VISUALIZATION OF NANOSCOPIC MEMBRANE SIGNALLING DOMAINS BY SINGLE MOLECULE LOCALISATION MICROSCOPY

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The distribution of proteins and their proximity at the nanometre scale critically determines cellular function but until recently such detail could not be observed with optical microscopy due to its limited resolution. Here we use optical super-resolution imaging which combines the strength of existing fluorescent labelling technology with greatly improved detail resolution as small as 10 nm.

Using this approach we have investigated the distribution of key calcium handling proteins in cardiac myocytes and their relationship to a nanoscopic signalling domain, the ~15 nm wide junctional space between the sarcolemmal membrane and the membranes of the sarcoplasmic reticulum, an intracellular calcium store. Cardiac excitation-contraction coupling in ventricular myocytes relies critically on the spatial proximity between several key calcium handling proteins in the vicinity of these junctional domains. We examined the distribution of ryanodine receptors (RyR) and related proteins in rat ventricular myocytes with ~30 nm resolution [1]. Using high-resolution antibody labelling data we show that the new imaging approach can give novel insight into the distribution of large proteins, with optical single channel resolution. Morphological analysis of peripheral RyR clusters in the surface membrane revealed a mean size of ~14 RyRs per cluster, almost an order of magnitude smaller than previously estimated by thin section electron microscopy. Clusters were typically not circular (as previously assumed) but elongated. Edge-to-edge distances between adjacent RyR clusters were often less than 50 nm suggesting that peripheral RyR clusters may exhibit strong inter-cluster signalling. Cluster sizes varied widely and followed a near-exponential distribution, compatible with a stochastic cluster assembly process. Based on the placement and morphology of RyR clusters we suggest that microscopic calcium release events observed in live muscle cells (calcium sparks) may be the result of the concerted activation of several clusters forming a functional supercluster whose gating is controlled by both cytosolic and sarcoplasmic reticulum luminal calcium levels.

Recently, we have extended our imaging methods to allow multi-colour imaging of protein distribution with ~30 nm resolution. This has allowed co-localization studies with much improved resolution. Proteins that appear to co-localize at diffraction-limited resolution often exhibit much lower coincidence when visualized with the super-resolution techniques. This will be illustrated with RyRs, the cardiac sodium-calcium exchanger (NCX), caveolin and the junctional protein junctophilin (JPH2). We also conducted similar signalling studies in rat hippocampal cultures and visualized the distribution of several synaptic proteins.