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EDITORIAL

AKAP18δ Puts CaMKII in the Right Place at the Right Time: Implications for Cardiac Ca^{2+} Handling

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A delicate control of myocyte Ca^{2+} handling is essential for efficient excitation-contraction coupling (ECC) in the heart, and its alteration is associated with decreased contractility, arrhythmias, hypertrophy, and heart failure. During ECC, entry of Ca^{2+} from the extracellular space occurs through L-type Ca^{2+} channels and mediates Ca^{2+}-dependent opening of RyR2 (ryanodine receptors) allowing massive movement of Ca^{2+} from the sarco-endoplasmic reticulum (SR) to the cytosol and ultimately triggering cell contraction. Thereafter, relaxation occurs primarily by the reuptake of Ca^{2+} into de SR by SERCA2a (SR Ca^{2+} ATPase 2a). Phosphorylation of proteins involved in Ca^{2+} cycling have critical functional consequences on ECC, including greater influx of Ca^{2+} through the L-type Ca^{2+} channels and a greater release of Ca^{2+} from the SR through RyR2 and a more efficient Ca^{2+} reuptake through SERCA2a as a result of phosphorylation of its regulatory protein PLN (phospholamban). Accumulating evidence indicates that spatial and temporal control of phosphorylation/dephosphorylation cycles are another crucial point of control of cardiac ECC. This control is achieved, at least in part, by a complex network of scaffolding, anchoring and adaptor proteins that recruit, compartmentalize, and regulate protein kinases in a location specific manner.[1] AKAPs (A-kinase anchoring proteins) are the paradigm of this integrated regulatory system that have been extensively shown to coordinate spatially restricted cAMP-PKA (protein kinase A)-dependent signaling that provides a high level of specificity, contributing to adrenergic modulation of cardiomyocyte function.[2] There are over 50 known AKAPs (including alternative-spliced forms) that target PKA to different sites within the cell. While AKAPs share their ability to bind PKA, they are remarkably diverse scaffolding proteins. Indeed, AKAPs couple PKA to different substrates, enhancing the rate and fidelity of their phosphorylation by the kinase. By bringing together different combinations of upstream and downstream signaling molecules, AKAPs provide the architectural infrastructure for specialization of the cAMP/PKA signaling network which is critical for the regulation of cardiac Ca^{2+} handling.

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CaMKII (calcium/calmodulin [CaM]-dependent protein kinase II) is also a key regulator of Ca^{2+} handling, ECC, electrophysiology, transcription, and other processes in the heart and its chronic activation is implicated in arrhythmias, heart failure, pathological remodeling, and other diseases.[3] However, only discrete evidence has been published on how this kinase targets to distinct nanodomains where Ca^{2+} handling proteins are localized. Initial work from Singh et al[4] provided the first evidence demonstrating a functional CaMKII anchoring protein that binds to SERCA2a and recruits CaMKII resulting in facilitated PLN phosphorylation. This CaMKII anchoring protein arises from the CAMK2A gene that encodes not only CaMKII but also a nonkinase peptide termed αKAP in skeletal and cardiac muscle.[5,6] αKAP has been reported to anchor, via its N-terminal hydrophobic sequence, to

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the SR membrane in skeletal muscle. In this way, αKAP recruits CaMKII through dimerization with the association domain and regulates SR Ca^{2} transport. There is evidence that αKAP and CaMKII are also enriched in cardiac SR membranes, suggesting that cardiac muscle shares with skeletal muscle a common regulatory role for these molecules. However, as yet, no direct evidence of the physiopathological impact of αKAP-dependent CaMKII-SERCA2a tethering on Ca^{2+} handling and ECC has been established.

In this issue of *Circulation Research*, Carlson et al[7] identify a CaM-KAP (CaM-kinase anchoring protein), AKAP18δ, that fine-tunes frequency-dependent activation of CaMKIIδ. They show that AKAP18δ adapts CaMKII response to increased frequency of Ca^{2+} cycling due to its unique capacity to make CaMKII more active at frequencies (1–4 Hz) at which Ca^{2+}-CaM is not able to fully activate the kinase.

Carlson et al provide significant structural insight on the anchoring and direct regulation of CaMKIIδ activity by AKAP18δ at PLN-SERCA2a and RyR2 vicinity. Using multiple experimental approaches ranging from bioinformatics, biochemical probes, microscopy, and surface plasmon resonance to study structural interaction and binding kinetics between AKAP18δ and CaMKII. Importantly, they identify 2 regions in AKAP18δ that inversely regulates CaMKII activity. The data strongly support that while N-terminal AKAP18δ inhibits CaMKII, C-terminal AKAP18δ activates CaMKII and promotes phosphorylation of both Thr17 of PLN and Ser2814 of RyR2.

The facilitation of the phosphorylation of SERCA2a and RyR2 due to AKAP18δ-CaMKII interaction are physiologically relevant given that they can adapt cardiomyocyte Ca^{2+} handling to pacing frequency. At low frequency, AKAP-N binds CaMKII by multiple contact sites and stabilizes its inactive conformation. However, at higher pacing frequencies, calcified CaM binds to both CaMKII and AKAP-N leading to displacement of CaMKII from the AKAP18δ-N region. Under this later condition, AKAP-C operates as a CaMKII activator by trapping CaM within the kinase and lowering the threshold for Ca^{2+}-dependent CaMKII activation. The dual phosphorylation induced by CaMKII would enhance Ca^{2+} reuptake and release velocity, accelerating relaxation, and increasing contractility. An interesting observation raised by the authors is that AKAP18δ seems to allow for an early CaMKII-dependent acceleration of cytosolic Ca^{2+} decline, which would otherwise occur at a higher pacing frequency in absence of AKAP-anchoring/regulatory function. The article provides experimental evidence showing that adult cardiomyocytes treated with the cell-permeant C-terminal region of AKAP18δ (AKAP18δ-C) have accelerated Ca^{2+} decline and that this effect is most prominent at frequencies between 1 and 4 Hz supporting that, by activating CaMKII, AKAP18δ is able to accelerate Ca^{2+} reuptake into the SR, enhancing relaxation.

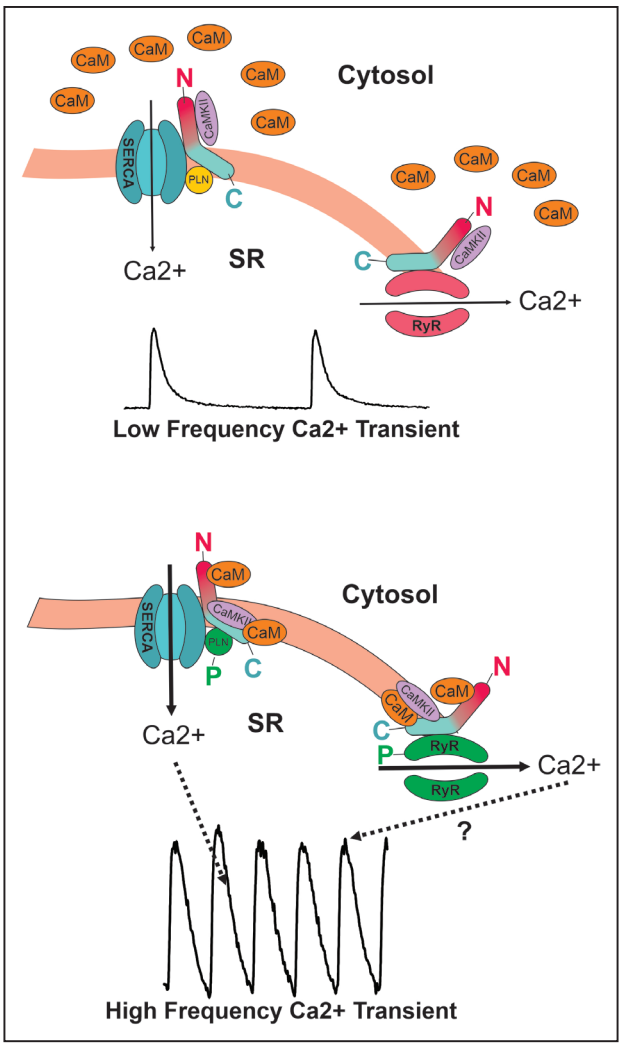

Figure. A-kinase anchoring protein 18δ (AKAP18δ) fine-tunes CaMKII (calcium/calmodulin-dependent protein kinase II) activation at the PLN (phospholamban)-SERCA2a (SR Ca2+ ATPase 2a) and RyR2 (ryanodine receptors) level to adjust Ca^{2+} transients during the force frequency response.
A, At low stimulation frequencies, AKAP18δ inhibits CaMKII and precludes PLN and RyR2 phosphorylation. **B**, When frequency increases, CaM accumulates and outcompetes AKAP18δ-CaMKII inhibition and favors CaM binding to CaMKII resulting in kinase activation. CaMKII is then able to phosphorylate PLN leading to enhanced Ca^{2+} uptake by SERCA2a thus contributing to frequency-dependent acceleration of relaxation and also the RyR2 enhancing SR Ca^{2+} release. This latter mechanism, although not studied, could impact on Ca^{2+} transient amplitude during the force frequency response.

Future studies to determine the impact AKAP18δ/CaMKII-dependent RyR2 phosphorylation on Ca^{2+}-transient amplitude and contractility would be an interesting follow-up of this seminal study (Figure).

Based on their results, the authors raised an important open possibility related to the use of AKAP-C as a booster of SERCA2a function in models of heart failure which are associated with reduced SERCA2a expression/activity. In this regard, future research tailoring the anchoring and regulation of RyR2 and SERCA2a/PLB

will be of primary importance to take advantage of AKAP-C as a therapeutic tool to accelerate Ca^{2+} uptake without promoting RyR2-mediated Ca^{2+} leak and arrhythmias.

In our opinion, the novel mechanistic insight provided by Carlson et al on the role of AKAP18δ as a CaM-KAP opens a completely new set of interesting questions to be resolved.

1. Does AKAP18δ regulate CaMKII activity at the level of other CaMKII target proteins (Na^+ and Ca^{2+}channels, mitochondrial proteins, HDACs, etc)?
2. Which is the role of AKAP18δ under conditions that promote CaMKII activation different from the Ca^{2+}-dependent mechanisms. For example, oxidation, glycosylation, etc?
3. Given that, mutations within CaM structure lead to arrhythmogenic conditions,[8] is it possible to speculate on altered AKAP function promoted by mutant CaMs? For example, a mutant CaM with low capacity to bind to AKAP-N would lead to a reduced capacity of Ca^{2+} uptake to be accelerated when it is required.
4. Beyond the force frequency relationship, Ca^{2+} handling by the SR is an important determinant for the occurrence of Ca^{2+} alternans at high frequency. Although the role of CaMKII activity is not completely clear in the development of Ca^{2+} alternans[9,10] AKAP18δ could be an additional player in this complex phenomenon.

Overall, in the cardiovascular field, there is an unmet need for novel therapeutic approaches that correct Ca^{2+} handling under different pathological situations and understanding the regulation of CaMKII at the level of its target proteins provides a new point of control to tailor specific therapeutic strategies. The present study sheds light on a novel regulation of CaMKII by AKAP18δ and will surely motivate future research leading to a better modulation of Ca^{2+} handling, arrhythmia prevention, and heart failure.

ARTICLE INFORMATION

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Disclosures

None.

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