REPLY TO ENTCHEVA:

The impact of T-tubules on action potential propagation in cardiac tissue

M. Scardigli[a,b], C. Crocini[a,b,1], C. Ferrantini[c], T. Gabbrielli[a], L. Silvestri[a,b], R. Coppini[d], C. Tesi[c], E. A. Rog-Zielinska[e], P. Kohl[e], E. Cerbai[d], C. Poggesi[c], F. S. Pavone[a,b,f], and L. Sacconi[a,b,2]

Since our observation that T-tubules can fail to propagate action potentials in diseased hearts (1), our studies have focused on understanding the consequences of these electrical defects for local Ca^{2+} release (2) and force production (3). More recently, we have started to explore the causes of electrical defects (4), focusing on the presence of electrical conduction failures, seemingly associated with a local drop in membrane excitability (5). The methodology developed through our work has allowed us to quantitatively assess T-tubular conductivity, thus permitting evaluation of the efficiency of passive spread of voltage changes across the cardiac cell. Although these passive electrical properties have been discussed in our article in some detail (4), the implications of the findings at the tissue level have been not been adequately explored.

In her letter, Entcheva (6) suggests a very inspiring scenario, carrying our finding to another level of complexity. Particularly interesting are the suggested implications of how voltage heterogeneities found in cells can affect the surrounding tissue: (*i*) electrical failure could be proarrhythmogenic, as supported by our previous works, and/or (*ii*) electrically heterogeneous myocytes could respond nonuniformly to external electric fields, such as applied via pacing leads and for defibrillation. The potential impact of T-tubules on intercellular propagation of action potentials is intriguing. So far, T-tubules have only been considered as a determinant of cell capacitance in propagation of passive depolarization (7). In fact, common and oversimplified schematizations generally neglect the extracellular electrical resistance. However, as demonstrated in our work, the complexity of the T-tubular system could yield regional extracellular resistances that exceed intracellular ones.

It is important in this context to underline that T-tubular conductivity strongly depends on the geometry of the T-tubular system itself. T-tubular diameter (8), T-tubular density (9), and ultrastructural organization giving rise to diffusion barriers (10) may well vary across species, perhaps explaining some of the differences in parameter ranges cited by Entcheva (6). In addition to differing phosphorylation levels of associated proteins (11), these changes can regionally modify T-tubular conductivity, making it hard to generalize the dynamic impact of T-tubules on propagation of passive depolarizations. Cardiac myocytes also deform with every cardiac cycle, and this is associated with alterations in subcellular membrane systems, such as T-tubules, which may further affect local ion gradients and diffusion.

We thank Emilia Entcheva for her insightful comments on how this complex network of membrane invaginations may affect action potential propagation at tissue levels, beyond conventional considerations related to the rapid spread of electrical activation throughout cells (6). Targeted FRAP experiments in intact beating tissue preparations may help to unravel the broader implications of extracellular electrical resistance across the transversal and axial tubular network for action potential propagation in physiological and pathological conditions.

[a]European Laboratory for Non-Linear Spectroscopy, 50019 Florence, Italy; [b]National Institute of Optics, National Research Council, 50019 Florence, Italy; [c]Division of Physiology, Department of Experimental and Clinical Medicine, University of Florence, 50121 Florence, Italy; [d]Division of Pharmacology, Department "NeuroFarBa," University of Florence, 50139 Florence, Italy; [e]Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg–Bad Krozingen, Faculty of Medicine, University of Freiburg, 79110 Freiburg, Germany; and [f]Department of Physics and Astronomy, University of Florence, 50019 Florence, Italy

Author contributions: M.S., C.C., C.F., T.G., L. Silvestri, R.C., C.T., E.A.R.-Z., P.K., E.C., C.P., F.S.P., and L. Sacconi wrote the paper.

The authors declare no conflict of interest.

[1]Present address: Department of Molecular, Cellular, and Developmental Biology and BioFrontiers Institute, University of Colorado, Boulder, CO 80309.

[2]To whom correspondence should be addressed. Email: sacconi@lens.unifi.it.

1 Sacconi L, et al. (2012) Action potential propagation in transverse-axial tubular system is impaired in heart failure. *Proc Natl Acad Sci USA* 109:5815–5819.
2 Crocini C, et al. (2014) Defects in T-tubular electrical activity underlie local alterations of calcium release in heart failure. *Proc Natl Acad Sci USA* 111:15196–15201.
3 Crocini C, et al. (2016) Novel insights on the relationship between T-tubular defects and contractile dysfunction in a mouse model of hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 91:42–51.
4 Scardigli M, et al. (2017) Quantitative assessment of passive electrical properties of the cardiac T-tubular system by FRAP microscopy. *Proc Natl Acad Sci USA* 114:5737–5742.
5 Crocini C, Ferrantini C, Coppini R, Sacconi L (2017) Electrical defects of the transverse-axial tubular system in cardiac diseases. *J Physiol* 595:3815–3822.
6 Entcheva E (2017) Uncovering an electrically heterogeneous cardiomyocyte by FRAP-quantified diffusion in the T-tubules. *Proc Natl Acad Sci USA*, 10.1073/pnas.1719550115.
7 Di Maio A, Ter Keurs HE, Franzini-Armstrong C (2007) T-tubule profiles in Purkinje fibres of mammalian myocardium. *J Muscle Res Cell Motil* 28:115–121.
8 Kong CHT, Rog-Zielinska EA, Orchard CH, Kohl P, Cannell MB (2017) Sub-microscopic analysis of t-tubule geometry in living cardiac ventricular myocytes using a shape-based analysis method. *J Mol Cell Cardiol* 108:1–7.
9 Ferrantini C, et al. (2013) The transverse-axial tubular system of cardiomyocytes. *Cell Mol Life Sci* 70:4695–4710.
10 Hong T, et al. (2014) Cardiac BIN1 folds T-tubule membrane, controlling ion flux and limiting arrhythmia. *Nat Med* 20:624–632.
11 Brandenburg S, et al. (2016) Axial tubule junctions control rapid calcium signaling in atria. *J Clin Invest* 126:3999–4015.