. 1996; 97:533–539. [PubMed: 8567978]

6. Meyer M, Schillinger W, Pieske B, Holubarsch C, Heilmann C, Posival H, Kuwajima G, Mikoshiba K, Just H, Hasenfuss G. Alterations of Sarcoplasmic Reticulum Proteins in Failing Human Dilated Cardiomyopathy. Circulation. 1995; 92:778 LP-784. [PubMed: 7641356]

7. Akin BL, Hurley TD, Chen Z, Jones LR. The structural basis for phospholamban inhibition of the calcium pump in sarcoplasmic reticulum. J Biol Chem. 2013; 288:30181–30191. [PubMed: 23996003]

8. Kimura Y, Kurzydlowski K, Tada M, MacLennan DH. Phospholamban Regulates the Ca2+-ATPase through Intramembrane Interactions. J Biol Chem. 1996; 271:21726–21731. [PubMed: 8702967]

9. Suckau L, Fechner H, Chemaly E, Krohn S, Hadri L, Kockskämper J, Westermann D, Bisping E, Ly H, Wang X, Kawase Y, Chen J, Liang L, Sipo I, Vetter R, Weger S, Kurreck J, Erdmann V, Tschope C, Pieske B, Lebeche D, Schultheiss H-P, Hajjar RJ, Poller WC. Long-Term Cardiac-Targeted RNA Interference for the Treatment of Heart Failure Restores Cardiac Function and Reduces Pathological Hypertrophy. Circulation. 2009; 119:1241 LP-1252. [PubMed: 19237664]

10. Schmitt JP, Kamisago M, Asahi M, Li GH, Ahmad F, Mende U, Kranias EG, MacLennan DH, Seidman JG, Seidman CE. Dilated Cardiomyopathy and Heart Failure Caused by a Mutation in Phospholamban. Science. 2003; 299:1410 LP-1413. [PubMed: 12610310]

11. Haghighi K, Kolokathis F, Pater L, Lynch RA, Asahi M, Gramolini AO, Fan G-C, Tsiapras D, Hahn HS, Adamopoulos S, Liggett SB, D GW II, MacLennan DH, Kremastinos DT, Kranias EG. Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human. J Clin Invest. 2003; 111:869–876. [PubMed: 12639993]

12. Haghighi K, Kolokathis F, Gramolini AO, Waggoner JR, Pater L, Lynch RA, Fan G-C, Tsiapras D, Parekh RR, Dorn GW, MacLennan DH, Kremastinos DT, Kranias EG. A Mutation in the Human Phospholamban Gene, Deleting Arginine 14, Results in Lethal, Hereditary Cardiomyopathy. Proc Natl Acad Sci U S A. 2006; 103:1388–1393. [PubMed: 16432188]

13. van der Zwaag PA, van Rijsingen IAW, Asimaki A, Jongbloed JDH, van Veldhuisen DJ, Wiesfeld ACP, Cox MGPJ, van Lochem LT, de Boer RA, Hofstra RMW, Christiaans I, van Spaendonck-Zwarts KY, Lekanne dit Deprez RH, Judge DP, Calkins H, Suurmeijer AJH, Hauer RNW, Saffitz JE, Wilde AAM, van den Berg MP, van Tintelen JP. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. Eur J Heart Fail. 2012; 14:1199–1207. [PubMed: 22820313]

14. Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. Circ Res. 2012; 110:1646–1660. [PubMed: 22679139]

15. Karakikes I, Stillitano F, Nonnenmacher M, Tzimas C, Sanoudou D, Termglinchan V, Kong C-W, Rushing S, Hansen J, Ceholski D, Kolokathis F, Kremastinos D, Katoulis A, Ren L, Cohen N, Gho JMIH, Tsiapras D, Vink A, Wu JC, Asselbergs FW, Li RA, Hulot J-S, Kranias EG, Hajjar RJ. Correction of human phospholamban R14del mutation associated with cardiomyopathy using targeted nucleases and combination therapy. Nat Commun. 2015; 6:6955. [PubMed: 25923014]

TABLE 1

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 or Hsp20	Inhibits PP1; ↓ PLN inhibition
AKAP / PKA	↑ P-PLN (S16); ↓ PLN inhibition
CAMKII	↑ P-PLN (T17); ↓ PLN inhibition

Gm: muscle glycogen targeting subunit of PP1; PP1: protein phosphatase 1; P-PLN: phosphorylated PLN; AKAP: protein kinase A anchoring protein (AKAP7δ/γ; AKAP15; AKAP18 δ); I-1: Inhibitor 1 of PP1