Re-evaluating sarcoplasmic reticulum function in heart failure

To the editor—Heart failure presently afflicts an estimated 5 million Americans with a 4 year mortality rate approaching 50% (ref. 1). The failing human heart transitions into a dilated state characterized by ventricular remodeling, collagen deposition and reduced contractility. The molecular etiology of this phenotype is closely associated with reduced calcium transients and prolongation of diastolic calcium re-sequestration leading to decreased cardiac contractility[2]. Critical intracellular regulators of calcium cycling within cardiac myocytes are the sarcoplasmic reticulum associated calcium ATPase pump, SERCA2, and its negative regulator phospholamban (PLB). The deterioration in calcium cycling characteristic of the failing human heart has been largely associated with a reduction in SERCA2 activity resulting from reduced protein concentrations or from enhanced inhibition mediated by PLB (ref. 2). Indeed, targeted ablation of PLB in the mouse allows unrestricted SERCA2 activity resulting in a dramatic increase in baseline calcium cycling and enhanced contractility in the unstimulated state[2].

A recent study by Minamisawa *et al.* used PLB knockout mice to investigate the causality between reduced SERCA2 activity and the initiation and propagation of dilated cardiomyopathy[3]. Ablation of the PLB gene was sufficient to rescue the dilated cardiomyopathic phenotype of the muscle lim protein (MLP) knockout mouse[3]. This observation suggested that dilated cardiomyopathy can be rescued by enhancing intracellular calcium cycling and that deterioration in calcium handling kinetics play a primary role in the pathogenesis of heart failure.

In contrast, our recent studies indicate that this postulate may not extend to all forms of dilated cardiomyopathy. Using a similar approach, we crossed PLB knockout mice with transgenic mice over-expressing the sarcomeric structural protein tropomodulin[4]. The tropomodulin over-expressing transgenic (TOT) mouse model of dilated cardiomyopathy shows many characteristics of human heart failure[4,5]. We observed that disruption of the PLB gene failed to rescue the overt dilated phenotype of TOT mice (Fig. 1*a*), nor did it rescue the increase in heart-to-body weight ratio characteristic of the TOT phenotype (Fig. 1*b*). Last, ablation of the PLB gene also failed to rescue juvenile lethality associated with tropomodulin over-expression, as 9 of 21 mice died by 28 days of life, comparable with reported levels of TOT mortality[5].

These data indicate that augmented calcium cycling may not benefit all forms of heart failure, especially if impaired intracellular calcium cycling is not associated with the end phenotype. Indeed, cardiac myocytes from TOT mice are actually characterized by enhanced calcium transients, despite the dilated cardiomyopathic state[5]. Minamisawa *et al.* proposed that PLB ablation rescued the pathology associated with the MLP gene disruption by a mechanism involving augmented calcium cycling resulting in greater cardiac contractility and reduced ventricular wall stress. This interpretation is further supported by the results of Rockman *et al.* in which enhanced contractility mediated by the βARKct transgene rescued certain aspects of the cardiomyopathic MLP phenotype[6]. Collectively, these data suggest that enhanced intracellular calcium cycling and the associated increase in cardiac contractility may benefit certain forms of heart failure. Our results, however, suggest that augmentation of cardiac calcium cycling may not benefit all forms of dilated heart failure. These results also underscore the potential complexity of associated molecular defects that may underlie human heart failure.

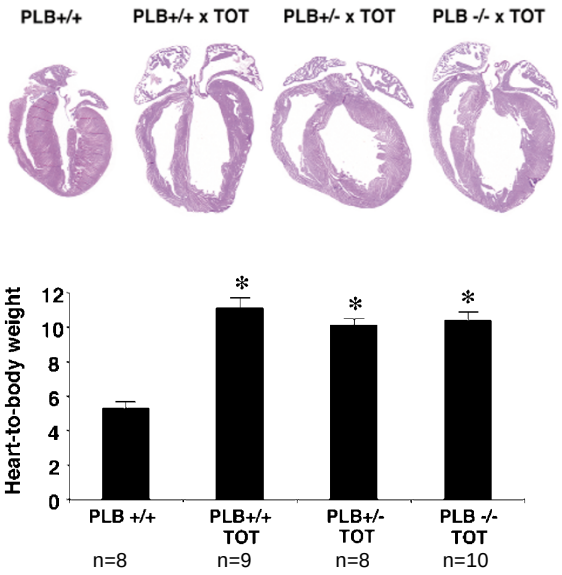

Fig. 1 ***a,*** Hematoxylin and eosin-stained histological sections demonstrate dilated cardiomyopathy induced by the tropomodulin transgene (TOT) which is not rescued by phospholamban ablation (PLB). Animals were analyzed at 22 days after birth, although phenotypic analysis at day 18 or 28 also failed to demonstrate any protective effect of PLB ablation. ***b,*** PLB ablation failed to protect TOT mouse hearts from increased heart-to-body weight ratio associated with the dilated cardiomyopathy. *$P < 0.05$ versus $PLB^{+/+}$

ULRIKE DELLING, MARK A. SUSSMAN, & JEFFERY D. MOLKENTIN
Division of Molecular Cardiovascular Biology
Children's Hospital Medical Center
Cincinnati, Ohio 45229
Email: molkj0@chmcc.org

Chien replies—Several complex signaling pathways can lead to dilated cardiomyopathy and heart failure (for review, see refs. 7,8). As noted in the letter, the ablation of phospholamban can completely arrest the progression of heart failure in a gene targeted mouse model of dilated cardiomyopathy[3], based on a deficiency in the muscle specific cytoskeletal protein, MLP. This model displays many conserved features of the human disease, and provided the first evidence that dilated cardiomyopathies might arise from mutations in Z disk components, a finding confirmed in human studies (for review, see ref. 9). However, cardiomyopathy is a heterogeneous disorder with diverse etiologies. Molkentin and colleagues appropriately argue for

caution, noting that defects in calcium cycling might not represent a universal pathway for dilated cardiomyopathy. Their results clearly indicate that phospholamban gene ablation cannot rescue the dilated cardiomyopathy that accompanies tropomodulin over-expression, using several independent endpoints, including survival.

The validity of their over-expressing tropomodulin mouse to human disease, however, is also an issue. Although one could argue that the MLP deficiency model reflects a subset of human disease that arises due to cytoskeletal mutations, the clinical relevance of the tropomodulin over-expression heart failure model to naturally occurring forms of human cardiomyopathy seems more tenuous. Most likely, the massive over-expression of tropomodulin produces cardiac injury and disruption of the stoichiometry that is required for normal sarcomeric assembly. Dilated cardiomyopathy results as a secondary effect, which, of course, would not be reflected in genetic or acquired human disease. Over 30 models of dilated cardiomyopathy have been reported through the cardiac over-expression of reporters (GFP) (ref. 10), signaling molecules, and channels to the amounts of alpha myosin heavy chain, raising the serious issue of the validity of any of these models to human disease[11].

Independent evidence has now confirmed and extended the results in the MLP model, documenting a critical role of calcium cycling defects in acquired forms of heart failure due to chronic pressure overload in normal animals[12]. Thus, calcium cycling defects may underlie some clinically relevant acquired forms of heart failure. Further work will be required to determine whether phospholamban inhibition will be beneficial after myocardial infarction, the most frequent etiology of human heart failure.

KENNETH R. CHIEN
University of California, San Diego-Salk Program in Molecular Medicine, Institute of Molecular Medicine
La Jolla, California 92093
Email: kchien@ucsd.edu

1. Ho, K.K., Pinsky, J.L., Kannel, W.B. & Levy, D. The epidemiology of heart failure: the Framingham Study. *J. Am. Coll. Cardiol*. **22**, 6–13 (1993).
2. Kiriazis. H., & Kranias, E.G. Genetically engineered models with alterations in cardiac membrane calcium-handling proteins. *Annu. Rev. Physiol*. **62**, 321–351 (2000).
3. Minamisawa, S., *et al.* Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell*. **99**, 313–322 (1999).
4. Sussman, M.A., *et al.* Myofibril degeneration caused by tropomodulin overexpression leads to dilated cardiomyopathy in juvenile *J. Clin. Invest*. **101**, 51–61 (1998).
5. Sussman, M.A., *et al.* Pathogenesis of dilated cardiomyopathy: molecular, structural, and population analyses in tropomodulin-overexpressing transgenic mice. *Am. J. Pathol*. **155**, 2101–2113 (1999).
6. Rockman, H.A., *et al.* Expression of a beta-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. *Proc. Natl. Acad. Sci. USA*. **95**, 7000–7005 (1998).
7. Chien, K.R. Stress pathways and heart failure. *Cell* 98, 555–558 (2000).
8. Olson, E.N. & Williams, R.S. Calcineurin signaling and muscle remodeling. *Cell* **101**, 689–692 (2000).
9. Chien, K.R. Genomic circuits and the integrative biology of complex cardiac diseases. *Nature*, in press, 2000.
10. Huang, WY *et al*. Transgenic expression of green fluorescence protein can cause dilated cardiomyopathy. *Nature Med*. **6**, 482–483 (2000).
11. Chien, K.R. Meeting Koch's postulates for calcium signaling in cardiac hypertrophy. *J. Clin. Invest*. **105**, 1339–1342 (2000).
12. Miyamoto, M. E. *et al*., Adenoviral gene transfer of SERCA2a improves left ventricular function in aortic-banded rats in transition to heart failure. *Proc. Natl. Acad. Sci. USA* **97**, 793–798 (2000).