

Alterations in T-tubule and dyad structure in heart disease: challenges and opportunities for computational analyses

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Abstract

Compelling recent experimental results make clear that sub-cellular structures are altered in ventricular myocytes during the development of heart failure, in both human samples and diverse experimental models. These alterations can include, but are not limited to, changes in the clusters of sarcoplasmic reticulum (SR) Ca^{2+} -release channels, ryanodine receptors, and changes in the average distance between the cell membrane and ryanodine receptor clusters. In this review, we discuss the potential consequences of these structural alterations on the triggering of SR Ca^{2+} release during excitation–contraction coupling. In particular, we describe how mathematical models of local SR Ca^{2+} release can be used to predict functional changes resulting from diverse modifications that occur in disease states. We review recent studies that have used simulations to understand the consequences of sub-cellular structural changes, and we discuss modifications that will allow for future modelling studies to address unresolved questions. We conclude with a discussion of improvements in both experimental and mathematical modelling techniques that will be required to provide a stronger quantitative understanding of the functional consequences of changes in sub-cellular structure in heart disease.

Keywords

Transverse tubule • Ca^{2+} spark • Mathematical modelling • Ryanodine receptor • Ventricular myocyte

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1. Introduction

The sub-cellular structure of cardiac myocytes is well suited to enable the function of these cells. As the ventricles are responsible for pumping blood, ventricular myocytes must be able to contract quickly and powerfully, which requires rapid and large increases in intracellular Ca^{2+} concentration. The increase in $[\text{Ca}^{2+}]$ that occurs with each heartbeat is initiated by Ca^{2+} entry through L-type Ca^{2+} channels and amplified by Ca^{2+} release from the sarcoplasmic reticulum (SR). Given the large size of these cells, however, diffusion would not be sufficient to rapidly transmit Ca^{2+} from the cell periphery to the cell interior. Thus, transverse tubules (T-tubules) penetrate into the cell and ensure that, in healthy cells, virtually all locations in the cytoplasm are close ($<1 \mu\text{m}$) to the cell membrane. Moreover, T-tubules bring together in close proximity the essential elements of excitation–contraction (EC) coupling: membrane L-type Ca^{2+} channels and Ca^{2+} -release channels, ryanodine receptors (RyRs), in

the SR membrane. Despite this optimized sub-cellular structure that allows for efficient function in healthy cells, recent evidence, discussed below and in other contributions to this special issue, indicates that deleterious structural changes occur in several disease states.^{1–3}

Mathematical modelling has been used to illuminate several important aspects of EC coupling. For instance, the local control hypothesis, the idea that SR Ca^{2+} release consists of many independent functional units, originated from simulations with models,⁴ and the predictions of these simulations were subsequently verified experimentally.^{5–7} Similarly, computational studies helped to develop the hypothesis that release termination relies on substantial local depletion of SR $[\text{Ca}^{2+}]$,⁸ and the conceptual framework established by the modelling helped to interpret later experimental data that supported the hypothesis.^{9–13}

Most modelling studies performed to date, however, have focussed on questions related to channel gating. For instance, simulations have been used to explore the mechanisms that allow for robust

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termination of Ca^{2+} release,^{8,14,15} and have examined how RyR sensitivity to Ca^{2+} influences the triggering of release by Ca^{2+} current.^{16–18} This has been a natural starting point, as these relevant questions can be addressed through approaches that follow the tradition established in studies of cellular action potentials, electrophysiology, and arrhythmias.^{19,20} It is clear, however, that to understand changes in EC coupling that occur in heart failure, simulation studies must consider not only changes in channel gating, but also changes in the sub-cellular structure.

In this brief review, we describe the structural changes to heart cells that occur in cardiac disease, and we discuss how simulation approaches will be an important complement to experiments for understanding dysfunctional changes to physiology. However, since the effects of structural alterations have been relatively less explored compared with changes in channel gating, many of the most important studies in this area have not yet been performed, and much of what follows will be prospective rather than retrospective.

2. Experimental evidence for T-tubule and dyad remodelling in disease

Numerous experimental studies have now shown that T-tubules and cardiac dyads become remodelled in disease states.^{21–32} Details differ depending on species and the particular disease model examined, but several observations appear to be robust and consistent between studies: (i) T-tubules become more disorganized and lose their regular structure as disease progresses; (ii) an increased fraction of T-tubules appears to be oriented longitudinally rather than transversely; and (iii) RyR clusters are more likely than T-tubules to maintain a regular structure, which means that many RyR clusters become ‘orphaned’ as the L-type Ca^{2+} channels in the cell membrane either move away or disappear along with the T-tubules. More recently, research has suggested that cardiomyopathy may also lead to changes in the morphology of the RyR clusters themselves, with smaller RyR clusters reported in a rat model of pressure overload-induced heart failure.³³

Several of these established and suggested structural alterations are illustrated schematically in *Figure 1*. In healthy cells (left), RyR clusters are relatively evenly spaced, and most RyR clusters sit in close proximity to T-tubule membranes. In disease (right), the following changes may occur: (i) local SR volumes and RyR clusters may become smaller than normal³³ (top); (ii) RyR clusters may move away from T-tubules³⁴ (middle); (iii) pinching off of T-tubules or changes in the regular T-tubule structure may lead to RyR clusters that lose contact with L-type Ca^{2+} channels in the T-tubule membrane, thereby becoming orphaned²³ (bottom).

The structural changes observed in disease occur in parallel with several alterations to SR Ca^{2+} release. Two early studies by Gomez *et al.* demonstrated reduced ‘gain’, or an impaired ability for L-type Ca^{2+} current to trigger SR Ca^{2+} release in cells from failing hearts.^{35,36} An additional important early study by Litwin and Bridge³⁷ in rabbits after myocardial infarction used confocal imaging to demonstrate a decrease in the synchrony of Ca^{2+} spark initiation, as several subsequent studies in a variety of heart failure models have confirmed.^{23–26} More recent studies have extended these initial observations. For instance, decreased ‘coupling fidelity’, or the capacity for L-type Ca^{2+} channel openings to trigger local Ca^{2+} release in the

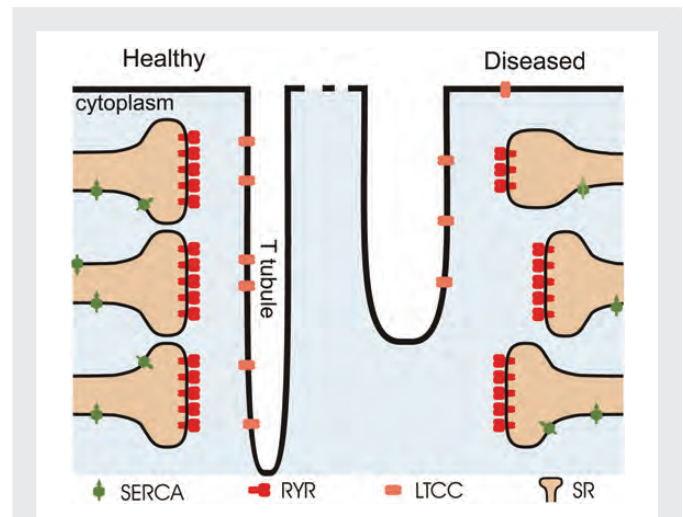


Figure 1 Schematic diagram of dyadic junctions in healthy and diseased cardiac cells. The left side of the diagram shows the regular organization of RyR clusters in healthy ventricular myocytes. In diseased cells (right), several possible forms of remodelling have been proposed: (i) RyR clusters and associated local SR volumes may become smaller (top)³³; (ii) the gap between the T-tubule membranes and RyR clusters may become larger as SR moves away from the Z-line (middle); and (iii) gross alterations in T-tubule structure may lead to ‘orphaning’ of RyR clusters (bottom).²³ The schematic is based on similar diagrams presented elsewhere.^{33,36}

form of Ca^{2+} sparks, has been shown at the level of individual dyads in a rat model of pressure overload-induced hypertrophy.³⁸ Additionally, in pigs after myocardial infarction, spontaneous Ca^{2+} sparks in sub-cellular regions near T-tubules have been shown to exhibit an increased frequency but decreased duration compared with sparks seen in regions far from T-tubules.³⁹

One of the functions of mathematical modelling is to explain these disparate experimental observations within a unified framework. As will be described below, this has been accomplished to a limited extent, but considerable additional research needs to be performed to fully understand the functional consequences of sub-cellular structural changes that occur in cardiac disease.

3. The importance of geometry in numerical simulations of Ca^{2+} sparks

Stochastic simulations of Ca^{2+} sparks provide an initial framework for understanding how changes in sub-cellular structure in disease states influence SR Ca^{2+} release and EC coupling in ventricular myocytes. Many studies have used Monte-Carlo methods to simulate local SR Ca^{2+} release,^{8,14,15,17,40,41} and contemporary models generally share the following features (*Figure 3A*): (i) a cluster of stochastically gating RyRs; (ii) a local dyadic space between the SR and T-tubule membranes; (iii) a local junctional SR (JSR) connected to a larger network SR (NSR); and (iv) Ca^{2+} buffering in both the dyadic and JSR spaces. Models differ in the assumptions made regarding geometry and RyR gating. Nonetheless, from simulations performed in several studies in the context of healthy myocytes, some general conclusions

seem to apply, and these can help us to predict the quantitative consequences of various alterations that may occur in disease states.

An increase in SR $[Ca^{2+}]$ will lead to larger Ca^{2+} sparks, and will increase the probability that a spontaneous RyR opening will trigger neighbouring channels to produce a spark. This latter effect results from two complementary factors: (i) RyRs become sensitized at higher SR $[Ca^{2+}]$ and (ii) single RyR openings carry more current, and this larger current is more effective at raising dyadic $[Ca^{2+}]$ and inducing neighbouring channels to open.⁴² A change in the number of RyRs in each cluster will alter spark morphology in expected ways: larger RyR clusters produce larger sparks. Changes in RyR quantity have relatively minor effects on spark amplitude, however, if JSR volume is kept constant.^{8,40} This is because more RyRs in a cluster will lead to faster depletion of local SR $[Ca^{2+}]$, which will mitigate the effects of having more channels in the cluster. An increase in either the local JSR volume or the JSR buffering power (concentration of calsequestrin, CSQ) will augment the amount of local Ca^{2+} available for release, and will also increase Ca^{2+} spark amplitude. Finally, changes in dyadic space volume are predicted to have little effect on spark amplitude or spark duration. This volume, however, can significantly influence the probability that individual RyR openings are able to trigger sparks. When a single RyR opens, the dyadic space volume is one of the main factors that determines the local dyadic $[Ca^{2+}]$, and this variable in turn determines the probability that neighbouring RyRs open. Thus, the effective cytosolic volume associated with a cluster of RyRs can determine whether that cluster primarily generates Ca^{2+} sparks or mostly produces isolated RyR openings, so-called 'invisible' SR Ca^{2+} leak.

In a recent study (Y.S. Lee, in preparation) we attempted to place these and related simulation results into a coherent quantitative context by performing a thorough parameter sensitivity analysis of an established Ca^{2+} spark model.^{8,41} We randomized parameters to generate a population of models, each with slightly different characteristics. After running simulations with each candidate model, we used statistical regression methods^{43,44} to relate the parameters to the simulation results. Results from this study (Y.S. Lee, in preparation) reproduced in *Figure 2*, show the following predictions: first, increases in the JSR Ca^{2+} capacity, through either CSQ concentration (parameter CSQ) or JSR volume (parameter V_{JSR}), increase both Ca^{2+} spark amplitude and Ca^{2+} spark duration. Secondly, changes in the number of RyRs per cluster (parameter N_{RyR}) or the Ca^{2+} flux through each open RyR (parameter D_{RyR}) have opposite effects on spark amplitude and duration. This occurs because an increase in the parameter causes faster JSR depletion, and hence shorter sparks. Local depletion also explains why the effects of D_{RyR} and N_{RyR} on amplitude are smaller than the effects of CSQ or V_{JSR} . Thirdly, the parameters controlling Ca^{2+} spark probability largely differ from those that determine spark morphology (amplitude and duration). These general rules, established qualitatively through simulations in many studies and more quantitatively here, help us to understand the changes in Ca^{2+} sparks that may occur in disease states.

4. How should one simulate a Ca^{2+} spark arising from a diseased dyad?

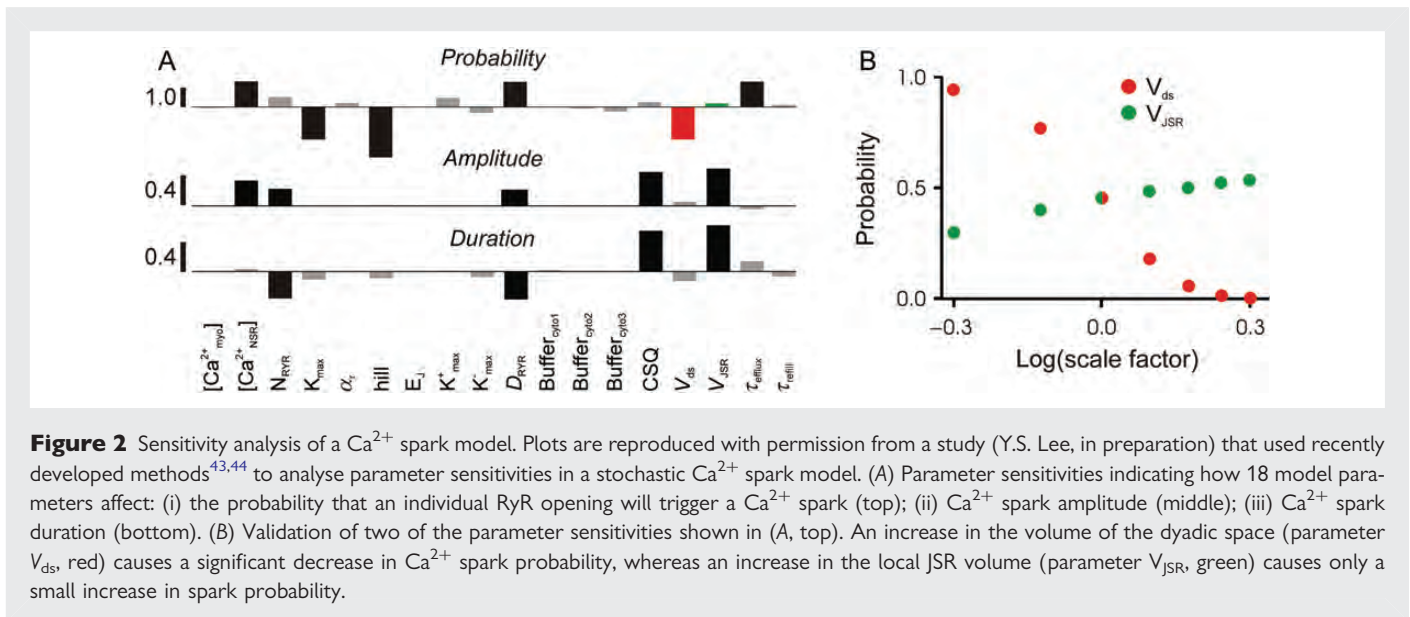
Given the structural alterations that have been observed in multiple studies as described above, what changes should one make in a mathematical model to simulate Ca^{2+} sparks arising from a diseased dyad?

We present results and discuss such a provisional simulation in *Figure 3*. As our baseline mathematical model we used identical parameters to those presented in Ramay *et al.*⁴¹ To simulate a diseased myocyte, we made the following alterations: (i) the number of RyRs per cluster was reduced by 50%, consistent with the recent finding of smaller dyads in disease³³; (ii) along with the decrease in the number of RyRs, local JSR and dyadic space volumes were also reduced by 50%³³; (iii) diastolic network SR and initial JSR $[Ca^{2+}]$ were each reduced by 20%; (iv) initial cytosolic and dyadic space $[Ca^{2+}]$ were each increased by 25% to simulate impaired relaxation and elevated diastolic $[Ca^{2+}]$; and (v) in a separate set of simulations dyadic space volume was increased by a factor of 2 to simulate orphaning of RyR clusters.²³ Results shown in *Figure 3* demonstrate that Ca^{2+} sparks originating from diseased RyR clusters may be expected to exhibit the following characteristics: (i) smaller amplitude due to the reduced Ca^{2+} capacity of the smaller dyads³³; (ii) slightly longer duration due to an enhanced ability for the network SR to refill the smaller JSR volume during the spark^{13,41,45}; (iii) in simulations that include the effect of orphaning, a substantially reduced ability for individual RyR openings to trigger sparks by activating neighbouring RyRs within the cluster.

Several alterations that are thought to take place in heart failure are not included in this simulation, and these excluded elements suggest avenues for future work (see also Sections 6 and 7). For instance, SERCA activity, frequently reported as decreased in heart failure,⁴⁶ is not explicitly included in this particular model. This alteration is included indirectly through the decrease in $[Ca^{2+}]_{NSR}$, but more complete models that simulate the balance between leak and SR Ca^{2+} reuptake^{17,18,47} can explore more thoroughly how SERCA influences SR $[Ca^{2+}]$, and how this in turn influences Ca^{2+} sparks. Secondly, intracellular $[Na^+]$ is not included as a variable in this Ca^{2+} spark model, although it is likely to be elevated in heart failure due to changes in Na^+ current⁴⁸ and/or Na^+-H^+ exchange.⁴⁹ Changes in intracellular $[Na^+]$ may influence Ca^{2+} sparks indirectly, although any such effect will also depend on the localization of RyR clusters relative to Na^+-Ca^{2+} exchangers, a topic that is currently being researched actively (see Sections 6 and 7).

5. What are the consequences of T-tubule remodelling at the cellular level?

In the previous section, we discussed the expected effects of sub-cellular structural changes on Ca^{2+} sparks, and we described how simulations can assist with predicting and understanding the quantitative effects of each alteration. At the cellular level, however, different categories of models must be used to understand the cellular-level consequences of the structural changes.⁵⁰ For instance, a few recent cellular models can simulate both stochastic triggering of sparks and Ca^{2+} flux balance between the cytosol and the SR.^{17,18,47} These new models can consider not only some of the structural changes observed in HF such as RyR orphaning, but also changes to cell-wide Ca^{2+} fluxes, such as increased NCX activity and decreased activity of the SR Ca^{2+} ATPase (SERCA). Although these whole-cell models have been used to understand SR Ca^{2+} leak in quiescent cells from diseased myocytes, most simulations performed to date have focussed on changes in RyR gating or SERCA function rather than on structural changes.^{18,47} Future studies will be expected to directly compare the



functional effects of altered channel gating vs. altered sub-cellular structures.

In simulations at the cellular level, we would expect the following changes to be observed: (i) in quiescent cells, since orphaning makes spontaneous RyR openings less likely to trigger sparks (Figure 3), we would expect a greater proportion of the SR Ca^{2+} leak to be invisible rather than in the form of Ca^{2+} sparks; (ii) during triggered release, orphaning should lead to the initiation of fewer Ca^{2+} sparks early in each action potential (AP) and reduced synchrony, as recently demonstrated in a cellular simulation¹⁷; (iii) prolonged APs will likely allow for increased Ca^{2+} entry through L-type Ca^{2+} channels, which may partially offset the lower SR Ca^{2+} load caused by reduced SERCA activity; (iv) NCX, although perhaps up-regulated at the cellular level, may be less efficient at extruding Ca^{2+} if fewer exchangers are located close to RyRs⁵¹ and if intracellular Na^+ is elevated^{48,49}; and (v) the altered AP shape may influence not only overall Ca^{2+} entry, but also the triggering of Ca^{2+} sparks early in the AP.^{52–54} Because of electrophysiological differences between species, this last effect will be expected to depend greatly on the specific disease model examined.

Figure 4 shows results from an important recent study that demonstrates the state-of-the-art in our quantitative understanding of how changes in T-tubules influence cellular Ca^{2+} handling.⁵⁵ These investigators used super-resolution fluorescence imaging (stimulated emission depletion microscopy, or STED) to examine the T-tubule system in cells from mouse hearts several weeks after myocardial infarction. Measurements with this advanced technique confirmed the increased longitudinal and oblique T-tubule elements in disease and provided the novel finding that T-tubule cross-sectional area is increased in this disease model. The experiments were complemented by numerical simulations of how structural changes, alterations in Ca^{2+} fluxes (SERCA and NCX) and AP prolongation conspire to affect triggered Ca^{2+} release in diseased myocytes. As expected, the simulations confirmed that longer APs in disease lead to greater Ca^{2+} entry during each EC coupling cycle, and that orphaning will decrease the synchrony of spark triggering early in the AP.¹⁷ The computations also generated the novel prediction that orphaning per se can increase total Ca^{2+} entry during each AP, thereby increasing SR Ca^{2+} load

and partially offsetting the reduced synchrony. This study⁵⁵ therefore demonstrates that structural remodelling can lead to compensatory as well as deleterious effects, and that mathematical modelling can be helpful for predicting the quantitative consequences of various alterations.

6. Future directions: experimental studies

The discussion above indicates several unresolved issues relevant to disease-induced structural changes that will require additional experimental study. One obvious question to address is: how do RyR clusters themselves change in disease, and are these alterations specific to particular forms of cardiomyopathy? A recent study³³ found that dyads are smaller in failing rat myocytes after aortic constriction, but it is not clear whether these results are generally applicable across different experimental models. It will be especially important in future studies to apply super-resolution optical techniques that allow researchers to determine the fine structure of RyR clusters.⁵⁶ When these techniques have been applied to healthy cells,⁵⁷ they have revealed an RyR cluster structure more complex and irregular than previously anticipated. It is not currently known, however, whether disease alters not only cluster size, but also the fine structure of these complexes.

It will also be important to determine how disease changes the localization of auxiliary proteins that normally reside close to RyR clusters and L-type Ca^{2+} channels and can indirectly modulate their function. One prominent example previously mentioned is the $\text{Na}^+ - \text{Ca}^{2+}$ exchanger. Intriguing recent results³⁹ suggest that NCX can reduce the duration of Ca^{2+} sparks in healthy myocytes, and that this modulation may be altered in disease. Structural studies, however, indicate that NCX is generally not co-localized with RyRs,⁵⁸ although a significant percentage of NCX proteins may reside within a few hundred nanometres of RyR clusters.⁵⁹ More precise localization of these proteins relative to one another, and possible alterations in disease, will help allow these issues to be explored more quantitatively through mathematical modelling. Other proteins

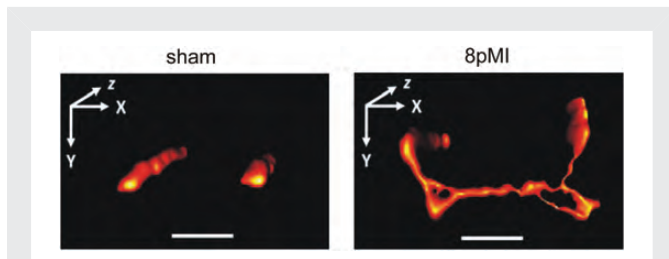
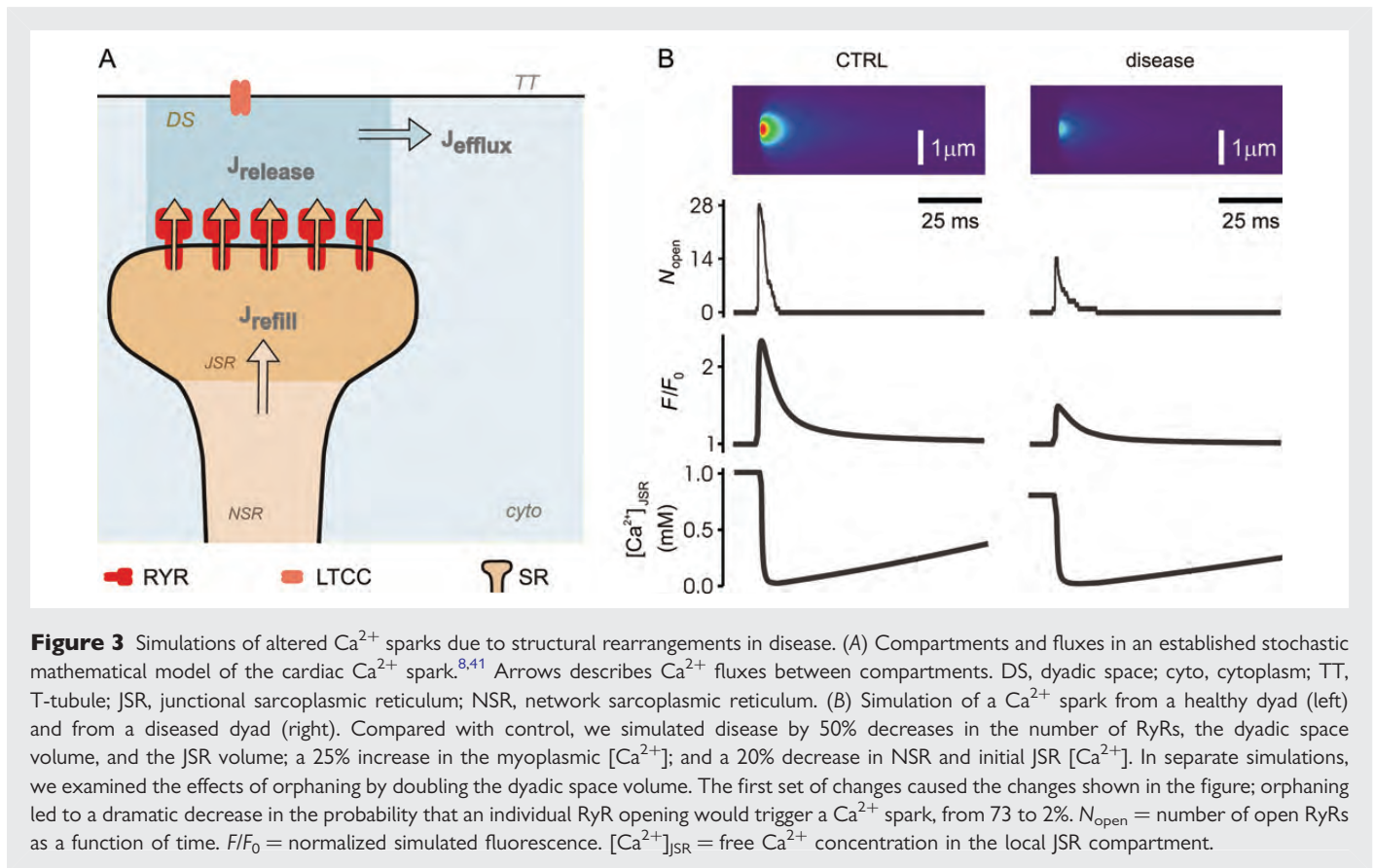


Figure 4 Experimental results demonstrating changes in T-tubule structure in disease. Three-dimensional reconstructions of T-tubules in healthy (left) and diseased mouse ventricular myocytes (right), obtained using STED microscopy. T-tubules in the diseased heart show increased cross-sections and more longitudinal elements running along the x-dimension. Scale bar = 1 μm . 8pMI = 8 weeks post myocardial infarction. Reproduced from Wagner *et al.*⁵⁵ with permission.

clearly important for the regulation of Ca^{2+} sparks and EC coupling are CSQ and SERCA. Recent simulation studies suggest that localization of either protein can affect the characteristics of localized Ca^{2+} sparks or cellular Ca^{2+} waves.^{60,61} Although SERCA is generally down-regulated during heart failure whereas overall CSQ levels are thought to remain constant,^{46,62} at present there is only limited information regarding whether these proteins significantly change their nanoscale localization.

Although much has been learned in recent years about the changes in T-tubule membranes during heart failure, much less is known about whether disease causes remodelling of the SR itself. SR alterations

have been demonstrated in two genetically modified mice,^{63,64} but it is not clear whether changes in the SR structure occur in other disease states. Improved methods to directly image the SR will be required to determine whether this is the case, but this is an important issue to address because Ca^{2+} diffusion within the SR is predicted to have significant functional consequences.^{45,61,65,66} Finally, the next several years are expected to bring significant new insight into the biochemical signalling mechanisms underlying changes in the T-tubule structure. Candidates identified to date include junctophilin^{34,67} and PI3-kinase,⁶⁸ but further mechanistic insight will certainly be uncovered in future studies.

7. Future directions: improvements to mathematical models

Besides the new experimental results that are likely to be obtained, the next several years will undoubtedly bring improvements to mathematical models, and the combination of experiments and simulations should advance our understanding and help to address unresolved questions regarding the functional effects of altered sub-cellular structure in cardiac disease.

To understand the functional consequences of altered sub-cellular structures, it seems clear that stochastic models of local SR Ca^{2+} release,^{18,41,47} which usually assume idealized geometries, will need to be combined with models that simulate Ca^{2+} movements in complex and realistic geometries.^{60,69,70} As discussed in more detail elsewhere,⁵⁰ these two categories of models have generally been developed separately, but the strengths of the two approaches must be combined to address how alterations to T-tubules and dyads,

which can sometimes be subtle, might affect EC coupling. Along these lines, an interesting question that has not yet been addressed through simulations concerns the functional consequences of irregularities in the structure of RyR clusters.⁵⁷ Similarly, mathematical modelling has not yet been used extensively to address how localization of mitochondria near RyR clusters affects either Ca^{2+} sparks or mitochondrial function. Models have integrated SR Ca^{2+} release with mitochondrial energetics at the cellular level,⁷¹ but stochastic gating of RyRs and greater spatial detail must be included to address the effects of changes in localization.

As representations of local geometry in mathematical models become more realistic, we expect that more simulation studies will address the consequences of changes in the localization of NCX proteins. Since this transporter can either import or extrude Ca^{2+} , depending on conditions, it can potentially influence both Ca^{2+} spark triggering and the decay of $[\text{Ca}^{2+}]$ after the initiation of release. NCX activity depends on $[\text{Na}^+]$ and $[\text{Ca}^{2+}]$ in addition to membrane potential, so simulations of local concentration changes are necessary to predict the consequences of altered NCX localization relative to L-type Ca^{2+} channels and RyRs. Although several studies, both experimental^{39,72,73} and computational,^{74–77} have addressed the potential importance of NCX, we expect that these issues will be examined in more detail in coming years, especially as novel experimental data regarding NCX localization in disease states become available.

More broadly, mathematical modellers will need to make a conceptual leap and become comfortable comparing the functional consequences of structural changes with the effects of altered ion channel expression and gating. Sensitivity analysis represents one method to consider biological changes within the same quantitative framework so that the effects of altered channel gating can be compared directly with the effects of remodelled structures (Y.S. Lee, in preparation).⁵⁰ This type of approach is only straightforward to implement, however, when simple geometries are assumed and a parameter such as compartment volume can easily be scaled. Novel methods will need to be developed in order to use such a strategy with models that consider complex geometries.

8. Conclusions

In this review, we have summarized alterations to T-tubules and dyads that occur in ventricular myocytes in disease, and we have described how mathematical modelling has provided important insight into the functional effects of these changes. Although work performed to date has improved our quantitative understanding of changes that occur with disease, further improvements, both experimental and computational, will be required to address unresolved questions. These include the effects of alterations in RyR cluster structure, and the nanoscale localization of auxiliary proteins such as NCX, CSQ, and SERCA. We expect that over the next several years, experimental measurements combined with simulations will provide significant new insights into these issues.

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