Although the quest to translate understanding at the molecular level to the prevention, diagnosis, and therapy of disorders of the heart has been ongoing in many laboratories for many years, the recently popular term “translational medicine” is useful to convey the reality of this objective to our leaders in government, industry, and the academy. In my judgment there is no better example of translational medicine than the identification of sarcomeric mutations linked to cardiomyopathies and sudden death. These myopathies identified by linkage analysis were the first cardiac conditions to be understood at the molecular level of organization.1 “Translation” requires determination of the primary effect of the mutation on function at the level of the cardiac sarcomeres and the cells, and integration of these findings at the level of the organ and the organism. This knowledge sets the stage for rational diagnosis, prevention, and therapy.

An extensive analysis of this sort in a mouse model has been performed by Du et al2 and is reported in the present issue. The focus of their work was on troponin T (TnT), a thin filament protein, which, together with troponin-I, troponin-C, and tropomyosin, imposes Ca2+-regulation on the actin-myosin reaction.3 Du et al generated a knock-in mouse model of a deletion mutant of TnT, TNNT2K210H11001K210, which had been linked to DCM.1 TnT-K210 is localized in a critical region of TnT that has multiple interactions with its neighbors on the thin filament and is critical for regulation by Ca2+.3 The transgenic mice demonstrated a DCM phenotype similar to that documented in patients with this mutation. There was no evidence of cellular or myofibrillar disarray, a feature of other myopathies,2 but there was an increase in interstitial fibrosis. Earlier in vitro studies had indicated that replacement of native TnT with a mutant TnT missing K210 induced a depression in sarcomeric response to Ca2+.4,5 Force generation by skinned fiber bundles (detergent extracted, membrane free strands of ventricular myocytes) isolated from the TNNT2K210mice also frequently died suddenly because of an abrupt induction of ‘Torsade de Pointes’ or long QT without overt heart failure symptoms. A significant question in this area of investigation is how a primary molecular alteration in a sarcomeric protein leads to these electrical abnormalities. Does the mutation lead to cellular abnormalities that trigger arrhythmias, or does it produce an arrhythmogenic substrate? Unfortunately, there are but a few studies that have performed relevant determinations of myocyte Ca2+-transients, action potentials, optical mapping for determination of spread of the action potentials, and vulnerability to tachycardias. Yet a reasonable conclusion from studies done so far is that altered cellular Ca2+ fluxes, either increased or decreased, link sarcomeric mutations to arrhythmias in DCM and HCM. There was an increase in the peak amplitude of the Ca2+ transient in myocytes isolated from hearts of the TNNT2K210 mice, associated with increased phosphorylation of ryanodine receptors and phospholamban (PLB). However, a decrease in Ca2+ transients and depressed decay kinetics are present in myocytes expressing the mutant TnT(I79N), which is classified as linked to HCM but in which there is little fibrosis and hypertrophy.5 These mice show a propensity to ventricular ectopy and nonsustained ventricular tachycardia in freely moving mice. Evidence generated by thorough study of excitation-contraction coupling in the TnT(I79N) model showed remodeling of the action potential profile, which was attributed to the alterations in the Ca2+ transients and to a suppression of IKr.6 Similar depressions in Ca2+ kinetics have been reported in myocytes expressing the HCM linked mutant, TnT(Δ160E).7 These altered Ca2+ fluxes appear to arise in part from altered expression of phospholamban and SERCA 2a. Although myocyte remodeling and phosphorylation of proteins regulating Ca2+ flows in the myocytes appears to be an important mechanism, altered buffering of Ca2+ by troponin C should be included in the possible mechanisms leading to the arrhythmias, especially in association with ischemia. An explicit test of the role of sarcomeric Ca2+ in triggered arrhythmias and Ca2+ waves strongly indicates that length changes of sarcomeres in the zone between normal and ischemic tissue induces Ca2+ waves triggered by a release of Ca2+ from troponin-C.8 Although not investigated extensively in the majority of studies on models of HCM and DCM, there is strong evidence that the changes in troponin Ca2+ binding are likely to occur in association with the altered myofilament response to Ca2+.9

**Translational Medicine With a Capital T, Troponin T, That Is**

R. John Solaro

Although the quest to translate understanding at the molecular level to the prevention, diagnosis, and therapy of disorders of the heart has been ongoing in many laboratories for many years, the recently popular term “translational medicine” is useful to convey the reality of this objective to our leaders in government, industry, and the academy. In my judgment there is no better example of translational medicine than the identification of sarcomeric mutations linked to cardiomyopathies and sudden death. These myopathies identified by linkage analysis were the first cardiac conditions to be understood at the molecular level of organization.1 “Translation” requires determination of the primary effect of the mutation on function at the level of the cardiac sarcomeres and the cells, and integration of these findings at the level of the organ and the organism. This knowledge sets the stage for rational diagnosis, prevention, and therapy. An extensive analysis of this sort in a mouse model has been performed by Du et al2 and is reported in the present issue. The focus of their work was on troponin T (TnT), a thin filament protein, which, together with troponin-I, troponin-C, and tropomyosin, imposes Ca2+-regulation on the actin-myosin reaction.3 Du et al generated a knock-in mouse model of a deletion mutant of TnT, TNNT2K210H11001K210, which had been linked to DCM.1 TnT-K210 is localized in a critical region of TnT that has multiple interactions with its neighbors on the thin filament and is critical for regulation by Ca2+.3 The transgenic mice demonstrated a DCM phenotype similar to that documented in patients with this mutation. There was no evidence of cellular or myofibrillar disarray, a feature of other myopathies,2 but there was an increase in interstitial fibrosis. Earlier in vitro studies had indicated that replacement of native TnT with a mutant TnT missing K210 induced a depression in sarcomeric response to Ca2+.4,5 Force generation by skinned fiber bundles (detergent extracted, membrane free strands of ventricular myocytes) isolated from the TNNT2K210mice also frequently died suddenly because of an abrupt induction of ‘Torsade de Pointes’ or long QT without overt heart failure symptoms. A significant question in this area of investigation is how a primary molecular alteration in a sarcomeric protein leads to these electrical abnormalities. Does the mutation lead to cellular abnormalities that trigger arrhythmias, or does it produce an arrhythmogenic substrate? Unfortunately, there are but a few studies that have performed relevant determinations of myocyte Ca2+-transients, action potentials, optical mapping for determination of spread of the action potentials, and vulnerability to tachycardias. Yet a reasonable conclusion from studies done so far is that altered cellular Ca2+ fluxes, either increased or decreased, link sarcomeric mutations to arrhythmias in DCM and HCM. There was an increase in the peak amplitude of the Ca2+ transient in myocytes isolated from hearts of the TNNT2K210 mice, associated with increased phosphorylation of ryanodine receptors and phospholamban (PLB). However, a decrease in Ca2+ transients and depressed decay kinetics are present in myocytes expressing the mutant TnT(I79N), which is classified as linked to HCM but in which there is little fibrosis and hypertrophy.5 These mice show a propensity to ventricular ectopy and nonsustained ventricular tachycardia in freely moving mice. Evidence generated by thorough study of excitation-contraction coupling in the TnT(I79N) model showed remodeling of the action potential profile, which was attributed to the alterations in the Ca2+ transients and to a suppression of IKr.6 Similar depressions in Ca2+ kinetics have been reported in myocytes expressing the HCM linked mutant, TnT(Δ160E).7 These altered Ca2+ fluxes appear to arise in part from altered expression of phospholamban and SERCA 2a. Although myocyte remodeling and phosphorylation of proteins regulating Ca2+ flows in the myocytes appears to be an important mechanism, altered buffering of Ca2+ by troponin C should be included in the possible mechanisms leading to the arrhythmias, especially in association with ischemia. An explicit test of the role of sarcomeric Ca2+ in triggered arrhythmias and Ca2+ waves strongly indicates that length changes of sarcomeres in the zone between normal and ischemic tissue induces Ca2+ waves triggered by a release of Ca2+ from troponin-C.8 Although not investigated extensively in the majority of studies on models of HCM and DCM, there is strong evidence that the changes in troponin Ca2+ binding are likely to occur in association with the altered myofilament response to Ca2+.9

**Induction of Arrhythmias and Sudden Death**

TNNT2K210H11001 mice also frequently died suddenly because of an abrupt induction of ‘Torsade de Pointes’ or long QT without overt heart failure symptoms. A significant question in this area of investigation is how a primary molecular alteration in a sarcomeric protein leads to these electrical abnormalities. Does the mutation lead to cellular abnormalities that trigger arrhythmias, or does it produce an arrhythmogenic substrate? Unfortunately, there are but a few studies that have performed relevant determinations of myocyte Ca2+-transients, action potentials, optical mapping for determination of spread of the action potentials, and vulnerability to tachycardias. Yet a reasonable conclusion from studies done so far is that altered cellular Ca2+ fluxes, either increased or decreased, link sarcomeric mutations to arrhythmias in DCM and HCM. There was an increase in the peak amplitude of the Ca2+ transient in myocytes isolated from hearts of the TNNT2K210 mice, associated with increased phosphorylation of ryanodine receptors and phospholamban (PLB). However, a decrease in Ca2+ transients and depressed decay kinetics are present in myocytes expressing the mutant TnT(I79N), which is classified as linked to HCM but in which there is little fibrosis and hypertrophy.5 These mice show a propensity to ventricular ectopy and nonsustained ventricular tachycardia in freely moving mice. Evidence generated by thorough study of excitation-contraction coupling in the TnT(I79N) model showed remodeling of the action potential profile, which was attributed to the alterations in the Ca2+ transients and to a suppression of IKr.6 Similar depressions in Ca2+ kinetics have been reported in myocytes expressing the HCM linked mutant, TnT(Δ160E).7 These altered Ca2+ fluxes appear to arise in part from altered expression of phospholamban and SERCA 2a. Although myocyte remodeling and phosphorylation of proteins regulating Ca2+ flows in the myocytes appears to be an important mechanism, altered buffering of Ca2+ by troponin C should be included in the possible mechanisms leading to the arrhythmias, especially in association with ischemia. An explicit test of the role of sarcomeric Ca2+ in triggered arrhythmias and Ca2+ waves strongly indicates that length changes of sarcomeres in the zone between normal and ischemic tissue induces Ca2+ waves triggered by a release of Ca2+ from troponin-C.8 Although not investigated extensively in the majority of studies on models of HCM and DCM, there is strong evidence that the changes in troponin Ca2+ binding are likely to occur in association with the altered myofilament response to Ca2+.9

**DCM, HCM, and Sarcomeric Protein Posttranslational Modifications**

In assessing the functional effects of mutations in sarcomeric proteins, there is now considerable evidence to indicate the importance of determination of posttranslational modifications that may worsen or lessen the effect of the mutation. A
particularly significant site close to K210 is Thr 206, which, when phosphorylated by protein kinase-C, induces a severe depression in sarcomeric response to Ca\(^{2+}\). Unpublished data from our laboratory indicate transient increases in the phosphorylation of this site in the transition of the spontaneously hypertensive rat to heart failure. Although there were measurements made in hearts of TNNT2\(^{AK210/AK210}\) mice regarding changes in troponin I phosphorylation using antibodies that detect Ser 23/24 phosphorylation, there was no detailed phospho-proteomic analysis of alterations in other sites on troponin-I and on sites in troponin-T. This leaves open the question as to whether these and other sites may have undergone altered phosphorylation. Another post-translational modification not fully investigated involves proteolysis associated with activation of caspases. Du et al note apoptosis as a frequent finding. Troponin-T is a robust substrate for caspase 3 as are actin and alpha-actinin.

**Rescue of the TNNT2\(^{AK210/AK210}\) Mice With an Activator of Sarcomeric Response to Ca\(^{2+}\)**

An exciting and potentially far-reaching aspect of the study by Du et al is their investigation of the effects of pimobendan on DCM in the TNNT2\(^{AK210/AK210}\) mice. Pimobendan was the first agent with documented combined activity as a phosphodiesterase III (PDE III) inhibitor and as a Ca\(^{2+}\)-sensitizer to make it into clinical use. Pimobendan increases the response of skinned fiber bundles to Ca\(^{2+}\), in a stereoselective manner by a mechanism involving enhanced binding of Ca\(^{2+}\) to troponin. Treatment of the TNNT2\(^{AK210/AK210}\) mice with pimobendan, but not amrinone (a pure PDE III inhibitor) or a beta-blocker, prolonged survival. Compared with vehicle, pimobendan treatment also significantly reduced end diastolic and end systolic dimensions and significantly elevated ejection fraction in the TNNT2\(^{AK210/AK210}\). Previous studies also indicated that pimobendan was more effective than amrinone in increasing ventricular function at rest and during exercise of conscious dogs with pacing induced heart failure. Although the utility of pharmacological modulators of sarcomeric function in cardiac disorders requires more agents and more studies, the evidence that an agent with demonstrated sensitization to Ca\(^{2+}\) on survival in this model is promising. In the case of HCM, we have recently reported a study that tested the hypothesis that an attenuation of the increased myofilament Ca\(^{2+}\) sensitivity would improve the pathology associated with an HCM model expressing the \(\alpha\)-tropomyosin (Tm) at amino acid 180 (Glu 180 Gly). Tm(E180G) HCM mice die between 4 to 6 months of age and have severely dysfunctional hearts. By cross-breeding the the Tm(E180G) with a mouse expressing chimeric \(\alpha\)/\(\beta\)-Tm protein, which induces a desensitization to Ca\(^{2+}\), we were able to rescue the HCM phenotype. The double transgenic mice demonstrated normal heart size and morphology, significantly improved cardiac function and normal myofilament Ca\(^{2+}\) sensitivity. The rescue of mice with DCM and HCM by modifying the sarcomeric response to Ca\(^{2+}\) together with the reality of developing such agents provides hope for rational and successful therapies. However, many challenges remain in terms of the diversity of clinical manifestations, as well as genetic background, and sex-related differences in the effects of the mutations.

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**Disclosures**

Dr Solaro is a member of the scientific advisory board of Cytokinetics.

**References**


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