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Visualizing CaMKII and CaM activity: a paradigm of compartmentalized signaling

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Abstract

Calcium (Ca^{2+}) has long been recognized as a crucial intracellular messenger attaining stimuli-specific cellular outcomes via localized signaling. Ca^{2+} binding proteins, such as calmodulin (CaM) and its target proteins are key to the segregation and refinement of these Ca^{2+} -dependent signaling events. This review summarizes the recent technological advances enabling the study of subcellular Ca^{2+} -CaM and Ca-CaM dependent protein kinase (CaMKII) signaling events but also highlights the outstanding challenges in the field.

Keywords

calcium; Calmodulin; Ca-calmodulin dependent protein kinase II (CaMKII)

Introduction

Intracellular Ca^{2+} is a quintessential intracellular messenger, and many of its cellular effects are transduced by calmodulin (CaM). The spatiotemporal characteristics of these intracellular Ca^{2+} signals hinge on a complex interplay between Ca^{2+} influx and efflux mechanisms, mobile vs. stationary Ca^{2+} binding proteins (such as CaM) and intracellular localization/partitioning or sequestration. Over the past decades, great strides have been made in understanding the local control mechanisms underlying the global Ca^{2+} oscillations (ranging from 0.1–1 μM) in the beating heart [1]. It has also become clear that multiple Ca^{2+} signaling events, independent of those governing cardiac contraction, occur simultaneously in the same myocyte and control myriad cellular outcomes [2]. Several microdomain Ca^{2+} signals leading to altered transcription have now been identified e.g. caveolae-localized L-type Ca channels provide the Ca^{2+} source for the Ca^{2+} -CaM-dependent activation of calcineurin-NFAT (nuclear factor of activated T-cells) signaling and inositol triphosphate receptors (InsP_3R) that release Ca^{2+} can activate Ca^{2+} -CaM dependent kinase (CaMKII) related to histone deacetylase (HDAC) signaling [3,4]. Here we report how recent advances in fluorescent biosensors can provide unique insights into the spatiotemporal dynamics of these local Ca^{2+} -CaM signals, how they are sensed and decoded to shape particular cellular responses.

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Disclosure

The authors declare that they have no conflict of interests.

Calmodulin: a tale of specificity and promiscuity

CaM acts as a pivotal transducer of Ca^{2+} signals for a wealth of cellular functions including excitation-contraction coupling, metabolism, cell survival and transcriptional regulation. How CaM regulates such a diversity of signaling pathways with distinct spatial and temporal outcomes remains a central question. Nonetheless key aspects of CaM signal transduction mechanisms can be explained by the biochemical properties of CaM: its Ca^{2+} binding properties, its localization, its concentration and mobility inside the myocyte and also its binding kinetics to target proteins.

CaM interactions

The three-dimensional crystal structure of Ca^{2+} -free CaM (apoCaM) reveals a dumbbell-like shape (Fig 1): a flexible, central α helix flanked by two globular lobes (the N- and C-lobes) [5,6]. Each lobe contains two coupled EF-hand motifs with the C-lobe having higher Ca^{2+} binding affinity ($K_d \sim 1 \mu\text{M}$) than the N-lobe ($K_d \sim 12 \mu\text{M}$) [7–9]. The lobes also differ in Ca^{2+} dissociation kinetics ($\sim 1000\text{s}^{-1}$ for the N-lobe and 100 times slower for the C-lobe). Upon Ca^{2+} binding, CaM undergoes a profound conformational change (becoming a more elongated structure), modulating the interaction with its target proteins [10]. The structural flexibility of CaM explains the abundance and structural heterogeneity of CaM interacting proteins or interactome [11–14]. These CaM binding targets differ in their preference for apoCaM and Ca^{2+} -CaM, can be activated or inhibited upon CaM binding and can themselves influence Ca^{2+} sensitivity of CaM [15,16]. Target proteins of note in the heart include ion channels (L-type Ca channels (LTCC) [17]), ryanodine receptors (RyR) [18], InsP_3R [19], the protein phosphatase calcineurin (CaN) [16], CaMKII, nitric oxide synthase (NOS), adenylyl cyclase (AC), phosphodiesterase (PDE), myosin light chain kinase (MLCK), etc. [20].

In vivo, CaM tethering to target proteins can be monitored with FRET (fluorescence resonance energy transfer), even with simultaneous $[\text{Ca}^{2+}]$ using Ca^{2+} indicators [21]. Our group used a variant of this approach in adult cardiac myocytes to map CaM binding to the RyR by monitoring FRET between fluorescently labeled FKBP12.6 bound to RyR and fluorescent CaM [22]. CaM binding was found to stabilize the zipped conformational state of the RyR (reducing RyR activation). Interdomain “unzipping” of the RyR by treating the cells with a peptide DPc10 that mimics one of the zipped regions, also reduced CaM binding and enhanced RyR gating, Ca sparks and waves that favor arrhythmogenic events and unloading Ca^{2+} from the SR, thereby mimicking aberrant channel activation as seen in heart failure [23].

CaM (trans)location and availability

Surprisingly, data regarding CaM location and redistribution remains scarce in adult cardiomyocytes. Total CaM concentration is $\sim 6 \mu\text{M}$ in isolated cardiac myocytes [24] and at resting $[\text{Ca}^{2+}]$ levels, most CaM is apoCaM (not Ca^{2+} -bound). Some of this apoCaM is already prebound to targets as structural elements of the protein or is poised nearby the target awaiting local activation (part of signaling scaffold). Using null-point titration methods with fluorescently labeled CaM, Wu and Bers found that, as in other cell types, free CaM is around 50–75 nM [25,26]. That is only $\sim 1\%$ of total CaM is free, which implies that dynamic competition for CaM among its targets is an integral part of the CaM signaling mechanism. In this “limiting CaM” scenario target proteins with higher Ca^{2+} -CaM affinities (e.g. CaN, PDE, MLCK with $K_d \sim 1 \text{nM}$) will be preferentially activated compared to those targets with lower affinity (e.g. CaMK, AC with $K_d > 10 \text{nM}$).

At resting Ca^{2+} (100nM $[\text{Ca}^{2+}]_i$) most intracellular CaM is bound to cellular constituents and appears highly localized to the Z-line (or transverse tubule) (Fig 1) [26]. High levels of CaM at the junction between the sarcolemma and SR membrane, where LTCCs and RyRs are both concentrated, facilitates CaM-dependent modulation of both Ca^{2+} current and SR Ca^{2+} release. Indeed, such regulatory CaM is already bound to the LTCC and RyR where upon LTCC Ca^{2+} influx and SR Ca^{2+} release, locally prebound apoCaM becomes Ca-CaM and mediates Ca^{2+} -dependent inactivation of Ca^{2+} current and favors RyR closure. Thus Ca-CaM is part of a highly local negative feedback system at the junctional cleft at the heart of excitation-contraction coupling.

Junctional targeting of CaM would likewise facilitate activation of lower CaM affinity targets such as CaMKII, by enhancing the latter's ability to compete with higher affinity global targets. This may relate to Ca-CaM-dependent activation of LTCC via CaMKII activity (Ca current facilitation) in which Ca current amplitude increases (and inactivation slows) in a stepwise manner during increases in stimulation rate [27–29]. This facilitation may help sustain Ca current amplitude at higher heart rates when Ca-dependent inactivation might otherwise limit it.

When $[\text{Ca}^{2+}]$ is elevated (from 100 to 500 nM), more CaM is bound at the Z-lines (where the junctional SR is) and from which CaM dissociation is extremely slow, but there is also a significant translocation of CaM into the nucleus [26]. Normal twitch Ca^{2+} oscillations did not trigger this nuclear redistribution [4]. This indicated that the kinetics of Ca-CaM signaling may be critical for this CaM translocation. Endothelin-1 binding to its G-protein coupled receptor (GPCR) also causes nuclear translocation of CaM, without triggering a detectable elevation of global $[\text{Ca}^{2+}]_i$ [4]. Analogous to the RyR environment above, it is possible that CaM at or near InsP_3R downstream of endothelin-1 might stimulate this CaM translocation. So chronic global Ca^{2+} elevation can trigger nuclear CaM accumulation, but certain local Ca^{2+} signals can achieve the same effect. Changes in nuclear CaM are likely to be of pathophysiological importance given its regulation of nuclear CaMKII activity (see below), but also because of direct effects on myocyte enhancer factor 2 –dependent transcription [30]. In neurons, nuclear CaM was also shown to affect phosphorylation of cAMP response element binding protein (CREB) [31]. How CaM translocates to the nucleus in cardiomyocytes remains to be identified [32]. In neurons this nuclear import occurs in association with partner proteins [33] and this is probably also true in the heart given the low free CaM levels (and competition for CaM binding). A more thorough understanding of what type of signals trigger CaM nuclear import could help to clarify things. Another outstanding question is how CaM expression and localization may be altered in cardiac disease. There are conflicting reports regarding CaM expression levels in human heart failure [34,35] and even less information on CaM localization. Alternatively, association of both apoCaM and Ca^{2+} -CaM with target proteins could be altered. For instance, Ai *et al.* demonstrated in their rabbit heart failure model that although the total CaM levels were unchanged, CaM association with the RyR was decreased (consistent with increased RyR activity or SR Ca leak) [36]. In that context, if in failing myocytes CaM was found to be redistributed to the nucleus, it could influence gene transcription and alter contractile function in the various etiologies of hypertrophy and heart failure. Gangopadhyay and Ikemoto, albeit in neonatal myocytes, suggested that this might be the case. Here, dantrolene treatment of neonatal myocytes, which ameliorates RyR unzipping and aberrant Ca^{2+} events following prolonged endothelin-1 exposure, reduced nuclear translocation of CaM and the development of hypertrophy [37].

Concept of “dedicated” vs. “promiscuous” CaM

An emerging theme for CaM modulation of ion channels in cardiac myocytes is the multiple modes of regulation mediated by distinct functional populations of CaM (Fig 1): a dedicated pool of CaM typically tethered to the channels which directly regulates channel activity (most often involved in inactivation) and a more promiscuous pool of CaM poised nearby to transduce local Ca^{2+} signals via activation of downstream effectors such as CaN and CaMKII (providing a negative or positive feedback loop). These intricate levels of CaM regulation of channel function were recently reviewed in detail [38] for the L-type Ca channel, the ryanodine receptor and the InsP_3 receptor but are beyond the scope of this review. In general the mechanisms of promiscuous CaM regulation are less clear but from an imaging perspective several of the remaining questions can be addressed such as: is this promiscuous CaM activated to act locally or does it diffuse from more distant sources? Are the Ca^{2+} signals transduced by the dedicated (tethered) and promiscuous (more global) CaM the same? What is the reservoir/source of this promiscuous CaM pool (e.g. which proteins release CaM with Ca^{2+} influx)? Several of these events are likely happening within the single resolvable volume of traditional confocal microscopy. The technical challenge to overcome here is the spatial resolution limitation and this may require novel experimental approaches or a combination thereof: TIRF (total internal reflection fluorescence microscopy which limits the fluorescence signal to ~ 100 nm from the coverslip), FRET imaging of dynamic protein interactions, subcellular targeting of biosensors, superresolution fluorescence microscopy (PALM and STORM).

Dynamics of CaM signals

As mentioned above, CaM target proteins vary greatly in their affinity for CaM (with dissociation constants ranging from 0.1nM to >100 nM [39–41]. The Ca-CaM affinity of the target protein will determine its Ca^{2+} dependence (greater for those that bind Ca-CaM tightly). The Persechini group developed FRET-based biosensors (BsCaMs) for dynamic measurements *in vivo* of Ca-CaM interaction with CaM target proteins in response to Ca^{2+} [42–46]. Changes in FRET are detected between the CFP and YFP fluorophores which flank a linker sequence derived from the Ca^{2+} -CaM binding domain of the smooth muscle MLCK (Fig). Site mutations of the linker sequence confer different Ca^{2+} -CaM sensitivities to the sensors [42]. In cardiomyocytes these sensors can be used to measure the real-time modulation of CaM targets by various Ca^{2+} signals including the beat-to-beat oscillations [24]. Sensors with higher affinity (like CaN) bind Ca^{2+} -CaM more tightly and therefore integrate the rhythmic Ca^{2+} signals, whereas sensors with lower affinity (like CaMKII) turn on and off more completely with each beat and display only minor integration [47] (Fig 2). These different behaviors could also be predicted quantitatively from a myocyte model [47,48] which implies that differences in Ca^{2+} -CaM affinities of CaM target proteins provide an important mechanism for accomplishing the CaM signaling diversity and complexity: Ca-CaM affinity confers specific Ca^{2+} -decoding ability to the CaM target protein thereby fine-tuning the cellular response.

Targeted versions of these biosensors are currently being used by our group to verify model projections of how local CaM signals might regulate CaMKII and CaN [48]. While large Ca-CaM oscillations are predicted to occur at the dyadic cleft on a beat-to-beat basis, the extensive CaM buffering and lower cytosolic Ca^{2+} in the cytosol would minimize the Ca-CaM fluctuations there. For CaMKII this would mean beat-dependent oscillations of activity in the dyadic cleft but also very little activation of CaMKII in the bulk cytosol because of the limited CaM signals in that compartment. It is hard to reconcile this prediction with known CaMKII dependent phosphorylation of cytosolic targets such as phospholamban, although this may be why CaMKII is routinely anchored nearby its targets –and might have a nearby pool of promiscuous CaM. CaN activity on the other hand is predicted to be

frequency-dependent in the cytosol and saturated in the dyad. This is consistent with observations that CaN/NFAT signaling correlates closely to global Ca^{2+} oscillation [49,50]. Since CaN is tethered to LTCCs [51] it will be important to assess whether CaN activity at the dyad is indeed constitutively active or remains somewhat dynamically regulated by Ca^{2+} signals.

CaMKII: a multifunctional kinase

While CaMKII is uniquely adapted to sense, integrate and transduce cellular Ca^{2+} (and Ca-CaM) signals, it is much more than merely a CaM effector. CaMKII is a key regulator of cardiac excitation-contraction coupling via its effects on ion channels and Ca^{2+} handling proteins, but is also important for more chronic cardiac responses via regulation of gene expression. Aside from this physiological role, CaMKII is also critically involved in the development of arrhythmias and heart failure. Several reviews have highlighted the role of CaMKII in various pathophysiological conditions and its potential as a therapeutic target [52–59].

Biochemistry of CaMKII

CaMKII is unique among protein kinases because it forms a dodecameric holoenzyme. It is encoded by four different genes (α , β , δ and γ) [60,61]. Of these, CaMKII δ is the predominant cardiac isoform and two splice variants are known to be present in the heart (δ_B and δ_C). Each subunit of the holoenzyme consists of a serine/threonine specific catalytic domain, followed by a regulatory segment that binds Ca-CaM (Fig 3). The latter is followed by a flexible linker of variable length (isoform-specific) that connects to the association or hub domain. The association domains assemble the subunits into two stacked hexameric rings. FRET experiments, where the catalytic domain was replaced with CFP or YFP, showed that CaMKII freely hetero-oligomerizes and that the variable linker influences the distance between N-termini [62]. The recent crystal structure of the auto-inhibited CaMKII holoenzyme indicated that this variable linker also provides a fine-tuning mechanism of the holoenzyme Ca^{2+} response: the variable linker alters the equilibrium between compact and more open docking states of the kinase domains against the central hub (with the CaM binding sites more accessible for the longer linkers) [63]. Indeed, different isoforms display slightly altered sensitivities to Ca^{2+} [64], but how this translates to signal-diversity in the cardiomyocyte remains to be established.

At baseline CaMKII is in an auto-inhibited state where the catalytic domain is constrained by the pseudosubstrate region within the regulatory domain [65] (Fig 3A–B). When Ca^{2+} rises, Ca^{2+} -CaM binds to the regulatory domain of CaMKII and triggers a conformational change that disrupts association with the catalytic domain and exposes an otherwise inaccessible phosphorylation site (T286) [65]. CaMKII deactivates again upon Ca-CaM dissociation. During prolonged $[\text{Ca}^{2+}]$ elevation, autophosphorylation at T286 prevents re-association of the regulatory and catalytic domains, resulting in autonomous activity that persists in the absence of Ca^{2+} -CaM [66,67]. Precisely how CaMKII activity responds to the intensity, duration and frequency of Ca^{2+} transients or how acute Ca^{2+} signaling and GPCR-linked Ca^{2+} signals are integrated on CaMKII are ongoing research foci. Reactive oxygen species (ROS) can also trigger autonomous CaMKII activity by oxidizing M280/281 residues after initial activation by Ca-CaM [68]. Both mishandling of Ca^{2+} and changes in ROS have been linked to cardiac disease, positioning CaMKII at a key interface [54].

Localization of CaMKII

CaMKII isoforms differ in subcellular targeting as well as interacting partners [69–71] and is purported as a key aspect of isoform signal specificity. In the heart, the CaMKII δ_B and δ_C

subtypes differ only by the presence of a nuclear localization sequence (NLS) and are thought to preferentially localize accordingly: δ_B mainly nuclear and δ_C mainly cytosolic [72–74]. Different functions had been attributed to each isoform, with nuclear δ_B involved in hypertrophic gene regulation and cytosolic δ_C in regulation of ion channels and Ca^{2+} handling. Initial findings in transgenic mice (TG) supported this since CaMKII δ_B transgenics develop cardiac hypertrophy, but this hypertrophy rapidly transitions to heart failure in the CaMKII δ_C mice (with extensive Ca^{2+} handling dysfunction) [74–78]. However, all CaMKII isoforms (α - δ , including these splice variants) appear to hetero-multimerize [62] which can explain in part why CaMKII δ_B is not restricted to the nucleus and CaMKII δ_C is not exclusively cytosolic [79]. Indeed, CaMKII δ_B and δ_C were found to have similar effects on MEF2 modulation and gene expression, but initially this was attributed to direct effects of CaMKII δ_B and δ_C subtypes on HDAC in the nucleus and cytosol respectively [80]. More recently Mishra *et al.* [79] upset this traditional dogma of isoform-specific location and postulated that spatial and functional specificity of CaMKII δ activation is obtained by mobilization of different Ca^{2+} stores [79]. This was based on their findings that even when expressed in CaMKII δ null background CaMKII δ_B and δ_C subtype targeting remained non-exclusive and both subtypes had compartment-specific activation profiles: a phenylephrine stimulus triggered nuclear CaMKII δ activation and HDAC phosphorylation, whereas caffeine elicited cytosolic CaMKII δ activation and phospholamban phosphorylation. To what extent these findings will impact the notion of CaMKII δ_B as having a cardioprotective role and δ_C as having a more deleterious role in cardiomyocyte survival and heart disease [81–84] remains to be established.

It is clear that CaMKII location matters and several direct interactions of CaMKII with target proteins have been described such as with the RyR [75,85], InsP₃R [86], LTCC subunits [28,29] and mitochondria [87]. Indeed, CaMKII overexpression potentiates SR Ca release and also enhances mitochondrial Ca uptake that can contribute to cell death [88]. Localization poises CaMKII near both a local Ca^{2+} pools and signaling microdomain thereby achieving signal specificity (e.g. the InsP₃R-CaMKII-HDAC signaling pathway [4]). This direct interaction pattern of CaMKII is in contrast to the scaffolding protein interactions found for PKA (A-kinase anchoring proteins, AKAPs) [89]). However α Kinase anchoring protein (α KAP) was found to serve as an “anchoring” protein for SERCA and CaMKII, positioning CaMKII for modulating PLB phosphorylation [90]. To what extent these interacting proteins modulate CaMKII function is still unclear. In neurons, α -actinin can act as a Ca^{2+} independent surrogate for CaM binding to CaMKII and also modulates CaMKII phosphorylation of specific substrates (such as glutamate receptor) [91]. In essence, α -actinin acted as a “phosphostat” for a subset of CaMKII targets, maintaining their basal phosphorylation. Other outstanding questions include to what extent CaMKII is translocated in cardiac myocytes? And whether CaMKII interactions, localization and translocation are altered in cardiac disease?

Imaging CaMKII activity and regulation

Until recently, the tools for measuring CaMKII activation in cardiomyocytes were very limited and destructive. These included snapshot measurements of CaMKII modifications (i.e. its autophosphorylation or oxidation state using western blotting or immunocytochemistry) or CaMKII target phosphorylation and kinase assays in cell lysate (³²P incorporations or fluorescence quenching). The Hayashi group developed Camui to study CaMKII activation in neurons [92]. This FRET based biosensor consists of the full length kinase flanked by the CFP/YFP fluorophores and takes advantage of the conformational changes of the kinase associated with activation. It is a powerful tool for detecting subtle, subcellular differences in CaMKII activation, so our group adapted it for use in cardiomyocytes [93]. The donor/acceptor fluorescence ratio ($F_{\text{CFP}}/F_{\text{YFP}}$) of camui is a

good reporter of CaMKII activation by Ca^{2+} -CaM, autophosphorylation and oxidation (since all result in an opening of the regulatory and catalytic CaMKII domains). That is, Camui is more than a readout of Ca-CaM binding dynamics to cellular CaMKII (which could be inferred from the BsCaM responses), it is a direct readout of the active conformation of the kinase.

It should be noted that although the Camui response is fairly linear over a wide range for autonomous CaMKII activity (via phosphorylation or oxidation), direct Ca-CaM activation produces a stronger FRET change for a given amount of enzyme activity. A second limitation is that the Camui FRET is not simple bimolecular FRET, but incorporates a structural element (the CFP on the catalytic domain can interact with more than one YFP on the association domain). This may contribute to some nonlinearity in $F_{\text{CFP}}/F_{\text{YFP}}$ increase as catalytic domains become activated. Nonetheless Camui is a good proxy for CaMKII activity. It can track both temporal and spatial changes in CaMKII activity *in vivo* and has the added benefit that the specific contributions of autophosphorylation and oxidation to CaMKII activation can be examined using Camui constructs lacking the target sites for these modifications. This makes camui uniquely suited to study the subcellular mechanisms driving CaMKII signaling pathways in cardiomyocytes. Indeed, initial experiments showed that while there is a significant role for oxidative activation of CaMKII in response to angiotensin II and endothelin-1 exposure, the isoproterenol and phenylephrine triggered activation of CaMKII was largely Ca-CaM and somewhat autophosphorylation dependent.

Camui studies examining the sensitivity of CaMKII function to varying beat-to-beat global Ca^{2+} signals are already ongoing, as are experiments assessing how CaMKII responds to multiple spatially unique Ca^{2+} signal inputs (global cytosolic Ca^{2+} and GPCR-linked signals). Improvements to the Camui sensor also continue to be made [94,95]: alternate CaMKII isoforms, improved FRET pairs, etc. An alternative to the Camui-style biosensors is a surrogate substrate based reporter (Kinase Activity Reporter family (KAR), [96]) where a forkhead association domain triggers a molecular switch upon phosphorylation by the target kinase and this would induce changes in fluorescence intensities of the FRET fluorophores. These KARs have the added benefit that they can be subcellularly targeted to signaling microdomains providing even more detailed spatial information of kinase function. Future studies combining Camui and various models of cardiac disease are likely to provide extensive mechanistic insight into how CaMKII signaling contributes to arrhythmias and heart failure.

Concluding Remarks and Future perspectives

Numerous studies have shown that Ca^{2+} -CaM-CaMKII signaling has a critical role in the regulation of both acute excitation-contraction and chronic excitation-transcription coupling in the heart. There is also abundant evidence that CaMKII has a ubiquitous role in pathophysiological conditions of the heart. Several fundamental questions regarding the signal processing and spatial intricacies of CaM/CaMKII signaling were highlighted here and deserve further investigation. The new generation of biosensors also provide unique opportunities to delineate the role and regulatory mechanisms of CaM and CaMKII in normal and diseased heart. This will greatly benefit future efforts to design effective therapeutics targeted at cardiac CaMKII.

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Figure 1. Ca²⁺-dependent localization of CaM in cardiomyocytes

(A) Structures of Ca²⁺-free CaM (apoCaM) and Ca²⁺-bound CaM where the arrows indicate the Ca²⁺ binding sites. Image was reproduced from ref [20]. (B–C) CaM binding increases at the nucleus and Z-lines with higher [Ca²⁺]_i. Fluorescent CaM (60nM) was washed into permeabilized myocytes at 100 and 500nM [Ca²⁺]_i. Confocal images were taken to monitor CaM binding. After 40 min of fluorescent CaM wash-in (shown in B) fluorescence intensities were quantified at M-lines, Z-lines and nucleus (normalized to fluorescence intensity at M-lines at 100nM [Ca²⁺]_i) for [Ca²⁺]_i of 100 and 500 nM. Image reproduced from ref [26] with permission. (D) Ca²⁺ regulates both functional populations of CaM: the dedicated CaM pool in complex with its target channels as well as the promiscuous CaM pool regulating CaMKII and CaN.

Figure 2. Visualizing Ca-CaM signals in cardiomyocytes

(A) Principle of the FRET-based Ca^{2+} -CaM biosensors that mimic either high (BsCaM2) vs. low (BsCaM45) affinity CaM targets such as CaN and CaMKII, respectively. (B–C) BsCaM FRET changes ($F_{\text{CFP}}/F_{\text{YFP}}$) in response to pacing reveal that both type of CaM targets can track the beat-to-beat intracellular Ca^{2+} changes, but in different ways: BsCaM2 integrates the oscillatory Ca^{2+} signals more strongly, whereas BsCaM45 turns on and off more completely with each Ca^{2+} transient. Reproduced with permission from ref [44].

Figure 3. Visualizing CaMKII signaling in cardiomyocytes

(A) Structural organization of the CaMKII holoenzyme (modified from ref [20]). The CaMKII monomer consists of 3 different domains: the association domain for assembly into the holoenzyme, the catalytic domain and the regulatory domain. CaMKII activation requires Ca-CaM binding to the regulatory domain relieving the autoinhibition of the kinase. The associated conformational change now permits activity-sustaining modifications of the kinase such as phosphorylation (T286) and oxidation (MM281/282). (B) The FRET sensor Camui consists of the full-length CaMKII sandwiched between a fluorophore FRET pair. Activating conformational changes result in a reduction of FRET. (C) Localization of camui in cardiomyocytes using CFP and YFP emission signals confirms targeting of Camui to Z and M lines (left panel). The right panel is a FRET ratio image of camui showing spatial differences in CaMKII activation (in the nucleus, perinucleus and cytosol). Panels B and C were from ref [90] with permission.