JGP Journal of General Physiology

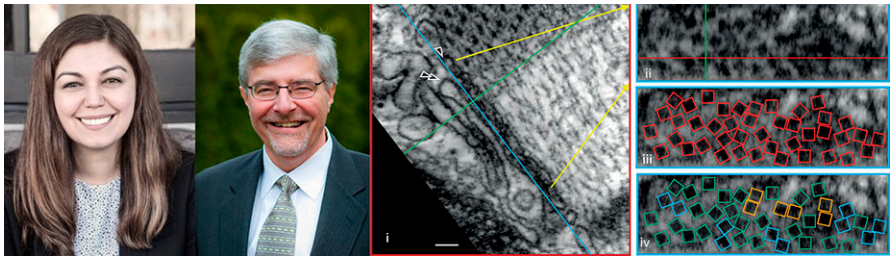
RESEARCH NEWS

RyR2 phosphorylation alters dyad architecture

Ben Short[1]

JGP study (Asghari et al. 2024. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202213108) indicates that β-adrenergic signaling enlarges dyads and reorganizes RyR2 tetramers in cardiomyocytes.

The type II ryanodine receptor (RyR2) is a large, homotetrameric Ca^{2+}-activated Ca^{2+} channel that is critical for excitation–contraction coupling (ECC) in muscle cells. In cardiomyocytes, RyR2 localizes to the junctional sarcoplasmic reticulum, and is positioned in close proximity to $Ca_V1.2$ channels in the sarcolemma/t-tubules at sites known as dyads, allowing ECC to occur with high efficiency during systole. In diastole, spontaneous opening of RyR2 channels can induce the activation of neighboring tetramers, resulting in Ca^{2+} sparks that can become dangerously elevated in patients with certain cardiac diseases. In this issue of *JGP*, Asghari et al. reveal that phosphorylation of RyR2 in response to β-adrenergic signaling can alter the size of dyads and the arrangement of RyR2 tetramers within them, which is likely to have important consequences for both Ca^{2+} spark generation and ECC (1).



Parisa Asghari (left), Edwin Moore (right), and colleagues reveal that β-adrenergic signaling and the phosphorylation of RyR2 dynamically regulate the organization of RyR2 tetramers (red boxes in panel iii). Clustered at the dyads formed by the apposition of the junctional SR (single arrowhead, panel i) and t-tubules (double arrowhead), RyR2 tetramers can be isolated (green boxes, panel iv) or arranged in side-by-side (orange) or checkerboard (blue) configurations. RyR2 phosphorylation expands the dyad and increases the proportion of tetramers in the checkboard arrangement, which likely alters ECC and Ca^{2+} sparking.

In recent years, it has become increasingly clear that dyads are far more dynamic than previously imagined. For example, Edwin Moore and colleagues at the University of British Columbia have found that most RyR2 tetramers are arranged in either side-by-side or checkerboard configurations within the dyad, and the proportion of tetramers in each configuration can be altered by a variety of factors (2). Factors that decrease the frequency of Ca^{2+} sparks increase the proportion of tetramers arranged side-by-side with each other. Factors that promote the checkerboard arrangement, in contrast, increase the probability of Ca^{2+} sparks, potentially due to some sort of positive allosteric effect on channel opening when the receptors are in this configuration (2, 3).

One of the factors found to promote the checkerboard arrangement was a phosphorylation cocktail containing numerous kinase activators and phosphatase inhibitors (2), but the exact kinases involved, and their downstream targets, remained unknown. "We wanted to determine whether phosphorylation of RyR2 itself was responsible for the rearrangements that we'd seen," Moore explains.

RyR2 is phosphorylated by both PKA and CamKII in response to β-adrenergic stimulation. Using dSTORM superresolution microscopy, Moore and colleagues previously observed that the β-adrenoceptor agonist isoproterenol increases the size of RyR2 clusters in wild-type cardiomyocytes (3). Now, using transmission electron microscopy, Moore's team, led by Research Associate Parisa Asghari, determined that this corresponds to an increase in dyad size (1). Moreover, using dual-tilt electron tomography, the researchers found that isoproterenol treatment also alters the organization of RyR2 in the enlarged dyads, increasing the proportion of tetramers in the checkerboard configuration.

To investigate whether the changes in dyad size and tetramer organization are directly linked to RyR2 phosphorylation, Moore and colleagues examined cardiomyocytes from mice carrying clinically relevant mutations in RyR2. Remarkably, one phosphomimetic mutation, S2814D, was sufficient to expand the dyad and promote the rearrangement of tetramers into the

[1]Science Writer, Rockefeller University Press, New York, NY, USA.

Correspondence to Ben Short: bshort@rockefeller.edu.

checkboard configuration, even in the absence of isoproterenol. In contrast, a nonphosphorylatable RyR2 mutant, S2030A, prevented isoproterenol from expanding the dyad and impaired the reorganization of tetramers into the checkerboard pattern.

"That implies there's a direct connection between the phosphorylation state of RyR2 and both the size of the dyad and the organization of tetramers," Moore says.

Overall, Asghari et al.'s observations suggest that phosphorylation of two RyR2 residues, S2030 and S2808, are required for the normal, structural response to isoproterenol but S2814 is not. Notably, phosphorylation of S2030 and S2808 are also required for isoproterenol's functional effects on ECC, whereas S2818 is dispensable (4–6). Based on their observations of tetramer organization, Asghari et al. were also able to correctly predict the effects of most of the phosphomutants on Ca^{2+} spark frequency.

β-adrenergic signaling and phosphorylation of RyR2 can therefore dynamically regulate the size and organization of RyR2 clusters in dyads, with important functional consequences. "In 20 years, we've gone from thinking of dyads as being static structures filled with beautifully organized tetramers to seeing the whole thing as being highly dynamic," Moore says. "We want to continue to investigate the dynamic nature of these structures and examine what happens under pathological conditions such as heart failure."

References

1. Asghari, P., et al. 2024. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202213108
2. Asghari, P., et al. 2014. *Circ. Res.* https://doi.org/10.1161/CIRCRESAHA.115.303897
3. Asghari, P., et al. 2020. *Elife*. https://doi.org/10.7554/eLife.51602
4. Ullrich, N.D., et al. 2012. *J. Mol. Cell. Cardiol.* https://doi.org/10.1016/j.yjmcc.2012.03.015
5. Potenza, D.M., et al. 2019. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201812155
6. Baier, M.J., et al. 2021. *FEBS Open Bio.* https://doi.org/10.1002/2211-5463.13274