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Resolving the Structural Basis of Cardiac Excitation-Contraction Coupling

by

Isuru Dilshan Jayasinghe

A thesis submitted in partial fulfillment for the degree of Doctor of Philosophy

in the
Faculty of Medical and Health Sciences
Department of Physiology

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Calcium (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. Clusters of ryanodine receptors (RyR) that are responsible for this Ca\(^{2+}\) release are known to reside on termini of the SR (located abundantly near z-lines) that form close junctions with the sarcolemmal membrane and invaginations known as t-tubules. Sarcolemma and t-tubules contain L-type Ca\(^{2+}\) channels and Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) proteins that may provide effective Ca\(^{2+}\) trigger currents if placed close to junctional RyRs. Using a novel protocol of immunofluorescence confocal microscopy, the architecture of SR and t-tubules in rat ventricular myocytes has been visualized at a resolution that was previously not achieved with optical techniques. This method revealed a subset of RyR clusters that were apparently non-junctional. Improved co-localization analysis methods were applied to confocal images and total internal reflection fluorescence images to quantify the co-localization of key trigger proteins (L-type Ca\(^{2+}\) channels and NCX) with clusters of RyR in the cell interior and near the surface. These confocal images also revealed that z-disks are non-planar. The three-dimensional topology of the z-disks was reconstructed from confocal images of the sarcomeric protein, α-actinin. 3D visualization of this data showed that adjacent sarcomeres may be misregistered. Some were arranged in helicoids that occupied large regions within the cell, effectively reducing the longitudinal distance between Ca\(^{2+}\) release sites. This was expected to improve the synchrony in the activation of contraction. Images of mammalian ventricular myocytes suggested that their t-tubules closely follow this z-disk topology although additional axial connections provided a more complex 3D architecture. Super-resolution images produced by single fluorophore localization were used for detailing the fine ultrastructure of RyR clusters that could underlie the variability observed previously in localized Ca\(^{2+}\) release events. An ~10-times finer resolution (compared to conventional confocal microscopy) allowed the quantification of junctional NCX that could participate in evoking Ca\(^{2+}\) release. A protein involved in junction formation, junctophilin, was strongly associated with the RyR cluster geometry, underscoring its role as a potential determinant and marker of RyR cluster size and shape. These new structural insights are discussed with respect to the formation and maintenance of junctions and the consequences for cardiac EC coupling.
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Abbreviations

3D three-dimensional
Ca$^{2+}$ calcium (ions)
Na$^+$ sodium (ions)
K$^+$ potassium (ions)
O$_2$ oxygen
NO nitric oxide
EC coupling Excitation-Contraction coupling
TATS Transverse Axial Tubular System
SR Sarcoplasmic Reticulum
ER Endoplasmic Reticulum
DHPR dihydropiridine receptor
NCX Na$^+$.Ca$^{2+}$ exchanger
CAV3 CAVeolin-3
TSR Terminal Sarcoplasmic Reticulum
NSR Network Sarcoplasmic Reticulum
JSR Junctional Sarcoplasmic Reticulum
RyR ryanodine receptor
WGA Wheat Germ Agglutinin
CICR Calcium-Induced Calcium Release
SERCA Sarcoplasmic Endoplasmic Reticular Ca$^{2+}$ ATPase
SHR Spontaneously Hypertensive Rats
JPH junctophilin
JnC junctin
Tr triadin
CSQ calsequestrin
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<tbody>
<tr>
<td>nNOS</td>
<td>neuronal Nitric Oxide Synthase</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial Nitric Oxide Synthase</td>
</tr>
<tr>
<td>PSF</td>
<td>Point Spread Function</td>
</tr>
<tr>
<td>EM</td>
<td>Electron Micrograph</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Micrograph</td>
</tr>
<tr>
<td>TIRF</td>
<td>Total Internal Reflection Fluorescence microscopy</td>
</tr>
<tr>
<td>STED</td>
<td>STimulated Emission Depletion microscopy</td>
</tr>
<tr>
<td>PALM</td>
<td>Photo-Activated Localization Microscopy</td>
</tr>
<tr>
<td>fPALM</td>
<td>fluorescence PALM</td>
</tr>
<tr>
<td>STORM</td>
<td>Stochastic Optical Reconstruction Microscopy</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>FWHM</td>
<td>Full Width at Half Maximum</td>
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<tr>
<td>DADs</td>
<td>Delayed After-Depolarizations</td>
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Symbols

$I_{Ca}$     sarcolemmal voltage-dependent inward $Ca^{2+}$ current
$I_{Na}$    sarcolemmal voltage-dependent inward $Na^{+}$ current
$I_{NaCa}$ sarcolemmal NCX current
$I_{SR}$    $Ca^{2+}$ flux from the SR into the cytoplasm
$[Ca^{2+}]_{i}$ cytoplasmic $Ca^{2+}$ concentration
$[Ca^{2+}]_{sm}$ $Ca^{2+}$ concentration in restricted ‘submembrane’ spaces
$[Na^{+}]_{i}$ cytoplasmic $Na^{+}$ concentration
$[Na^{+}]_{sm}$ $Na^{+}$ concentration in restricted ‘submembrane’ spaces
$V_m$       membrane potential
$E_{Na}$    Nernst potential for sodium
$E_{Ca}$    Nernst potential for calcium
$E_{NaCa}$ reversal potential for NCX