New and Notable

Cellular Cartography

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The electronic chips that have revolutionized our world are architectural marvels. Transistors, ~50 nm long, are etched in silicon with remarkable precision. We who work with floppy biological cells in a watery world might be forgiven for thinking that such precision and order are not achievable and may not be necessary for the proper functioning of living cells. Immunofluorescence images of cells often give one the impression that proteins are cast about willy-nilly. A precision of a few thousand nanometers seems to be good enough. Call this biological sloppiness or robustness. But nanometers matter. The recent history of cardiac muscle tells us why. The ryanodine receptor (RyR, the intracellular Ca^{2+} release channel of the sarcoplasmic reticulum, SR) has been known since 1970 to possess the property of Ca^{2+}-induced Ca^{2+} release: Ca^{2+} entering the cell through the L-type Ca^{2+} channel ($Ca_V1.2$) increases the open probability of the nearby RyRs causing Ca^{2+} release from SR, further increasing the Ca^{2+} concentration at the mouth of RyR.

One would immediately predict that a wave of propagating Ca^{2+} release would occur and that Ca^{2+} release would be all-or-none. Paradoxically, the strength of contraction (largely a function of the amount of Ca^{2+} released from the SR) is smoothly graded with the membrane potential. The key to resolving this paradox was provided by Michael Stern, whose "local control theory" (1) posits that $Ca_V1.2$ and RyR are clustered into spatially separated units called "couplons" and each couplon, by virtue of their separation, acts more or less independently. Gradation of contraction is a statistical outcome of the number of couplons activated. A year later, Cheng et al. (2) showed us how local control looks like with their discovery of Ca^{2+} sparks. That was 17 years ago. Now muscle researchers all think of cell signaling as local. In retrospect, it seems obvious that the spatial distribution of molecules would affect signaling, but what is not obvious is how sensitively the communication between molecules depends on their spatial distribution. Surprisingly, changes in the spatial separation of RyR clusters as small as 100 nm can qualitatively alter cellular Ca^{2+} dynamics (3,4).

The goal of cellular cartographers is to map the position of molecules in the three-dimensional space in the cell. Knowing the position of the molecules, one can estimate how many molecules are in a cluster, how far apart the clusters are from each other, and who are neighbors. These data are essential for understanding cellular physiology. Scriven et al. (5), whose work appears in this issue, have mapped the positions of RyR and $Ca_V1.2$ in the rat ventricular myocyte. This is familiar territory for them as they have done the mapping before but this time they use three-dimensional-object (called a "blob") colocalization metrics they recently developed (5) to define where molecules are, how large the clusters are, and how far clusters are from each other. Defining the position of a molecule isn't as easy as looking at an image. Scriven et al. used fluorescence confocal and wide-field microscopy to image RyR and $Ca_V1.2$ labeled with fluorophore-tagged antibodies. Diffraction always introduces ambiguities; a point object appears like an American football in a three-dimensional image. Scriven et al. reduced ambiguities by deconvolution and also, by standing the roughly cylindrical myocyte on-end to take advantage of the better resolution in the *x-y* plane than along the optical axis.

Despite these efforts, ambiguities in the positions of RyR and CaV1.2 persist and there must be some way of deciding on whether these molecules occupy the same point in space, i.e., colocalized. In this article, they first threshold the raw image, eliminating all voxels that are below some threshold value. However, instead of using voxel colocalization as in their earlier works, in this new work they use blob colocalization. One of the most important benefits of using blob colocalization is the ability to determine whether the colocalization value is statistically different from what you would expect if the proteins were randomly scattered. While the threshold value affects the percent colocalization, importantly and remarkably, the statistical significance of blob colocalization is largely unaffected by the threshold level.

Using their blob metrics, Scriven et al. (5) find that 65% of the RyR clusters and 75% of the $Ca_V1.2$ clusters are colocalized with each other, that is, in couplons. Based on the cluster sizes and data from earlier binding studies, they estimate that the RyR clusters contain ~74 RyR tetramers and the $Ca_V1.2$ clusters ~10 $Ca_V1.2$ molecules. The RyR numbers are within the low range of estimates by others (referenced in (5)). These numbers are important. The probability that any one RyR or $Ca_V1.2$ channel will open, P_o, must be low enough to minimize spontaneous openings. Yet upon receiving the appropriate signal these channels must reliably open. The probability that at least one channel in the cluster opens (assuming independence) is $1-(1-P_o)^N \approx 1-e^{NP_o}$, where N is the number of molecules in the cluster. Thus, the reliability of a cluster opening scales exponentially with N. Using a similar analysis, Inoue et al. (see reference in (5)) estimate that 3–11 $Ca_V1.2$ molecules per couplon are necessary to account for the reliability they measured. What about the 35% of RyRs and 25% of $Ca_V1.2$ that are not in couplons?

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The RyR clusters could be part of spiral arrangement of RyRs discovered by Soeller et al. (4) that lies between the z-lines. Further experiments are needed to answer this question. The sizes of noncoupled clusters are smaller than their dyadic counterparts. Scriven et al. speculate that these clusters may be too small to link together to form a couplon, or they could be nascent couplons on their way to being assembled or degraded. Assuming that the ensemble collection of RyR and $Ca_V1.2$ clusters equals the average flow over time, their data suggests that 35% of the RyRs and 25% of the $Ca_V1.2$ molecules are being overturned at any moment. Many heady ideas come from looking at a map.

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