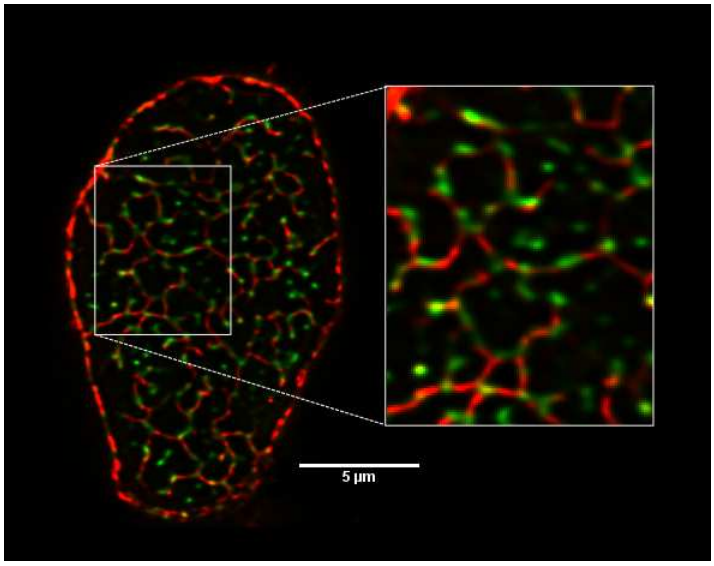
Visualization of the 3-D distribution of proteins involved in cardiac excitation-contraction coupling using a novel optical approach

I.D. Jayasinghe, M.B. Cannell and C. Soeller, Department of Physiology, Faculty of Medical and Health Sciences, 85 Park Road, Grafton, Auckland, New Zealand.

Ca^{2+} induced release of Ca^{2+} from the sarcoplasmic reticulum (SR) triggered by voltage-dependent trans-sarcolemmal Ca^{2+} fluxes is thought to form the basis of excitation-contraction (EC) coupling in cardiac myocytes. Close apposition of the sarcolemmal membrane with the SR Ca^{2+} release channels or ryanodine receptors (RyRs) is essential to achieving the rapid Ca^{2+} release that ensures cardiac contraction. The sarcolemmal membrane architecture is complex, with the presence of caveolar invaginations and a dense network of t-tubules near each z-disk. Such complexity in the organization of membrane structures and the compact arrays of key proteins central to EC coupling is only partially resolved when imaged by confocal microscopy. The resolution can be effectively improved by using a recently-demonstrated technique (Chen-Izu *et al.*, 2006) to image these fine elements of the sarcolemma in single cells oriented vertically on the microscope stage. We have used this approach to visualize the distribution of the Na-Ca exchanger (NCX), Caveolin-3 (Cav3, a marker for caveolar membrane invaginations) and RyRs in enzymatically isolated and fixed rat myocytes at high resolution. The distribution of Caveolin-3 (red) and RyR (green) labelling in a single z-disk of a rat ventricular myocyte is shown in the figure below illustrating the level of detail that is achieved.



Combined with digital 3-D deconvolution to further improve contrast and signal-to-noise ratio, we employed this novel method to resolve the distribution of NCX relative to the sites of SR Ca^{2+} release that are apparent as clusters of RyR labelling (Soeller & Cannel, 2007). It has been suggested that reverse mode exchange via NCX may contribute towards triggering SR Ca^{2+} release in rat myocytes (Bridge *et al.*, 2003, Lines *et al.*, 2006), but the putative junctional localisation of NCX implied by these studies has been somewhat unclear due to the lack of techniques with sufficient spatial resolution. While NCX labelling generally followed the geometry of the t-system (as outlined by Cav3 labelling), more intense labelling of the exchanger was observed at regularly-spaced domains along z-line-associated t-tubules. Analysis of double-labelled cells indicated that some but not all RyR clusters colocalize with NCX labelling and at least 10% of the total NCX labelling was located at or near junctions.

Recently, it has been suggested that some RyR clusters found distal to z-lines are not associated with junctions (Lukyanenko *et al.*, 2007). Our data show that RyR clusters located between z-lines are generally closely associated with longitudinal elements of the tsystem (Soeller & Cannell, 1999) as identified by NCX and Cav3 labelling. Thus these RyR clusters are likely in junctions with the longitudinal t-system.

Our data provide 3D data sets for detailed modelling studies to improve our understanding of the potential role of NCX in modulating SR release.

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