UNIVERSITY OF AUCKLAND

Resolving the Structural Basis of Cardiac Excitation-Contraction Coupling

by

Isuru Dilshan Jayasinghe

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Abstract

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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 $\sim$ 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	**E**xcitation-**C**ontraction coupling
TATS	**T**ransverse **A**xial **T**ubular **S**ystem
SR	**S**arcoplasmic **R**eticulum
ER	**E**ndoplasmic **R**eticulum
DHPR	**dih**ydro**p**iridine **r**eceptor
NCX	**N**a^{+}-**C**a^{2+} e**x**changer
CAV3	**CAV**eolin-**3**
TSR	**T**erminal **S**arcoplasmic **R**eticulum
NSR	**N**etwork **S**arcoplasmic **R**eticulum
JSR	**J**unctional **S**arcoplasmic **R**eticulum
RyR	**ry**anodine **r**eceptor
WGA	**W**heat **G**erm **A**gglutinin
CICR	**C**alcium-**I**nduced **C**alcium **R**elease
SERCA	**S**arcoplasmic **E**ndoplasmic **R**eticular **C**a^{2+} **A**TPase
SHR	**S**pontaneously **H**ypertensive **R**ats
JPH	junctophilin
JnC	junctin
Tr	triadin
CSQ	calsequestrin

nNOS	**n**euronal **N**itric **O**xide **S**ynthase
eNOS	**e**ndothelial **N**itric **O**xide **S**ynthase
PSF	**P**oint **S**pread **F**unction
EM	**E**lectron **M**icrograph
TEM	**T**ransmission **E**lectron **M**icrograph
TIRF	**T**otal **I**nternal **R**eflection **F**luorescence microscopy
STED	**ST**imulated **E**mission **D**epletion microscopy
PALM	**P**hoto-**A**ctivated **L**ocalization **M**icroscopy
fPALM	**f**luorescence **PALM**
STORM	**S**tochastic **O**ptical **R**econstruction **M**icroscopy
IgG	**I**mmuno**g**lobulin **G**
FWHM	**F**ull **W**idth at **H**alf **M**aximum
DADs	**D**elayed **A**fter-**D**epolarizations

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
$[\mathbf{Ca}^{2+}]_i$	cytoplasmic Ca^{2+} concentration
$[\mathbf{Ca}^{2+}]_{sm}$	Ca^{2+} concentration in restricted 'submembrane' spaces
$[\mathbf{Na}^{+}]_i$	cytoplasmic Na^{+} concentration
$[\mathbf{Na}^{+}]_{sm}$	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