

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

Jeffery D. Molkentin

*J Clin Invest.* 2006;116(3):623-626. <https://doi.org/10.1172/JCI27824>.

## Commentary

$\text{Ca}^{2+}$  plays a pivotal role in both excitation-contraction coupling (ECC) and activation of  $\text{Ca}^{2+}$ -dependent signaling pathways. One of the remaining questions in cardiac biology is how  $\text{Ca}^{2+}$ -dependent signaling pathways are regulated under conditions of continual  $\text{Ca}^{2+}$  transients that mediate cardiac contraction during each heartbeat.  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase II (CaMKII) activation and its ability to regulate histone deacetylase 5 (HDAC5) nuclear shuttling represent a critical  $\text{Ca}^{2+}$ -dependent signaling circuit for controlling cardiac hypertrophy and heart failure, yet the mechanism of activation by  $\text{Ca}^{2+}$  is not known. In this issue of the *JCI*, Wu et al. convincingly demonstrate that the inositol 1,4,5-trisphosphate receptor (InsP<sub>3</sub>R) is involved in local control of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  pool that is disparate or geometrically insensitive to cytosolic  $\text{Ca}^{2+}$  underlying each contractile cycle.

**Find the latest version:**

<http://jci.me/27824-pdf>





# Dichotomy of Ca<sup>2+</sup> in the heart: contraction versus intracellular signaling

Jeffery D. Molkentin

Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA.

**Ca<sup>2+</sup> plays a pivotal role in both excitation-contraction coupling (ECC) and activation of Ca<sup>2+</sup>-dependent signaling pathways. One of the remaining questions in cardiac biology is how Ca<sup>2+</sup>-dependent signaling pathways are regulated under conditions of continual Ca<sup>2+</sup> transients that mediate cardiac contraction during each heartbeat. Ca<sup>2+</sup>-calmodulin-dependent protein kinase II (CaMKII) activation and its ability to regulate histone deacetylase 5 (HDAC5) nuclear shuttling represent a critical Ca<sup>2+</sup>-dependent signaling circuit for controlling cardiac hypertrophy and heart failure, yet the mechanism of activation by Ca<sup>2+</sup> is not known. In this issue of the *JCI*, Wu et al. convincingly demonstrate that the inositol 1,4,5-trisphosphate receptor (InsP<sub>3</sub>R) is involved in local control of Ca<sup>2+</sup> 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<sup>2+</sup> pool that is disparate or geometrically insensitive to cytosolic Ca<sup>2+</sup> underlying each contractile cycle.**

The physiologic relevance of “reactive” Ca<sup>2+</sup> signaling in the heart is uncertain, given the highly specialized manner in which Ca<sup>2+</sup> 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<sup>2+</sup> enters through the voltage-dependent L-type Ca<sup>2+</sup> channel, which directly stimulates adjacent ryanodine receptors embedded within the sarcoplasmic reticulum (SR). This priming Ca<sup>2+</sup> from the L-type channel induces a much larger release of Ca<sup>2+</sup> from ryanodine receptors that increase intracellular Ca<sup>2+</sup> concentration by more than 10-fold to induce contraction. During diastole, Ca<sup>2+</sup> is removed from the cytoplasm by resequestration back into the SR through the action of SR/ER Ca<sup>2+</sup>-ATPase (SERCA) as well as extrusion from the cell through the action of the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger within the sarcolemma (Fig-

ure 1). Thus, ECC-mediated Ca<sup>2+</sup> cycling accounts for nearly all routinely detectable Ca<sup>2+</sup> alterations that occur within a cardiomyocyte, which makes it difficult to explain how Ca<sup>2+</sup>-activated signaling proteins function in this background. Also to be considered, it would make little sense to regulate Ca<sup>2+</sup>-dependent signaling proteins through changes in Ca<sup>2+</sup> cycling associated with ECC since such a mechanism would not afford a disconnection between inotropy and signaling. Indeed, near maximal Ca<sup>2+</sup> 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<sup>2+</sup> for intracellular signaling pathways (1). Thus, specialized pools of Ca<sup>2+</sup> that are location specific or somehow buffered from cytoplasmic Ca<sup>2+</sup> need to be evoked to account for the regulation of Ca<sup>2+</sup>-sensitive signaling proteins, such as Ca<sup>2+</sup>-calmodulin-dependent protein kinase (CaMK), calcineurin, and PKC. However, the existence of microdomains or region-specific buffering of Ca<sup>2+</sup> 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<sub>3</sub>R-based) alterations in Ca<sup>2+</sup> levels regulate CaMKII activation and its ability to control histone deacetylase 5 (HDAC5) translocation, thus suggesting a Ca<sup>2+</sup>-dependent reactive signaling circuit that is controlled outside of ECC-based Ca<sup>2+</sup> cycling (2).

## Ca<sup>2+</sup>-sensitive reactive signaling pathways in cardiac myocytes

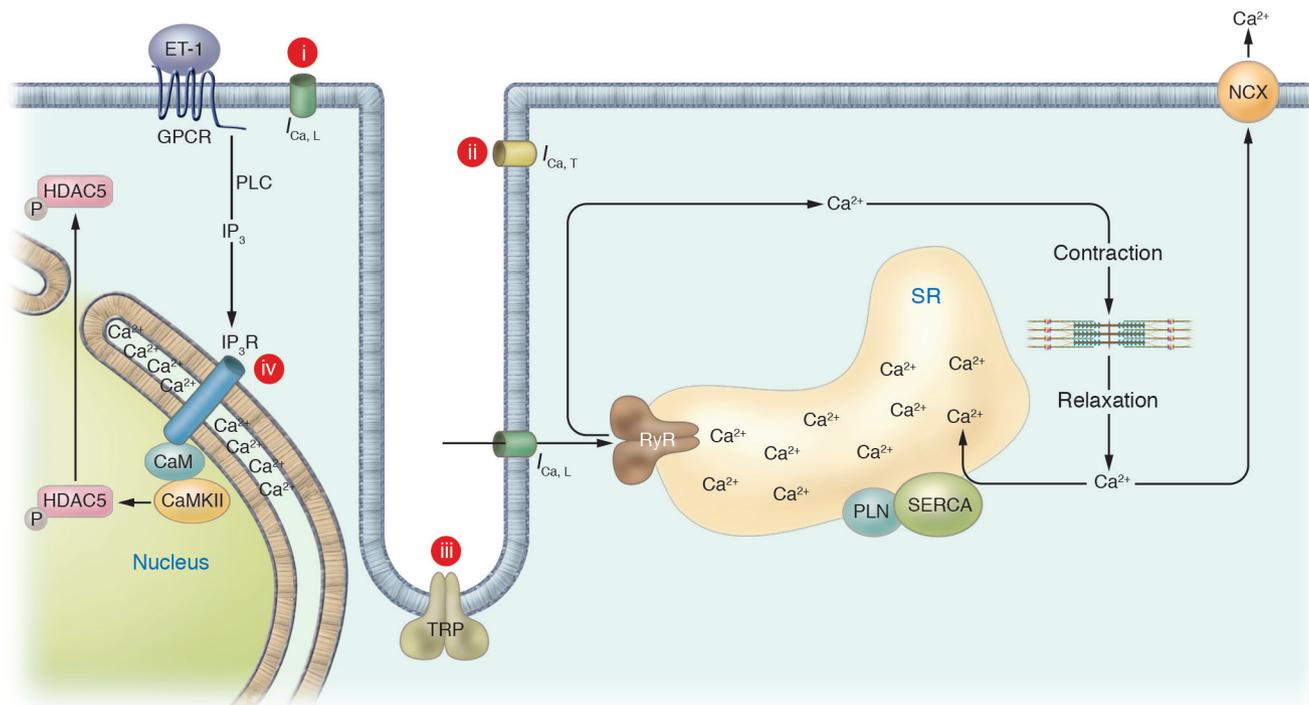
In response to disease-causing stimuli, the myocardium is directly influenced by neuroendocrine secreted growth factors and/or cytokines that induce ventricular remodeling, hypertrophic enlargement of myocytes, and alterations in the viability of myocytes. Many of these neuroendocrine factors (e.g., 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<sub>3</sub> and diacylglycerol (DAG) (3). In most cell types, InsP<sub>3</sub> generation in turn leads to release of Ca<sup>2+</sup> from the endoplasmic reticulum through the InsP<sub>3</sub>R channel, although it is uncertain whether InsP<sub>3</sub>R activity significantly regulates Ca<sup>2+</sup> release in adult ventricular cardiac myocytes. It is known that the heart generates InsP<sub>3</sub> and that the type 2 InsP<sub>3</sub>R (InsP<sub>3</sub>R2) is present and localized around the nucleus of ventricular myocytes while atrial myocytes also likely contain InsP<sub>3</sub>Rs 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 PKC $\alpha$ ,  $\beta$ , and  $\gamma$ , require increases in Ca<sup>2+</sup> to become activated although, as discussed above, the source of such Ca<sup>2+</sup> in cardiac myocytes has yet to be determined. In most other cell types, InsP<sub>3</sub>R-regulated Ca<sup>2+</sup> mobili-

**Nonstandard abbreviations used:** CaMKII, Ca<sup>2+</sup>-calmodulin-dependent protein kinase II; ECC, excitation-contraction coupling; HDAC, histone deacetylase; InsP<sub>3</sub>, inositol 1,4,5-trisphosphate; InsP<sub>3</sub>R, InsP<sub>3</sub> receptor; PLC, phospholipase C; SR, sarcoplasmic reticulum.

**Conflict of interest:** The author has declared that no conflict of interest exists.

**Citation for this article:** *J. Clin. Invest.* 116:623–626 (2006). doi:10.1172/JCI27824.



**Figure 1**

Schematic of potential  $\text{Ca}^{2+}$  sources that might be specialized to regulate reactive signaling pathways in cardiac myocytes. (i) Some L-type  $\text{Ca}^{2+}$  channels ( $I_{\text{Ca,L}}$ ) are not associated with the junctional complex and hence could be involved in providing a local  $\text{Ca}^{2+}$  signal in specific membrane-associated compartments. (ii) T-type  $\text{Ca}^{2+}$  channels ( $I_{\text{Ca,T}}$ ) are reexpressed in hypertrophic states where they could provide  $\text{Ca}^{2+}$  in specific microenvironments associated with the sarcolemma to affect reactive signaling pathways. (iii) Capacitative or store-operated  $\text{Ca}^{2+}$  entry through transient receptor potential (TRP) channels, alone or in conjunction with (iv)  $\text{InsP}_3\text{R}$ -mediated release of  $\text{Ca}^{2+}$  from the ER/nuclear envelope, could also provide a highly localized  $\text{Ca}^{2+}$  pool for controlling reactive signaling pathways in cardiac myocytes. Signaling from G protein-coupled receptors (GPCRs) activates PLC and generates  $\text{InsP}_3$ , causing a perinuclear  $\text{Ca}^{2+}$  signal through the  $\text{InsP}_3\text{R}$ , resulting in  $\text{CaMKII}$  activation and HDAC5 nuclear export, as proposed by Wu et al. (2).  $\text{IP}_3$ ,  $\text{InsP}_3$ ;  $\text{IP}_3\text{R}$ ,  $\text{InsP}_3\text{R}$ ; PLN, phospholamban; NCX,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger; RyR, ryanodine receptor; CaM, calmodulin; SERCA, SR/ER  $\text{Ca}^{2+}$ -ATPase; P, phosphate.

zation in association with store-operated  $\text{Ca}^{2+}$  entry is required for activation of  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  that function in reactive signaling.

**Potential  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  regulatory domains that function outside of ECC and serve a signaling function. At least 4 different regulatory mechanisms for compartmentalizing  $\text{Ca}^{2+}$  outside of ECC have been hypothesized. One possible source of reactive  $\text{Ca}^{2+}$  is the voltage-regulated L-type  $\text{Ca}^{2+}$  channel itself, which normally triggers  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release and contraction. However, a percentage of L-type  $\text{Ca}^{2+}$  channels are not associated with the junctional complex and  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channel in the hearts of transgenic mice led to late-onset cardiac hypertrophy (10). Another potential source of  $\text{Ca}^{2+}$  for reactive signaling is the voltage-dependent current mediated by

T-type  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  in myocytes is associated with members of the transient receptor potential channel that are thought to mediate store-operated  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  entry once depleted with PLC-dependent agonists (16–18). Moreover, Hunton and colleagues showed that general inhibitors of store-operated



Ca<sup>2+</sup> entry could reduce calcineurin activation in cardiac myocytes (17). Indeed, in nonmyocytes, calcineurin signaling is prominently regulated by store-operated Ca<sup>2+</sup> entry (19). In general, store-operated Ca<sup>2+</sup> entry is associated or coupled with PLC-dependent InsP<sub>3</sub>R Ca<sup>2+</sup> release. Thus, the fourth potential source of Ca<sup>2+</sup> for reactive signaling is InsP<sub>3</sub>R dependent (Figure 1, iv). In adult ventricular cardiac myocytes, InsP<sub>3</sub>R2 is expressed and primarily localized to the nuclear envelope (5). Interestingly, InsP<sub>3</sub>R2 is physically associated with CaMKII and thus could serve a more specialized role in providing a local pool of Ca<sup>2+</sup> to activate this kinase in or around the nucleus (Figure 1, iv).

### InsP<sub>3</sub>R signaling may be the missing link

In this issue of the *JCI*, Wu et al. provide convincing evidence that Ca<sup>2+</sup> from an InsP<sub>3</sub>R-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<sup>2+</sup> concentration and that this export was blocked with an InsP<sub>3</sub>R inhibitor or stimulated directly with an InsP<sub>3</sub>R 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<sub>3</sub>R signaling. Assessment of Ca<sup>2+</sup> levels within the cell using Fluo-5N suggested that an InsP<sub>3</sub> signal induces Ca<sup>2+</sup> mobilization in the perinuclear area where the InsP<sub>3</sub>R2 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<sub>3</sub>-dependent pathway through a region-specific pool of

Ca<sup>2+</sup> within or near the nuclear envelope. This movement of HDAC5 in association with InsP<sub>3</sub>R-dependent Ca<sup>2+</sup> 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<sup>2+</sup> can regulate a hypertrophic signaling circuit within a ventricular cardiac myocyte independent of contractile Ca<sup>2+</sup>.

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<sup>2+</sup> fluxing has little effect on this regulatory pathway (2). In contrast, pacing of skeletal muscle myotubes enhanced Ca<sup>2+</sup> 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<sup>2+</sup> can set in motion 2 separate Ca<sup>2+</sup>-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<sup>2+</sup> such that the ECC-based pool is distinct from the pool that regulates reactive signaling.

One such Ca<sup>2+</sup> microenvironment in the adult ventricular cardiac myocytes appears to be dependent on the InsP<sub>3</sub>R localized to the perinuclear region. Indeed, CaMKII is directly complexed with the InsP<sub>3</sub>R in cardiac myocytes, suggesting a mechanism whereby CaMKII might only respond to exceedingly high Ca<sup>2+</sup> levels in the microenvironment of the InsP<sub>3</sub>R and hence be insensitive to total intracellular Ca<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup>, 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<sup>2+</sup> within the cardiac myocyte so that a hierarchy or specialization of Ca<sup>2+</sup> microdomains might be established. Thus, the results of Wu et al. have not only established 1 Ca<sup>2+</sup>-dependent regulatory paradigm, but they have also provided the conceptual framework for further parsing contractile versus reactive signaling Ca<sup>2+</sup> in the heart.

Address correspondence to: Jeffery D. Molkentin, Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, Ohio 45229, USA. Phone: (513) 636-3557; Fax: (513) 636-5958. E-mail: jeff.molkentin@cchmc.org.

1. Kiriazis, H., et al. 2002. Hypertrophy and functional alterations in hyperdynamic phospholamban-knockout mouse hearts under chronic aortic stenosis. *Cardiovasc. Res.* **53**:372-381.
2. Wu, X., et al. 2006. Local InsP<sub>3</sub>-dependent perinuclear Ca<sup>2+</sup> signaling in cardiac myocyte excitation-transcription coupling. *J. Clin. Invest.* **116**:675-682. doi:10.1172/JCI27374.
3. Molkentin, J.D., and Dorn, G.W., 2nd. 2001. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu. Rev. Physiol.* **63**:391-426.
4. Woodcock, E.A., and Matkovich, S.J. 2005. Ins(1,4,5)P<sub>3</sub> receptors and inositol phosphates in the heart—evolutionary artefacts or active signal transducers? *Pharmacol. Ther.* **107**:240-251.
5. Bare, D.J., Kettlun, C.S., Liang, M., Bers, D.M., and Mignery, G.A. 2005. Cardiac type 2 inositol 1,4,5-trisphosphate receptor: interaction and modulation by calcium/calmodulin-dependent protein kinase II. *J. Biol. Chem.* **280**:15912-15920.
6. Schulz, I., and Krause, E. 2004. Inositol 1,4,5-trisphosphate and its co-players in the concert of Ca<sup>2+</sup> signalling—new faces in the line up. *Curr. Mol. Med.* **4**:313-322.
7. Molkentin, J.D., et al. 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell.* **93**:215-228.
8. Passier, R., et al. 2000. CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo. *J. Clin. Invest.* **105**:1395-1406.
9. Zhang, T., et al. 2002. The cardiac-specific nuclear delta(B) isoform of Ca<sup>2+</sup>/calmodulin-dependent protein kinase II induces hypertrophy and dilated cardiomyopathy associated with increased protein phosphatase 2A activity. *J. Biol. Chem.* **277**:1261-1267.
10. Muth, J.N., Bodi, I., Lewis, W., Varadi, G., and Schwartz, A. 2001. A Ca(2+)-dependent transgenic model of cardiac hypertrophy: a role for protein kinase Calpha. *Circulation.* **103**:140-147.
11. Nuss, H.B., and Houser, S.R. 1993. T-type Ca<sup>2+</sup> current is expressed in hypertrophied adult feline left ventricular myocytes. *Circ. Res.* **73**:777-782.
12. Sen, L., and Smith, T.W. 1994. T-type Ca<sup>2+</sup> channels are abnormal in genetically determined cardiomyopathic hamster hearts. *Circ. Res.* **75**:149-155.
13. Martinez, M.L., Heredia, M.P., and Delgado, C. 1999. Expression of T-type Ca(2+) channels in ventricular cells from hypertrophied rat hearts. *J. Mol. Cell. Cardiol.* **31**:1617-1625.
14. Yunker, A.M., and McEnery, M.W. 2003. Low-voltage-activated ("T-Type") calcium channels in



- review. *J. Bioenerg. Biomembr.* **35**:533–575.
15. Freichel, M., et al. 1999. Store-operated cation channels in the heart and cells of the cardiovascular system. *Cell. Physiol. Biochem.* **9**:270–283.
  16. Hunton, D.L., Zou, L., Pang, Y., and Marchase, R.B. 2004. Adult rat cardiomyocytes exhibit capacitative calcium entry. *Am. J. Physiol. Heart Circ. Physiol.* **286**:H1124–H1132.
  17. Hunton, D.L., et al. 2002. Capacitative calcium entry contributes to nuclear factor of activated T-cells nuclear translocation and hypertrophy in cardiomyocytes. *J. Biol. Chem.* **277**:14266–14273.
  18. Uehara, A., Yasukochi, M., Imanaga, I., Nishi, M., and Takeshima, H. 2002. Store-operated Ca<sup>2+</sup> entry uncoupled with ryanodine receptor and junctional membrane complex in heart muscle cells. *Cell Calcium.* **31**:89–96.
  19. Lewis, R.S. 2001. Calcium signaling mechanisms in T lymphocytes. *Annu. Rev. Immunol.* **19**:497–521.
  20. Vega, R.B., et al. 2004. Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol. Cell. Biol.* **24**:8374–8385.
  21. McKinsey, T.A., Zhang, C.L., Lu, J., and Olson, E.N. 2000. Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature.* **408**:106–111.
  22. Zhang, C.L., et al. 2002. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell.* **110**:479–488.
  23. Chang, S., et al. 2004. Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Mol. Cell. Biol.* **24**:8467–8476.
  24. Liu, Y., Cseresnyes, Z., Randall, W.R., and Schneider, M.F. 2001. Activity-dependent nuclear translocation and intranuclear distribution of NFATc in adult skeletal muscle fibers. *J. Cell Biol.* **155**:27–39.
  25. Liu, Y., Randall, W.R., and Schneider, M.F. 2005. Activity-dependent and -independent nuclear fluxes of HDAC4 mediated by different kinases in adult skeletal muscle. *J. Cell Biol.* **168**:887–897.
  26. Parsons, S.A., et al. 2004. Genetic loss of calcineurin blocks mechanical overload-induced skeletal muscle fiber type switching but not hypertrophy. *J. Biol. Chem.* **279**:26192–26200.
  27. Bers, D.M. 2002. Cardiac excitation-contraction coupling. *Nature.* **415**:198–205.

## A unique role for Stat5 in recovery from acute anemia

Gregory D. Longmore

Departments of Medicine and Cell Biology, Division of Hematology, Washington University, St. Louis, Missouri, USA.

**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 developmen-

tal 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).

### 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-

**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.

**Conflict of interest:** The author has declared that no conflict of interest exists.

**Citation for this article:** *J. Clin. Invest.* **116**:626–628 (2006). doi:10.1172/JCI27988.