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Dichotomy of Ca^{2+} in the heart: contraction versus intracellular signaling

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Commentary

Ca^{2+} plays a pivotal role in both excitation-contraction coupling (ECC) and activation of Ca^{2+}-dependent signaling pathways. One of the remaining questions in cardiac biology is how Ca^{2+}-dependent signaling pathways are regulated under conditions of continual Ca^{2+} transients that mediate cardiac contraction during each heartbeat. Ca^{2+}-calmodulin–dependent protein kinase II (CaMKII) activation and its ability to regulate histone deacetylase 5 (HDAC5) nuclear shuttling represent a critical Ca^{2+}-dependent signaling circuit for controlling cardiac hypertrophy and heart failure, yet the mechanism of activation by Ca^{2+} is not known. In this issue of the*JCI*, Wu et al. convincingly demonstrate that the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) is involved in local control of Ca^{2+} for activating CaMKII in the nuclear envelope of adult ventricular cardiac myocytes (see the related article beginning on page 675). The overall paradigm that is demonstrated is the best example of a molecular mechanism whereby signaling is directly regulated by a local Ca^{2+} pool that is disparate or geometrically insensitive to cytosolic Ca^{2+} underlying each contractile cycle.

Dichotomy of Ca^{2+} in the heart: contraction versus intracellular signaling

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Ca^{2+} plays a pivotal role in both excitation-contraction coupling (ECC) and activation of Ca^{2+}-dependent signaling pathways. One of the remaining questions in cardiac biology is how Ca^{2+}-dependent signaling pathways are regulated under conditions of continual Ca^{2+} transients that mediate cardiac contraction during each heartbeat. Ca^{2+}-calmodulin–dependent protein kinase II (CaMKII) activation and its ability to regulate histone deacetylase 5 (HDAC5) nuclear shuttling represent a critical Ca^{2+}-dependent signaling circuit for controlling cardiac hypertrophy and heart failure, yet the mechanism of activation by Ca^{2+} is not known. In this issue of the *JCI*, Wu et al. convincingly demonstrate that the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) is involved in local control of Ca^{2+} for activating CaMKII in the nuclear envelope of adult ventricular cardiac myocytes (see the related article beginning on page 675). The overall paradigm that is demonstrated is the best example of a molecular mechanism whereby signaling is directly regulated by a local Ca^{2+} pool that is disparate or geometrically insensitive to cytosolic Ca^{2+} underlying each contractile cycle.

The physiologic relevance of "reactive" Ca^{2+} signaling in the heart is uncertain, given the highly specialized manner in which Ca^{2+} cycling is tightly controlled through the membrane compartments/domains, channels, and pumps that underlie excitation-contraction coupling (ECC). For example, in response to depolarization of the sarcolemma, Ca^{2+} enters through the voltage-dependent L-type Ca^{2+} channel, which directly stimulates adjacent ryanodine receptors embedded within the sarcoplasmic reticulum (SR). This priming Ca^{2+} from the L-type channel induces a much larger release of Ca^{2+} from ryanodine receptors that increase intracellular Ca^{2+} concentration by more than 10-fold to induce contraction. During diastole, Ca^{2+} is removed from the cytoplasm by resequestration back into the SR through the action of SR/ER Ca^{2+}-ATPase (SERCA) as well as extrusion from the cell through the action of the Na^+/Ca^{2+} exchanger within the sarcolemma (Figure 1). Thus, ECC-mediated Ca^{2+} cycling accounts for nearly all routinely detectable Ca^{2+} alterations that occur within a cardiomyocyte, which makes it difficult to explain how Ca^{2+}-activated signaling proteins function in this background. Also to be considered, it would make little sense to regulate Ca^{2+}-dependent signaling proteins through changes in Ca^{2+} cycling associated with ECC since such a mechanism would not afford a disconnection between inotropy and signaling. Indeed, near maximal Ca^{2+} cycling due to *phospholamban* deletion in mice does not induce cardiac hypertrophy or otherwise predispose the heart to dysfunction that would be associated with activation of reactive signaling pathways, indicating that inotropic drive is not a direct source of Ca^{2+} for intracellular signaling pathways (1). Thus, specialized pools of Ca^{2+} that are location specific or somehow buffered from cytoplasmic Ca^{2+} need to be evoked to account for the regulation of Ca^{2+}-sensitive signaling proteins, such as Ca^{2+}-calmodulin–dependent protein kinase (CaMK), calcineurin, and PKC. However, the existence of microdomains or region-specific buffering of Ca^{2+} to control signaling proteins has not been convincingly demonstrated to date in cardiac myocytes. In this issue of the *JCI*, Wu et al. report on their employment of a series of pharmacologic, molecular, and genetic manipulations to convincingly demonstrate that inositol 1,4,5-trisphosphate receptor–based ($InsP_3R$-based) alterations in Ca^{2+} levels regulate CaMKII activation and its ability to control histone deacetylase 5 (HDAC5) translocation, thus suggesting a Ca^{2+}-dependent reactive signaling circuit that is controlled outside of ECC-based Ca^{2+} cycling (2).

Ca^{2+}-sensitive reactive signaling pathways in cardiac myocytes

In response to disease-causing stimuli, the myocardium is directly influenced by neuroendocrine secreted growth factors and/or cytokines that induce ventricular remodeling, hypertrophic enlargement of myocytes, and alterations in the viability of myocytes. Many of these neuroendocrine factors (e.g., angiotensin II and endothelin-1) signal through G protein–coupled receptors on cardiac myocytes to induce phospholipase C (PLC) activation, which in turn generates $InsP_3$ and diacylglycerol (DAG) (3). In most cell types, $InsP_3$ generation in turn leads to release of Ca^{2+} from the endoplasmic reticulum through the $InsP_3R$ channel, although it is uncertain whether $InsP_3R$ activity significantly regulates Ca^{2+} release in adult ventricular cardiac myocytes. It is known that the heart generates $InsP_3$ and that the type 2 $InsP_3R$ ($InsP_3R2$) is present and localized around the nucleus of ventricular myocytes while atrial myocytes also likely contain $InsP_3Rs$ in the junctional SR where they can affect ECC (4, 5).

Neuroendocrine factors and cytokines promote activation of a number of intracellular signaling pathways in cardiac myocytes, including MAPK, calcineurin, PKC, CaMK, and IGF-1 pathway constituents (3). Three of these signaling factors, calcineurin, CaMK, and PKCα, -β, and -γ, require increases in Ca^{2+} to become activated although, as discussed above, the source of such Ca^{2+} in cardiac myocytes has yet to be determined. In most other cell types, $InsP_3R$-regulated Ca^{2+} mobili-

Nonstandard abbreviations used: CaMKII, Ca^{2+}-calmodulin–dependent protein kinase II; ECC, excitation-contraction coupling; HDAC, histone deacetylase; $InsP_3$, inositol 1,4,5-trisphosphate; $InsP_3R$, $InsP_3$ receptor; PLC, phospholipase C; SR, sarcoplasmic reticulum.

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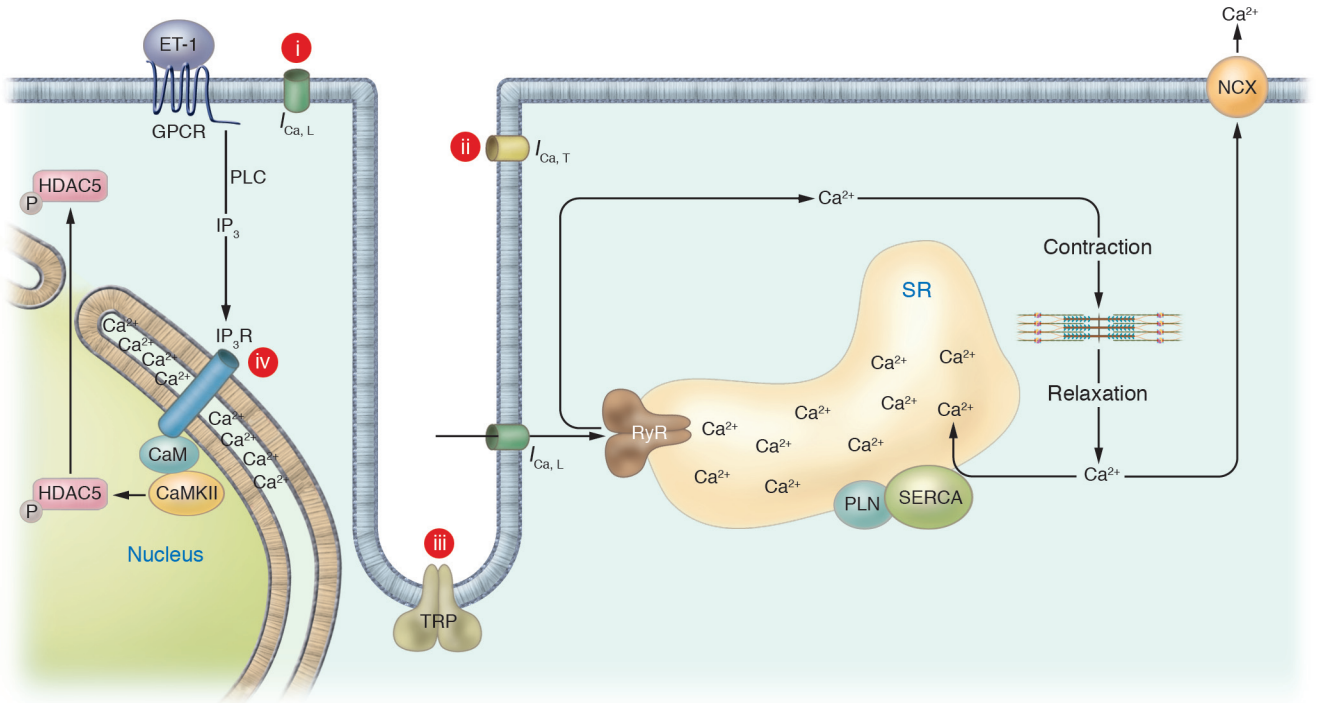

Figure 1
Schematic of potential Ca^{2+} sources that might be specialized to regulate reactive signaling pathways in cardiac myocytes. (i) Some L-type Ca^{2+} channels ($I_{Ca,L}$) are not associated with the junctional complex and hence could be involved in providing a local Ca^{2+} signal in specific membrane-associated compartments. (ii) T-type Ca^{2+} channels ($I_{Ca,T}$) are reexpressed in hypertrophic states where they could provide Ca^{2+} in specific microenvironments associated with the sarcolemma to affect reactive signaling pathways. (iii) Capacitative or store-operated Ca^{2+} entry through transient receptor potential (TRP) channels, alone or in conjunction with (iv) $InsP_3R$-mediated release of Ca^{2+} from the ER/nuclear envelope, could also provide a highly localized Ca^{2+} pool for controlling reactive signaling pathways in cardiac myocytes. Signaling from G protein–coupled receptors (GPCRs) activates PLC and generates $InsP_3$, causing a perinuclear Ca^{2+} signal through the $InsP_3R$, resulting in CamKII activation and HDAC5 nuclear export, as proposed by Wu et al. (2). IP_3, $InsP_3$; IP_3R, $InsP_3R$; PLN, phospholamban; NCX, Na^+/Ca^{2+} exchanger; RyR, ryanodine receptor; CaM, calmodulin; SERCA, SR/ER Ca^{2+}-ATPase; P, phosphate.

zation in association with store-operated Ca^{2+} entry is required for activation of Ca^{2+}-dependent signaling effectors (6). The importance of understanding such regulation in cardiac myocytes is underscored by the observation that both calcineurin and CaMK are potent inducers of the hypertrophic response (7–9). Hence, careful elucidation of the mechanism whereby calcineurin and CaMK become activated in cardiac myocytes should suggest the pool(s) of Ca^{2+} that function in reactive signaling.

Potential Ca^{2+} pools for mediating reactive signaling

The cardiac myocyte contains an organized array of sarcolemmal invaginations referred to as the T tubule network that closely apposes the SR as a means of distributing membrane depolarization and coordinated Ca^{2+} release throughout the cytoplasm. The complexity of this T tubule network as well as regions of the SR/ER that includes the nuclear envelope could easily accommodate more specialized Ca^{2+} regulatory domains that function outside of ECC and serve a signaling function. At least 4 different regulatory mechanisms for compartmentalizing Ca^{2+} outside of ECC have been hypothesized. One possible source of reactive Ca^{2+} is the voltage-regulated L-type Ca^{2+} channel itself, which normally triggers Ca^{2+}-induced Ca^{2+} release and contraction. However, a percentage of L-type Ca^{2+} channels are not associated with the junctional complex and Ca^{2+}-induced Ca^{2+} release, but instead could be dedicated to special membrane domains, such as lipid rafts to mediate a signaling function (Figure 1, i). Indeed, overexpression of the pore-forming subunit of the L-type Ca^{2+} channel in the hearts of transgenic mice led to late-onset cardiac hypertrophy (10). Another potential source of Ca^{2+} for reactive signaling is the voltage-dependent current mediated by T-type Ca^{2+} channels (Figure 1, ii). T-type channels are not normally expressed in mature ventricular myocytes, but expression is present in immature myocytes as well as in adult ventricular myocytes from hypertrophied and failing hearts (11–13). In other cell types, T-type currents play important roles in regulating reactive signaling and cellular proliferation, and more interestingly, these channels are associated with specialized membrane structures such as lipid rafts (14). A third possible source of reactive Ca^{2+} in myocytes is associated with members of the transient receptor potential channel that are thought to mediate store-operated Ca^{2+} entry and are expressed in the heart (15) (Figure 1, iii). Indeed, previous work has suggested that neonatal and/or adult rat cardiac myocytes are capable of store-operated Ca^{2+} entry once depleted with PLC-dependent agonists (16–18). Moreover, Hunton and colleagues showed that general inhibitors of store-operated

Ca^{2+} entry could reduce calcineurin activation in cardiac myocytes (17). Indeed, in nonmyocytes, calcineurin signaling is prominently regulated by store-operated Ca^{2+} entry (19). In general, store-operated Ca^{2+} entry is associated or coupled with PLC-dependent $InsP_3R$ Ca^{2+} release. Thus, the fourth potential source of Ca^{2+} for reactive signaling is $InsP_3R$ dependent (Figure 1, iv). In adult ventricular cardiac myocytes, $InsP_3R2$ is expressed and primarily localized to the nuclear envelope (5). Interestingly, $InsP_3R2$ is physically associated with CaMKII and thus could serve a more specialized role in providing a local pool of Ca^{2+} to activate this kinase in or around the nucleus (Figure 1, iv).

$InsP_3R$ signaling may be the missing link

In this issue of the *JCI*, Wu et al. provide convincing evidence that Ca^{2+} from an $InsP_3R$-dependent store regulates activation of HDAC5 nuclear export through CaMKII in adult ventricular cardiomyocytes (2). HDAC4/5 activity and nuclear occupancy are directly regulated by CaMK-, PKC-, and protein kinase D–mediated (PKD-mediated) phosphorylation (20, 21). That this regulatory relationship is central to the cardiac hypertrophic response is consistent with the observation that *Hdac9*- and *Hdac5*-null mice each develop exaggerated hypertrophy following pressure overload or when crossed with the calcineurin transgene (22, 23). Here, the authors demonstrate that the G protein–coupled agonist (PLC-activating) endothelin-1 induced export of HDAC5 from adult cardiac myocytes without affecting total cytosolic Ca^{2+} concentration and that this export was blocked with an $InsP_3R$ inhibitor or stimulated directly with an $InsP_3R$ agonist (2). More convincingly, HDAC5 nuclear export after endothelin-1 stimulation is completely absent in adult cardiac myocytes from *Ip3r2* gene–targeted mice. These results unequivocally demonstrate that HDAC5 regulation depends in part on $InsP_3R$ signaling. Assessment of Ca^{2+} levels within the cell using Fluo-5N suggested that an $InsP_3$ signal induces Ca^{2+} mobilization in the perinuclear area where the $InsP_3R2$ is localized in the nuclear envelope. Thus, HDAC5 nuclear export, which permits gene activation and induction of the hypertrophic response, can be directly regulated by an $InsP_3$-dependent pathway through a region-specific pool of Ca^{2+} within or near the nuclear envelope. This movement of HDAC5 in association with $InsP_3R$-dependent Ca^{2+} mobilization was partially mediated by CaMK activation since pharmacologic inhibition blocked approximately half of the HDAC5 nuclear export. Thus, the current work by Wu et al. establishes at least 1 paradigm whereby a specialized pool of Ca^{2+} can regulate a hypertrophic signaling circuit within a ventricular cardiac myocyte independent of contractile Ca^{2+}.

As with most landmark studies, Wu et al. raises a number of important issues. First, it is interesting that pacing of adult cardiac myocytes up to 2 Hz did not cause HDAC5 nuclear export, suggesting that ECC-mediated Ca^{2+} fluxing has little effect on this regulatory pathway (2). In contrast, pacing of skeletal muscle myotubes enhanced Ca^{2+} transients enough to induce nuclear export of HDAC4 as well as nuclear factor of activated T cells (NFAT), a sensor of calcineurin signaling (24, 25). These later observations suggest that contractile Ca^{2+} can set in motion 2 separate Ca^{2+}-dependent signaling pathways in skeletal muscle myotubes although this paradigm does not seem to apply to adult cardiac myocytes. Indeed, calcineurin does not even regulate hypertrophy in skeletal muscle as it does in heart muscle, demonstrating profound differences in signaling between these tissues (26). Hence, cardiac myocytes appear to be unique in their ability to compartmentalize or control Ca^{2+} such that the ECC-based pool is distinct from the pool that regulates reactive signaling.

One such Ca^{2+} microenvironment in the adult ventricular cardiac myocytes appears to be dependent on the $InsP_3R$ localized to the perinuclear region. Indeed, CaMKII is directly complexed with the $InsP_3R$ in cardiac myocytes, suggesting a mechanism whereby CaMKII might only respond to exceedingly high Ca^{2+} levels in the microenvironment of the $InsP_3R$ and hence be insensitive to total intracellular Ca^{2+} levels that cannot reach such a putative threshold. For example, such a microenvironment exists at the junction between the sarcolemma and SR, where local Ca^{2+} regulates the independent activation of each ryanodine receptor (27). While Wu et al. only evaluated HDAC5 nuclear export as regulated by CaMKII (2), this paradigm should be evaluated for other signaling circuits that are controlled by Ca^{2+}, such as other class II HDACs, calcineurin, and/or PKC isoforms. Similarly, it would also be interesting to determine if CaMKII and HDAC5 can be regulated by other potential microdomains of Ca^{2+} within the cardiac myocyte so that a hierarchy or specialization of Ca^{2+} microdomains might be established. Thus, the results of Wu et al. have not only established 1 Ca^{2+}-dependent regulatory paradigm, but they have also provided the conceptual framework for further parsing contractile versus reactive signaling Ca^{2+} in the heart.

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A unique role for Stat5 in recovery from acute anemia

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The precise role of erythropoietin receptor–activated (EpoR-activated) Stat5 in the regulation of erythropoiesis remains unclear. In this issue of the *JCI,* Menon and colleagues present new experimental data that indicate a distinct role for Stat5 in the regulation of stress-induced erythropoiesis, such as during acute anemic states (see the related article beginning on page 683). A critical function for Stat5 is to promote cell survival, possibly through transcriptional induction of the antiapoptotic protein Bcl-x. In the present experimental system, erythropoietin-Stat5 signals did not induce Bcl-x expression but did induce oncostatin-M. Moreover, oncostatin-M was found to enhance survival of erythroid progenitors. This work differentiates between steady-state (or homeostatic) erythropoiesis and stress-induced erythropoiesis at the level of EpoR signaling.

In adults, red blood cell production by bone marrow progenitors maintains the steady-state level of circulating cells. But during times of "stress," such as acute anemia, the erythropoietic response is predominantly generated by hematopoietic progenitors residing in the spleen, at least in mice. In both circumstances, erythropoietin (Epo) and SCF are the central regulators, albeit that compensatory circulating Epo levels are higher during recovery from acute anemia. The study of developmental hematopoiesis has revealed that fetal erythropoiesis and adult erythropoiesis occur in distinct anatomic sites and with distinct regulatory features (1, 2), and it has been suggested that adult splenic erythropoiesis more closely approximates fetal liver erythropoiesis than adult bone marrow erythropoiesis. Do these differences between steady-state erythropoietic regulation and the stress-induced erythropoietic response reflect differences in the erythroid progenitors in adult bone marrow versus spleen, differences in Epo/SCF signaling, the activity of other cytokines, or differences in the tissue microenvironment? In this issue of the *JCI*, Menon and coworkers provide evidence implicating the Epo receptor–activated (EpoR-activated) Stat5 signal as a unique contributor to the stress-induced erythropoietic response and thereby distinguishing homeostatic and stress-induced erythropoiesis at the level of EpoR signaling (3).

Nonstandard abbreviations used: BMP, bone morphogenic protein; Epo, erythropoietin; EpoR, Epo receptor; EpoR-H mice, mice expressing a truncated form of the EpoR containing the single Stat5-interacting tyrosine residue Y343; EpoR-HM mice, mice expressing a truncated form of the EpoR that is tyrosine null (Y343F); *f, flexed-tail*; PY, phosphotyrosine.

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Stat5 activation is required for the stress-induced erythropoietic response

Following engagement of the EpoR, the preassociated tyrosine kinase Jak2 is activated, which then mediates phosphorylation of 8 conserved tyrosine motifs to generate phosphotyrosine (PY) recognition sites within the cytoplasmic tail of the EpoR (Figure 1). These PY sites serve to recruit both positive and negative regulators that effect erythroid progenitor expansion through both prosurvival and proliferative signals. The most proximal PY site, Y343, serves to recruit and activate Stat5; however, the role of Stat5 in the regulation of erythropoiesis remains controversial (4–8). Menon et al. (3) used genetically engineered mice, developed in the laboratory of James N. Ihle (St. Jude Children's Research Hospital, Memphis, Tennessee, USA), that express a truncated form of the EpoR containing the single Stat5-interacting tyrosine residue Y343 (EpoR-H mice) or express a similarly truncated EpoR that is tyrosine null (Y343F) (EpoR-HM mice) (8). Since these mutants were knocked in to the *EpoR* gene locus, this allows for physiologic mutant receptor expression in appropriate tissues and cells, in the absence of endogenous WT receptor expression. These mice were subjected to 3 stress conditions: phenylhydrazine-induced acute hemolytic anemia, acute anemia secondary to 5-fluorouracil–induced reduction in number of maturing erythroid progenitors, and anemia follow-