Analysis of ryanodine receptor clusters in rat and human cardiac myocytes at high optical resolution

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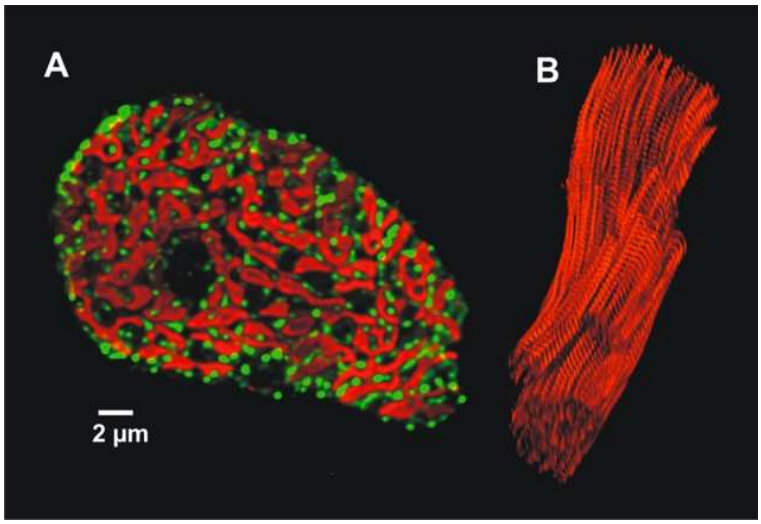
The molecular basis of 'local control' of cardiac excitation-contraction (E-C) coupling resides in the gating of sarcoplasmic reticulum (SR) Ca^{2+} release channels or ryanodine receptors (RyRs) which are clustered in the junctions between terminal SR and the transverse tubular system. We have determined the localization of RyR clusters and simultaneously visualized the location of the contractile apparatus by antibody labelling. Using a novel confocal imaging protocol (Chen-Izu *et al.*, 2006) we were able to resolve all the clusters within a z-disk at very high optical resolution (~250 nm) in single rat ventricular myocytes and human ventricle tissue sections. For optimal resolution it was critical to maximize image contrast by refractive index matching and deconvolution. The RyR label formed discrete puncta representing clusters of RyRs or 'couplons' around the edges of the myofilaments (see Figure, panel A) with a nearest neighbour spacing of 0.66 ± 0.06 μm in rat and 0.78 ± 0.07 μm in human (Soeller *et al.*, 2007). Each bundle of myofibrils was served by ~6 couplons which supplied a cross-sectional area of ~0.6 μm^2 in rat and ~0.8 μm^2 in human. While the couplons were in reasonable registration with z-lines, the α-actinin labeling (see Figure, Panel B) revealed discontinuities in the longitudinal position of sarcomeres across the cell so that dislocations in the order of RyR clusters occurred. By quantifying the labelling intensity in rat ventricular myocytes based on antibody binding, a lower limit of 78 RyRs per cluster (on average) was obtained. With a new calibration method that uses optically resolved clusters to calibrate the intensity signal and assuming a geometry where couplons wrap as a disk around a t-tubule, 95% of couplons contained between 120 and 260 RyRs. Our data can explain the spherical propagation of Ca^{2+} waves and provide the first quantitative three-dimensional data sets needed for accurate modelling of cardiac CICR and the transition to regenerative release. The 3D information should help improve our understanding of the regulation of cardiac Ca^{2+} metabolism.



Chen-Izu Y, McCulle S, Ward C, Soeller C, Allen B, Rabang C, Cannell MB, Balke C & Izu L. (2006) *Biophysical Journal*, **91:** 1-13.

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