Real-time nanoscopy reveals sub-resolution building blocks of elementary Ca^{2+} signaling in adult cardiomyocytes

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In cardiac myocytes, fast Ca^{2+} release via clusters of Ryanodine Receptor 2 (RyR2) located in the membrane of sarcoplasmic reticulum is the key step that couples the electrical excitation to the mechanical contraction. Conventional confocal microscopy has enabled thorough investigations of Ca^{2+} sparks[1], the elementary Ca^{2+} release events that can be visualized with fluorescent probes, e.g. Fluo-4. Traditionally it is believed that, one RyR2 cluster is activated and responsible for the release of Ca^{2+} ions underlying single Ca^{2+} sparks[2]. However, super-resolution microscopy employing immunolabeling has suggested that in rat ventricular myocytes the average distance between RyR2 clusters is about 1.0 μm[3]. Noteworthy, this distance is much smaller than the full width at half maximum of single sparks (2.8 μm)[1], proposing that multiple RyR2 clusters / fundamental Ca^{2+} Release Bursts (fCRB) are involved in the generation of a microscopic Ca^{2+} spark.

To functionally investigate elementary Ca^{2+} release in cardiac myocytes, we employed real-time nanoscopy (180 Hz, 130 nm lateral and 270 nm along the z-axis) using a novel instant SIM microscope (iSIM, VisiTech Ltd, Sunderland, UK) equipped with a Hamamatsu ORCA Flash4 sCMOS camera to follow the spatiotemporal properties of individual Ca^{2+} sparks in permeabilized cardiomyocytes from rats or mice. By utilizing the state-of-art CaCLEAN algorithm[4] that considers the release and the diffusion processes of Ca^{2+} ions, we were capable of identifying isolated fCRBs that build up individual Ca^{2+} sparks. Our results demonstrate that, in permeabilized cardiomyocytes, spontaneous Ca^{2+} sparks are comprised on average of 6.2 (n>15) isolated FCRBs. When the activity of the RyR2 was modulated, e.g. the accessory protein FKBP was removed by application of FK506, the number of fCRB in the Ca^{2+} sparks was reduced to 4.7 (n>15). In contrast, ß-adrenergic stimulation of RyR2 resulted in an augmented number of fCRBs (9/spark, n>15). Temporally, these fCRBs fired in a sequential manner, but spatially the active fCRBs appears to jump to its nearest neighbors. Registration of these FCRBs resulted in a map of active but well organized RyR2 clusters in cardiac myocytes.

By employing innovative real-time nanoscopy, we were – for the first time – able to reveal the as of yet unknown nanoscopic structure of elementary signals of Ca^{2+} signaling in cardiac myocytes. Each Ca^{2+} spark actually comprises not only the Ca^{2+} released from a single stereotypic cluster of RyR2 but instead results from the recruitment of multiple clusters acting in concert whereby the number of participating clusters appears to determine the overall Ca^{2+} spark properties. We therefore believe that our novel approach and the resulting data represent another fundamental breakthrough after 25 years of Ca^{2+} spark research.

[1] H. Cheng, *et.al*, "Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle." *Science*, 262, 740–744, (1993).
[2] H. Cheng and W. J. Lederer, "Calcium Sparks." *Physiological Reviews*, 88, 1491–1545 (2008).
[3] C. Soeller, *et.al*, "Analysis of ryanodine receptor clusters in rat and human cardiac myocytes." *Proc Natl Acad Sci USA*, 104, 14958–14963 (2007).
[4] T. Qinghai, *et.al*, "An adaptation of astronomical image processing enables characterization and functional 3D mapping of individual sites of excitation-contraction coupling in rat cardiac muscle." *Elife*, 6, 665 (2017).