Calcium (Ca^{2+}) is a ubiquitous key signaling element in all eukaryotic cell types. It regulates diverse essential physiological processes such as cell differentiation, proliferation and motility, apoptosis, secretion, excitation, contraction, and neuronal plasticity. In the heart, the most obvious crucial role of Ca^{2+} is its involvement in electric activity and cardiac contractility, acting as the central player of excitation–contraction (EC) coupling. Besides being directly involved in EC coupling, Ca^{2+} is an important element in various signaling cascades, regulating the activity of diverse downstream effectors. Thereby, Ca^{2+} can exert acute effects but also influence the regulation of gene expression, called excitation–transcription (ET) coupling. These ET coupling pathways play a critical role not only in cardiac homeostasis but also in cardiac disease development, since diseased cardiomyocytes show vast alterations in Ca^{2+}-handling and Ca^{2+}-dependent transcriptional patterns. Among these pathways, especially signaling cascades involving CaMKII (Ca^{2+}/calmodulin-dependent kinase II) and the Ca^{2+}/calmodulin-dependent serine/threonine phosphatase calcineurin have been extensively characterized on their role in cardiac hypertrophy and remodeling processes. Yet, we are just beginning to understand how the multiple ET coupling pathways are differentially activated, how they interrelate, and how the multiple targets in this complex network relatively contribute to failure of cardiomyocytes.

This review gives an overview on Ca^{2+}-dependent transcriptional mechanisms in cardiomyocytes and their role in cardiac disease. First, we discuss cytosolic and nuclear Ca^{2+} dynamics in cardiomyocytes with respect to their impact on Ca^{2+}-dependent signaling, and give an overview on Ca^{2+}-dependent transcriptional pathways in cardiomyocytes, and discuss implications of excitation–transcription coupling in the diseased heart. (Circ Res. 2017;121:1000-1020. DOI: 10.1161/CIRCRESAHA.117.310355.)

Key Words: calcium ■ calcium-calmodulin dependent protein kinase II ■ calcineurin ■ calmodulin ■ excitation transcription coupling

Calcium Signaling Series
Donald M. Bers, Guest Editor

Calcium Signaling and Transcriptional Regulation in Cardiomyocytes
Matthias Dewenter,* Albert von der Lieth,* Hugo A. Katus, Johannes Backs

Abstract: Calcium (Ca^{2+}) is a universal regulator of various cellular functions. In cardiomyocytes, Ca^{2+} is the central element of excitation–contraction coupling, but also impacts diverse signaling cascades and influences the regulation of gene expression, referred to as excitation–transcription coupling. Disturbances in cellular Ca^{2+}-handling and alterations in Ca^{2+}-dependent gene expression patterns are pivotal characteristics of failing cardiomyocytes, with several excitation–transcription coupling pathways shown to be critically involved in structural and functional remodeling processes. Thus, targeting Ca^{2+}-dependent transcriptional pathways might offer broad therapeutic potential. In this article, we (1) review cytosolic and nuclear Ca^{2+} dynamics in cardiomyocytes with respect to their impact on Ca^{2+}-dependent signaling, (2) give an overview on Ca^{2+}-dependent transcriptional pathways in cardiomyocytes, and (3) discuss implications of excitation–transcription coupling in the diseased heart. (Circ Res. 2017;121:1000-1020. DOI: 10.1161/CIRCRESAHA.117.310355.)
Ca²⁺ stores, in particular the sarcoplasmic reticulum (SR). A critical cellular microdomain for cytosolic Ca²⁺ cycling is the dyadic junction, where invaginations of the plasma membrane (T-tubules) and the SR are in close proximity. On depolarization of the cell membrane, LTCC (L-type Ca²⁺ channels; also known as dihydropyridine receptors) in the plasma membrane are activated, allowing Ca²⁺ to enter the cytosol. This Ca²⁺ entry raises local Ca²⁺ concentration from 0.1 to >10 µmol/L in the junctional cleft, which triggers the activation of Ca²⁺ release channels (RyR [ryanodine receptors]) in the SR membrane. The SR Ca²⁺ release raises local cleft Ca²⁺ to >100 µmol/L and global cytosolic Ca²⁺ concentration from 0.1 µmol/L during diastole to 1 µmol/L during systole. This increase in cytosolic Ca²⁺ causes contraction via Ca²⁺–binding–induced conformational changes in the troponin–tropomyosin complex, which allows the myofilaments actin and myosin to slide past one another. For relaxation to occur, Ca²⁺ must be removed from the cytosol. The 2 main mechanisms are Ca²⁺ transport back into the SR by SERCA (SR Ca²⁺ ATPase) and Ca²⁺ extrusion from the cell via NCX (Na+/Ca²⁺ exchanger) in the plasma membrane. A small amount of Ca²⁺ is also removed by Ca²⁺-ATPases in the plasma membrane and by mitochondrial Ca²⁺ uniporters.

These Ca²⁺ cycling processes are finely tuned in response to extra- or intracellular stimuli by various regulators that affect the activity state of Ca²⁺-handling proteins. Central modulators of cardiomyocyte Ca²⁺-handling proteins are PKA (protein kinase A) and CaMKII. PKA is activated in response to sympathetic stimulation of β-AR (β-adrenergic receptors) and phosphorylates LTCC, RyR, and the SERCA inhibitor PLN (phospholamban), thereby increasing Ca²⁺ influx, SR Ca²⁺ release, and SR Ca²⁺ reuptake. CaMKII shares common targets with PKA and is also activated in response to sympathetic activation; however, CaMKII-dependent mechanisms have been suggested to exert more long-term effects on Ca²⁺ cycling. These kinase-mediated effects on EC coupling proteins are counterbalanced by protein phosphatases, especially protein phosphatase 1 and 2A. In addition, the activity state of Ca²⁺-handling proteins and also of their upstream regulatory kinases can be modulated by multiple other posttranslational modifications, such as oxidation, nitrosylation, and glycosylation.

Besides the above-mentioned elements of Ca²⁺-handling, other Ca²⁺ cycling proteins have been characterized in cardiomyocytes that are suggested to play a less pivotal role in the classical concept of EC coupling but are implicated in Ca²⁺-dependent signaling mechanisms; in particular, IP3 (inositol 1,4,5-trisphosphate) receptors, TRP (transient receptor potential) channels, and SOCE (store-operated Ca²⁺ entry) controlled by STIM1 (stromal interaction molecule 1). IP3 receptors are Ca²⁺ channels activated by IP3, which is generated through PLC (phospholipase C)–dependent hydrolysis of phosphatidylinositol 4,5-bisphosphate after Gq protein–coupled receptor stimulation. Typical activators of IP3 signaling in cardiomyocytes are ET-1 (endothelin-1), catecholamines, and ANGII (angiotensin 2), which presumably modulate specialized pools of Ca²⁺ via this mechanism. Localized preferentially in the perinuclear region and the nuclear envelope, IP3 receptors are suggested to play a major role in local nuclear Ca²⁺ cycling. Further details in this regard will be discussed below. TRP channels are ubiquitously expressed nonselective cation channels with variable Ca²⁺ permeability. In cardiomyocytes, TRP channels, especially TRPCs, have been suggested to act as fine-tuners of Ca²⁺ cycling and were demonstrated to control Ca²⁺-dependent signaling in response to neurohumoral stimulation. Besides the activation via Gq protein–coupled receptor stimulation (receptor-operated Ca²⁺ entry), TRPCs also participate in SOCE. SOCE involving STIM1 and Ca²⁺ channel Orai1 has been characterized as a key element of Ca²⁺-dependent signaling in nonexcitable cells. In cardiomyocytes, STIM1-dependent Ca²⁺ entry was observed to coexist with the global Ca²⁺ transients. Increasing evidence indicates that STIM1 is important for Ca²⁺ response signaling within microdomains, inducing changes in cardiac transcriptional profiles. Apart from association with Orai1 and TRPC channels, recent data suggest that STIM1 interacts with...
several other Ca\textsuperscript{2+} cycling proteins, including LTCC, SERCA, plasma membrane Ca\textsuperscript{2+} ATPases, and RyR\textsuperscript{24-27}.

This highly complex system of cardiomyocyte Ca\textsuperscript{2+} cycling on the one hand allows global continuous intracellular Ca\textsuperscript{2+} oscillations and on the other hand controls local pools of Ca\textsuperscript{2+} within spatial microdomains, and the regulation of nuclear Ca\textsuperscript{2+} adds an additional layer of complexity.

**Nuclear Ca\textsuperscript{2+}**

The nucleus is a subcellular compartment surrounded by 2 phospholipid bilayers, referred to as the nuclear envelope. This nuclear envelope separates cytoplasm from nucleoplasm and is structurally and functionally subdivided. The outer nuclear membrane faces the cytosol and merges with the membrane of the SR. Therefore, both the outer nuclear membrane and the SR membrane share the same characteristics on their composition, and the perinuclear space, which is in between the outer nuclear membrane and the inner nuclear membrane, resembles the SR in ion and protein content\textsuperscript{28,29}. In contrast to the outer nuclear membrane, the inner nuclear membrane, which is directed to the nucleoplasm, shows a distinct composition unique to the nucleus. The nuclear envelope forms nuclear invaginations that reach deep into the nucleus, creating complex structures that in their entirety are termed the nuclear reticulum.\textsuperscript{30,33} In close proximity to the nuclear envelope, T-tubules reaching deep into the cytosol and mitochondria can be found, constituting microdomains for Ca\textsuperscript{2+} cycling and signal responsiveness.\textsuperscript{31}

The nuclear membranes are poorly permeable to ions and water-soluble molecules. Transport and diffusion is facilitated by nuclear pore complexes (NPCs), which are distributed throughout the nuclear envelope.\textsuperscript{31,32} Proteins and RNAs ≤39 nm in diameter are actively shuttled in and out of the nucleus by a transport system associated with the NPC.\textsuperscript{34,35} Ions and molecules smaller than 9 nm and 40 kDa can diffuse through the NPC.\textsuperscript{34} Thus, the NPCs function as both barriers and selective filters for the substances trafficking between nucleoplasm and cytoplasm.

In cardiomyocytes, every cytosolic Ca\textsuperscript{2+} transient is accompanied by a nuclear Ca\textsuperscript{2+} transient.\textsuperscript{36} However, nuclear Ca\textsuperscript{2+} transients, compared with cytosolic transients, seem to have a slower and delayed upstroke, a lower peak, and a prolonged return back to baseline.\textsuperscript{37,38} These findings led to the hypothesis that cytosolic Ca\textsuperscript{2+} passively diffuses into the nucleus during systole, thereby causing the nuclear Ca\textsuperscript{2+} transients. Interestingly, to date none or only inconsiderable numbers of primary active pumps for Ca\textsuperscript{2+} reuptake have been found on the inner nuclear membrane.\textsuperscript{33,39} Therefore, the idea is that Ca\textsuperscript{2+} mostly diffuses out of the nucleus via NPCs and is then taken up by SERCA on the outer nuclear membrane and the junctional SR, by nearby mitochondria or is extruded by NCX on T-tubules in proximity to the nuclear envelope.\textsuperscript{31,40,41} This would also explain the observation that the nuclear baseline [Ca\textsuperscript{2+}] increases with higher beating frequencies. Because the decay of nucleoplasmic Ca\textsuperscript{2+} transients is slower compared with cytoplasmic Ca\textsuperscript{2+} transients, Ca\textsuperscript{2+} noticeably builds up within the nucleus when diastole is shortened.\textsuperscript{37}

Nuclear Ca\textsuperscript{2+} not only follows whole-cell Ca\textsuperscript{2+} oscillation but also small perinuclear Ca\textsuperscript{2+} release events. Lipp et al showed that even Ca\textsuperscript{2+} puffs in the perinuclear area within 3 µm of the nuclear envelope can lead to an increase in nuclear Ca\textsuperscript{2+} while leaving the rest of the cytosol unaffected.\textsuperscript{42} This clearly states the importance of the perinuclear SR when considering nuclear Ca\textsuperscript{2+} regulation and already implies that nuclear Ca\textsuperscript{2+} is influenced by many aspects beyond whole-cell Ca\textsuperscript{2+} oscillation.

However, passive diffusion is only 1 aspect of nuclear Ca\textsuperscript{2+} cycling. Ion diffusion can also be subject to regulation at the level of NPCs. Ambient Ca\textsuperscript{2+} and ATP are essential for the maintenance of NPC diffusion capacity; thus, decreasing Ca\textsuperscript{2+} or ATP in the area surrounding the NPC slows down its conductance, but not to the extent of a full diffusion blockade.\textsuperscript{43} Furthermore, the distribution and position of NPCs on the nuclear envelope, as well as their distance to nuclear Ca\textsuperscript{2+} channels on the outer and inner nuclear membrane, seem to have an important effect on local Ca\textsuperscript{2+} diffusion capacity varies accordingly.\textsuperscript{44,45}

Functional and structural evidence further indicates that nuclear Ca\textsuperscript{2+} can be actively controlled. IP3 receptor type 2, the predominant subtype in cardiomyocytes, is concentrated in the perinuclear area and can be found on the junctional SR, the outer and the inner nuclear membrane.\textsuperscript{46} These nuclear IP3 receptors seem to account for the widely described observation of a diastolic nucleoplasmic-to-cyttoplasmic [Ca\textsuperscript{2+}] gradient.\textsuperscript{47} Experiments on isolated nuclei, permeabilized, and intact cardiac myocytes consistently show that the activation of IP3 receptors preferably increases nuclear Ca\textsuperscript{2+}.\textsuperscript{48,49} IP3, generated after stimulation of Gq protein–coupled receptors in the plasma membrane, acts not only on plasmalemmal IP3 receptors but can also diffuse to the nucleus and activate IP3 receptors in the outer and inner nuclear membrane.\textsuperscript{48,49} In addition, there is increasing evidence that IP3 can also be directly generated at the inner nuclear membrane and, therefore, selectively act on nuclear IP3 receptors to increase nuclear Ca\textsuperscript{2+}, especially in response to ET-1, ANGII, and α-adrenergic stimuli.\textsuperscript{47,50-52} Notably, ET-1 receptors, ANGII receptors, and α-AR have been identified not only on the plasma membrane but also on the nuclear envelope.\textsuperscript{50-53}

Several more receptors were proposed to play a role in nuclear Ca\textsuperscript{2+} regulation. For example, it has been demonstrated that activation of the IGF-1 (insulin-like growth factor 1) receptor, a receptor tyrosine kinase localized deep within the T-tubules in proximity to the nucleus, leads to PLC-dependent production of perinuclear IP3.\textsuperscript{54} This consequently causes a selective increase in nuclear Ca\textsuperscript{2+}. Moreover, the existence of β\textsubscript{3}- and β\textsubscript{7}-AR on the nuclear membrane was demonstrated,\textsuperscript{55} suggesting that β-adrenergic signaling might have an influence on nuclear [Ca\textsuperscript{2+}] within microdomains via localized perinuclear PKA activation and subsequent phosphorylation of PKA target structures.\textsuperscript{56} Although there is substantial evidence for the influence of nuclear β-ARs on transcriptional processes, the exact mechanisms involved remain unknown.\textsuperscript{57} Another suggested regulator of nuclear Ca\textsuperscript{2+}-handling is the RyR. It is primarily located on the SR and only to a lesser extent on the outer...
nuclear membrane.\textsuperscript{58} Intranuclear RyR localization remains controversial, yet it was described for neonatal cardiac myocytes.\textsuperscript{58} Perinuclear RyRs, however, were reported to play a pivotal role in generation of Ca\textsuperscript{2+} waves originating from the perinuclear region.\textsuperscript{59}

In conclusion, nuclear Ca\textsuperscript{2+} is basically subject to cytosolic Ca\textsuperscript{2+} oscillations. The mere passive character of cytosolic to nuclear Ca\textsuperscript{2+} diffusion is, however, enriched by different modifying mechanisms incorporating influences and specific pathways of various origins. Moreover, nuclear, perinuclear, and cytosolic structures, such as nuclear invaginations, NPCs, perinuclear reticulum, T-tubules, and the distribution of, for example, receptors, channels, and pumps, create Ca\textsuperscript{2+} microdomains that offer a vast array of possibilities for spatially and temporally restricted Ca\textsuperscript{2+} control.

Thus, cytosolic and nuclear Ca\textsuperscript{2+} cycling are part of a highly sophisticated system which controls Ca\textsuperscript{2+}-dependent signaling by modifications of global Ca\textsuperscript{2+} oscillations as well as Ca\textsuperscript{2+} regulation within microdomains. In the following, we focus on pathways that link cardiomyocyte Ca\textsuperscript{2+} cycling with transcriptional regulation.

Ca\textsuperscript{2+}-Dependent Regulation of Gene Expression in Cardiomyocytes

Several Ca\textsuperscript{2+}-dependent pathways are described that modulate gene expression by signal transduction to transcriptional regulators. In cardiomyocytes, well-characterized signaling patterns in this regard involve calmodulin, CaMKII, and calcineurin. Figure 1 illustrates the major findings summarized in this section.

Calmodulin

Calmodulin is a highly conserved Ca\textsuperscript{2+} sensor protein in eukaryotic cells with no innate enzymatic function. It is a small 16 kDa protein that consists of a C-terminal and an N-terminal lobe with 2 Ca\textsuperscript{2+}-binding EF-hands each. Different Ca\textsuperscript{2+} affinities of the N-terminal and C-terminal EF-hands and intramolecular structures cooperatively facilitate a differentiated response to a broad range in Ca\textsuperscript{2+} concentrations. When Ca\textsuperscript{2+} binds to the lobes, conformational changes occur and thereby protein interaction sites are released.\textsuperscript{60} To date, >300 proteins have been shown to bind to calmodulin. Various binding motifs and mechanisms of interaction have been identified.
besides its regulatory function in activating Ca2+-sensitive enzymes such as CamkII and calcineurin, calmodulin exerts effects on gene expression via CAMTA (calmodulin-binding transcriptional activator).63 In humans, 2 different CAMTA genes, CAMTA1 and CAMTA2, have been characterized. Their expression seems to be most prominent in heart and brain tissue.64 In the heart, CAMTA2 raised interest because of its noteworthy ability to induce the expression of atrial natriuretic peptide—a surrogate parameter for cardiac hypertrophy and marker of heart failure–related diseases. Mechanistically, it was shown that CAMTA2 binds to the homeobox protein NKX2-5, which is also implicated in hypertrophic response, and coactivates NKX2-5–dependent transcription. Furthermore, the Olson laboratory demonstrated that HDAC5 (histone deacetylase 5) represses CAMTA2 through a direct interaction. With PKD (protein kinase D)–dependent HDAC5 phosphorylation and consequent nuclear export, CAMTA2 is released and executes its coactivating function.65,66 Therefore, CAMTA is not only directly regulated by Ca2+ via binding of Ca2+/calmodulin but also indirectly via its interaction with HDAC5. The relevance of CAMTA2 in human was only recently confirmed by showing that certain genetic polymorphisms in the coding region of CAMTA2 alter the risk of developing cardiac hypertrophy.66

**CamkII**

CamkII is a serine/threonine kinase with the isoforms CaMKIIb and CaMKIIγ being expressed in the heart. The splice variant CaMKIIbC primarily localizes within the cytosol, whereas CAMKIIbB has a nuclear localization sequence. CaMKII becomes activated upon Ca2+/calmodulin binding with Kd ≈50 nmol/L.67 An important regulatory mechanism of CaMKII activity dynamics is its autophosphorylation, which is sensitive to the frequency and duration of Ca2+ spikes.68.69 Autophosphorylation at Thr286/287 enhances the affinity of CaMKII toward calmodulin and prevents autoinhibition of CaMKII, thereby maintaining enzyme activity independent of Ca2+/calmodulin binding.69,70 Analogous to autophosphorylation, several other posttranslational modifications, such as oxidation, glycosylation, and nitrosylation, were described to further increase CaMKII activity after initial opening by Ca2+/calmodulin binding.67,71,72 CaMKII exerts its molecular effects by binding and phosphorylation of target proteins. Regarding the regulation of transcriptional processes, CaMKII has been shown to phosphorylate transcription factors, epigenetic regulators, and histones.

To date, several transcription factors that are activated in a CaMKII-dependent manner have been identified. CREB (cAMP response element binding protein) is a ubiquitous transcription factor involved in cardiac integrity, inflammation processes, and metabolic signaling.73–75 CREB activity is regulated via various signaling pathways, and especially PKA–mediated phosphorylation of Ser-133 has been shown to be critical for its activation. CaMKII either increases activation of CREB by phosphorylation of Ser-133 or decreases CREB activity by phosphorylation of Ser-142, suggesting a modulatory role beside cAMP/PKA-dependent CREB regulation.76 Notably, CaMKII has recently been demonstrated to mediate CREB activation in response to neurohumoral stimulation by ET-1 and phenylephrine.77 In addition, ATF1 (activating transcription factor 1), that shares huge homology with CREB and that is also modulated by cAMP/PKA, can be phosphorylated by CaMKII, remarkably only at its activation site Ser-63 (corresponding to Ser-133 of CREB).78 However, up to now the specific functional consequences of CaMKII–dependent modulation of these cAMP-regulated transcription factors in cardiac myocytes remain elusive.79

The functional role of other CamkII–dependent transcription factor activation events has been more specifically characterized, for example, on apoptosis and inflammation. Interestingly, whereas the CaMKIIb splice variant CaMKIIbC has been suggested to mediate stress-induced proapoptotic effects via classical mitochondrial pathways,80,81 the splice variant CaMKIIbB seems to play an antiapoptotic role. Mechanistically, CaMKIIbB has been suggested to activate the transcription factor heat shock factor 1 by phosphorylation at Ser-230, inducing increased transcription of cell-protective factor inducible heat shock protein 70.82 This putatively protective role of the CaMKIIbB splice variant is supported by the observation that it is required for binding of transcription factor GATA4 to the promoter region of antiapoptotic protein Bcl-2 and consequently is crucial for reducing doxorubicin-mediated cardiotoxicity.83

Emerging evidence points to an important role of CamkII in inflammation. For instance, CaMKIIb has been described to mediate activation of the transcription factor NFκB (nuclear factor κ B).84,85 The underlying mechanism most likely involves a more indirect pathway via inhibitor of κB kinase activation and subsequent phosphorylation and degradation of Inhibitor of κB, leading to nuclear translocation of NFκB. This pathway has been investigated, for example, in the setting of ischemia/reperfusion injury, suggesting a detrimental proinflammatory role of CaMKIIb.86 In the same setting of ischemia/reperfusion injury, we found that CaMKII induces transcription and secretion of the chemokines CCL2 and CCL3 from cardiomyocytes, thereby triggering an intrinsic chemoattractant cardiomyocyte signaling cascade that is associated with scar formation and cardiac fibrosis.87

In addition, CamkII was demonstrated to interact with SRF (serum response factor), a transcription factor implicated in cardiac integrity as genetic models have revealed.88,89 It has been shown that CaMKII phosphorylates SRF at Ser103 and Thr160 in skeletal muscle90; however, the functional role of these events in cardiomyocytes is not clear yet. Furthermore, myocardin, a transcriptional coactivator of SRF that has been causatively associated with cardiac hypertrophy,91 is activated via Ca2+-dependent signaling involving CaMKII.92 CaMKII modulates ion currents not only by direct interaction and phosphorylation of ion channels but also by transcriptional regulation. Studies reported that CaMKII is critical for β-AR–stimulated upregulation of NCX1 by transcription factor AP-1 (activator protein 1) activation.93 Moreover, CaMKII has been demonstrated to repress the expression of LTCC by activating downstream regulatory element binding transcription factor DREAM, thus constituting a negative feedback mechanism on Ca2+ influx.94
CaMKII may also affect transcriptional patterns on a more global scale by regulating transcription factor MeCP2 (methyl CpG binding protein 2). MeCP2, which was implicated in regulating the genome-wide chromatin state, was shown to be phosphorylated by CaMKII in neurons. Notably, MeCP2 is also expressed in the heart, where its relevance in human and experimental heart failure was recently demonstrated. Looking at these studies, it is worth considering that CaMKII signaling to MeCP2 may depict another important Ca²⁺-dependent aspect of transcriptional regulation in cardiac myocytes.

Besides the phosphorylation of transcription factors, CaMKII is also involved in chromatin modification. On the one hand, CaMKIIδB can directly phosphorylate histones, in particular histone 3 at Ser10 (H3S10). This phosphorylation event has been associated with induction of hypertrophic growth, based on increased chromatin accessibility for prohypertrophic gene regulation. It will be interesting to see in the future whether CaMKII-mediated histone phosphorylation at H3S10 or at other potential histone phosphorylation sites directly results in the regulation of specific gene programs. Chromatin immunoprecipitation of CaMKII-phosphorylated histones followed by massive genomic sequencing might enable one to study this fascinating possibility. On the other hand, CaMKII has been identified to interact with chromatin modifying enzymes, in particular HDAC4. HDAC4 is a class IIa HDAC that itself has only a weak deacetylase activity and exerts transcriptional regulation by binding of transcription factors or recruitment of other chromatin modifying enzymes. One of the critical transcription factors regulated by HDAC4 is MEF2 (myocyte enhancer factor 2). MEF2 activation has been implicated in cardiac remodeling, fetal gene reprogramming, and inflammation, and HDAC4 acts as a repressor in this scenario. CaMKII has been identified as a specific kinase for HDAC4, since HDAC4 has a unique docking site for CaMKII that is absent in other HDACs. Nuclear CaMKIIδB-dependent phosphorylation of HDAC4 (at Ser-467 and Ser-632) promotes chaperone 14-3-3 binding and subsequent nuclear export of HDAC4, whereas cytosolic CaMKIIδC-dependent phosphorylation of HDAC4 blocks nuclear import (Figure 2). As a consequence, HDAC4-dependent repression of MEF2 is diminished, resulting in enhanced MEF2-dependent transcriptional activity. Aside from MEF2, HDAC4 also acts as a repressor of the above-mentioned SRF, indicating a more complex functional consequence of CaMKII-mediated HDAC4 shuttling. Via HDAC4 binding, CaMKII is also capable of regulating HDAC5. Although HDAC5 alone is not responsive to CaMKII, hetero-oligomerization of HDAC5 with HDAC4 enables CaMKII to phosphorylate HDAC5, resulting in nuclear export and subsequent activation of MEF2. CaMKII-regulated nuclear export of HDAC4 seems to affect transcriptional patterns even further. For example, it has been demonstrated that methylation of H3K9 at the promoter site of atrial natriuretic peptide depends on HDAC4 shuttling. Mechanistically, HP1 (heterochromatin protein 1) and histone methyltransferase SUV39H1 associate with class IIa HDACs to build a corepressor complex. HDAC4, when shuttled out of the nucleus, releases the corepressor complex and, therefore, prevents further methylation. Subsequently, the H3K9 site becomes demethylated, which relieves chromatin condensation and facilitates gene expression.

**Calcineurin**
Calcineurin (protein phosphatase 2B) is a serine/threonine phosphatase that is structurally composed of 2 subunits: CnA (calmodulin-binding catalytic subunit A) and CnB (Ca²⁺-binding regulatory subunit B). Like CaMKII, calcineurin is activated by binding of Ca²⁺/calmodulin, notably with a much higher affinity (Kₐ<1 nmol/L). The classical calcineurin-dependent transcriptional regulation pathway involves NFAT (nuclear factor of activated T cells). On cytosolic dephosphorylation by calcineurin, NFAT is imported into the nucleus, where it acts in conjunction with transcription factor GATA4. This calcineurin-dependent pathway has been elegantly demonstrated to play a crucial role in cardiac development and in the adult cardiac hypertrophic response. Mechanistically, nuclear calcineurin was shown to suppress exportin protein Crm1 binding to NFAT, with nuclear NFAT consequently accumulating.

Downstream effectors of calcineurin signaling may also include MEF2, as this is implied by findings from skeletal

---

**Figure 2.** Proposed interplay between Ca²⁺/calmodulin-dependent kinase II and calcineurin and their downstream signaling pathways in cardiomyocytes. CaMKII indicates Ca²⁺/calmodulin dependent kinase II; CaN, calcineurin; HDAC4, histone deacetylase 4; MEF2, myocyte enhancer factor 2; Mrj, mammalian relative of DnaJ; and NFAT, nuclear factor of activated T cells.
muscle cells, neurons, and lymphocytes.\textsuperscript{116–118} As an underlying mechanism, it was proposed that calcineurin is able to dephosphorylate MEF2, thereby blocking its sumoylation and consequently promoting MEF2-dependent transcription.\textsuperscript{119} In addition, it was shown that calcineurin possesses the ability to enhance MEF2 transcriptional activity via a calcineurin/mAKAP/MEF2 complex formation.\textsuperscript{120} Another possible pathway involves prominent calcineurin downstream target NFAT, since Blaeser et al described activated calcineurin to induce the formation of a MEF2/NFATc2 transcriptional complex.\textsuperscript{118} Irrespective of the exact mechanism, the functional improvement of MEF2 antagonization in a calcineurin-induced heart failure model argues for a relevant calcineurin-dependent MEF2 effect also in cardiomyocytes.\textsuperscript{121} Yet, one has to consider that MEF2 activation in this model of calcineurin overexpression may also be a secondary phenomenon during the development of heart failure, which is not based on a direct signaling of calcineurin to MEF2. Another aspect of interrelation between the HDAC/MEF2 axis and NFAT was emphasized by Dai et al,\textsuperscript{122} showing that NFATc3 is negatively regulated by class II HDACs through the DnaJ-Related Factor Mrj. Notably, calcineurin activity can be negatively regulated by direct CaMKII-dependent phosphorylation, causing diminished NFAT translocation.\textsuperscript{123} This phenomenon has been demonstrated by us to account for hypertrophic cardiac growth in CaMKII\(δ\)/CaMKII\(γ\) double knockout (KO) mice.\textsuperscript{124} It has moreover been suggested that calcineurin-dependent dephosphorylation of CaMKII at its autophosphorylation site vice versa constitutes another negative crosstalk pathway between these 2 enzymes.\textsuperscript{125} This pathway may also involve a more indirect mechanism via calcineurin-dependent dephosphorylation of protein phosphatase 1 inhibitor-1.\textsuperscript{126} Thereby, protein phosphatase 1 activity is increased, which would consequently reduce autophosphorylation of CaMKII.\textsuperscript{127} Figure 2 illustrates the potential interplay between CaMKII and calcineurin and their downstream signaling pathways.

In addition to CaMKII, calcineurin via NFAT was also reported to have an impact on the transcriptional coactivator myocardin.\textsuperscript{128} Li et al\textsuperscript{129} could further show that NFAT binds to the promotor region of myocardin, induces its expression, and synergistically with myocardin enhances expression of the LTCC \(α\) subunit.

Beyond the regulation of NFAT, calcineurin was revealed to interact with the transcription factor nuclear factor \(Y\) and affect gene expression of TXNIP (thioredoxin-interacting protein). Nuclear factor \(Y\) binds to the CCAAT element in promoter regions, and depending on the particular DNA sequence either is an activator or repressor of gene expression.\textsuperscript{130} It has been proposed that calcineurin dephosphorylates nuclear factor \(Y\), therefore, decreases its affinity to the DNA and attenuates its transcriptional repression of one of its targets—TXNIP.\textsuperscript{131} TXNIP, an endogenous inhibitor of antioxidant thioredoxin, is considered to be a relevant player in controlling energy metabolism and reactive oxygen species in cardiac pathologies.\textsuperscript{132,133}

\textbf{Calpain}

The nonlysosomal cysteine protease calpain gets only activated at relatively high ambient Ca\(^{2+}\) concentrations of about 1 \(\mu\)mol/L.\textsuperscript{134} Otherwise, it is regulated by its endogenous inhibitor calpastatin. Although some physiological functions have been demonstrated, calpain is mainly described to cleave proteins in pathological settings. In regard to cardiomyocytes, especially ischemia reperfusion settings and heart failure conditions depict situations of Ca\(^{2+}\) overload where calpain was shown to be more active.\textsuperscript{135}

Calpain was described to cleave PKC\(α\), the most abundant PKC isoform in the heart, in its V3 hinge region, thereby separating the C-terminal catalytic from the N-terminal regulatory domain.\textsuperscript{116–118} Accordingly, without this regulatory domain permanently inhibiting the catalytic function, the C-terminal domain is constitutively active. Furthermore, the C-terminal fragment of PKC\(α\) gains the ability to phosphorylate additional targets and to translocate to the nucleus. Here, the catalytic fragment leads to HDAC5 phosphorylation, which results in nuclear export of HDAC5, and consequent activation of MEF2-dependent genes.\textsuperscript{136} Aside from PKC, 2 other prominent cleavage targets of calpain have been described in regard to their influence on transcriptional regulation. For the phosphatase calcineurin, calpain was reported to cleave the autoinhibitory domain, which results in translocation of calcineurin to the nucleus, causing increased and prolonged NFAT-dependent gene transcription.\textsuperscript{137} In addition, the calpain-mediated calcineurin cleavage product was described to be independent of Ca\(^{2+}\)/calmodulin binding and, therefore, constitutively active in its function.\textsuperscript{138} Moreover, calpain cleavage of LTCC was proposed. Its distal C-terminal fragment was shown to localize to the nucleus and alter transcriptional activity.\textsuperscript{121} Via this mechanism, the LTCC, for example, autoregulates its own expression in cardiomyocytes.\textsuperscript{142}

\textbf{Direct Effects of Ca}\(^{2+}\)

An interesting finding in terms of direct interaction between Ca\(^{2+}\) and chromatin is the observation that certain GC sequences in the DNA have the ability to bind Ca\(^{2+}\), which leads to local changes in the DNA structure.\textsuperscript{144} The physiological relevance of this finding is still unclear, yet one has to consider that Ca\(^{2+}\), locally enhanced in nuclear microdomains, might bind to a very specific region of the DNA and recruit Ca\(^{2+}\)-dependent enzymes to spatially modify chromatin structure and potentially change transcription.

\textbf{ET Coupling in the Diseased Heart}

\textbf{Ca}\(^{2+}\) Cycling in Heart Failure

Defects in cellular Ca\(^{2+}\)-handling and alterations in Ca\(^{2+}\)-dependent signaling pathways are molecular hallmarks of heart failure. The underlying mechanisms of cardiac dysfunction may vary depending on the different causes and stages of the disease, yet several consistent findings on the dysregulation of Ca\(^{2+}\) cycling have been characterized. On the one hand, structural changes in Ca\(^{2+}\)-handling microdomains regularly occur such as decreases in T-tubule and nuclear invagination density and rearrangements of the nuclear transport machinery.\textsuperscript{135,145–148} On the other hand, several Ca\(^{2+}\)-handling proteins show altered expression patterns and activity states. In particular, defective SR Ca\(^{2+}\)-handling is considered an important aspect of heart failure pathophysiology. On a molecular level,
Ca2+ load is decreased because of diminished SR filling and amplitude decreases, the time to peak prolongs, the rate of Ca2+ influx in failing cardiomyocytes show distinct alterations: the amplitude decreases, the time to peak prolongs, the rate of Ca2+ influx decreases, and the resting [Ca2+]i is elevated. NCX is increased in expression and activity in various heart failure conditions and may influence these alterations in different ways. Shifting the balance of cytosolic Ca2+ removal toward enhanced Ca2+ extrusion via NCX might further impair SR Ca2+ load. In contrast, NCX can switch into reversed mode at high intracellular Na+ as observed in heart failure, causing Ca2+ influx which may contribute to diastolic Ca2+ overload. Besides these global Ca2+ disturbances, distinct local changes in Ca2+ have been observed in heart failure. Ljubojevic et al demonstrated that nuclear Ca2+ transients display pathological patterns earlier in disease development than the cytosolic Ca2+ transients, suggesting an important role for Ca2+-dependent maladaptive gene program activation. Consistent with this observation, changes in nucleocytoplasmic trafficking processes were observed to precede cardiac functional deterioration in experimental heart failure. Moreover, increasing evidence indicates that other Ca2+ channels, less implicated in EC coupling, significantly contribute to disturbances in Ca2+-dependent pathways, as IP3 receptors and several TRPC channels have been demonstrated to be upregulated in experimental and human heart failure.

Changes in Ca2+-Dependent Transcriptional Regulation in Heart Failure

Ca2+-dependent transcriptional pathways involving key regulators such as CaMKII and calcineurin are overactive in the diseased heart and suggested to drive structural changes and functional deterioration by inducing remodeling processes. Table 1 gives an overview on in vivo studies on proteins involved in these pathways with respect to their impact on cardiac hypertrophy, cardiac function, cardiac fibrosis, and cardiac inflammation.

CaMKII activity is consistently increased in human and in experimental heart failure, and activation of CaMKII has been observed in response to multiple neurohumoral signals that are enhanced in heart failure, including ANGII, ET-1, α-AR, and β-AR agonists. This neurohumoral activation of CaMKII has been linked to the induction of hypertrophic and fetal gene programs. In addition, CaMKII was shown to act as a critical downstream mediator of noncanonical Wnt signaling via dishevelled, which represents a widely unrecognized but central pathway in myocardial remodeling processes. A causative role of CaMKII in heart failure development has been derived from cardiomyocyte-specific genetic mouse models. Cardiac-specific overexpression of cytosolic CaMKIIδC and nuclear CaMKIIδB induces dilated cardiomyopathy and cardiac hypertrophy, respectively. Given the multiple targets of CaMKII in cardiomyocytes, specifically estimating the relevance of effects based on Ca2+-cycling protein modification versus effects on transcriptional regulation components is challenging. However, deciphering the role of nuclear versus cytosolic CaMKII might deliver insights into distinct mechanisms. By comparative approach, it was shown that overexpression of both splice variants induces activation of MEF2, mechanistically via phosphorylation of HDAC4, whereas SR Ca2+ cycling is only affected by cytosolic CaMKIIδC. Accordingly, CaMKIIδB KO mice are protected from cardiac remodeling upon pressure overload.

In our studies on CaMKIIδB KO mice, we found a marked reduction in CaMKII-dependent HDAC4 phosphorylation and attenuated induction of the fetal gene program, while we could not see any genotype-related disturbances in intracellular Ca2+ transients under either unstressed conditions or in the presence of transverse aortic constriction. These results indicate that effects on Ca2+-handling are not absolutely required for the protective CaMKIIδB–dependent phenotype. In contrast, Ling et al demonstrated in a different CaMKIIδ KO mouse model that the increased SR Ca2+ leak after transverse aortic constriction is completely abolished via prevention of RyR2-S2814 hyperphosphorylation, suggesting that restored SR Ca2+ might underlie the beneficial effects of CaMKIIδ deletion. However, attempts to rescue the cardiomyopathic phenotype in CaMKIIδC TG mice by restoring SR function to our knowledge failed to date. SR-targeted CaMKII inhibition indeed significantly reduced the SR Ca2+ leak but accelerated the development of cardiac remodeling in the TG. In addition, restoring SR load in CaMKIIδC TG by genetic KO of PLN showed no rescue effect but resulted in exacerbation of cardiac dysfunction and mortality in the TG. These findings strongly indicate that even cytosolic CaMKIIδC experts maladaptive effects in heart failure via SR-independent mechanisms.

The critical involvement of the class IIa HDAC/MEF2 axis in the induction of cardiac hypertrophy and remodeling has also been demonstrated in genetic mouse models. HADC9 KO mice are sensitized to hypertrophic signals including calcineurin overexpression, show an increased activation of the fetal gene program, and display a hypersensitive MEF2 activity. Similar findings have been reported for HADC5 KO mice. Regarding MEF2, characterization of MEF2A and MEF2C TG revealed a dosage-dependent cardiomyopathic phenotype and reduction in ventricular performance, as well as an increased susceptibility to calcineurin overexpression-induced hypertrophy. In addition, MEF2D overexpression induces pathological cardiac remodeling and drives the fetal gene program, whereas MEF2D KO are protected against remodeling and exhibit attenuated fetal gene activation in response to pressure overload and chronic β-adrenergic stimulation.

Calcineurin is overactive not only in human heart failure but also in compensated cardiac hypertrophy. Notably, IRF8 (interferon regulatory factor 8), which acts as an inhibitor of calcineurin downstream target NFATc1, has been described to be downregulated in dilated and hypertrophic cardiomyopathy. In vitro data consistently indicate that pharmacological and genetic calcineurin inhibition attenuates cardiomyocyte hypertrophy induced by neurohumoral stimulation. This anti hypertrophic effect was
Table 1. Overview on In Vivo Studies Investigating Ca\(^{2+}\)-Dependent Signaling Proteins Involved in Transcriptional Pathways With Respect to Their Impact on Cardiac Hypertrophy, Cardiac Function, Cardiac Fibrosis, and Cardiac Inflammation

<table>
<thead>
<tr>
<th>Protein/Mutant Name</th>
<th>Genetic Model</th>
<th>Pharmacological/Peptide-Based Inhibition</th>
<th>Intervention</th>
<th>Cardiac Growth</th>
<th>Cardiac Function</th>
<th>Cardiac Fibrosis</th>
<th>Cardiac Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaMKIIδC</td>
<td>TG</td>
<td>none</td>
<td>MI</td>
<td>↑ (156,157)</td>
<td>↓↓ (156–160)</td>
<td>↑ (156)</td>
<td>↑ (156)</td>
</tr>
<tr>
<td>CaMKIIδB</td>
<td>TG</td>
<td>none</td>
<td>MI</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
<td>↓↓ (156)</td>
<td>↑ (156,157)</td>
</tr>
<tr>
<td>CaMKIIδ</td>
<td>KO</td>
<td>TAC</td>
<td>MI</td>
<td>↑ (156)</td>
<td>↓ (161)</td>
<td>↑ (156)</td>
<td>↓ (161)</td>
</tr>
<tr>
<td>CaMKIIδ/ CaMKIIγ</td>
<td>DKO</td>
<td>TAC</td>
<td>MI</td>
<td>↑ (161)</td>
<td>↓ (161)</td>
<td>↑ (161)</td>
<td>↓ (161)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>AC3-I TG</td>
<td>MI</td>
<td>TAC</td>
<td>↑ (167)</td>
<td>↓ (167)</td>
<td>↑ (167)</td>
<td>↓ (167)</td>
</tr>
<tr>
<td>HDAC5</td>
<td>KO</td>
<td>none</td>
<td>MI</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
</tr>
<tr>
<td>HDAC5/ CnA (constitutively active CnA)</td>
<td>KO/TG</td>
<td>none</td>
<td>MI</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
</tr>
<tr>
<td>HDAC9</td>
<td>KO</td>
<td>none</td>
<td>MI</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
</tr>
<tr>
<td>HDAC9/ CnA (constitutively active CnA)</td>
<td>KO/TG</td>
<td>none</td>
<td>MI</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
<td>↑ (169)</td>
<td>↓ (169)</td>
</tr>
<tr>
<td>MEF2A</td>
<td>TG</td>
<td>none</td>
<td>MI</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
</tr>
<tr>
<td>MEF2C</td>
<td>TG</td>
<td>none</td>
<td>MI</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
</tr>
<tr>
<td>MEF2D</td>
<td>Kp</td>
<td>TAC</td>
<td>MI</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
</tr>
<tr>
<td>CnAB</td>
<td>KO</td>
<td>TAC</td>
<td>MI</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
</tr>
<tr>
<td>dnCnA</td>
<td>TG</td>
<td>TAC</td>
<td>MI</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
<td>↑ (172)</td>
<td>↓ (172)</td>
</tr>
</tbody>
</table>

(Continued)
confirmed in various in vivo models. Further evidence for a causative role of calcineurin and its downstream effector NFAT in heart failure was obtained from cardiac-specific genetic mouse models. Calcineurin and NFAT3 TG develop cardiac hypertrophy and heart failure, with NFAT and GATA4 synergistically activating the fetal gene program. Of note, calcineurin TG mice were shown to exhibit cardiac remodeling despite increased SR function. The critical involvement of NFAT in cardiac hypertrophy was additionally investigated by studying NFATc3 KO mice, revealing that NFATc3 deletion protects against calcineurin overexpression-induced, pressure overload–induced, and ANGII-induced cardiac hypertrophy.

There are, however, some inconsistent findings about the role of the calcineurin/NFAT pathway in physiological versus pathological hypertrophy because several studies also suggest calcineurin-dependent pathways to be involved in nonmaladaptive growth. Interestingly, calcineurin upregulation is more pronounced in compensated cardiac hypertrophy compared with heart failure. Eto et al reported calcineurin

<table>
<thead>
<tr>
<th>Protein/Mutant Name</th>
<th>Genetic Model</th>
<th>Pharmacological/Peptide-Based Inhibition</th>
<th>Intervention</th>
<th>Cardiac Growth</th>
<th>Cardiac Function</th>
<th>Cardiac Fibrosis</th>
<th>Cardiac Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaN</td>
<td></td>
<td></td>
<td>TAC</td>
<td>↓↓ (183–186)</td>
<td>↓ (184–185)</td>
<td>↑ (188)</td>
<td></td>
</tr>
<tr>
<td>CnA TG</td>
<td></td>
<td></td>
<td>MI</td>
<td>↓ (187–189)</td>
<td>↓ (185,186)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FK506</td>
<td></td>
<td></td>
<td>TAC</td>
<td>↓ (184)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔCain TG</td>
<td></td>
<td></td>
<td>TAC</td>
<td>↓ (189)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔAKAP TG</td>
<td></td>
<td></td>
<td>TAC</td>
<td>↓ (189)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCIP1 TG</td>
<td></td>
<td></td>
<td>TAC</td>
<td>↓ (189)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaki-4$eta$ TG</td>
<td></td>
<td></td>
<td>TAC</td>
<td>↓ (201)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFATc3 Δ317 TG</td>
<td>TG</td>
<td></td>
<td>none</td>
<td>↑ (113)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFATc3 KO</td>
<td></td>
<td></td>
<td>TAC</td>
<td>↓ (173)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFATc4 TG</td>
<td>none</td>
<td></td>
<td>↑ (177)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFATc4 KO</td>
<td>CnA TG</td>
<td></td>
<td>none</td>
<td>↑↑↑ (174)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAMTA2 TG</td>
<td></td>
<td></td>
<td>none</td>
<td>↑↑↑ (65)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAMTA2 KO</td>
<td>MI</td>
<td></td>
<td>↑ (48)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANGII indicates chronic angiotensin II infusion; AKAP, A-kinase anchoring protein; CaMKII, Ca$^{2+}$/calmodulin-dependent kinase II; CAMTA, calmodulin-binding transcriptional activator; CaN, calcineurin; CnA, calcineurin subunit A; CsA, cyclosporin A; DKO, double knockout; dnCnA, dominant negative calcineurin subunit A; DVL, dishevelled; HDAC, histone deacetylase; ISO, chronic isoprenaline infusion; KO, knockout; MCIP, myocyte-enriched calcineurin-interacting protein; MEF2, myocyte enhancer factor 2; MI, myocardial infarction; NFAT, nuclear factor of activated T cells; PE+ANGII, chronic application of phenylephrine and angiotensin II; TAC, transverse aortic constriction; TG, transgenic overexpression; ↑, <2-fold increase compared with respective control; ↑↑, >2-fold increase; ↑↑↑, >3-fold increase (Compared with the wild-type control group); →, no significant alterations; ↓, <50% decrease; and ↓↓, >50% decrease.
upregulation in adaptive cardiac hypertrophy upon exercise training in rats.\textsuperscript{218} In line with this finding, calcineurin inhibition by MCPI1 (myocyte-enriched calcineurin-interacting protein 1) overexpression in mice has been shown to attenuate exercise-induced cardiac hypertrophy.\textsuperscript{214} Moreover, calcineurin activity was suggested to be a requirement for adaptive hypertrophy during pregnancy.\textsuperscript{219} Another observation that might challenge the view of calcineurin as an overall detrimental ET coupling player is related to its role in dilated cardiomyopathy. KO mice lacking the stress responsive isoform of calcineurin exhibit enhanced cardiomyocyte apoptosis and cardiac dysfunction in a genetic mouse model of muscle LIM protein deficiency. Conversely, expression of activated calcineurin improves function and adverse remodeling in this model, suggesting a rather protective role in this scenario.\textsuperscript{175}

Also findings in the context of the interrelation between CaMKII and calcineurin signaling indicate calcineurin activation in nonpathological cardiac growth. In line with the above-mentioned detrimental role of overactivated CaMKII, CaMKIIb/CaMKIIγ double KO mice are protected against cardiac dysfunction and interstitial fibrosis upon pressure overload. However, these mice do develop cardiac hypertrophy, but remarkably without signs of pathological remodeling, establishing a dissociation of cardiac hypertrophy on the one hand and pathological remodeling on the other hand. In the same study, these mice also show an increased activity of endogenous calcineurin and exaggerated cardiac hypertrophy upon exercise, again without signs of heart failure. Mechanistically, calcineurin activation was shown to account for cardiac growth in this model because of completely diminished CaMKII-dependent phosphorylation at the inhibitory calcineurin phosphosite Ser-411 in the CaMKIIδ/CaMKIIγ double KO mice. Notably, this striking effect was only observed in the CaMKIIδ/CaMKIIγ double KO model, which lacks any measurable CaMKII activity in the heart, but not in the CaMKII single KO models, because CaMKIIδ and CaMKIIγ are able to compensate for each other.\textsuperscript{124}

In contrast to the indicated crucial involvement of the calcineurin/NFAT pathway activation in adaptive cardiac growth, Haines et al reported no effect of calcineurin inhibitor cyclosporin A on exercise-induced cardiac hypertrophy in wild-type mice.\textsuperscript{220} Other data even suggest that cardiac antiremodeling effects of exercise training involve deactivation of the calcineurin/NFAT pathway.\textsuperscript{221} Consistent with the latter, Wilkins et al also observed a decrease in NFAT activity upon exercise.\textsuperscript{222}

An aspect that should be taken into consideration when trying to explain the discrepancies on the role of calcineurin in nonmaladaptive growth versus decompensated heart failure is the applied genetic model. The studies investigating calcineurin TG mice used a constitutively active form of calcineurin for the transgenic overexpression. These mice show severe cardiac remodeling accompanied by contractile dysfunction and increased collagen deposition. However, the fact that the overexpressed calcineurin is constitutively active might account for some divergences in the results compared with studies investigating the modulation of endogenous calcineurin. Taking all of the studies on genetic and pharmacological calcineurin and NFAT inhibition into account (Table 1), the relevance of the calcineurin/NFAT axis for cardiac hypertrophy is striking, but not so the evidence for its role in pathological remodeling and contractile dysfunction. Also the time course of calcineurin activation during the compensated versus the decompensated phase of heart failure supports this perspective. For instance, our laboratory showed that calcineurin activation precedes CaMKII activation upon the induction of pathological pressure overload and is, in contrast to CaMKII, down-regulated during the transition to heart failure.\textsuperscript{214} These data may indicate that increased calcineurin/NFAT activity plays an important role in compensated and early cardiac hypertrophy rather than in long-term cardiac remodeling processes. The observation that calcineurin is upregulated in exercise-induced hypertrophy and required for adaptive cardiac growth supports from our point of view this conclusion.

Taken together, it will be important in the future to detect dynamic changes of different Ca\textsuperscript{2+}-dependent signaling molecules in the course of heart failure development but also to increase our understanding of the distinct Ca\textsuperscript{2+} sources that account for the differential regulation in adaptive versus maladaptive signaling cascades.

Increasing evidence indicates that IP3 receptors, TRPC channels, and SOCE dysregulation significantly contribute to detrimental enhancement of the above-mentioned transcriptional pathways in heart failure. Table 2 gives an overview on in vivo studies investigating these Ca\textsuperscript{2+}-handling proteins with respect to their impact on cardiac hypertrophy, cardiac function, cardiac fibrosis, and the suggested involvement in transcriptional regulation.

IP3R2 TG mice develop cardiac hypertrophy in response to chronic isoproterenol infusion, Gqα overexpression, and exercise stimulation, which is blocked via genetic calcineurinAβ deletion.\textsuperscript{17} Moreover, local nuclear envelope Ca\textsuperscript{2+} release via IP3 receptors in response to ET-1 has been shown to affect the HDAC5/MEF2 axis independent of the global Ca\textsuperscript{2+} transients.\textsuperscript{211}

Also Ca\textsuperscript{2+} entry via TRPC channels has been linked to calcineurin activation. TRPC3 TG develop hypertrophy and contractile dysfunction in response to pressure overload, which is abrogated in a calcineurinAβ KO background.\textsuperscript{225} In addition, overexpression of TRPC6 was demonstrated to induce cardiomyopathy and accelerate cardiac remodeling, accompanied by an increase in NFAT-dependent expression of the β-myosin heavy chain.\textsuperscript{132} Of note, Seo et al demonstrated that only combined deletion of TRPC3 and TRPC6 reduces the hypertrophic response to pressure overload, whereas individual gene deletion is not protective.\textsuperscript{226} Moreover, studies on dominant-negative TRPC3, TRPC6, and TRPC4 revealed that inhibition of TRPCs reduces the activity of the calcineurin/NFAT pathway and attenuates hypertrophic response in vitro and in vivo.\textsuperscript{228} Interestingly, protection against neurohumoral-induced cardiac hypertrophy in TRPC1/C4 KO mice was shown to involve a reduction not only in calcineurin activity but also in MEF2-dependent gene activation.\textsuperscript{227}

Recent experimental data also suggest SOCE to play an important role in activating transcriptional pathways involved in cardiomyocyte hypertrophy. STIM1-dependent Ca\textsuperscript{2+} signaling was revealed to activate the calcineurin/NFAT pathway and CaMKII signaling in vitro.\textsuperscript{232,233} Hulot et al emphasized the in vivo relevance of STIM1 in cardiac hypertrophy,
demonstrating that shRNA-mediated STIM1 gene silencing protects against cardiac growth in response to pressure overload, associated with a reduction in nuclear NFATc3 translocation. Conversely, it was shown that overexpression of STIM1 results in cardiac hypertrophy and contractile dysfunction. The cardiac-specific STIM1 KO mice from Parks et al exhibited similar results with regard to attenuation of hypertrophic response, but additionally presented impaired baseline contractility and rearrangements of cytoskeletal structures under unstressed conditions. The important role of STIM1 in cardiac integrity was further illustrated by Collins et al, demonstrating that cardiac-restricted deletion of STIM1 leads to increased ER stress, mitochondrial disorganization, and progressive cardiac remodeling in mice. This shapes an overall complex picture of STIM1-dependent cellular effects with broad implications that are up to now still poorly understood.

<table>
<thead>
<tr>
<th>Protein/Mutant Name</th>
<th>Genetic Model</th>
<th>Intervention</th>
<th>Cardiac Growth</th>
<th>Cardiac Function</th>
<th>Cardiac Fibrosis</th>
<th>Suggested Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP3R2</td>
<td>TG</td>
<td>none</td>
<td>↑ (17)</td>
<td>→ (17)</td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TAC</td>
<td>↑ (17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ISO</td>
<td>↑ (7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP3R2</td>
<td>KO</td>
<td>TAC</td>
<td>→ (227)</td>
<td>→ (229)</td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td>TRPC1</td>
<td>KO</td>
<td>TAC</td>
<td>↓ (229)</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td></td>
</tr>
<tr>
<td>TRPC3</td>
<td>TG</td>
<td>none</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TAC</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE+ANGII</td>
<td>↑ (229)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPC3</td>
<td>KO</td>
<td>TAC</td>
<td>→ (229)</td>
<td>→ (229)</td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td>TRPC6</td>
<td>TG</td>
<td>none</td>
<td>↑ (229)</td>
<td></td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TAC</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPC6</td>
<td>KO</td>
<td>TAC</td>
<td>→ (229)</td>
<td>→ (229)</td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td>TRPC3/ TRPC6</td>
<td>DKO</td>
<td>TAC</td>
<td>→ (229)</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ISO</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANG</td>
<td>→ (229)</td>
<td></td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td>TRPC1/ TRPC4</td>
<td>DKO</td>
<td>TAC</td>
<td>↓ (229)</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ISO</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td>CaMKII-MEF2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANG</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dnTRPC3</td>
<td>TG</td>
<td>TAC</td>
<td>↓ (229)</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE+ANGII</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td>dnTRPC4</td>
<td>TG</td>
<td>TAC</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td>dnTRPC6</td>
<td>TG</td>
<td>TAC</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE+ANGII</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIM1</td>
<td>TG</td>
<td>none</td>
<td>↑↑ (229)</td>
<td>↓ (229)</td>
<td></td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TAC</td>
<td>↑ (229)</td>
<td>↓ (229)</td>
<td></td>
<td>CaMKII-MEF2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ISO</td>
<td>↑ (229)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIM1</td>
<td>KO</td>
<td>none</td>
<td>→ (229)</td>
<td>↓ (229)</td>
<td>↑ (229)</td>
<td>Calcineurin-NFAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TAC</td>
<td>↓ (229)</td>
<td></td>
<td></td>
<td>FAK-Akt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE+ANGII</td>
<td>↑ (229)</td>
<td></td>
<td></td>
<td>ERK1/2</td>
</tr>
</tbody>
</table>

ANGII indicates chronic angiotensin II infusion; CaMKII, Ca2+/calmodulin dependent kinase II; DKO, double knockout; dnTRPC, dominant negative transient receptor potential C; IP3R, inositol-1,4,5-trisphosphate (IP3) receptor; ISO, chronic isoprenaline infusion; KO, knockout; MEF2, myocyte enhancer factor 2; NFAT, nuclear factor of activated T cells; PE+ANGII, chronic application of phenylephrine and angiotensin II; STIM1, Stromal interaction molecule-1; TAC, transverse aortic constriction; TG, transgenic overexpression; TRPC, transient receptor potential C; ↑, <2-fold increase compared with respective control; ↑↑, >2-fold increase; →, no significant alterations; ↓, <50% decrease; and ↓↓, >50% decrease.
In conclusion, Ca\(^{2+}\)-dependent transcriptional regulators are evidently involved in heart failure pathophysiology. The majority is overactive in the state of heart disease and in experimental in vivo models, and numerous studies emphasized that Ca\(^{2+}\)-dependent ET effectors and pathways are able to dramatically change the cardiac phenotype. Nevertheless, whereas some few downstream targets are well characterized, most of them remain elusive just as the interaction of different Ca\(^{2+}\)-dependent pathways and the question whether 1 pathway is solely maladaptive or does also possess some adaptive functions.

**Therapeutic Implications**

Current pharmacological treatment options in heart failure (beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone inhibitors) are in line with the paradigm of neurohumoral overactivation. Indeed, angiotensin, endothelin, and catecholamines are reportedly upregulated in heart failure, and exposure to excessive amounts has detrimental effects on the cellular level. Conversely, neurohumoral blockade effectively enhances heart function, improves clinical outcome, and reduces hospitalization. Despite this, the overall mortality of heart failure is still high. In addition, tolerability of the optimal doses of standard medications is often limited because of contraindications, interactions, and adverse effects. This underlines the necessity to find novel and relevant therapeutic targets.

Different neurohumoral stimuli were indeed shown to affect cellular Ca\(^{2+}\), and neurohumoral inhibition consequently improves Ca\(^{2+}\)-handling, possibly thereby contributing to clinical amelioration of contractility. The fact that Ca\(^{2+}\) is regulated by a multitude of pathways, and the finding that Ca\(^{2+}\) itself affects various cellular functions such as excitation, contraction, metabolism, and transcription, turns it into a nexus of signaling cascades in cardiomyocytes. On the background of altered Ca\(^{2+}\)-handling in heart failure, it was thus proposed that restoring Ca\(^{2+}\) cycling back to the physiological state is a promising therapeutic goal. However, defining a potent target turned out to be rather challenging.

As mentioned above, increased SR Ca\(^{2+}\) leak is a characteristic finding in failing hearts; thus, restabilization of the RyR is thought to be a rationale concept. Still, the functional role and the relative impact of the PKA- and CaMKII-dependent phosphorylation of RyR remain controversial. Studies on PKA-phospho-resistant RyR mutant mice could not consistently demonstrate the relevance of this phosphorylation site for SR Ca\(^{2+}\) leak or cardiac function in experimental heart failure models. In contrast, the association of CaMKII-dependent RyR phosphorylation with Ca\(^{2+}\) sparks is well established. Findings on RyR mutants that prevent or mimic CaMKII-dependent phosphorylation offered insights into the functional relevance of this phosphorylation site at S2814. The phoso-resistant S2814A mutation was shown to inhibit Ca\(^{2+}\) leakage from the SR, improve SR Ca\(^{2+}\) load, and to be protective in the setting of transverse aortic constriction. In contrast, however, this RyR mutation had no protective impact on cardiac dysfunction upon myocardial infarction. Conversely, the S2814D mutation depicts a constitutively activated form of the CaMKII-dependent RyR phosphosite. Mice with this mutation exhibit pathological SR Ca\(^{2+}\) release events and reduced SR Ca\(^{2+}\) load. Interestingly, this seems to primarily result in an increase in arrhythmia susceptibility but causes only a slight decrease in contractile function with aging. Thus, it seems evident that CaMKII-dependent RyR phosphorylation at the S2814 site causes pathological alterations in SR Ca\(^{2+}\)-handling and arrhythmias. However, a clear picture with respect to the pathogenesis of all aspects of heart failure including contractile dysfunction cannot yet be drawn. Given the complexity of RyR regulation in a macromolecular complex with multiple posttranslational modifications, targeting proteins and interaction partners, future research will need to shape an integrated view on RyR regulation, causes of RyR dysfunction, and their relevance in the pathogenesis of heart failure.

Because decreased Ca\(^{2+}\) uptake into the SR is a characteristic observation in heart failure, another promising approach may be enhancement of SR Ca\(^{2+}\) reuptake to rescue pathologically altered Ca\(^{2+}\) cycling. In this regard, gene therapy approaches may offer yet unexplored possibilities. Especially SERCA, SUMO-1, and S100A1 vectors proved to have beneficial effects on heart failure in animal models. However, the outcome of the latest clinical trial for adeno-associated virus 1–delivered SERCA gene therapy (CUPID 2) could not yet successfully establish this approach in the clinical setting. Although these results do not argue against SERCA as a promising target, ongoing difficulties with the gene therapy approach regarding vector design, delivery methods, and issues with humoral immunity need to be solved to use gene therapy for the re-expression of downregulated Ca\(^{2+}\)-handling proteins such as SERCA or S100A1.

Moreover, the major Ca\(^{2+}\)-dependent signaling enzymes, CaMKII and calcineurin, are focus of attempts to pharmacologically treat heart failure. In case of CaMKII, it has been demonstrated that induced genetic KO slows down the progression of heart failure development in response to pressure overload, herewith providing further evidence for its therapeutic potential. In addition, recent findings suggest that CaMKII blockade would complement the effect of the current standard therapy. However, given the fundamental role of CaMKII in various physiological cellular functions involving, for example, synaptic plasticity, fertility, and immunologic memory, pharmacologically inhibiting global CaMKII might presumably be accompanied by serious adverse effects. Thus, for a successful application, the issues of selective organ targeting and CaMKII isoform specificity have to be addressed as well as the issue of target-specific inhibition, which requires a deeper understanding of the critical CaMKII downstream mechanisms in heart failure. Inhibitor development programs with focus on myocardial CaMKII have been launched by several companies, but the way into clinical application is not clear to date.

Cardiac calcineurin is predominantly described to be involved in pathological settings. However, this picture is controversial and has been challenged repeatedly, as discussed above. Referring to Table 1, the evidence for calcineurin being involved in cardiac hypertrophy is indeed overwhelming, but especially with regard to contractile function, the evidence
for the benefit of calcineurin inhibition is not consistent. In part, this could be related to different models of experimental heart failure, inhibitor strategies, doses, treatment time points, and treatment periods used in the various studies. These inconsistencies might also explain why, despite the accessibility of established pharmaceutical compounds, translation of the findings around calcineurin inhibition into the clinics was not successful yet. A recent phase III trial with single application of CsA in patients after myocardial infarction somewhat depicts this translational dilemma and shows no improvement in clinical outcomes or ventricular remodeling compared with the control group.264

Even though the recent clinical trials following the premise to rescue cellular Ca2+ cycling did not yet meet the expectations, and also pharmacological inhibition of the major Ca2+-dependent CaMKII and calcineurin pathways could not be translated into clinical application to date, these Ca2+-regulated enzymes were convincingly shown to be important players in cardiac pathologies and, therefore, their cascades present promising therapeutic targets. Concurrently, these findings illustrate the major problems we face when dealing with the paradigm of globally disturbed Ca2+ cycling and Ca2+-dependent pathways, that is, deciphering (1) how Ca2+-dependent pathways influence different pathological features, and (3) how Ca2+-dependent pathways interrelate.

To circumvent the complexity of CaMKII or calcineurin signaling and their time-dependent and isoform-specific peculiarities, another therapeutic approach would be to systematically search for downstream effectors, elucidate their pathophysiological relevance, and focus on the targets solely mediating maladaptive features. Pharmacological interventions further downstream bear the potential to offer several advantages like a higher specificity in the mechanism of action and, therefore, a lower possibility of on-target adverse effects.

Given the various disease causes of heart failure and, therefore, a lower possibility of on-target adverse effects, another therapeutic approach would be to systematically search for downstream effectors, elucidate their peculiarities, another therapeutic approach would be to systematically search for downstream effectors, elucidate their pathophysiological relevance, and focus on the targets solely mediating maladaptive features. Pharmacological interventions further downstream bear the potential to offer several advantages like a higher specificity in the mechanism of action and, therefore, a lower possibility of on-target adverse effects. Given the various disease causes of heart failure and, therefore, a lower possibility of on-target adverse effects.

Acknowledgments

We thank Dr Christiane Vettel for her valuable suggestions.

Sources of Funding

H.A. Katus and J. Backs were supported by grants from the DZHK (Deutsches Zentrum für Herz-Kreislauferforschung—German Centre for Cardiovascular Research) and by the BMBF (German Ministry of Education and Research).

Disclosures

None.

References


CaMKII activation through NF-αβ and TNF-δ

Miyamoto S, Westenbrink BD, Brown JH. CaMKII

fusion injury.


Dewenter et al. Calcium Signaling and Transcription 1017


Oliveira RS, Ferreira JC, Gomes ER, Paixao NA, Rolim NP, Medeiros A, Guatimosim S, Brum PC. Cardiac anti-remodelling effect of aerobic...


Calcium Signaling and Transcriptional Regulation in Cardiomyocytes
Matthias Dewenter, Albert von der Lieth, Hugo A. Katus and Johannes Backs

Circ Res. 2017;121:1000-1020
doi: 10.1161/CIRCRESAHA.117.310355

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/121/8/1000

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/