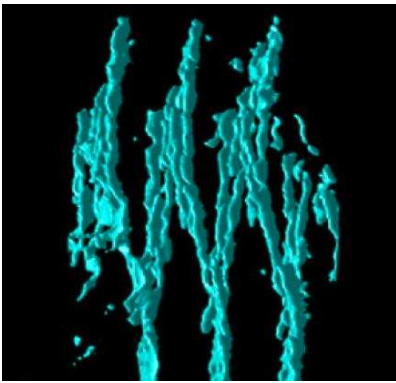


A new twist in cardiac muscle: dislocated and helicoid arrangements of myofibrillar z-disks in mammalian ventricular myocytes

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The general structure of cardiac muscle cells has been established by light and electron microscopy for some time, but detailed information on 3D organization is less common as is the organization of proteins that enable high speed signal transduction to take place. To provide information for detailed modeling of cardiac cell function we have been examining the organization of myofibrils, sarcoplasmic reticulum, t-tubules and associated proteins by confocal fluorescence microscopy. These studies show that the basic 3D organization of the myofilaments is complex, with dislocations and helicoidal structures evident (see figure). Such a complex structure may arise during normal growth but it also has important implications for arrhythmogenesis *via* calcium wave propagation. While the sarcoplasmic reticulum calcium release channel (RyR) is generally close to z-lines and separated laterally by $\sim 0.6 \mu\text{m}$, the longitudinal separation should be set by the sarcomere length which is considerably longer $1.9\text{-}2.2 \mu\text{m}$. Therefore one would expect that a Ca wave would be able to propagate more easily in the transverse direction than longitudinally. However, it is well known that calcium waves propagate throughout the cell rather uniformly (*e.g.* Berlin, Cannell & Lederer, 1989). Our observation (Jayasinghe *et al.*, 2010) of dislocated z-lines that have RyRs associated with them helps explain this paradox; the dislocations coupled with the jitter in longitudinal RyR spacing will assist longitudinal calcium wave propagation.



For normal excitation-contraction coupling, calcium influx *via* l-type calcium channels (DHPRs) triggers the release of calcium from RyRs and 2D analysis of the colocalization of DHPRs and RyR in rat suggested that most ($\sim 60\%$) of the DHPRs were grouped opposite the RyRs (Scriven, Dan & Moore, 2000). However, when analysis is performed in 3D, the colocalization is significantly lower ($\sim 45\%$) (Fletcher *et al.*, 2010). This recent finding underscores the importance of studying structure in 3D especially when the underlying resolution of the microscope is insufficient at the spatial scale of interest. In human, we find an even lower level of colocalization which suggests that normal EC coupling may arise from a combination of tightly coupled release triggered by DHPR activation plus a component due to local calcium diffusion from nearby DHPRs and Ryrs.

At the near molecular scale, we find that RyRs are not packed into junctional regions as a single cluster but rather as smaller groups of RyRs forming super clusters. This again suggests that local diffusion between release sites plays an important role in normal excitation-contraction coupling. It is possible that this partial uncoupling of RyRs from DHPRs serves to protect excitation contraction coupling during the normal trafficking of proteins and sub-cellular remodeling which would be important as the heart never has a chance to rest and rebuild. On the other hand, this looser coupling makes arrhythmogenesis *via* calcium waves more likely and may help explain the propensity of the remodeled, diseased heart to develop arrhythmias.

1. Berlin, J.R., Cannell, M.B. & Lederer, W.J. (1989) ITI in single rat cardiac ventricular cells: Relationship to fluctuations in intracellular calcium. *Circulation Research* **65**, 115-126.
2. Jayasinghe, I.D., Crossman, D.J., Soeller, C., Cannell, M.B. (2010) A new twist in cardiac muscle: dislocated and helicoid arrangements of myofibrillar Z-Disks in mammalian ventricular myocytes. *Journal of Molecular and Cellular Cardiology* **48(5)**, 964-971.
3. Scriven, D.L., Dan, P., Moore, E.D.W. (2000) Distribution of proteins implicated in excitation-contraction coupling in rat ventricular myocytes. *Biophysical Journal* **79**, 2682-2691.
4. Fletcher, P.A., Scriven, D.L., Schulson, M.N., Moore, E.D.W. (2010) Multi-image colocalization and its statistical significance. *Biophysical Journal* **99**, 1996-2005.