inability to distinguish closely related viruses. Aside from the specificity of the toehold sensor itself, the authors bring to bear a novel technique based upon CRISPR/Cas9 to distinguish between ZIKV strains that differ only by a single nucleotide. This method of discrimination would be valuable in genotyping and determining the origin of infection because some genetic variants of ZIKV may result in different clinical manifestations of infection. The NASBA amplification proceeds via a double-stranded (ds)DNA intermediate, which the authors exploit as a substrate for the Cas9 endonuclease. If Cas9 cuts this dsDNA intermediate, the NASBA cycle is broken, and the target RNA is not sufficiently amplified to trigger the toehold switch. This is the first example we are aware of where CRISPR/Cas9 is used in tandem with a nucleic acid detection assay. Of particular significance, the technique could be adapted to add specificity to other amplification techniques that result in dsDNA intermediates or products.

Based on available clinical data, ZIKV infection leads to a relatively short viremia (i.e., viral RNA detectable in plasma by standard PCR assays for ~5–7 days), but viral RNA can remain detectable in urine after viremia has waned to undetectable levels [9]. While peak levels of $>10^7$ ZIKV RNA copies/ml have been reported for plasma and urine, most reported Zika viral loads range from 10^3 to 10^6 RNA copies/ml [9]. Of note, because most people exhibit mild or no symptoms and, thus, may not report to the clinic, there is still insufficient knowledge of individual time-courses of virus levels, including the range of peak levels that might be seen in a population. Given the sensitivity of the NASBA/toehold switch detection method ($\geq 10^6$ RNA copies/ml), its clinical utility will be limited to the relatively narrow window of peak virus replication. This implies that, while a positive test result is indicative of an ongoing infection, a negative test result remains inconclusive, because it does not preclude an active infection that might be associated with risk of transmission via known routes (mosquito-borne, transplacental, sexual, or blood transfusion).

In conclusion, the development of easy and relatively cheap diagnostic kits is a promising step forward toward monitoring the spread of ZIKV, both for identifying what may be a large number of undiagnosed cases of ZIKV infection, and also for mobilizing public-health responses, such as vector control, to stop the spread of ZIKV. As with any new diagnostic technique, validation on human samples from areas with ZIKV outbreaks will be required to assess the clinical utility of the new assay. Further development of this methodology to increase its sensitivity, by coupling it to methods that concentrate viral RNA before amplification, for example, may improve the sensitivity of ZIKV detection in samples harboring low viral loads. The authors have demonstrated that their methodology can be adapted rapidly to new targets by using rational design and screening of toehold sensors, meaning that this approach could be generalized to the detection of other RNA viruses, or multiplexed to detect multiple pathogens in parallel, for which there is an urgent need as new or re-emerging pathogens, such as ZIKV, place new populations at risk worldwide.

Acknowledgments

Sandia National Laboratories is a multiprogram laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the US Department of Energy's National Nuclear Security Administration under Contract DE-AC04-94AL85000.

[1]Sandia National Laboratories, Biotechnology and Bioengineering Department, Livermore, CA 94551, USA
[2]California National Primate Research Center, University of California, Davis, CA 95616, USA

*Correspondence: rmeaghe@sandia.gov (R.J. Meagher).

http://dx.doi.org/10.1016/j.molmed.2016.05.009

References

1. Perkins, M.D. and Kessel, M. (2015) What Ebola tells us about outbreak diagnostic readiness. *Nat. Biotech.* 33, 464–469
2. Pardee, K. *et al.* (2016) Rapid, low-cost detection of Zika virus using programmable biomolecular components. *Cell. Published online May* 165, 1255–1266
3. Pardee, K. *et al.* (2014) Paper-based synthetic gene networks. *Cell* 159, 940–954
4. Compton, J. (1991) Nucleic acid sequence-based amplification. *Nature* 350, 91–92
5. Kievits, T. *et al.* (1991) NASBA^{TM} isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. *J. Virol. Methods* 35, 273–286
6. Craw, P. and Balachandran, W. (2012) Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review. *Lab. Chip* 12, 2469–2486
7. Ball, C.S. *et al.* (2016) Quenching of unincorporated amplification signal reporters in reverse-transcription loop-mediated isothermal amplification enabling bright, single-step, closed-tube, and multiplexed detection of RNA viruses. *Anal. Chem.* 88, 3562–3568
8. Faye, O. *et al.* (2015) Development and deployment of a rapid recombinase polymerase amplification Ebola virus detection assay in Guinea in 2015. *Euro Surveill.* 20, 30053
9. Musso, D. and Gubler, D.J. (2016) Zika Virus. *Clin. Microbiol. Rev.* 29, 487–524

Spotlight

Sarcomeres and Cardiac Growth: Tension in the Relationship

Saptarsi M. Haldar[1,2,*] and Deepak Srivastava[1,3,*]

Genetic mutations in the cardiomyocyte contractile apparatus cause aberrant cardiac growth categorized morphologically as hypertrophic or dilated. A recent study leverages an array of mutant mouse models to extrapolate a new integrated parameter: the myofilament 'tension index', which predicts patterns of cardiac growth resulting from individual sarcomeric mutations. These findings may inform genotype-specific therapies.

Inherited cardiomyopathies, largely caused by mutations in genes encoding sarcomeric proteins, affect 1:500 people in the general population, causing considerable morbidity and mortality from heart contractile dysfunction and an increased risk of sudden cardiac death [1]. Phenotypic abnormalities resulting from specific sarcomeric mutations have been broadly categorized into two morphologic

subsets: hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). HCM is characterized by thick ventricular walls, encroachment on the left ventricular (LV) cavity, increased myocyte force generation, impaired myocyte relaxation kinetics, and cardiomyocyte growth due to increased cellular width. By contrast, DCM is characterized by LV chamber enlargement, decreased myocyte force generation, and eccentric myocyte growth as a result of increased cellular length. Specific examples include the human MYH7 R663H mutation which causes HCM, and the TNNT2 R173W mutation causing DCM. In fact, different point mutations in the same sarcomeric protein can lead to HCM or DCM, as in the case of cardiac troponin C (cTnC), a proximal calcium-binding protein of the sarcomere.

Despite our ability to catalog genotype–phenotype correlations for several causal sarcomere mutations, current pharmacologic treatments are limited to a 'one-size-fit-all' use of neurohormonal antagonists (i.e., β1-adrenergic receptor and renin–angiotensin axis inhibition). This underscores a fundamental gap in our understanding of how individual sarcomeric mutations trigger defined signaling events culminating in HCM versus DCM growth patterns. In the context of a mutant sarcomere, could a biologically-relevant mechanical parameter be 'sensed' by a cardiomyocyte, predicting the type of cardiac remodeling that ensues? The identification of such a parameter would not only provide a first step towards an integrated model of cardiac growth, but might also inform genotype-specific therapeutic strategies for inherited cardiomyopathies. While sarcomere mutations are known to alter calcium dynamics [2], attempts to model patterns of cardiac growth based solely on perturbations in the cardiomyocyte calcium transient have generally lacked discriminatory power [3]. Now, a study published in *Cell* by Davis and colleagues leverages a large array of mutant mouse models to extrapolate a new integrated physiologic parameter, termed the myofilament 'tension index', that is applied to accurately predict patterns of cardiac growth [4] (Figure 1).

The investigators studied two variants of cardiac troponin C (cTnC): L48Q, which increases myofilament calcium sensitivity and causes HCM; and I61Q, which decreases myofilament calcium sensitivity and causes DCM [5,6]. The study of mutations in cTnC allowed a direct manipulation of the calcium–tension relationship while minimizing potential confounding effects. Introduction of the L48Q at physiologic levels increased myofilament calcium sensitivity, as evidenced by increased force generation despite lower amplitude of the calcium transient. This variant redistributed calcium unto the myofilament and away from the cytosolic pool, increasing force generation and prolonging relaxation. The net physiological effects increased the 'area under the curve' of the force versus time profile (the myofilament tension integral). Mice harboring the L48Q variant had a hypercontractile heart with a predisposition to HCM. The HCM in these mice was grossly exacerbated by blunting the high baseline adrenergic tone upon administration of β1-adrenergic receptor antagonists or the negative chronotropic agent, ivabridine. Because these pharmacologic manipulations delayed myofilament calcium release and prolonged myocyte relaxation, they increased the tension integral. This suggested that ratcheting-up the tension integral was somehow related to the unmasking of an overt HCM phenotype. By contrast, the I61Q variant decreased myofilament calcium sensitivity and dynamically redistributed calcium into the cytosolic pool, resulting in a net decrease in the tension integral. *In vivo*, the I61Q variant led to DCM with depressed LV systolic function and early lethality. Based on these observations, the authors hypothesized that increasing the tension integral promoted HCM, while decreasing it led to DCM.

To test this, they used I61Q-expressing mice, which exhibit a decreased tension integral and DCM. They assessed whether further perturbations in the tension integral in either direction could push patterns of cardiac growth in a predicted manner. Introduction of a αMHC R403Q$^{+/-}$ genetic background – which results in a high tension integral and HCM – partially rescued key features of the I61Q DCM phenotype, including LV function, cavity dilation, and death. Similarly, intercrossing I61Q mutant mice with SERCA2A haploinsufficient mice – effectively increasing the tension integral – ameliorated features of DCM. By contrast, further decreasing the tension integral by crossing I61Q mice to phospholamban-null mice – with increased SR calcium ATPase function – significantly exacerbated DCM. Together, these compound mutant data support a model where manipulations in SR calcium flux that increase the tension integral promote HCM-type remodeling, whereas those that decrease the tension integral promote DCM.

The authors tested whether directional changes in the tension integral correlated with the activity of two nodal effectors of cardiac growth, calcineurin A [7] and MEK1-ERK1/2 [8]. When I61Q mice were crossed into a calcineurin Aβ-null background, a decrease in cardiac mass but no effect on cavity dilation or LV systolic function were observed. Conversely, when I61Q mice were crossed to MEK1-overexpressing mice, there was a marked improvement in LV dysfunction and cavity dilation, with no effect on overall cardiac mass. Hence, the authors concluded that calcineurin functions as a regulator of overall cardiac growth (but not type of growth) that is activated in response to any directional perturbation in the tension integral. By contrast, decreases in the tension integral lead to suppression of ERK1/2 activity and a subsequent tendency to DCM-type remodeling.

Finally, the investigators inquired whether the tension integral could be harnessed to