Calcium Cycling and Signaling in Cardiac Myocytes

Donald M. Bers

Department of Physiology and Cardiovascular Institute, Stritch School of Medicine,
Loyola University Chicago, Maywood, Illinois 60153; email: dbers@lumc.edu

Key Words

Ca channels, sarcoplasmic reticulum, mitochondria, contraction,
calmodulin

Abstract

Calcium (Ca) is a universal intracellular second messenger. In mus-
cle, Ca is best known for its role in contractile activation. However,
in recent years the critical role of Ca in other myocyte processes
has become increasingly clear. This review focuses on Ca signaling
in cardiac myocytes as pertaining to electrophysiology (including
action potentials and arrhythmias), excitation-contraction coupling,
modulation of contractile function, energy supply-demand balance
(including mitochondrial function), cell death, and transcription reg-
ulation. Importantly, although such diverse Ca-dependent regula-
tions occur simultaneously in a cell, the cell can distinguish distinct
signals by local Ca or protein complexes and differential Ca signal
integration.

INTRODUCTION

Calcium (Ca) is a ubiquitous intracellular second messenger throughout biology, involved in regulating diverse functions such as fertilization, electrical signaling, contraction, secretion, memory, gene transcription, and cell death. Some important systems in which Ca is important in cardiac myocytes are discussed here: (*a*) electrophysiology [via ion currents and exchangers, the regulation of other channels or exchangers, the mediation of action potential (AP) shape, arrhythmogenic mechanisms, and the regulation of cell-cell communication]; (*b*) excitation-contraction (E-C) coupling, which governs the Ca transient that drives contraction; (*c*) contraction itself, in which Ca is the activating switch of the myofilaments and a modulator of key contractile properties (e.g., cooperativity, length-dependent activation, and frequency-dependent acceleration of relaxation); (*d*) energy consumption (by contraction and Ca transport) and production (via the regulation of mitochondrial ATP production); (*e*) cell death by apoptosis or necrosis; and (*f*) transcriptional regulation via, e.g., calmodulin (CaM)-dependent activation of calcineurin and the nuclear translocation of factors such as histone deacetylases. This is only a fraction of Ca-dependent systems in cardiac myocytes, but this scope is already large and will allow neither uniform depth nor comprehensiveness in references (1). However, here I attempt to integrate this array of Ca signaling in cardiac myocytes and identify some unanswered questions to encourage further work.

Many of the Ca-dependent processes described above involve an orchestrated symphony that produces the acute and reliable contractile function of the heart to perform the tasks of pumping blood on a beat-to-beat basis and the short-term modulation of this function upon changes in heart rate or workload (topics *a–d* above). Acute malfunctions of these systems can result in both mechanical and electrical dysfunction (e.g., reduced cardiac output and arrhythmias). Longer-term adaptations and maladaptations to altered conditions involve altered protein expression, cell growth, and cell death (topics *e* and *f* above).

Ca-dependent regulation often works via specific Ca-binding proteins [such as CaM and troponin C (TnC)] and is also frequently mediated by very local intermolecular signaling in restricted spaces, rather than changes in global cytosolic [Ca] ($[Ca]_i$). Indeed, local Ca signaling allows multiple Ca-dependent pathways to sense spatially unique Ca signals simultaneously in the same cell.

ELECTROPHYSIOLOGICAL Ca SIGNALING IN MYOCYTES

Ca carries and regulates ionic currents via channels and exchangers, thereby influencing AP configuration, arrhythmogenesis, and cell-cell communication (**Figure 1**).

Ca Current (I_{Ca})

The two classes of membrane potential (E_m)-dependent I_{Ca} in cardiac myocytes, L-type ($I_{Ca,L}$) and T-type ($I_{Ca,T}$), can contribute to pacemaker activity, the AP upstroke and plateau phases, and arrhythmias [particularly early afterdepolarizations (EADs)]. Cardiac $I_{Ca,T}$ (encoded by α_{1G} or α_{1H} genes) activates at more negative E_m than $I_{Ca,L}$ (encoded by α_{1C} or α_{1D} genes) but also inactivates more rapidly than $I_{Ca,L}$ and independently of Ca influx. $I_{Ca,T}$ is not present in most ventricular myocytes but is expressed mainly in conducting and pacemaker cells in the heart [and also in immature or developing ventricular myocytes (2)]. Even in myocytes that express $I_{Ca,T}$, the current density is often small in comparison with $I_{Ca,L}$. $I_{Ca,L}$ and $I_{Ca,T}$ apparently serve different functional roles.

The α_{1D} gene is expressed at relatively high levels in pacemaker-type cells (3), and it activates at an intermediate E_m between that for $I_{Ca,T}$ and $I_{Ca,L}$ derived from α_{1C} (where the latter is the vast majority of cardiac I_{Ca} and the only type in most ventricular myocytes).
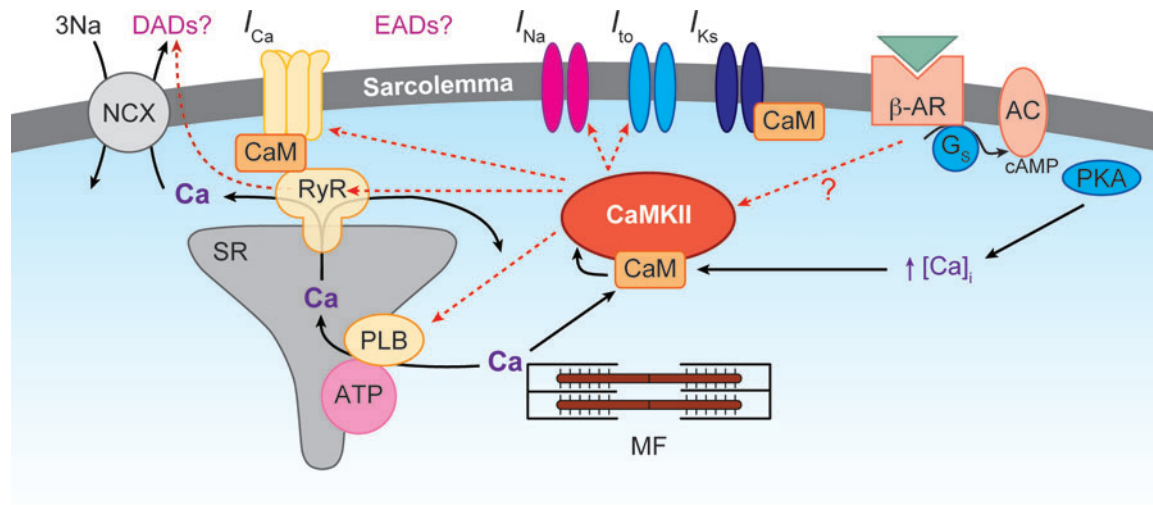
Figure 1

Ca-dependent signaling to cardiac myocyte ion channels. Ca entry via I_{Ca} activates sarcoplasmic reticulum (SR) Ca release via the ryanodine receptor (RyR), resulting in the activation of contraction. SR Ca uptake via the SR Ca-ATPase (ATP) and extrusion via Na/Ca exchange (NCX) allow relaxation. Calcium-calmodulin-dependent protein kinase (CaMKII) can phosphorylate phospholamban (PLB), causing enhanced SR Ca uptake, and also the RyR, enhancing spontaneous diastolic SR Ca release. That Ca release activates inward NCX current and arrhythmogenic delayed afterdepolarizations (DADs). CaMKII can also phosphorylate Ca and Na channel subunits, thereby altering I_{Ca} and I_{Na} gating, thereby prolonging APD and increasing the propensity for early afterdepolarizations (EADs). CaMKII can also modulate I_{to}, whereas calmodulin (CaM) itself can modulate RyR, I_{Ca}, and I_{Ks} gating. Activation of β-adrenergic receptors (β-AR) activates adenylate cyclase (AC) to produce cyclic AMP (cAMP) and activate PKA. PKA phosphorylates PLB and regulates SR Ca uptake, $I_{Ca,}$ I_{Ks}, and RyR, with a net increase in Ca transient amplitude. This is accepted to contribute to CaM and CaMKII activation, but there may also be a more direct, Ca-independent pathway by which β-AR can activate CaMKII. MF, myofilaments.

During diastolic pacemaker depolarization in spontaneously active cardiomyocytes [e.g., sinoatrial (SA) node cells], $I_{Ca,T}$ can become activated relatively early in diastole and contribute to diastolic depolarization. As depolarization proceeds, $I_{Ca,L}$ from α_{1D} and then $I_{Ca,L}$ from α_{1C} are recruited and further contribute to depolarization, and $I_{Ca,L}$ via α_{1C} drives the upstroke of the regenerative AP in pacemaker cells (vs the Na current I_{Na}, which dominates in atrial and ventricular myocytes).

Ca is also involved in pacemaker activity in other ways (4). Inward Na/Ca exchange current (I_{NCX}) during early diastolic depolarization is activated by [Ca]$_i$ from the previous beat as [Ca]$_i$ decline outlasts the SA node AP duration. Later during diastole, Ca is re-leased from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyRs), which can be triggered by either $I_{Ca,T}$ (5) or early $I_{Ca,L}$ or can even occur spontaneously by an SR Ca release clock (6). That is, as the SR refills with Ca from the previous beat and the RyR recovers from refractoriness, the high luminal SR [Ca] causes local SR Ca release events (Ca sparks) during diastolic depolarization. The SR Ca release itself does not produce a transsarcolemmal current, but the local rise in [Ca]$_i$ activates a second window of inward I_{NCX} that contributes to late diastolic depolarization. Notably, this is mechanistically similar to the accepted mechanism of delayed afterdepolarizations (DADs), a major class of triggered arrhythmias (see below). There is also a finite

fraction ($\leq$1%) of I_f (typically considered a monovalent-selective channel) that is carried by Ca, and this can produce a detectable rise in [Ca]$_i$ (7). β-Adrenergic agonists, which increase heart rate, accelerate I_f-dependent depolarization in SA nodal cells but also shift I_{Ca} gating to more negative potentials (recruiting I_{Ca} earlier in diastolic depolarization) and increase peak [Ca]$_i$ and the rate of SR Ca reuptake (thereby speeding the SR Ca release clock). Thus, several different currents carried by Ca can contribute to normal and abnormal pacemaker activity in the heart. Normal pacemaker activity includes all the above and also some other Ca-independent currents (4). Questions remain about the relative influence of these functionally overlapping and somewhat redundant mechanisms in normal heart rate control.

In ventricular myocytes $I_{Ca,L}$ does not contribute substantially to the rapid rising phase of the AP. Although the I_{Na}-dependent rapid depolarization activates $I_{Ca,L}$ quickly, the amplitude of $I_{Ca,L}$ is not maximal near the AP peak. This phenomenon occurs partly because $I_{Ca,L}$ activation is time dependent, but it also results from a trade-off of the coexistence of relatively high channel conductance (g_{Ca}) and low driving force (E_m-E_{Ca}), where $I_{Ca,L}$ is the product of the two factors (8). Indeed, the maximum AP overshoot ($\sim$+40 mV) is relatively close to the I_{Ca} reversal potential (E_{Rev}) that is typically measured under physiological conditions (+50 to +60 mV), so driving force is low at the AP peak. In contrast, the thermodynamic Ca equilibrium potential (E_{Ca}) is closer to +125 mV, and substantial Ca influx via I_{Ca} occurs at potentials between E_{Rev} and E_{Ca} (9), but at E_{Rev} the inward current carried by Ca is exactly counterbalanced by outward K current through the channel. Thus, the electrophysiologically important measured I_{Ca} becomes negligible near the AP peak, despite some Ca influx that may be important in E-C coupling. After the AP peak, there is an early repolarization phase owing to I_{Na} inactivation and transient outward K currents (I_{to}). This serves to

increase I_{Ca} driving force, such that peak I_{Ca} occurs after the peak of g_{Ca}. So one can almost consider that that I_{Ca} activates in two phases: a rapid rise in conductance and then an increase in driving force. This may be valuable in synchronizing SR Ca release during E-C coupling, and this synchrony is critical for optimal contractility, but the Ca release also influences I_{Ca} during the early AP.

$I_{Ca,L}$ turnoff during the AP is mediated by both voltage- and Ca-dependent inactivation, but the latter is by far predominant (1). Indeed, when monovalent cations carry the charge through Ca channels in myocytes, inactivation is extremely slow (the time constant τ is hundreds of milliseconds at 0 mV). It is now clear that Ca-dependent inactivation of $I_{Ca,L}$ is mediated by CaM, which preassociates with the C terminus of the α_{1C} subunit even at diastolic [Ca]$_i$ (10–12). When local [Ca]$_i$ near the inner channel mouth rises owing to I_{Ca} and/or SR Ca release, Ca binds to the preassociated CaM (at high-affinity Ca sites on the carboxy end). This action causes Ca-CaM to bind to the α_{1C} at a critical IQ motif that is near the apoCaM-binding site, thereby accelerating inactivation. DeMaria et al. (13) reported similar results for neuronal Ca channels (α_{1A}), except that these researchers found that Ca-dependent inactivation was caused by Ca binding to the low-affinity amino end of prebound CaM, whereas the carboxy lobe was important for Ca-dependent I_{Ca} facilitation.

Cardiac myocytes also exhibit Ca-dependent I_{Ca} facilitation, which depends on Ca-CaM-dependent protein kinase (CaMKII) (14–17). I_{Ca} facilitation happens over a longer timescale (from one beat to the next) and results in a moderate increase in I_{Ca} amplitude and slowing of inactivation, which accumulates over 5–10 pulses at 1 Hz. The molecular mechanism is not fully resolved, but it seems unlikely that the CaM that activates CaMKII in this process is the same CaM that causes Ca-dependent inactivation. CaMKII can associate directly with the Ca channel (on the carboxy tail of α_{1C}), and phosphorylation sites on both the

α_{1C} itself and the accessory β_2 subunit have been implicated in mediating I_{Ca} facilitation (18, 19).

Ca-dependent I_{Ca} inactivation may function as a feedback system to limit Ca influx under conditions in which Ca influx or SR Ca release is high, and this may be a protection against cellular Ca overload, which can cause both arrhythmias and cell death. I_{Ca} facilitation may function as a minor component of a positive force-frequency relationship but may also serve to offset the negative effect of increased frequency on Ca channel availability (see below).

The amount of Ca entry via I_{Ca} during the cardiac AP varies among cell types and species and with the AP configuration and SR Ca release. In rat vs rabbit ventricular myocytes, the same square voltage clamp pulse admits more Ca in rat, but when species-appropriate APs are used, there is less integrated I_{Ca} in the rat (14 vs 21 μmol/l cytosol) because of the shorter AP duration (20). Although Ca entry via I_{Ca} induces substantial Ca-dependent inactivation, SR Ca release causes a further 50% reduction of integrated I_{Ca} during steady-state APs (21). I_{Ca} may typically become 90–95% inactivated during a normal AP with SR Ca release (22). As such, I_{Ca} deactivation (vs inactivation) only contributes appreciably to I_{Ca} decline during the late stages of repolarization (as E_m approaches −40 mV and below). This also means that I_{Ca} must recover from inactivation between APs.

The time constant τ for recovery from inactivation depends on both E_m and [Ca]$_i$ (typically 100–200 ms at −80 mV and low [Ca]$_i$). The E_m dependence has been more frequently characterized, but [Ca]$_i$ decline is incomplete at the time the AP has repolarized, such that the Ca dependence of recovery may be important (although harder to study). During increases in heart rate, the time at diastolic E_m shortens and may approach the recovery time for $I_{Ca,L}$. This shortening of diastole is partially mitigated by the intrinsic action potential duration (APD) shortening at higher heart rates (so repolarization happens sooner)

and also by frequency-dependent acceleration of relaxation (FDAR) and [Ca]$_i$ decline, which lowers [Ca]$_i$ sooner. Under physiological conditions, these factors probably allow I_{Ca} to recover fully between beats, although this may not be true at extremely high heart rates. However, in heart failure (HF), in which the APD is prolonged and [Ca]$_i$ declines more slowly, the frequency at which I_{Ca} availability becomes limiting may well occur at physiological heart rates (22, 23).

β-Adrenergic agonists [via cAMP and protein kinase A (PKA)] increase $I_{Ca,L}$ amplitude and shift activation to more negative E_m. This may all occur via a local signaling complex in which β-adrenergic receptors, adenylate cyclase, cAMP, and PKA are all right at the channel (24, 25). Again, there is some controversy about whether the key phosphorylation site is on the α_{1C} subunit in the C terminus, possibly S1928 (26), or perhaps elsewhere on the C terminus (27) or on the β subunit (28). Although β-adrenergic agonists can greatly increase $I_{Ca,L}$, the current also inactivates more rapidly (owing to the greater Ca influx and release), such that the integrated $I_{Ca,L}$ during the AP is increased by a smaller factor. In conclusion, I_{Ca} contributes to inward current during the AP but is under dynamic control by local [Ca]$_i$, E_m, and regulatory kinases.

Na/Ca Exchange

Na/Ca exchange (NCX) is the Ca transporter in the heart that is largely responsible for extruding the Ca that enters via I_{Ca}. NCX stoichiometry is generally accepted to be 3 Na:1 Ca (1; but see also References 29 and 30), such that the NCX transporter is electrogenic (the extrusion of 1 Ca is coupled to inward flux of 3 Na and one net charge) and carries ionic current (I_{NCX}). I_{NCX} is reversible, and its direction and amplitude are controlled by [Na] and [Ca] on both sides of the membrane as well as by E_m (and I_{NCX} reverses at $E_{NCX} = 3E_{Na}-2E_{Ca}$, analogous to ion channels). At rest E_m is negative to E_{NCX} (typically −50 mV), such that Ca extrusion is favored thermodynamically, even

though the low $[Ca]_i$ limits the absolute rate of Ca extrusion and diastolic inward I_{NCX}. During the AP upstroke, E_m passes E_{NCX} so that Ca influx and outward I_{NCX} are favored and can occur briefly (31). However, this period is very short-lived because as soon as $I_{Ca,L}$ is activated and SR Ca release ensues, the very high local $[Ca]_i$ near the membrane drives E_{NCX} back above E_m such that I_{NCX} becomes inward and extrudes Ca again. Notably, the higher the Ca transient is and the further repolarization proceeds, the greater the inward current. Thus, I_{NCX} is an inward current throughout most of the AP under normal conditions, driven by $[Ca]_i$ but tempered by the positive E_m during the AP. However, if I_{Ca} does not occur in a certain cellular region, Ca entry and outward I_{NCX} can continue. In HF, in which SR Ca release is low, $[Na]_i$ is elevated, and APD is long, the situation can be reversed, such that outward I_{NCX} and Ca influx occur throughout most of the AP (32).

In addition to the above-described regulation of NCX flux by the thermodynamics of ion gradients and the kinetic constraints related to substrate (Na and Ca) concentrations, there is also allosteric regulation mediated by $[Ca]_i$ and $[Na]_i$ (33). At high $[Na]_i$ the I_{NCX} is inactivated, much like E_m-dependent ion channels. This effect is most prominent at high $[Na]_i$, beyond the physiological range (>20 mM), but it is unknown whether this Na-dependent inactivation occurs to a significant extent under any relevant physiological conditions. In contrast, it is easy to see how this might occur and be beneficial in pathophysiological settings of glycoside toxicity or hypoxia, or ischemia and reperfusion, when $[Na]_i$ can rise to a very high level. That is, inactivation of NCX at very high $[Na]_i$ may prevent or limit a consequent cellular Ca overload (driven by outward I_{NCX}).

Allosteric activation of NCX results from elevated $[Ca]_i$, with an apparent $K_{0.5} \sim 100$ nM $[Ca]_i$ in myocytes (33, 34). Without $[Ca]_i$, mammalian NCX does not work at all, even in the Ca influx direction. It makes sense that a Ca efflux mechanism would be turned off

to prevent $[Ca]_i$ from falling too low and that Ca efflux should be more active when cellular $[Ca]_i$ is high (to avoid Ca overload). However, the actual physiological importance of this Ca-dependent regulation is not well understood. The kinetics of Ca-dependent activation are faster than those for deactivation (35), so in normal physiological conditions NCX may be fully activated at this site or have only a moderate residual range of regulation with alterations of heart rate or inotropic state. NCX is also regulated by intracellular phosphatidylinositol bisphosphate (PIP_2) and ATP (36), but like Na-dependent inactivation, this may only be functionally important in heart under pathophysiological conditions. Protein kinase C (PKC) can activate NCX (37), and PKA also has been reported to stimulate NCX, but that remains highly controversial (38, 39).

I_{NCX} is sometimes inappropriately considered to be a minor current during the cardiac AP. I_{NCX} can significantly influence AP shape, and the late plateau phase of most atrial and rodent ventricular APs can be almost entirely due to inward I_{NCX}. The ability of NCX to carry both inward and outward I_{NCX} during the same AP and the central role of NCX in Ca fluxes, pacemaker action, and arrhythmias make it very important to understand this transporter in detail.

Other Ca-Dependent Currents

There are numerous other Ca-regulated currents that are relevant in cardiac electrophysiology. There is a Ca-activated Cl current ($I_{Cl(Ca)}$), which is activated by high local $[Ca]_i$ during I_{Ca} and SR Ca release and contributes a transient outward current early during the AP, when local submembrane $[Ca]_i$ is high and E_m is very positive to E_{Cl} (40, 41). There have been reports of Ca-activated cation-nonselective current in cardiac myocytes (42), but we have been unable to detect this current in rabbit, rat, or human myocytes. Gap junction channels (connexons), which electrically connect all myocytes in the heart,

are inhibited by prolonged high $[Ca]_i$, and CaM and local Ca signaling may be involved (43, 44). This may serve a valuable protective mechanism whereby potentially dying myocytes in severe Ca overload can electrically isolate themselves from the rest of the heart, avoiding the depolarizing and Ca-overloading influences on neighboring cells. It is less clear how much dynamic Ca-dependent regulation of gap junctions occurs in normal physiological conditions or how such regulation may influence electrical propagation in the heart.

The slowly activating delayed rectifier K current (I_{Ks}) is increased by elevated $[Ca]_i$ (45), and the channel protein KCNQ1 (or Kv7.1) is known to bind CaM, which appears to serve as the Ca sensor. Although the molecular mechanism of this Ca-CaM-dependent modulation of I_{Ks} is becoming better clarified (46, 47), there is still very little information about how physiologically dynamic changes in local $[Ca]_i$ during the AP influence the behavior of I_{Ks} during that or the next AP.

I_{Na} can also be modulated by Ca via CaM directly as well as by CaMKII-dependent phosphorylation of the SNC5A channel–forming subunit. The direct Ca-CaM effect appears to involve an enhancement of slow inactivation (48, 49), although details remain to be worked out and some controversy remains (50). CaMKII also associates with the cardiac Na channel, phosphorylates the channel, and alters I_{Na} gating (51). CaMKII shifts I_{Na} availability to more negative E_m, increases the accumulation of channels in intermediate inactivation, and slows recovery from inactivation. These are loss-of-function effects that would reduce I_{Na}, especially at high heart rates (seen in, e.g., Brugada syndrome). CaMKII also increases late-inactivating or slowly inactivating I_{Na}, which is a gain in function, and may prolong APD and cause long QT syndrome (LQTS), especially at low heart rates. Intriguingly, there is a human genetic mutation in *SCN5A* that causes almost the identical I_{Na} gating changes, and patients with this mutation exhibit combined Brugada syndrome and LQTS (52). Moreover, CaMKII is up-regulated in HF. Thus, CaMKII-dependent I_{Na} regulation may create an acquired form of LQTS or Brugada syndrome in HF patients.

Cardiac I_{to} can also be modulated by CaMKII (53), and recent work has shown that CaMKII may slow I_{to} inactivation (54), and enhance recovery from inactivation for both the fast and slow components of I_{to}, mediated by Kv4.2/4.3 and Kv1.4, respectively (55). These effects would enhance I_{to} and shorten APD. Ca-CaM also can control I_{to} channel expression via calcineurin- and CaMKII-dependent pathways, decreasing Kv4.2/4.3 expression and fast I_{to} function but enhancing Kv1.4 expression and slow I_{to} function (55, 56). Thus, Ca modulates numerous ion channels in the heart and can have complex electrophysiological effects.

Arrhythmogenic Mechanisms

Triggered arrhythmias can occur as DADs, EADs, or increased automaticity, and all these events can be attributed in part to Ca signaling or transport. DADs, which take off from the resting E_m after AP repolarization, are widely accepted as being caused by spontaneous SR Ca release events that occur at relatively high SR Ca levels. This SR Ca release causes an aftercontraction and a transient inward current (I_{ti}) that depolarizes E_m toward threshold for an AP. Several Ca-activated currents (I_{NCX}, $I_{Cl(Ca)}$, and a monovalent-nonselective current) are candidates for I_{ti}, but in human and rabbit ventricular myocytes, I_{NCX} appears to account fully for I_{ti} (57, 58). For a substantial DAD (approaching the AP threshold), the spontaneous SR Ca release probably needs to propagate in the cell as a Ca wave (vs simply an enhanced Ca spark frequency). A DAD in a single cell cannot cause a heart arrhythmia because neighboring cells would provide a current sink, dissipating the I_{ti}. However, if DADs occur in a cluster of neighboring cells (e.g., the cells are relatively synchronized by the prior beat), the impulse can escape and propagate through the heart. This is

an important initiator of arrhythmias because human RyR and calsequestrin mutations associated with CPVT (catecholaminergic polymorphic ventricular tachycardia) make SR Ca release more readily activated at diastolic $[Ca]_i$ (59), and animals with these mutations show enhanced DADs and aftercontractions as well as CPVT (60, 61).

Inositol 1,4,5 trisphosphate ($InsP_3$) receptors ($InsP_3Rs$) are low in number in ventricular myocytes (and are mainly in the nuclear envelope; see below). However, in atrial myocytes $InsP_3Rs$ are much more numerous and coexist with RyR on the SR (62, 63). This allows G protein–coupled receptors (e.g., endothelin-1) to induce $InsP_3$-dependent local SR Ca release, which activates neighboring RyRs and precipitates DAD-like arrhythmias (see **Figure 3,** below). In HF, in which $InsP_3Rs$ are upregulated in the ventricle, this atrial arrhythmogenic mechanism may also be relevant to ventricular myocytes.

EADs are depolarizations that take off during the AP plateau or phase 3 repolarization and are more likely during long APDs, LQTS, and bradycardia. EADs are thought to be mediated by the reactivation of I_{Ca} that has recovered from Ca-dependent inactivation during long APs near plateau E_m once $[Ca]_i$ has significantly declined (64, 65). Even in LQT3, during which mutant Na channels result in late I_{Na} and long APD, I_{Ca} (and not I_{Na}) reactivation may be responsible for EADs (66). Although I_{Ca} recovery remains widely accepted as the mechanism for EADs, some events that look like EADs are initiated by SR Ca release and are thus mechanistically like DADs (67, 68).

Electrical alternans (in which APD alternates between long and short) is thought to be a diagnostic precursor for more severe arrhythmias such as ventricular tachycardia or ventricular fibrillation. Although there are, in principle, many electrophysiological prospects to explain APD alternans, in many, if not most cases, the APD alternans is secondary to and dictated by Ca transient-amplitude alternans (69). Several aspects of Ca handling have been implicated: incomplete I_{Ca} or RyR recovery and alternating SR Ca content. However, our recent work suggests that alternans induced by increasing pacing frequency are due mainly to the slow restitution of RyR availability (70). That is, slow RyR recovery after a large SR Ca release results in a smaller SR Ca release at the next beat such that the RyRs that did not fire are ready for activation on the next beat, which again has larger SR Ca release. When the larger Ca releases and Ca transients occur, there is more Ca extrusion via NCX and less Ca entry via I_{Ca} (and NCX) because of greater Ca-dependent inactivation of I_{Ca}. This leads to an alternation of SR Ca content that accompanies and can enhance the extent of Ca alternans. I_{Ca} availability can also be involved, but probably only at even higher heart rates. So, how do Ca transient alternans cause APD alternans? Many Ca-dependent currents may contribute, but I_{Ca} and I_{NCX} are probably key. At the larger beat, there is more inward I_{NCX}, which would prolong APD, but also less integrated I_{Ca} (and more inactivation), which would shorten APD. Normally in rabbit myocytes concordant alternans are seen (a large Ca transient and long APD coincide), implying that at the big beat, the greater I_{NCX} predominates over the smaller I_{Ca} integral (71). The terms concordant and discordant are also used to describe alternans spatially, for which spatially concordant means that longer APDs happen everywhere at the same beat. During spatially discordant alternans, which is a more immediate precursor of dangerous arrhythmias, APs in different regions are out of synch.

Ca AND EXCITATION-CONTRACTION COUPLING

Each junction between the sarcolemma (T-tubule and surface) and SR, where 10–25 L-type Ca channels and 100–200 RyRs are clustered, constitutes a local Ca signaling complex, or couplon. When a Ca channel opens, local $[Ca]_i$ rises in <1 ms in the

junctional cleft to 10–20 μM, and this activates RyRs to release Ca from the SR. A single Ca channel opening can trigger Ca release, but typically several channels open during an AP, creating a safety margin to ensure effective coupling. Approximately 6–20 RyRs probably open at each couplon (1), on the basis of estimates of the SR Ca release flux and single-channel current. This release, which is synchronized by local Ca-induced Ca release among RyRs [and maybe by physical coupling between RyRs (72)] raises cleft [Ca]$_i$ to 200–400 μM. Ca diffuses from the cleft to the cytosol to activate the myofilaments. However, under normal conditions Ca released from one couplon does not activate the neighboring junction (which is ~1 μm away radially or 2 μm away longitudinally). This is because [Ca]$_i$ declines over that distance (i.e., the released Ca is diluted in the much larger volume). This independent function of couplons (each is under local control) means that for synchronous contractile activation, all 20,000 couplons in the cell must be simultaneously activated, which is normally accomplished by the AP and I_{Ca} activation.

When the SR Ca content is elevated, the RyR is more sensitive to [Ca]$_i$. During systole this increased sensitivity can result in a greater fractional release for a given I_{Ca} trigger (73). During diastole it can result in increased SR Ca leak (74), frequency of Ca sparks [spontaneous Ca releases from individual couplons (75, 76)], and Ca waves, in which Ca release from one couplon activates neighboring couplons and propagates the length of the cell (and can lead to DADs). The increase in RyR sensitivity with higher SR Ca content has been attributed to the binding of Ca to calsequestrin, which binds to triadin and RyR inside the SR in a Ca-sensitive manner (77). In contrast, in calsequestrin knock-out mice fractional release is still enhanced by increased SR Ca content (78). Thus, RyR regulation by intra-SR Ca is important, but the details remain elusive.

Within a couplon, Ca release is positive feedback, but the fractional SR Ca release is

only approximately 50–60% (79). So, what turns off SR Ca release before the SR is emptied? The two leading explanations are (*a*) RyR inactivation or adaptation that depends somehow on cleft [Ca]$_i$ (80) and (*b*) RyR regulation by luminal SR [Ca] (79, 81, 82). The issue is not resolved, but our working hypothesis is that luminal SR [Ca] is more critical, because enhanced SR Ca buffering capacity prolongs release (83), I_{Ca} cannot trigger release when the SR Ca load is approximately half-normal (73, 79), and direct measures of local intra-SR free [Ca] show a nadir at SR [Ca] ~ 0.4 mM, almost regardless of initial value (82). Thus, as release proceeds and [Ca]$_{SR}$ declines, the RyR gating properties strongly favor closure. This ensures that the SR never is fully depleted of Ca physiologically, although direct activation of RyR by caffeine can lead to full depletion.

Although it is clear now that $I_{Ca,L}$ is the main physiological trigger of E-C coupling (for other proposed mechanisms, see Reference 1), it seems that NCX can modulate the process in three ways. First, submembrane [Na]$_i$ rises as a result of I_{Na}, which may drive Ca influx via NCX, thereby adding to the I_{Ca} trigger. We measured how much I_{Na} influences NCX, and this effect seems to be small (84) but may contribute functionally (85). Second, at the early AP peak, Ca channels are just getting activated, but NCX can bring Ca in right away (potentially pre-elevating cleft [Ca]$_i$). Here, it is important to appreciate that once a Ca channel opens, Ca entry via NCX will normally reverse because of high local [Ca]. Third, if a Ca channel fails to open at a given couplon, Ca entry via NCX may eventually raise cleft [Ca]$_i$ to the point at which SR Ca release is triggered. Two factors that limit triggering via NCX are that (*a*) the unitary Ca flux rate via NCX is ~1000 times smaller than for I_{Ca} (so many NCX molecules would need to be in the right place) and (*b*) ultra-structural data suggest that, although L-type Ca channels and RyR are colocalized at couplons, neither NCX nor Na channels colocalize with RyR, or each other (86). More work

is required to understand fully how I_{Na} and NCX modulate E-C coupling.

Both PKA and CaMKII can phosphorylate and regulate I_{Ca} and RyR. Also, both PKA and CaMKII can regulate SR Ca uptake via phospholamban phosphorylation and relief of the tonic SERCA2a inhibition by phospholamban. These kinases phosphorylate different sites on these proteins but in general activate each process. However, in our hands, PKA robustly activates I_{Ca} and SR Ca uptake but has modest effects on RyR gating (87, 88). As such, the PKA-dependent inotropy is due mainly to increased I_{Ca} and enhanced SR Ca uptake and content (where both enhanced I_{Ca} and SR Ca content enhance fractional release). In contrast, CaMKII seems to activate RyR robustly (enhancing fractional release directly) while more modestly stimulating I_{Ca} (facilitation) and SR Ca uptake [owing to more limited phospholamban phosphorylation (89)]. Notably, there is some controversy about the effects of PKA and CaMKII on RyR, which require further clarification (90–92). Physiologically, sympathetic activation stimulates both PKA and CaMKII, which thus function synergistically. CaMKII is typically considered to be downstream of PKA and elevated Ca transients. However, this may be overly simplistic because (*a*) β-adrenergic agonists appear to activate CaMKII effects on RYR independently of PKA or Ca (93) and (*b*) CaMKII mediates long-term inotropic effects of β-adrenergic agonists after PKA loses efficacy (94).

Both Ca influx and SR Ca release contribute to the Ca transient, and Ca removal from the cytosol is by both the SR Ca-ATPase (SERCA) and sarcolemmal extrusion (mainly via NCX, with a very minor contribution by the plasma membrane Ca-ATPase). In the steady state, the amount of Ca taken up by the SR during relaxation must equal the amount released, and the amount that enters by I_{Ca} and NCX must equal the amount extruded. The fraction of activating Ca that crosses the SR vs sarcolemma varies among species. In mouse and rat ventricles, 90–95% of the activator Ca cycles through the SR, with only 5–8% transported via I_{Ca} and NCX. In contrast, in rabbit, dog, cat, guinea pig, ferret, and human ventricles, this balance is closer to 70% SR and 25–28% sarcolemmal (1). In HF, in which typically SERCA function is decreased and NCX function is enhanced, this balance can be shifted closer to 50–50% (57, 95). This balance has important implications for understanding and modulating Ca transients in HF. It also places constraints on a popular index of E-C coupling, namely gain. Gain is experimentally defined in different ways, but in the strict sense, it is the amount of SR Ca release divided by the amount of trigger Ca influx (total Ca release/integrated I_{Ca}). Thus, the detailed quantitative analysis of Ca fluxes, which is easier to do on the extrusion side (1), indicates that the physiological gain of SR Ca release is 2.5–4 in most mammalian ventricular myocytes but is much higher in rat and mouse (in the range of 12 to 20). Rat and mouse ventricular myocytes also have an unusually short APD, lacking a prominent plateau, as well as a different balance of K currents and substantially higher $[Na]_i$. These species differences in E-C coupling are important to keep in mind, especially because mouse and rat are frequently used animal models for cardiovascular studies.

NCX is crucial for extruding Ca from cardiac myocytes. So it was a surprise that the conditional knockout of NCX in adult mouse myocyte has a remarkably mild phenotype (96). However, these mice show an intriguing molecular adaptation to NCX ablation. There is no functional upregulation of sarcolemmal Ca-ATPase (the other Ca extrusion mechanism in myocytes). Instead, myocytes reduce $I_{Ca,L}$ and shorten the APD (via enhanced I_{to}), which further decreases total Ca influx. Although this experiment has been done only in mouse, I wonder if a more human-like species with longer APD and greater transsarcolemmal Ca fluxes would make the same adaptation, and if it would suffice. Follow-up studies in these NCX knockout mice suggested that I_{Ca} was reduced because of

elevated cleft $[Ca]_i$, raising again the possibility that NCX is important in modulating cleft $[Ca]_i$ (97).

Ca-DEPENDENT CONTRACTILE ACTIVATION

The rise of $[Ca]_i$ activates contraction by the binding of Ca to TnC (which is similar to CaM), a part of the thin-filament regulatory complex (**Figure 2**). Upon Ca binding, TnC binds troponin I (TnI) more strongly, pulling TnI off its actin-binding site, such that the troponin/tropomyosin complex rolls deeper into the groove of the actin filament, allowing myosin heads to interact with actin. When a myosin head attaches, forming a crossbridge, it can push the troponin/tropomyosin complex even deeper into the groove, which facilitates the formation of crossbridges at neighboring actin sites and the binding of Ca at neighboring TnC sites. This contributes to the strong cooperativity observed in myofilament Ca sensitivity curves [Hill coefficients of 6–8 have been reported (98)]. Notably, the Ca-force interaction is reciprocal because crossbridge binding and force generation enhance the affinity of Ca binding to TnC, slowing Ca dissociation and thereby prolonging the active state.

It has been suggested that Ca dissociates rapidly from the myofilaments (paralleling $[Ca]_i$ decline) and that Ca-independent myofilament processes fully dictate relaxation kinetics and even maintain maximal systolic pressure (99) because of long-lived, Ca-free crossbridge cycling. However, this is based on indirect inferences about myofilament Ca binding, and true measures of TnC-bound Ca during normal contractions would be needed to test this hypothesis. I suspect that the increase in Ca affinity (and slowed Ca off-rate) during contraction can largely explain the time lag between $[Ca]_i$ decline and relaxation. Indeed, an acceleration of $[Ca]_i$ decline accelerates relaxation proportionally, whereas PKA-dependent TnI phosphorylation (accelerating TnC-Ca off-rate without accelerating $[Ca]_i$ decline) has a much weaker lusitropic effect (100). Nevertheless, altering the off-rate of Ca binding to TnC does influence relaxation rate, especially under relatively isometric conditions (100–103). Overall, then, there is a dynamic interplay between Ca binding, crossbridge cooperativity, and myofilament deactivation, in which $[Ca]_i$ decline, TnC Ca affinity, and intrinsic crossbridge properties all influence relaxation.

The Frank-Starling law of the heart (greater diastolic filling leads to stronger contraction) was classically explained by sarcomere anatomy. That is, with increasing sarcomere length (SL), the overlap of thick and thin filaments is more optimal, allowing more crossbridges to form and greater force development. Furthermore, cardiac muscle becomes very stiff as SL increases to the optimum overlap, such that physiologically, the declining limb of the length-developed tension curve at which myofilament overlap would progressively decline would never be reached. In skeletal muscle SL is typically limited from entering the descending limb of the curve by the range of motion around joints (e.g., the biceps/elbow).

Although myofilament overlap contributes to the Frank-Starling law, increasing SL also dramatically increases myofilament Ca sensitivity (104, 105), and this Ca sensitization is an even more important factor (106). This raises the question, why does increasing SL increase myofilament Ca sensitivity? An attractive hypothesis, based on constancy of myocyte volume, is the following (107–109). As myocyte and SL increase, the width and filament lattice spacing decrease, bringing thick and thin filaments closer together and increasing the likelihood of crossbridge formation and cooperative Ca binding. Indeed, osmotic compression of the myofilament lattice can mimic the enhanced myofilament Ca sensitivity seen by increasing SL. Although lattice spacing is probably the dominant factor enhancing myofilament Ca sensitivity at longer SL, this may not be the whole story (109, 110), and the
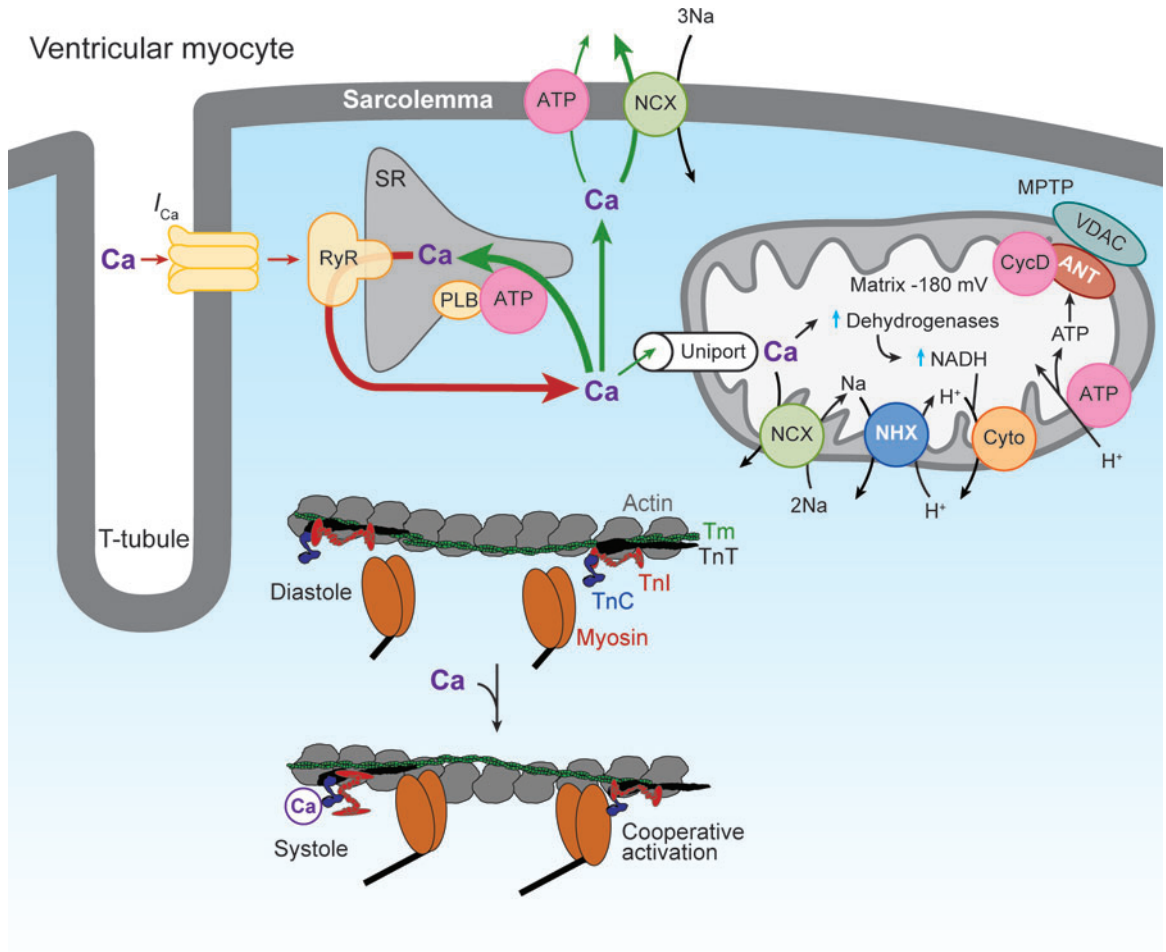
Figure 2

Ca transport, myofilament Ca activation, and mitochondrial Ca handling. Ca influx and Ca-induced Ca release from the sarcoplasmic reticulum (SR) activate the myofilaments. Ca binding to troponin C (TnC) at its N terminus causes TnC to bind to the C terminus of troponin I (TnI), pulling TnI off its site on actin and allowing tropomyosin (Tm) and the third part of the troponin complex (TnT) to roll deeper into the groove between actin monomers. This allows myosin to bind to actin, and this further shifts Tm-TnT deeper into the groove, enhancing Ca binding and crossbridge formation at neighboring sites and resulting in cooperativity (myofilament depiction is modified from a version kindly supplied by Dr. R.J. Solaro, University of Illinois, Chicago). Ca can also enter mitochondria via a Ca uniporter and is extruded by a Na/Ca antiporter (NCX). This mitochondrial NCX is different from sarcolemmal NCX, and its stoichiometry is controversial (2–3Na:1Ca). Na is extruded from mitochondria by electroneutral Na/H exchange (NHX), and protons (H) are extruded by the electron transport chain [including cytochromes (Cyto)]. The mitochondrial F_0F_1-ATPase uses the energy in the inward H gradient to couple H influx to ATP synthesis. Increases in mitochondrial [Ca] activate dehydrogenases that supply reducing equivalents (as NADH) to stimulate ATP synthesis. The mitochondrial permeability transition pore (MPTP) is thought to be composed of the voltage-dependent anion channel (VDAC), adenine nucleotide translocator (ANT), and cyclophilin D (CycD). PLB, phospholamban; RyR, ryanodine receptor.

molecular details of this effect have yet to be delineated.

Frequency-dependent acceleration of relaxation (FDAR) is likely a physiologically important mechanism in allowing ventricular refilling at increasing heart rates, but the molecular mechanism has been elusive. Early work showed that accelerated SR Ca uptake was involved and made a plausible case for frequency- and CaMKII-dependent phospholamban phosphorylation at Thr 17, which may match the time course of FDAR accumulation and washout (111, 112). However, FDAR was still present in phospholamban knockout mice but appeared to be CaMKII dependent, suggesting that CaMKII is involved but phospholamban phosphorylation is not required (113, 114). Whereas our studies have implicated CaMKII in FDAR [at least in part (115)] and shown parallel effects on $[Ca]_i$ decline and relaxation, some groups have failed to see effects of CaMKII inhibitors (116–118) and also raised the possibility of altered myofilament properties (118). It is fair to say that at this point the molecular mechanism remains unresolved. In a physiological context, heart rate increases via sympathetic activation, so intrinsic FDAR would synergize with PKA-dependent acceleration of $[Ca]_i$ decline (via phospholamban phosphorylation at Ser 16) and reduced Ca affinity of TnC to accelerate relaxation.

Ca AND ENERGY BALANCE (MITOCHONDRIA)

The two biggest consumers of myocyte ATP during the cardiac cycle are the Ca-dependent myofilament ATPase and ion transport ATPases (e.g., SR Ca-ATPase and Na/K-ATPase). Because more Na enters the myocyte via NCX (to extrude Ca) vs via Na channels and extrusion of that Na requires Na/K-ATPase activity, a vast majority of ATP consumption is either regulated by Ca or involved in transporting Ca. Mitochondria are of course the main producers of myocyte ATP, and the details of regulation of ATP production are beyond the scope of this review (**Figure 2**). However, micromolar intramitochondrial [Ca] ($[Ca]_{Mito}$) can activate the mitochondrial F_1F_0ATPase (119) and several key mitochondrial dehydrogenases (pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and NAD-dependent isocitrate dehydrogenase) (120). Thus, mitochondrial Ca may provide important feedback between energy supply and demand. Indeed, when cardiac muscle work is increased (with glucose as the substrate), [NADH] abruptly decreases, reflecting ATP consumption. However, if the increased workload was associated with a higher amplitude or frequency of Ca transients, [NADH] recovered gradually, matching the time course of the slow rise in $[Ca]_{Mito}$. In contrast, if the imposed workload was not Ca dependent (e.g., mediated by increased SL), NADH declined but failed to recover (121, 122). This suggests that $[Ca]_{Mito}$-dependent activation of dehydrogenases may be important in balancing energy supply to demand when increases in workload mediated by Ca occur (i.e., most inotropic mechanisms, but not Frank-Starling).

So how is myocyte $[Ca]_{Mito}$ regulated? Ca can enter mitochondria via a Ca uniporter, but the rate is extremely low under physiological conditions, such that during a normal Ca transient only ~1% of the Ca removed from the cytosol is taken up by mitochondria (123). At a steady-state beat this small amount of Ca must also be extruded from mitochondria to the cytosol, presumably late in diastole, because Ca extrusion via the Na/Ca antiporter (which differs from sarcolemmal NCX) is very slow. Indeed, mitochondria take many tens of seconds to extrude a moderate Ca load. Such a Ca load can be generated by increasing either the frequency or amplitude of Ca transients (such that efflux cannot keep up with the increased influx) or by the application of caffeine with NCX blocked (disabling the two dominant competitors of the uniporter in myocytes, SR Ca-ATPase and NCX). Moreover, this extrusion is up a huge electrochemical driving force for Ca entry (–180 mV

inside-vs-cytosolic potential; diastolic $[Ca]_{Mito}$ is similar to $[Ca]_i$) and is thus very expensive energetically.

This framework, in which beat-to-beat changes in $[Ca]_{Mito}$ are small but cumulative changes in $[Ca]_{Mito}$ over several or tens of seconds cause regulatory changes in ATP production, fits most of our data and much historical data from isolated mitochondria. However, Ca uptake via the Ca uniporter can be dramatically increased when $[Ca]_i$ levels are chronically elevated to greater than 1 μM ($K_m \sim 30$ μM), although this activation seems to take time at high $[Ca]_i$. This may limit uniporter-mediated Ca uptake during normal E-C coupling conditions (allowing Ca use in contractile activation) but also permit mitochondria to accumulate large amounts of Ca during cellular Ca overload (to protect the cytosol from Ca overload).

This working model may be overly simplistic, and several critical controversies need to be resolved. First, $[Ca]_{Mito}$ may increase as fast as or faster than $[Ca]_i$ upon SR Ca release (124–127). One explanation for this is that the end of a mitochondrion is typically in close proximity to the site of SR Ca release, where local $[Ca]_i$ is much higher than bulk $[Ca]_i$. Indeed, mitochondria, like couplons, are packed around the myofilaments (whose course cannot be interrupted without mechanical consequence). This explanation may be correct, but none of these $[Ca]_{Mito}$ measurements have been calibrated, so it is difficult to know whether the phasic signals correspond to only the 1% of cytosolic Ca removal mentioned above. However, Maack et al. (127) suggested that mitochondrial Ca uptake may be even higher, possibly sufficient to limit the $[Ca]_i$ transient and contraction. Quantitative measurements will be required to resolve this issue. Second, the $[Ca]_{Mito}$ decline signal in some of these studies was comparable to or only 2.5 times slower than $[Ca]_i$ decline. This is more worrisome because it is inconsistent with known mitochondrial Ca efflux mechanisms (which are much slower than SR Ca-ATPase). This issue will be important

to resolve. Other issues requiring resolution include the still unknown molecular identities of the Ca uniporter and Na/Ca antiporter and a provocative, but controversial, report of a skeletal muscle RyR present in mitochondria that may mediate Ca fluxes (128).

Although mitochondria make most of the myocyte ATP, glycolytic enzymes cluster around both the SR Ca-ATPase and Na/K-ATPase [and the ATP-sensitive K channel (e.g., Reference 129)]. This is notable because glycolysis can keep local [ATP] high and local [ADP] low, both of which help these pumps develop high transmembrane [Ca] and [Na]/[K] gradients (as these gradients depend on ΔG_{ATP} and hence on $[ADP][P_i]/[ATP]$).

Ca AND CELL DEATH

Cell death can be by either necrosis, programmed cell death (apoptosis), or autophagy, but I restrict discussion here to the first two (130, 131). Myocyte and particularly mitochondrial Ca overload can be involved in both necrosis and apoptosis. As indicated above, when myocytes are overloaded with Ca (e.g., in reperfusion after ischemia), mitochondria (which occupy $\sim$35% of cell volume) can temporarily take up large amounts of Ca, thereby limiting hypercontracture, the activation of Ca-dependent proteases (calpains), and arrhythmias. However, mitochondrial Ca uptake occurs at the expense of ATP production. That is, the same mitochondrial membrane potential is used to drive uncoupled Ca influx as is used for proton influx that is coupled to ATP synthesis. In the extreme case, Ca uptake can depolarize mitochondria entirely. Thus, this is only a temporary solution because if most mitochondria in a myocyte have taken up an excess of Ca, the myocyte cannot function energetically.

High $[Ca]_{Mito}$ activates the mitochondrial permeability transition pore (MPTP), which is thought to be composed of the adenine nucleotide translocator (ANT), voltage-dependent anion channel (VDAC), and cyclophilin D (**Figure 2**). However, recent work

with knockouts of ANT, VDAC, and cyclophilin D has failed to abolish MPTP function (132–134), indicating that we do not understand adequately either which proteins comprise the MPTP or how it works functionally. The MPTP allows molecules of up to ~1500 Da to flow freely across the mitochondrial membrane. This immediately dissipates the mitochondrial membrane potential and releases Ca to the cytosol because the electrical gradient had driven $[Ca]_{Mito} > [Ca]_i$. This exacerbation of cytosolic Ca overload results in hypercontracture, ATP depletion, and cell death. For Ca-overload-induced cell death, what may distinguish necrosis from apoptosis is simply whether the cell runs out of ATP (leading to necrosis) or has sufficient ATP to follow the apoptotic pathway. The MPTP opening also uncouples oxidative phosphorylation and causes swelling of the internal mitochondrial matrix, leading to rupture of the outer mitochondrial membrane. This allows mitochondrial cytochrome *c* release, contributing to caspase activation and apoptosis [although cytochrome *c* release does not require MPTP activation (130)].

Ca-dependent proteases (calpains) may also be activated during Ca overload during ischemia-reperfusion, cleaving key proteins such as TnI and others, and this may be involved in long-term contractile dysfunction associated with myocardial stunning (135, 136). In ischemia-reperfusion, calpain also cleaves the proapoptotic BH3-only Bcl-2 family member Bid, which can trigger mitochondrial cytochrome *c* release and apoptosis (137). Thus, Ca-dependent signaling can be critically involved in cell death pathways.

Ca IN TRANSCRIPTIONAL REGULATION AND HYPERTROPHY

Ca-dependent signaling is also involved in transcriptional regulation and hypertrophic signaling in the heart. Transgenic overexpression of CaM causes hypertrophy (138), and CaM inhibition can block myocyte hypertrophy (**Figure 3**) (139). This implicates Ca-CaM-dependent pathways in cardiac hypertrophic signaling. Two major Ca-dependent hypertrophic signaling pathways are Ca-CaM-CaMKII-HDAC (histone deacetylase) and Ca-CaM-calcineurin-NFAT (nuclear factor of activated T cells) (140–143).

Ca-CaM-CaMK-HDAC Pathway

CaMK has been implicated in the regulation of various transcription factors, e.g., activation protein-1 (AP-1), activating transcription factor-1 (ATF-1), serum response factor (SRF), cAMP-response element binding protein (CREB), and myocyte enhancer factor 2 (MEF2) (144). CREB can be phosphorylated by CaMKII and CaMKIV, but CREB phosphorylation levels were unaltered in transgenic mice overexpressing CaMKIIδ_B or CaMKIV that develop hypertrophy and HF (140, 145). This implied that CREB was not critical for CaMKII-dependent cardiac hypertrophy. In contrast, CaMK-dependent MEF2 activation is strongly implicated in hypertrophy (140), but not by phosphorylating MEF2. Rather, the CaMKII- MEF2 link seems to be via class II HDACs (as transcriptional repressors) (142, 143).

Most class II HDACs (HDAC4, -5, -7, and -9) are expressed in the heart and have a unique MEF2-binding domain in the N-terminal extension that is not in other HDACs (143). This N-terminal extension also contains two conserved serines that, when phosphorylated, bind to the chaperone 14-3-3 (masking a nuclear localization signal), and the complex is exported from the nucleus via CRM1 (chromosomal region maintenance protein 1) (thereby relieving MEF2 repression). HDAC activity is opposed by histone acetyltransferases (HATs), which compete with HDACs for the same binding site on MEF2. HATs acetylate core histones and relax chromatin structure, allowing transcriptional activation. Although Ca-CaM can bind to the MEF2-binding domain of HDACs and dissociate the MEF2-HDAC complex,
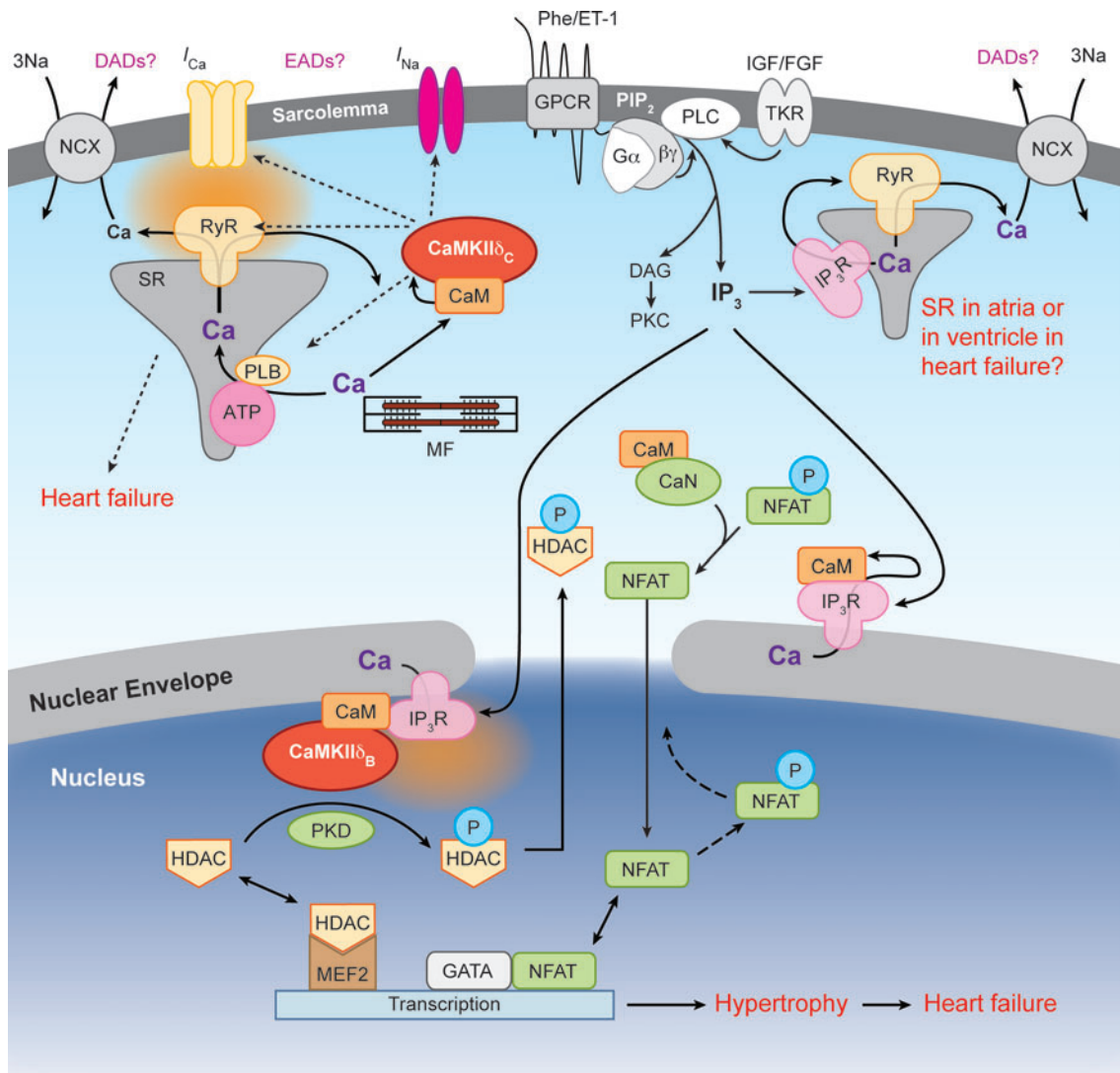
Figure 3

Ca-dependent transcriptional activation in cardiac myocytes. In addition to the Ca regulation in excitation-contraction coupling (*top left*, as in **Figure 1**), and potential role of InsP$_3$R (IP$_3$R) in triggered arrhythmias (*top right*), Ca-dependent signaling to the nucleus can modulate transcription. G protein–coupled receptors (GPCR) such as those for endothelin-1 (ET-1) or α-adrenergic agonists [phenylephrine (Phe)] and tyrosine kinase receptors (TKR) such as those activated by insulin-like growth factor (IGF) and fibroblast growth factor (FGF) can activate phospholipase C (PLC) to produce diacylglycerol (DAG) (which can activate PKC) and InsP$_3$ (IP$_3$). IP$_3$R in the nuclear envelope associate with calcium-calmodulin-dependent kinase IIδ (CaMKIIδ), which can be activated by local IP$_3$R-mediated Ca release, resulting in histone deacetylase (HDAC) phosphorylation (which also occurs by PKD) and nuclear export. This relieves HDAC-dependent suppression of myocyte enhancer factor 2 (MEF2)-driven transcription. Calcineurin (CaN) is activated by Ca-CaM and can dephosphorylate NFAT (nuclear factor of activated T cells). NFAT is then translocated to the nucleus, where, along with the transcription factor GATA, it can stimulate the transcription of genes contributing to hypertrophy. The orange clouds indicate where local [Ca] elevation is critical for signaling near RyR and InsP$_3$R. P indicates phosphorylation, MF denotes myofilaments, and Gα and βγ are subunits of the G protein G$_q$.

phosphorylation of the two conserved HDAC serines is probably the more important physiological mechanism to derepress MEF2, allowing transcriptional activation. CaMK is a class II HDAC kinase (143, 144).

Olson's group has characterized how G protein–coupled receptors (e.g., endothelin-1 and phenylephrine) regulate HDAC4 and HDAC5 phosphorylation in Cos cells and cultured neonatal rat ventricular myocytes (143, 145–147). HDAC4 has a CaMKII docking site near the key phosphorylation sites, making CaMKII a privileged activator of HDAC4 nuclear export and HDAC4 a key CaMKII-dependent regulator of hypertrophy (148). Indeed, CaMKII-dependent HDAC4 phosphorylation causes hypertrophy, whereas overexpression of HDAC4 limits agonist-induced hypertrophy. However, there is no information about what specific Ca signals are involved in CaMKII-dependent HDAC4 phosphorylation. In their cellular expression systems, HDAC5 (which lacks the CaMKII docking site and a third phosphorylation site) does not seem to be activated by CaMKII. Rather, protein kinase D (PKD) mediates HDAC5 phosphorylation and export for both endothelin-1 and phenylephrine. Moreover, for phenylephrine, PKC is responsible for PKD-dependent HDAC5 phosphorylation, whereas for endothelin-1 PKD activation appears to be PKC independent (although how PKD is activated in this case is unclear) (147).

We studied HDAC5 translocation in adult rabbit and mouse ventricular myocytes and found that endothelin-1-induced HDAC5 nuclear export depends equally on CaMKII and PKD (149). Part of the difference may be that myocyte PKD expression dramatically decreases as neonatal myocytes mature to adult myocytes (150), and CaMKII may have greater access to HDAC5 in adult myocytes. We also examined the detailed Ca signaling in endothelin-1-induced HDAC5 phosphorylation in adult ventricular myocytes. Nuclear export required InsP$_3$ (and type 2 InsP$_3$Rs), Ca release from stores, CaM, and HDAC5 phosphorylation but was completely insensitive to the Ca transients associated with E-C coupling (cytosolic or nuclear) or PKC inhibition (149). Adult ventricular myocytes express almost only type 2 InsP$_3$Rs (which form a strong complex with CaMKIIδ), and these receptors are localized mainly on the nuclear envelope (151). Moreover, CaMKII-dependent InsP$_3$R phosphorylation inhibits InsP$_3$R channel activity (a type of negative feedback). We concluded that endothelin-1 induces InsP$_3$ production and diffusion to the nuclear InsP$_3$R, causing locally very high [Ca]$_i$ at the mouth of the channel to activate local CaMKII. CaMKII can then phosphorylate HDAC5. In this way myocytes can distinguish simultaneous local and global Ca signals involved in contractile activation from those targeting gene expression.

Ca-CaM-Calcineurin-NFAT Pathway

Calcineurin (phosphatase 2B) has two subunits, CnA and CnB. CnA includes the CaM-binding and phosphatase domains but requires CnB for activity. Calcineurin has much higher Ca-CaM affinity than does CaMKII ($K_m \sim 0.1$ vs 50 nM). Thus, whereas CaMKII may respond to high-amplitude (or frequent) Ca oscillations and require autophosphorylation for true signal integration, calcineurin may be better at sensing smaller sustained [Ca]$_i$ elevations, and the low off-rate allows intrinsic signal integration (152). Upon activation by Ca-CaM, calcineurin dephosphorylates NFAT, causing NFAT import into the nucleus, where NFAT can work cooperatively with the cardiac-restricted zinc finger transcription factor GATA4 to activate hypertrophic gene transcription (153). Cardiac overexpression of activated calcineurin or NFAT causes massive cardiac hypertrophy and HF. In contrast, calcineurin inhibition (with numerous inhibitors and approaches) can prevent hypertrophy induced by a wide variety of stimuli in vivo and in vitro, and cardiac expression of dominant negative calcineurin or CnAβ knockout impairs

hypertrophic responses to pressure overload, angiotensin II, and isoproterenol infusion (141, 153–155). Whereas knockout of NFATc4 does not block hypertrophy, knockout of NFATc3 inhibits the hypertrophic response to aortic banding, angiotensin II infusion, or active calcineurin overexpression (156). Several kinases that are also implicated in hypertrophic signaling (p38, JNK, and glycogen synthase kinase-3β) can phosphorylate NFAT (153). Thus, the calcineurin-NFATc3 pathway is also a Ca-dependent nodal point in cardiac hypertrophic signaling.

Although calcineurin-NFAT is a Ca-CaM-driven pathway, there is remarkably little information in adult cardiac myocytes regarding the specific types of Ca signals that activate this system. In some lymphocyte studies, sustained global $[Ca]_i$ elevation seems key in activating NFAT signals (152, 157), whereas in others $[Ca]_i$ oscillations are more efficient NFAT activators (158, 159). In hippocampal neurons, L-type I_{Ca} induces NFAT translocation (160). In skeletal muscle, electrical stimulation with patterns typical of slow-twitch (but not fast-twitch) muscle caused calcineurin-dependent NFATc1 translocation to the nucleus (161). It will be valuable to get a better understanding of what specific subcellular Ca signals activate cardiac myocyte calcineurin and drive nuclear translocation of the different NFAT isoforms.

Finally, a novel Ca-related transcriptional regulatory pathway in neurons has been described. This pathway involves a cleaved C-terminal fragment of the L-type Ca channel (162). Maneuvers that lower $[Ca]_i$ cause nuclear translocation of this fragment, whereas I_{Ca} activity and elevated $[Ca]_i$ diminish nuclear localization. In the nucleus this fragment appears to bind to proteins known to regulate transcription, and many genes are differentially regulated when this fragment is expressed. It will be interesting to see if this pathway is exhibited in cardiac my-

ocytes [where L-type Ca channels are similarly cleaved (163)] and how it is regulated by Ca.

These Ca-CaM-dependent excitation-transcription coupling pathways outlined above may alter the transcription of key Ca transport and regulatory proteins such as SERCA, phospholamban, NCX, RyR2, CaM, and CaMKII. This Ca-dependent regulation of Ca handling protein expression may be part of a long-term feedback loop. This may work by altered Ca signaling that triggers the altered expression of genes that may feedback to normalize cardiac myocyte function or contribute to the exacerbation of hypertrophic or HF phenotypes.

CONCLUSIONS

In summary, Ca participates as a critical signaling molecule in many different myocyte systems and on various timescales (milliseconds to hours or days) and in different spatial environments that allow a myocyte to distinguish between Ca signals that activate SR Ca release in E-C coupling, DADs, contraction, and gene transcription. I raise here a number of specific issues for which our understanding of Ca-dependent regulation is incomplete, and these individual questions merit further investigation. With the complex pleiotropic effects of Ca in cardiac myocytes, it also becomes more important to consider the integrative influences at the cellular and tissue levels of these individual molecular regulatory mechanisms. For example, how do the multiple effects of altered Ca handling and signaling impact the AP shape (and functional expression of key proteins) of different cells in the heart, and how do these predispose one to whole-heart arrhythmias in HF? This requires additional experimental work at multiple levels. Our understanding of these issues may also be assisted by improved mechanistic computational models to guide experiments and test our understanding.

LITERATURE CITED

1. Bers DM. 2001. *Excitation-Contraction Coupling and Cardiac Contractile Force*. Dordrecht, Neth.: Kluwer Acad. 427 pp. 2nd ed.

2. Chen X, Wilson RM, Kubo H, Berretta RM, Harris DM, et al. 2007. Adolescent feline heart contains a population of small, proliferative ventricular myocytes with immature physiological properties. *Circ. Res.* 100:536–44

3. Zhang Z, Xu Y, Song H, Rodriguez J, Tuteja D, et al. 2002. Functional roles of $Ca_v1.3$ (α_{1D}) calcium channel in sinoatrial nodes: insight gained using gene-targeted null mutant mice. *Circ. Res.* 90:981–87

4. Bers DM. 2006. The beat goes on: diastolic noise that just won't quit. *Circ. Res.* 99:921–23

5. Lipsius SL, Huser J, Blatter LA. 2001. Intracellular Ca^{2+} release sparks atrial pacemaker activity. *News Physiol. Sci.* 16:101–6

6. Vinogradova TM, Zhou YY, Maltsev V, Lyashkov A, Stern M, et al. 2004. Rhythmic ryanodine receptor Ca^{2+} releases during diastolic depolarization of sinoatrial pacemaker cells do not require membrane depolarization. *Circ. Res.* 94:802–9

7. Yu X, Chen XW, Zhou P, Yao L, Liu T, et al. 2007. Calcium influx through I_f channels in rat ventricular myocytes. *Am. J. Physiol. Cell Physiol.* 292:C1147–55

8. Sah R, Ramirez RJ, Oudit GY, Gidrewicz D, Trivieri MG, et al. 2003. Regulation of cardiac excitation-contraction coupling by action potential repolarization: role of the transient outward potassium current (I_{to}). *J. Physiol.* 546:5–18

9. Zhou Z, Bers DM. 2000. Ca^{2+} influx via L-type Ca^{2+} channel during tail current and above current reversal potential in ferret ventricular myocytes. *J. Physiol.* 523:57–66

10. Pitt GS, Zühlke RD, Hudmon A, Schulman H, Reuter H, et al. 2001. Molecular basis of calmodulin tethering and Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels. *J. Biol. Chem.* 276:30794–802

11. Erickson MG, Alseikhan BA, Peterson BZ, Yue DT. 2001. Preassociation of calmodulin with voltage-gated Ca^{2+} channels revealed by FRET in single living cells. *Neuron* 31:973–85

12. Pate P, Mochca-Morales J, Wu Y, Zhang JZ, Rodney GG, et al. 2000. Determinants for calmodulin binding on voltage-dependent Ca^{2+} channels. *J. Biol. Chem.* 275:39786–92

13. DeMaria CD, Soong TW, Alseikhan BA, Alvania RS, Yue DT. 2001. Calmodulin bifurcates the local Ca^{2+} signal that modulates P/Q-type Ca^{2+} channels. *Nature* 411:484–89

14. Yuan W, Bers DM. 1994. Ca-dependent facilitation of cardiac Ca current is due to Ca-calmodulin dependent protein kinase. *Am. J. Physiol. Heart Circ. Physiol.* 267:H982–93

15. Anderson ME, Braun AP, Schulman H, Premack BA. 1994. Multifunctional Ca/calmodulin-dependent protein kinase mediates Ca-induced enhancement of the L-type Ca current in rabbit ventricular myocytes. *Circ. Res.* 75:854–61

16. Xiao RP, Cheng H, Lederer WJ, Suzuki T, Lakatta EG. 1994. Dual regulation of Ca/calmodulin kinase II activity by membrane voltage and by calcium influx. *Proc. Natl. Acad. Sci. USA* 91:9659–63

17. Dzhura I, Wu Y, Colbran RJ, Balser JR, Anderson ME. 2000. Calmodulin kinase determines calcium-dependent facilitation of L-type calcium channels. *Nat. Cell Biol.* 2:173–77

18. Hudmon A, Schulman H, Kim J, Maltez JM, Tsien RW, et al. 2005. CaMKII tethers to L-type Ca^{2+} channels, establishing a local and dedicated integrator of Ca^{2+} signals for facilitation. *J. Cell Biol.* 171:537–47

19. Grueter CE, Abiria SA, Dzhura I, Wu Y, Ham AJ, et al. 2006. L-type Ca^{2+} channel facilitation mediated by phosphorylation of the β subunit by CaMKII. *Mol. Cell* 23:641–50

20. Yuan W, Ginsburg KS, Bers DM. 1996. Comparison of sarcolemmal Ca channel current in rabbit and rat ventricular myocytes. *J. Physiol.* 493:733–46

21. Puglisi JL, Yuan W, Bassani JWM, Bers DM. 1999. Ca^{2+} influx through Ca^{2+} channels in rabbit ventricular myocytes during action potential clamp: influence of temperature. *Circ. Res.* 85:e7–16

22. Altamirano J, Bers DM. 2007. Effect of intracellular Ca^{2+} and action potential duration on L-type Ca^{2+} channel inactivation and recovery from inactivation in rabbit cardiac myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 293:H563–73

23. Sipido KR, Stankovicova T, Flameng W, Vanhaecke J, Verdonck F. 1998. Frequency dependence of Ca^{2+} release from the sarcoplasmic reticulum in human ventricular myocytes from end-stage heart failure. *Cardiovasc. Res.* 37:478–88

24. Davare MA, Avdonin V, Hall DD, Peden EM, Burette A, et al. 2001. A β_2 adrenergic receptor signaling complex assembled with the Ca^{2+} channel $Ca_v1.2$. *Science* 293:98–101

25. Hall DD, Feekes JA, Arachchige Don AS, Shi M, Hamid J, et al. 2006. Binding of protein phosphatase 2A to the L-type calcium channel $Ca_v1.2$ next to Ser1928, its main PKA site, is critical for Ser1928 dephosphorylation. *Biochemistry* 45:3448–59

26. Hulme JT, Westenbroek RE, Scheuer T, Catterall WA. 2006. Phosphorylation of serine 1928 in the distal C-terminal domain of cardiac $Ca_v1.2$ channels during β1-adrenergic regulation. *Proc. Natl. Acad. Sci. USA* 103:16574–79

27. Ganesan AN, Maack C, Johns DC, Sidor A, O'Rourke B. 2006. β-adrenergic stimulation of L-type Ca^{2+} channels in cardiac myocytes requires the distal carboxyl terminus of α1C but not serine 1928. *Circ. Res.* 98:e11–18

28. Bunemann M, Gerhardstein BL, Gao T, Hosey MM. 1999. Functional regulation of L-type calcium channels via protein kinase A-mediated phosphorylation of the β_2 subunit. *J. Biol. Chem.* 274:33851–54

29. Egger M, Niggli E. 2000. Paradoxical block of the Na^+-Ca^{2+} exchanger by extracellular protons in guinea-pig ventricular myocytes. *J. Physiol.* 523:353–66

30. Kang TM, Hilgemann DW. 2004. Multiple transport modes of the cardiac Na^+/Ca^{2+} exchanger. *Nature* 427:544–48

31. Weber CR, Piacentino V III, Ginsburg KS, Houser SR, Bers DM. 2002. Na/Ca exchange current and submembrane [Ca] during the cardiac action potential. *Circ. Res.* 90:182–89

32. Weber CR, Piacentino V III, Houser SR, Bers DM. 2003. Dynamic regulation of Na/Ca exchange function in human heart failure. *Circulation* 108:2224–29

33. Philipson KD, Nicoll DA. 2000. Sodium-calcium exchange: a molecular perspective. *Annu. Rev. Physiol.* 62:111–33

34. Weber CR, Ginsburg KS, Philipson KD, Shannon TR, Bers DM. 2001. Allosteric regulation of Na/Ca exchange current by cytosolic Ca in intact cardiac myocytes. *J. Gen. Physiol.* 117:119–31

35. Reeves JP, Condrescu M, Urbanczyk J, Chernysh O. 2007. New modes of exchanger regulation: physiological implications. *Ann. NY Acad. Sci.* 1099:64–77

36. Hilgemann DW, Ball R. 1996. Regulation of cardiac Na^+,Ca^{2+} exchange and K_{ATP} potassium channels by PIP_2. *Science* 273:956–59

37. Iwamoto T, Pan Y, Nakamura TY, Wakabayashi S, Shigekawa M. 1998. Protein kinase C-dependent regulation of Na^+/Ca^{2+} exchanger isoforms NCX1 and NCX3 does not require their direct phosphorylation. *Biochemistry* 37:17230–38

38. Ginsburg KS, Bers DM. 2005. Isoproterenol does not enhance Ca-dependent Na/Ca exchange current in intact rabbit ventricular myocytes. *J. Mol. Cell Cardiol.* 39:972–81

39. Lin X, Jo H, Sakakibara Y, Tambara K, Kim B, et al. 2006. β-adrenergic stimulation does not activate Na^+/Ca^{2+} exchange current in guinea pig, mouse, and rat ventricular myocytes. *Am. J. Physiol. Cell Physiol.* 290:C601–8

40. Zygmunt AC. 1994. Intracellular calcium activates a chloride current in canine ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 267:H1984–95

41. Trafford AW, Díaz ME, Eisner DA. 1998. Ca-activated chloride current and Na-Ca exchange have different timecourses during sarcoplasmic reticulum Ca release in ferret ventricular myocytes. *Pflügers Arch.* 435:743–45

42. Colquhoun D, Neher E, Reuter H, Stevens CF. 1981. Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature* 294:752–54

43. Peracchia C. 2004. Chemical gating of gap junction channels; roles of calcium, pH and calmodulin. *Biochim. Biophys. Acta* 1662:61–80

44. Dakin K, Zhao Y, Li WH. 2005. LAMP, a new imaging assay of gap junctional communication unveils that Ca^{2+} influx inhibits cell coupling. *Nat. Methods* 2:55–62

45. Tohse N. 1990. Calcium-sensitive delayed rectifier potassium current in guinea pig ventricular cells. *Am. J. Physiol. Heart Circ. Physiol.* 258:H1200–7

46. Ghosh S, Nunziato DA, Pitt GS. 2006. KCNQ1 assembly and function is blocked by long-QT syndrome mutations that disrupt interaction with calmodulin. *Circ. Res.* 98:1048–54

47. Shamgar L, Ma L, Schmitt N, Haitin Y, Peretz A, et al. 2006. Calmodulin is essential for cardiac IKS channel gating and assembly: impaired function in long-QT mutations. *Circ. Res.* 98:1055–63

48. Tan HL, Kupershmidt S, Zhang R, Stepanovic S, Roden DM, et al. 2002. A calcium sensor in the sodium channel modulates cardiac excitability. *Nature* 415:442–47

49. Kim J, Ghosh S, Liu H, Tateyama M, Kass RS, et al. 2004. Calmodulin mediates Ca^{2+} sensitivity of sodium channels. *J. Biol. Chem.* 279:45004–12

50. Shah VN, Wingo TL, Weiss KL, Williams CK, Balser JR, et al. 2006. Calcium-dependent regulation of the voltage-gated sodium channel hH1: Intrinsic and extrinsic sensors use a common molecular switch. *Proc. Natl. Acad. Sci. USA* 103:3592–97

51. Wagner S, Rasenack ECL, Dybkova1 N, Zhang T, Heller-Brown J, et al. 2006. Ca/calmodulin-dependent protein kinase II (CaMKII) reduces cardiac Na channel availability and inactivation rate. *J. Clin. Invest.* 116:3127–38

52. Veldkamp MW, Viswanathan PC, Bezzina C, Baartscheer A, Wilde AA, et al. 2000. Two distinct congenital arrhythmias evoked by a multidysfunctional Na^+ channel. *Circ. Res.* 86:E91–97

53. Tessier S, Karczewski P, Krause EG, Pansard Y, Acar C, et al. 1999. Regulation of the transient outward K^+ current by Ca^{2+}/calmodulin-dependent protein kinases II in human atrial myocytes. *Circ. Res.* 85:810–19

54. Colinas O, Gallego M, Setien R, Lopez-Lopez JR, Perez-Garcia MT, et al. 2006. Differential modulation of Kv4.2 and Kv4.3 channels by calmodulin-dependent protein kinase II in rat cardiac myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 291:H1978–87

55. Wagner S, Hacker E, Dybkova N, Fabritz L, Kirchhof P, et al. 2006. Ca/calmodulin kinase II differentially modulates transient outward potassium current in heart failure. *Circulation* 114:II-60

56. Perrier E, Perrier R, Richard S, Benitah JP. 2004. Ca^{2+} controls functional expression of the cardiac K^+ transient outward current via the calcineurin pathway. *J. Biol. Chem.* 279:40634–39

57. Pogwizd SM, Schlotthauer K, Li L, Yuan W, Bers DM. 2001. Sodium-calcium exchange contributes to mechanical dysfunction and triggered arrhythmias in heart failure. *Circ. Res.* 88:1159–67

58. Bers DM, Pogwizd SM, Schlotthauer K. 2002. Upregulated Na/Ca exchange is involved in both contractile dysfunction and arrhythmogenesis in heart failure. *Basic Res. Cardiol.* 97(Suppl. 1):36–42

59. George CH, Jundi H, Thomas NL, Fry DL, Lai FA. 2007. Ryanodine receptors and ventricular arrhythmias: emerging trends in mutations, mechanisms and therapies. *J. Mol. Cell Cardiol.* 42:34–50

60. Liu N, Colombi B, Memmi M, Zissimopoulos S, Rizzi N, et al. 2006. Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a RyR2 R4496C knock-in mouse model. *Circ. Res.* 99:292–98

61. Terentyev D, Nori A, Santoro M, Viatchenko-Karpinski S, Kubalova Z, et al. 2006. Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. *Circ. Res.* 98:1151–58

62. Mackenzie L, Bootman MD, Laine M, Berridge MJ, Thuring J, et al. 2002. The role of inositol 1,4,5-trisphosphate receptors in Ca^{2+} signalling and the generation of arrhythmias in rat atrial myocytes. *J. Physiol.* 541:395–409

63. Zima AV, Blatter LA. 2004. Inositol-1,4,5-trisphosphate-dependent Ca^{2+} signalling in cat atrial excitation-contraction coupling and arrhythmias. *J. Physiol.* 555:607–15

64. January CT, Riddle JM. 1989. Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca^{2+} current. *Circ. Res.* 64:977–90

65. Sipido KR, Callewaert G, Carmeliet E. 1995. Inhibition and rapid recovery of Ca^{2+} current during Ca^{2+} release from sarcoplasmic reticulum in guinea pig ventricular myocytes. *Circ. Res.* 76:102–9

66. Clancy CE, Rudy Y. 2002. Na^+ channel mutation that causes both Brugada and long-QT syndrome phenotypes: a simulation study of mechanism. *Circulation* 105:1208–13

67. Volders PG, Kulcsar A, Vos MA, Sipido KR, Wellens HJ, et al. 1997. Similarities between early and delayed afterdepolarizations induced by isoproterenol in canine ventricular myocytes. *Cardiovasc. Res.* 34:348–59

68. Choi BR, Burton F, Salama G. 2002. Cytosolic Ca^{2+} triggers early afterdepolarizations and Torsade de Pointes in rabbit hearts with type 2 long QT syndrome. *J. Physiol.* 543:615–31

69. Pruvot EJ, Katra RP, Rosenbaum DS, Laurita KR. 2004. Role of calcium cycling vs restitution in the mechanism of repolarization alternans. *Circ. Res.* 94:1083–90

70. Picht E, DeSantiago J, Blatter LA, Bers DM. 2006. Cardiac alternans does not rely on diastolic sarcoplasmic reticulum calcium content fluctuations. *Circ. Res.* 99:740–48

71. Weiss JN, Karma A, Shiferaw Y, Chen PS, Garfinkel A, et al. 2006. From pulsus to pulseless: the saga of cardiac alternans. *Circ. Res.* 98:1244–53

72. Kaftan E, Marks AR, Ehrlich BE. 1996. Effects of rapamycin on ryanodine receptor Ca^{2+}-release channels from cardiac muscle. *Circ. Res.* 78:990–97

73. Shannon TR, Ginsburg KS, Bers DM. 2000. Potentiation of fractional SR Ca release by total and free intra-SR Ca concentration. *Biophys. J.* 78:334–43

74. Shannon TR, Ginsburg KS, Bers DM. 2002. Quantitative assessment of SR Ca leak-load relationships. *Circ. Res.* 91:594–600

75. Cheng H, Lederer WJ, Cannell MB. 1993. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* 262:740–44

76. Satoh H, Blatter LA, Bers DM. 1997. Effects of $[Ca^{2+}]_i$, SR Ca^{2+} load, and rest on Ca^{2+} spark frequency in ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 272:H657–68

77. Gyorke I, Hester N, Jones LR, Gyorke S. 2004. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys. J.* 86:2121–28

78. Knollmann BC, Chopra N, Hlaing T, Akin B, Yang T, et al. 2006. Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca^{2+} release, and catecholaminergic polymorphic ventricular tachycardia. *J. Clin. Invest.* 116:2510–20

79. Bassani JWM, Yuan W, Bers DM. 1995. Fractional SR Ca release is altered by trigger Ca and SR Ca content in cardiac myocytes. *Am. J. Physiol. Cell Physiol.* 268:C1313–19

80. Sham JS, Song LS, Chen Y, Deng LH, Stern MD, et al. 1998. Termination of Ca^{2+} release by a local inactivation of ryanodine receptors in cardiac myocytes. *Proc. Natl. Acad. Sci. USA* 95(25):15096–101

81. Terentyev D, Viatchenko-Karpinski S, Valdivia HH, Escobar AL, Gyorke S. 2002. Luminal Ca^{2+} controls termination and refractory behavior of Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. *Circ. Res.* 91:414–20

82. Shannon TR, Guo T, Bers DM. 2003. Ca^{2+} scraps: Local depletions of free $[Ca^{2+}]$ in cardiac sarcoplasmic reticulum during contractions leave substantial Ca^{2+} reserve. *Circ. Res.* 93:40–45

83. Terentyev D, Viatchenko-Karpinski S, Gyorke I, Volpe P, Williams SC, et al. 2003. Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: mechanism for hereditary arrhythmia. *Proc. Natl. Acad. Sci. USA* 100:11759–64

84. Weber CR, Ginsburg KS, Bers DM. 2003. Cardiac submembrane $[Na^+]$ transients sensed by Na^+-Ca^{2+} exchange current. *Circ. Res.* 92:950–52

85. Lines GT, Sande JB, Louch WE, Mørk HK, Grøttum P, et al. 2006. Contribution of the Na^+/Ca^{2+} exchanger to rapid Ca^{2+} release in cardiomyocytes. *Biophys. J.* 91:779–92

86. Scriven DRL, Dan P, Moore EDW. 2000. Distribution of proteins implicated in excitation-contraction coupling in rat ventricular myocytes. *Biophys. J.* 79:2682–91

87. Li Y, Kranias EG, Mignery GA, Bers DM. 2002. Protein kinase A phosphorylation of the ryanodine receptor does not affect Ca sparks in permeabilized mouse ventricular myocytes. *Circ. Res.* 90:309–16

88. Ginsburg KS, Bers DM. 2004. Modulation of excitation-contraction coupling by isoproterenol in cardiomyocytes with controlled SR Ca load and I_{Ca} trigger. *J. Physiol.* 556:463–80

89. Huke S, Bers DM. 2007. Temporal dissociation of frequency-dependent acceleration of relaxation and protein phosphorylation by CaMKII. *J. Mol. Cell Cardiol.* 42:590–99

90. Guo T, Zhang T, Mestril R, Bers DM. 2006. Ca/calmodulin-dependent protein kinase II phosphorylation of ryanodine receptor does affect calcium sparks in mouse ventricular myocytes. *Circ. Res.* 99:398–406

91. Yang D, Zhu WZ, Xiao B, Brochet DX, Chen SR, et al. 2007. Ca^{2+}/calmodulin kinase II-dependent phosphorylation of ryanodine receptors suppresses Ca^{2+} sparks and Ca^{2+} waves in cardiac myocytes. *Circ. Res.* 100:399–407

92. Bers DM, Eisner DA, Valdivia HH. 2003. Sarcoplasmic reticulum Ca and heart failure: roles of diastolic leak and Ca transport. *Circ. Res.* 93:487–90

93. Curran J, Ríos E, Bers DM, Shannon TR. 2007. β-Adrenergic enhancement of sarcoplasmic reticulum Ca leak in cardiac myocytes is mediated by Ca-calmodulin-dependent protein kinase. *Circ. Res.* 100:391–98

94. Wang W, Zhu W, Wang S, Yang D, Crow MT, et al. 2004. Sustained β1-adrenergic stimulation modulates cardiac contractility by Ca^{2+}/calmodulin kinase signaling pathway. *Circ. Res.* 95:798–806

95. Piacentino V III, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, et al. 2003. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ. Res.* 92:651–58

96. Pott C, Philipson KD, Goldhaber JI. 2005. Excitation-contraction coupling in Na^+-Ca^{2+} exchanger knockout mice: reduced transsarcolemmal Ca^{2+} flux. *Circ. Res.* 97:1288–95

97. Pott C, Yip M, Goldhaber JI, Philipson KD. 2007. Regulation of cardiac L-type Ca^{2+} current in Na^+-Ca^{2+} exchanger knockout mice: functional coupling of the Ca^{2+} channel and the Na^+-Ca^{2+} exchanger. *Biophys. J.* 92:1431–37

98. Yue DT, Marbán E, Wier WG. 1986. Relationship between force and intracellular $[Ca^{2+}]$ in tetanized mammalian heart muscle. *J. Gen. Physiol.* 87:223–42

99. Hinken AC, Solaro RJ. 2007. A dominant role of cardiac molecular motors in the intrinsic regulation of ventricular ejection and relaxation. *Physiology* 22:73–80

100. Li L, DeSantiago J, Chu G, Kranias EG, Bers DM. 2000. Phosphorylation of phospholamban and troponin-I in β-adrenergic-induced acceleration of cardiac relaxation. *Am. J. Physiol. Heart Circ. Physiol.* 278:H769–79

101. Pena JR, Wolska BM. 2004. Troponin I phosphorylation plays an important role in the relaxant effect of β-adrenergic stimulation in mouse hearts. *Cardiovasc. Res.* 61:756–63

102. Kentish JC, McCloskey DT, Layland J, Palmer S, Leiden JM, et al. 2001. Phosphorylation of troponin I by protein kinase A accelerates relaxation and crossbridge cycle kinetics in mouse ventricular muscle. *Circ. Res.* 88:1059–65

103. Pi Y, Kemnitz KR, Zhang D, Kranias EG, Walker JW. 2002. Phosphorylation of troponin I controls cardiac twitch dynamics: evidence from phosphorylation site mutants expressed on a troponin I-null background in mice. *Circ. Res.* 90:649–56

104. Hibberd MG, Jewell BR. 1982. Calcium-and length-dependent force production in rat ventricular muscle. *J. Physiol.* 527–40

105. Kentish JC, ter Keurs HEDJ, Ricciardi L, Bucx JJJ, Noble MIM. 1986. Comparison between the sarcomere length-force relations of intact and skinned trabeculae from rat right ventricle. *Circ. Res.* 58:755–68

106. Moss RL, Buck SH. 2002. Regulation of cardiac contraction by Ca^{2+}. In *Handbook of Physiology*, ed. E Page, HA Fozzard, RJ Solaro, pp. 420–54. New York: Oxford Univ. Press

107. Fuchs F, Wang YP. 1996. Sarcomere length vs interfilament spacing as determinants of cardiac myofilament Ca^{2+} sensitivity and Ca^{2+} binding. *J. Mol. Cell Cardiol.* 28:1375–83

108. McDonald KS, Moss RL. 1995. Osmotic compression of single cardiac myocytes eliminates the reduction in Ca^{2+} sensitivity of tension at short sarcomere length. *Circ. Res.* 77:199–205

109. Fuchs F, Martyn DA. 2005. Length-dependent Ca^{2+} activation in cardiac muscle: some remaining questions. *J. Muscle Res. Cell Motil.* 26:199–212

110. Farman GP, Walker JS, de Tombe PP, Irving TC. 2006. Impact of osmotic compression on sarcomere structure and myofilament calcium sensitivity of isolated rat myocardium. *Am. J. Physiol. Heart Circ. Physiol.* 291:H1847–55

111. Schouten VJ. 1990. Interval dependence of force and twitch duration in rat heart explained by Ca^{2+} pump inactivation in sarcoplasmic reticulum. *J. Physiol.* 431:427–44

112. Bassani RA, Mattiazzi A, Bers DM. 1995. CaMKII is responsible for activity-dependent acceleration of relaxation in rat ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 268:H703–12

113. Li L, Chu G, Kranias EG, Bers DM. 1998. Cardiac myocyte calcium transport in phospholamban knockout mouse: relaxation and endogenous CaMKII effects. *Am. J. Physiol. Heart Circ. Physiol.* 274:H1335–47

114. DeSantiago J, Maier LS, Bers DM. 2002. Frequency-dependent acceleration of relaxation (FDAR) in heart depends on CaMKII, but not phospholamban. *J. Mol. Cell Cardiol.* 34:975–84

115. Picht E, DeSantiago J, Huke S, Kaetzel MA, Dedman JR, et al. 2007. CaMKII inhibition targeted to the sarcoplasmic reticulum inhibits frequency dependent acceleration of relaxation and Ca^{2+} current facilitation. *J. Mol. Cell Cardiol.* 42:196–205

116. Hussain M, Drago GA, Colyer J, Orchard CH. 1997. Rate-dependent abbreviation of Ca^{2+} transient in rat heart is independent of phospholamban phosphorylation. *Am. J. Physiol. Heart Circ. Physiol.* 273:H695–706

117. Layland J, Kentish JC. 1999. Positive force- and [Ca^{2+}]$_i$-frequency relationships in rat ventricular trabeculae at physiological frequencies. *Am. J. Physiol. Heart Circ. Physiol.* 276:H9–18

118. Kassiri Z, Myers R, Kaprielian R, Banijamali HS, Backx PH. 2000. Rate-dependent changes of twitch force duration in rat cardiac trabeculae: property of the contractile system. *J. Physiol.* 524:221–31

119. Territo PR, Mootha VK, French SA, Balaban RS. 2000. Ca^{2+} activation of heart mitochondrial oxidative phosphorylation: role of F$_0$/F$_1$ ATPase. *Am. J. Physiol. Cell Physiol.* 278:C423–35

120. Denton RM, McCormack JG. 1990. Ca^{2+} as a second messenger within mitochondria of the heart and other tissues. *Annu. Rev. Physiol.* 52:451–66

121. Brandes R, Bers DM. 1997. Intracellular [Ca^{2+}] increases the mitochondrial NADH concentration during elevated work in intact cardiac muscle. *Circ. Res.* 80:82–87

122. Brandes R, Bers DM. 2002. Simultaneous measurements of mitochondrial NADH and Ca^{2+} during increased work in intact rat heart trabeculae. *Biophys. J.* 83:587–604

123. Bassani JWM, Bassani RA, Bers DM. 1994. Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. *J. Physiol.* 476:279–93

124. Trollinger DR, Cascio WE, Lemasters JJ. 1997. Selective loading of Rhod 2 into mitochondria shows mitochondrial Ca^{2+} transients during the contractile cycle in adult rabbit cardiac myocytes. *Biochem. Biophys. Res. Commun.* 236:738–42

125. Sharma VK, Ramesh V, Franzini-Armstrong C, Sheu SS. 2000. Transport of Ca^{2+} from sarcoplasmic reticulum to mitochondria in rat ventricular myocytes. *J. Bioenerg. Biomembr.* 32:97–104

126. Robert V, Gurlini P, Tosello V, Nagai T, Miyawaki A, et al. 2001. Beat-to-beat oscillations of mitochondrial [Ca^{2+}] in cardiac cells. *EMBO J.* 20:4998–5007

127. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, et al. 2006. Elevated cytosolic Na$^+$ decreases mitochondrial Ca^{2+} uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. *Circ. Res.* 99:172–82

128. Beutner G, Sharma VK, Lin L, Ryu SY, Dirksen RT, Sheu SS. 2005. Type 1 ryanodine receptor in cardiac mitochondria: transducer of excitation-metabolism coupling. *Biochim. Biophys. Acta* 1717:1–10

129. Xu KY, Zweier JL, Becker LC. 1995. Functional coupling between glycolysis and sarcoplasmic reticulum Ca^{2+} transport. *Circ. Res.* 77:88–97

130. Crow MT, Mani K, Nam YJ, Kitsis RN. 2004. The mitochondrial death pathway and cardiac myocyte apoptosis. *Circ. Res.* 95:957–70

131. Murphy E, Steenbergen C. 2007. Preconditioning: the mitochondrial connection. *Annu. Rev. Physiol.* 69:51–67

132. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, et al. 2004. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 427:461–65

133. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, et al. 2005. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 434:658–62

134. Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD. 2007. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat. Cell Biol.* 9:550–55

135. Bolli R, Marban E. 1999. Molecular and cellular mechanisms of myocardial stunning. *Physiol. Rev.* 79:609–34

136. Barta J, Toth A, Edes I, Vaszily M, Papp JG, et al. 2005. Calpain-1-sensitive myofibrillar proteins of the human myocardium. *Mol. Cell Biochem.* 278:1–8

137. Chen M, He H, Zhan S, Krajewski S, Reed JC, et al. 2001. Bid is cleaved by calpain to an active fragment in vitro and during myocardial ischemia/reperfusion. *J. Biol. Chem.* 276:30724–28

138. Gruver CL, DeMayo F, Goldstein MA, Means AR. 1993. Targeted developmental overexpression of calmodulin induces proliferative and hypertrophic growth of cardiomyocytes in transgenic mice. *Endocrinology* 33:376–88

139. McDonough PM, Glembotski CC. 1992. Induction of natriuretic factor and myosin light chain-2 gene expression in cultured ventricular myocytes by electrical stimulation. *J. Biol. Chem.* 267:11665–68

140. Passier R, Zeng H, Frey N, Naya FJ, Nicol RL, et al. 2000. CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo. *J. Clin. Invest.* 105:1395–1406

141. Molkentin JD. 2000. Calcineurin and beyond: cardiac hypertrophic signaling. *Circ. Res.* 87:731–38

142. Zhang T, Brown JH. 2004. Role of Ca^{2+}/calmodulin-dependent protein kinase II in cardiac hypertrophy and heart failure. *Cardiovasc. Res.* 63:476–86

143. Backs J, Olson EN. 2006. Control of cardiac growth by histone acetylation/deacetylation. *Circ. Res.* 98:15–24

144. Linseman DA, Bartley CM, Le SS, Laessig TA, Bouchard RJ, et al. 2003. Inactivation of the myocyte enhancer factor-2 repressor histone deacetylase-5 by endogenous Ca^{2+}/calmodulin-dependent kinase II promotes depolarization-mediated cerebellar granule neuron survival. *J. Biol. Chem.* 278:41472–81

145. McKinsey TA, Zhang CL, Olson EN. 2000. Activation of myocyte enhancer factor-2 transcription factor by calcium/calmodulin-dependent protein kinase-stimulated binding of 14-3-3 to histone deacetylase 5. *Proc. Natl. Acad. Sci. USA* 97:14400–5

146. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, et al. 2002. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* 110:479–88

147. Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, et al. 2004. Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol. Cell Biol.* 24:8374–85

148. Backs J, Song K, Bezprozvannaya S, Chang S, Olson EN. 2006. CaM kinase II selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. *J. Clin. Invest.* 116:1853–64

149. Wu X, Zhang T, Bossuyt J, Li X, McKinsey T, et al. 2006. Local $InsP_3$-dependent perinuclear Ca^{2+} signaling in cardiac myocytes excitation-transcription coupling. *J. Clin. Invest.* 116:675–82

150. Haworth RS, Goss MW, Rozengurt E, Avkiran M. 2000. Expression and activity of protein kinase D/protein kinase Cμ in myocardium: evidence for α1-adrenergic receptor- and protein kinase C-mediated regulation. *J. Mol. Cell Cardiol.* 32:1013–23

151. Bare DJ, Kettlun CS, Liang M, Bers DM, Mignery GA. 2005. Cardiac type-2 inositol trisphosphate receptor: interaction and modulation by CaMKII. *J. Biol. Chem.* 280:15912–20

152. Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI. 1997. Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. *Nature* 386:855–58

153. Molkentin JD. 2004. Calcineurin-NFAT signaling regulates the cardiac hypertrophic response in coordination with the MAPKs. *Cardiovasc. Res.* 63:467–75

154. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, et al. 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93:215–28

155. Zou Y, Hiroi Y, Uozumi H, Takimoto E, Toko H, et al. 2001. Calcineurin plays a critical role in the development of pressure overload-induced cardiac hypertrophy. *Circulation* 104:97–101

156. Wilkins BJ, De Windt LJ, Bueno OF, Braz JC, Glascock BJ, et al. 2002. Targeted disruption of NFATc3, but not NFATc4, reveals an intrinsic defect in calcineurin-mediated cardiac hypertrophic growth. *Mol. Cell Biol.* 22:7603–13

157. Timmerman LA, Clipstone NA, Ho SN, Northrop JP, Crabtree GR. 1996. Rapid shuttling of NF-AT in discrimination of Ca^{2+} signals and immunosuppression. *Nature* 383:837–40

158. Dolmetsch RE, Xu K, Lewis RS. 1998. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* 392:933–36

159. Li W, Llopis J, Whitney M, Zlokarnik G, Tsien RY. 1998. Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression. *Nature* 392:936–41

160. Graef IA, Mermelstein PG, Stankunas K, Neilson JR, Deisseroth K, et al. 1999. L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons. *Nature* 401:703–8

161. Liu Y, Cseresnyes Z, Randall WR, Schneider MF. 2001. Activity-dependent nuclear translocation and intranuclear distribution of NFATc in adult skeletal muscle fibers. *J. Cell Biol.* 155:27–39

162. Gomez-Ospina N, Tsuruta F, Barreto-Chang O, Hu L, Dolmetsch R. 2006. The C terminus of the L-type voltage-gated calcium channel $Ca_V1.2$ encodes a transcription factor. *Cell* 127:591–606

163. Hulme JT, Yarov-Yarovoy V, Lin TW, Scheuer T, Catterall WA. 2006. Autoinhibitory control of the $Ca_V1.2$ channel by its proteolytically processed distal C-terminal domain. *J. Physiol.* 576:87–102

Contents

SPECIAL TOPIC, OBESITY, *Joel Elmquist and Jeffrey Flier, Special Topic Editors*

Indexes

Errata

An online log of corrections to *Annual Review of Physiology* articles may be found at http://physiol.annualreviews.org/errata.shtml