Calcineurin Enhances MAPK Phosphatase-1 Expression and p38 MAPK Inactivation in Cardiac Myocytes*

Received for publication, January 17, 2001
Published, JBC Papers in Press, February 22, 2001, DOI 10.1074/jbc.M100452200

Hae W. Lim‡, Liguo New§, Jiahuai Han§, and Jeffery D. Molkentin¶

From the ‡Department of Pediatrics, University of Cincinnati, Children’s Hospital Medical Center, Cincinnati, Ohio 45229-3039 and the ¶Department of Immunology, The Scripps Research Institute, La Jolla, California 92037

Multiple intracellular signaling pathways have been shown to regulate the hypertrophic growth of cardiac myocytes including mitogen-activated protein kinase (MAPK) and calcineurin-nuclear factor of activated T-cells. However, it is uncertain if individual regulatory pathways operate in isolation or if interconnectivity between unrelated pathways is required for the orchestration of the entire hypertrophic response. To this end, we investigated the interconnectivity between calcineurin-mediated cardiac myocyte hypertrophy and p38 MAPK signaling in vitro and in vivo. We show that calcineurin promotes down-regulation of p38 MAPK activity and enhances expression of the dual specificity phosphatase MAPK phosphatase-1 (MKP-1). Transgenic mice expressing activated calcineurin in the heart were characterized by inactivation of p38 and increased MKP-1 expression during early postnatal development, before the onset of cardiac hypertrophy. In vitro, cultured neonatal cardiomyocytes infected with a calcineurin-expressing adenovirus and stimulated with phenylephrine demonstrated reduced p38 phosphorylation and increased MKP-1 protein levels. Activation of endogenous calcineurin with the calcium ionophore A23187 decreased p38 phosphorylation and increased MKP-1 protein levels. Inhibition of endogenous calcineurin with cyclosporin A decreased MKP-1 protein levels and increased p38 activation in response to agonist stimulation. To further investigate potential cross-talk between calcineurin and p38 through alteration in MKP-1 expression, the MKP-1 promoter was characterized and determined to be calcineurin-responsive. These data suggest that calcineurin enhances MKP-1 expression in cardiac myocytes, which is associated with p38 inactivation.

Cardiac hypertrophy is broadly defined as an abnormal enlargement of the heart characterized by an increase in cardiac myocyte cell volume and the re-expression of genes encoding fetal protein isoforms. Current pharmacologic treatment strategies for cardiac hypertrophy utilize general inhibitors of certain neuroendocrine stimulatory pathways (such as those mediated by angiotensin II, endothelin-1, and catecholamines), which are thought to mediate hypertrophic growth of cardiac myocytes, in part, by activating specific intracellular signal transduction cascades (1).

In recent years, a number of intracellular signaling pathways have been implicated as important downstream transducers of neuroendocrine stimuli and generalized stress stimuli in cardiac myocytes. Specifically, activation of certain G-protein-coupled receptors and receptor tyrosine kinases has been shown to promote Ras activity, the three major branches of the MAPK signaling cascade (ERKs, JNKs, and p38), protein kinase C, and calcineurin (reviewed in Refs. 2 and 3). Activation of these various intracellular signaling pathways/factors has, in many instances, been directly associated with the induction of the cardiac hypertrophic response in either the hearts of transgenic mice or in cultured neonatal rat cardiac myocytes (2, 3).

Data implicating p38 MAPK as a regulator of the hypertrophic response have largely been obtained in cultured neonatal rat cardiomyocytes. The G-protein-coupled receptor agonists PE and endothelin-1 are potent activators of p38 MAPK in cardiomyocytes (4). Overexpression of activated MKK3 or MKK6 (upstream activators of p38) in neonatal cardiomyocytes was shown to induce hypertrophy and atrial natriuretic factor expression in vitro (5–7). Pharmacologic inhibition of p38 kinase activity with the antagonists SB203580 or SB202190 was shown to attenuate agonist-stimulated cardiomyocyte hypertrophy in culture (4, 5, 7). In addition, pharmacologic or dominant negative inhibition of p38 signaling significantly reduced agonist-induced b-type natriuretic peptide promoter activity in vitro (8, 9). However, three additional studies reported that p38 inhibition was not sufficient to attenuate agonist-induced cardiomyocyte hypertrophy under certain conditions, suggesting a more specialized role for p38 MAPK signaling in vitro (4, 10, 11).

Within the last 2 years, a number of reports have implicated a calcineurin-dependent signaling pathway in the cardiac hypertrophic response. Calcineurin is a calcium/calmodulin-dependent phosphatase that directly regulates the nuclear translocation of a family of transcription factors referred to as NFAT (reviewed in Ref. 2). Cardiac-specific expression of either constitutively active calcineurin or constitutively nuclear NFATc4 mutant protein in transgenic mouse hearts resulted in a dramatic hypertrophic response that quickly transitioned to heart failure (12). Inhibition of calcineurin activity with either cyclo-

* This work was supported by a National Institutes of Health (NIH) grant, by the Pew charitable trust foundation (to J. D. M., a Pew Scholar), and an NIH postdoctoral award (to H. W. L). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Division of Molecular, Cardiovascular Biology, Dept. of Pediatrics, Children’s Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229-3039. E-mail: jeff.molkentin@chmcc.org.

The abbreviations used are: MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T-cells; ERK1/2, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; AdCnA, calcineurin adenovirus; Adjgal, β-galactosidase adenovirus; PE, phenylephrine; CsA, cyclosporin A; MKP, MAPK phosphatase; MKK, MAPK kinase; MEK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase.

This paper is available on line at http://www.jbc.org

15913
Calcineurin Promotes p38 MAPK Inactivation

Calcineurin Transgenic Hearts Show Reduced p38 MAPK Activation—Expression of activated calcineurin in the hearts of transgenic mice induces a striking hypertrophy response that is detectable by 9–14 days after birth and results in a 3-fold increase in heart size by 6 weeks of age (12). It was of interest to examine the status of other intracellular signaling pathways in calcineurin transgenic hearts to characterize the mechanisms that might underlie this dramatic increase in heart size. We focused an analysis on the p38 branch of the MAPK signaling pathway, since it has been shown to play a modulatory role in the cardiac hypertrophic process (3). Hearts from calcineurin transgenic or wild-type littermate mice were analyzed at 4 days of age before any signs of cardiac hypertrophy were observed, guarding against secondary effects associated with hypertrophy itself. Western blot analysis of cardiac protein extracts from 4-day old hearts demonstrated a 2.9-fold down-regulation of p38 MAPK phosphorylation (Fig. 1A, asterisks). ERK1/2 phosphorylation status was not affected at this time point (Fig. 1A).

RESULTS

Calcineurin Transgenic Hearts Show Reduced p38 MAPK Activation—Expression of activated calcineurin in the hearts of transgenic mice induces a striking hypertrophy response that is detectable by 9–14 days after birth and results in a 3-fold increase in heart size by 6 weeks of age (12). It was of interest to examine the status of other intracellular signaling pathways in calcineurin transgenic hearts to characterize the mechanisms that might underlie this dramatic increase in heart size. We focused an analysis on the p38 branch of the MAPK signaling pathway, since it has been shown to play a modulatory role in the cardiac hypertrophic process (3). Hearts from calcineurin transgenic or wild-type littermate mice were analyzed at 4 days of age before any signs of cardiac hypertrophy were observed, guarding against secondary effects associated with hypertrophy itself. Western blot analysis of cardiac protein extracts from 4-day old hearts demonstrated a 2.9-fold down-regulation of p38 MAPK phosphorylation from calcineurin transgenic mice without a change in total p38 MAPK protein (Fig. 1A, asterisks). ERK1/2 phosphorylation status was not affected at this time point (Fig. 1A). A similar down-regulation of p38 MAPK was also seen at 8 and 14 days of age, but such down-
Calcineurin Promotes p38 MAPK Inactivation—MAPK activation occurs through the dual phosphorylation of a threonine and adjacent tyrosine residue (TXY). A large family of at least nine individual dual specificity protein phosphatases (MKPs) has been shown to specifically recognize this unique phosphorylation motif in MAPK factors, causing their dephosphorylation (reviewed in Ref. 20). Each of these dual specificity MKPs differs in substrate specificity (p38 versus ERKs versus JNKs), tissue distribution, subcellular localization, or inducible expression profile (20). In addition to the dual specificity phosphatases, protein phosphatase 2A, a serine/threonine phosphatase has also been shown to specifically dephosphorylate ERK1/2 (21–23). This observation suggested that the serine/threonine phosphatase calcineurin might directly inactivate p38 MAPK. To test this possibility, bacterial generated p38 MAPK or a kinase-dead mutant of p38 (KM) was phosphorylated with purified MKK6 and subsequently incubated with purified calcineurin protein in an excess of calmodulin and calcium. The data demonstrate that calcineurin is unable to directly dephosphorylate p38 MAPK in vitro, while a control utilizing calf intestinal alkaline phosphatase (CIP) (Fig. 1A) or calf intestinal alkaline phosphatase (CIP) (Fig. 1B) significantly reduced p38 MAPK phosphorylation (0.54×) and increased MKP-1 protein levels (6.29×). The data indicate that calcineurin requires an additional signal to promote efficient MKP-1 expression. MKP-2 levels were also stimulated by PE, but AdCnA infection did not promote a further increase.

CsA Reverses p38 MAPK Inactivation and Decreases MKP-1 Protein Levels—To examine the association between calcineurin and p38 MAPK activation in more detail, CsA was used to pharmacologically inhibit calcineurin in cultured cardiomyocytes. CsA treatment promoted greater p38 MAPK phosphorylation at both 30 min and 1 h of PE stimulation compared with PE stimulation alone (Fig. 3). Specifically, MKP-1 protein levels were decreased by −2.5-fold after 1 h of PE stimulation in the presence of CsA compared with PE stimulation alone (Fig. 3). These data indicate that calcineurin inhibition is associated with greater p38 MAPK phosphorylation. CsA treatment also significantly decreased MKP-1 protein expression at 30 min and 1 h (Fig. 3).

Cultured cardiomyocytes were infected with either AdCnA or Adβgal (24 h) and treated with PE for 1 h to induce p38 MAPK phosphorylation. The data demonstrate that PE augmented p38 MAPK phosphorylation by −1.8- and 1.4-fold in uninfected and Adβgal-infected cultures, respectively (Fig. 2B). In contrast, AdCnA infection was associated with a 3.5-fold reduction in p38 MAPK phosphorylation in cardiac myocytes compared with myocytes stimulated with PE alone (Fig. 2B). Interestingly, MKP-1 protein expression was most potently induced by the combination of AdCnA infection and PE treatment (Fig. 2B). While these data suggest that calcineurin induces MKP-1 protein expression, control experiments consisting of either AdCnA or Adβgal alone (no PE stimulation) did not show induction of MKP-1 expression at 12, 24, 48, or 72 h postinfection (data not shown). These results suggest that calcineurin requires an additional signal to promote efficient MKP-1 expression. MKP-2 levels were also stimulated by PE, but AdCnA infection did not promote a further increase.

Calcineurin Indirectly Promotes p38 MAPK Inactivation—Western blotting was performed to characterize the dual specificity phosphatases MKP-1 and MKP-2 as well as the upstream MAPK kinase factors MEK1/2 and MKK3/6 (Fig. 1, B and C). MEK1/2 or MKK3/6 phosphorylation status was not significantly affected, suggesting that the upstream activation cascade was not influenced by calcineurin. However, a 2.3-fold increase in MKP-1 protein levels, but not MKP-2, was observed in 4-day-old calcineurin transgenic hearts. These data suggest that p38 MAPK inactivation is associated with augmented MKP-1 protein levels in calcineurin transgenic hearts.

Calcineurin Promotes p38 MAPK Inactivation—Western blot analysis of phosphorylated p38 MAPK and MKP-1 protein levels from cultured neonatal cardiomyocytes infected with AdCnA or Adβgal and treated with PE for 1 h. Under these conditions, AdCnA significantly reduced p38 MAPK phosphorylation (0.54×) and increased MKP-1 protein levels (6.29×). Similar results were observed in two independent experiments.
Calcineurin promotes p38 MAPK inactivation

Calcineurin promotes p38 MAPK inactivation in COS-7 cells—To determine if the identified association between calcineurin and p38 MAPK inactivation was specific to cardiac myocytes, we performed transient transfection experiments in another cell type, COS-7. An expression vector encoding the upstream activator of p38 MAPK, MKK6, was transfected into COS-7 cells, resulting in significant p38 MAPK phosphorylation assessed by Western blotting (Fig. 5A). However, co-transfection of an activated calcineurin expression vector along with the MKK6 encoding vector significantly reduced p38 MAPK phosphorylation (Fig. 5A).

While expression of activated calcineurin in COS-7 cells promoted p38 MAPK inactivation, the potential association with MKP-1 expression in this cell type was uncertain. Accordingly, calcineurin transfection by itself did not result in a significant increase in MKP-1 protein in COS-7 cells (Fig. 5B). However, MKP-1 protein levels increased when calcineurin was co-transfected with MAPK kinase expression vectors (MEK1, MKK6, or MKK7), suggesting that calcineurin requires MAPK co-stimulation to promote efficient MKP-1 expression (Fig. 5B). Such results are consistent with the observations in cardiac myocytes whereby calcineurin promoted the greatest increase in MKP-1 protein levels when co-stimulated by PE (Fig. 2B). Taken together, these data suggest that efficient MKP-1 induction requires coordinate input from calcineurin and MAPK signaling pathways.

Calcineurin augments MKP-1 promoter activity—The mouse MKP-1 genomic organization was previously characterized, including the upstream regulatory region and transcription start site (18). A 633 base pair fragment of the MKP-1 promoter was cloned and fused to a luciferase reporter (Fig. 6A). The MKP-1 promoter contains a TATA box, Sp1 element, CAAT box, serum response factor binding site, and multiple AP1 and cAMP-response element-binding protein stress-responsive elements (29). However, consensus or near consensus NFAT binding elements were not identified within the minimal MKP-1 promoter (see “Discussion”). The 633 base pair MKP-1 promoter was transiently co-transfected with an expression vector encoding activated calcineurin or wild-type and activated NFAT expression vectors. We determined that calcineurin augmented promoter activity 4–5-fold in multiple experiments, suggesting that calcineurin directly regulates MKP-1 promoter activity (Fig. 6B). Calcineurin-mediated induction of the MKP-1 promoter was blocked by cotransfection with an expression vector encoding a 194-amino acid calcineurin-inhibitory domain from the protein chain (13).

NFAT transcription factors are direct downstream mediators of calcineurin-induced alterations in gene expression in multiple cell types. However, not all calcineurin-induced transcriptional effects are mediated by NFAT factors. Likewise, we observed that co-transfection of the MKP-1 promoter
construct with full-length NFATc4 or NFATc3 expression vectors or constitutively nuclear forms of these factors (∆NFATc4 and ∆NFATc3) did not significantly augment promoter activity (Fig. 6B). We also failed to identify any additional increase in promoter activity when calcineurin was co-transfected in conjunction with NFATc4 or NFATc3 (data not shown), which is consistent with the lack of an identifiable consensus NFAT binding site within the −633 base pair MKP-1 promoter. Collectively, these data indicate that calcineurin regulates the minimal MKP-1 promoter in an NFAT-independent manner.

Last, we also investigated the potential cooperation between calcineurin and MAPK signaling in the activation of the MKP-1 promoter. Expression vectors encoding activated forms of the MAPK kinase signaling factors MEK1 (activates ERKs), MKK6 (activates p38), and MKK7 (activates JNK) each stimulated MKP-1 promoter activity in co-transfection experiments, similar to calcineurin (Fig. 6C). Furthermore, co-transfection experiments between calcineurin and individual MAPK kinase factors promoted an additive or greater than additive increase in MKP-1 promoter activity (Fig. 6C). These data indicate that the calcineurin regulates MKP-1 promoter activity in coordination with MAPK stress-responsive signaling pathways. This interpretation is consistent with the coordinated increase in MKP-1 protein level that was observed between calcineurin and MAPK signaling responses in cardiomyocytes and COS-7 cells.

**DISCUSSION**

A wide array of intracellular signaling pathways have been implicated in the regulation of cardiac myocyte hypertrophic growth. Intracellular signaling pathways such as calcineurin/NFAT and MAPK represent central mediators of reactivity in cardiac myocytes as well as other diverse cell types. For example, calcineurin and MAPK signaling pathways are critical mediators of T-cell reactivity where they regulate proliferation and cytokine production in response to antigen. A challenge that remains is to elucidate the potential interconnectivity between calcineurin and MAPK signaling pathways in cardiac myocytes as well as other cell types. Here we show that calcineurin activation promotes a decrease in p38 MAPK activity in both transgenic mice and cultured cardiomyocytes through a potential mechanism involving the dual specificity phosphatase MKP-1.

**Interconnection between Calcineurin and p38 MAPK in Cardiac Hypertrophy**—Transgenic mice expressing an activated calcineurin cDNA in their hearts were characterized by a significant reduction in basal p38 MAPK phosphorylation at early developmental times. However, this decrease in p38 MAPK activation gradually diminished with age so that by 24 days postnatal, altered activation was less obvious. Such regulation might be explained by the following observations. First, p38 MAPK activation is itself dynamically regulated during postnatal development such that greater activation is observed during early developmental periods when the heart is undergoing developmental hypertrophy (30). In older hearts, basal p38 MAPK activation status is probably reduced so that a further reduction in activity (as mediated by calcineurin) would be less obvious. Second, as calcineurin transgenic hearts progressively hypertrophy with age, secondary effects associated with pathologic hypertrophy might influence the activity of various intracellular signaling pathways. In this respect, the observed reduction in p38 MAPK activity in very young calcineurin transgenic hearts suggests a more direct interconnection between calcineurin and p38 MAPK, without the potential secondary effects associated with cardiac hypertrophy itself.

**In vitro**, we observed that prolonged stimulation of cardiac myocytes with PE, which potently activates calcineurin (13), promoted a gradual reduction of p38 phosphorylation (Fig. 3). This reduction in p38 phosphorylation after just 1 h of PE treatment was largely prevented with CsA, a calcineurin inhibitor (Fig. 3). These data suggest that prolonged agonist stimulation can lead to p38 inactivation, in part, through a calcineurin-regulated pathway. This interpretation is consistent with a recent report by Hines et al. (11) in which prolonged electrical pacing of cardiac myocytes in culture resulted in p38 inactivation despite a progressive hypertrophy response. Interestingly, electrical pacing of cultured cardiomyocytes was also recently shown to directly activate calcineurin/NFAT signaling (31). Taken together, these data suggest that calcineurin activation can promote p38 MAPK inactivation in cultured cardiomyocytes.

**Calcineurin Promotes p38 MAPK Inactivation in Association with MKP-1 Expression**—Calcineurin is a serine/threonine phosphatase that has not been reported to act on MAPK proteins. In contrast, a specialized family of immediate early genes encoding dual specificity phosphatases have been identified that act as specific negative regulators of ERKs, JNKs, and p38. MKPs are largely regulated at the transcriptional level such that stress stimuli or mitogen stimulation induce gene expression (reviewed in Ref. 20). MKP-1 is most selective for p38 MAPK at physiologic levels of expression, although high levels of expression are also associated with JNK and even mild
ERK1/2 inactivation (24, 25, 32). Indeed, at physiologic expression levels MKP-1 directly binds to p38 kinases but not ERKs or JNKs (24, 25). These reports are consistent with our observation that an approximate 2-fold increase in MKP-1 protein in day 4 cardiomyocytic transgenic hearts is associated with p38 MAPK inactivation, but not ERKs or JNKs (Fig. 1, and data not shown).

The inability of purified calcineurin to directly dephosphorylate p38 MAPK in vitro suggested that calcineurin might potentially act indirectly through MKP-1. Indeed, cardiomyocytic transgenic hearts at 4 days of age and AdCnA infection of PE-stimulated cardiac myocytes demonstrated a dramatic increase in MKP-1 protein levels but no change in MKP-2 (Fig. 2B). In addition, inhibition of calcineurin with CsA led to a loss of MKP-1 induction and even promoted a decrease in basal MKP-1 protein levels in cardiac myocytes (Fig. 3). These data are consistent with the recent observation that MAPK transcriptional responses in a neuronal-like cell line were inhibited with a calcium ionophore through a mechanism involving enhanced MKP-1 expression (28).

To examine the ability of calcineurin to directly regulate MKP-1 expression, we characterized the mouse minimal MKP-1 promoter. Co-transfection of an activated calcineurin expression vector with an MKP-1 promoter-luciferase fusion reporter resulted in a 4–5-fold increase in promoter activity compared with reporter alone, suggesting that calcineurin might regulate MKP-1 at the transcriptional level. This interpretation is further supported by the observation that MKP-1 protein levels were not altered by 5 or 15 min of PE stimulation in cardiac myocytes, but levels were augmented in a Ca2+-sensitive manner by 30 and 60 min. Despite a potential direct role for calcineurin in regulating MKP-1 expression, we also observed that calcineurin acted in concert with MAPK signaling pathways to more efficiently up-regulate MKP-1 expression, suggesting that calcineurin and MAPK coordinate maximal MKP-1 induction.

To more carefully evaluate the transcriptional mechanism whereby calcineurin might regulate MKP-1 promoter activity, we co-transfected full-length or constitutively nuclear expression vectors for NFATc4 and NFATc3. However, NFAT factors did not significantly induce MKP-1 promoter activity; nor were good consensus sites present within the −633 base pair region that was analyzed. We did identify a site at +25 base pairs (−5′-AGGAAAGCCG-3′) in the 5′-untranslated region that weakly resembled a NFAT element, but this site failed to interact with in vitro translated NFATc4 protein by gel shift assay, while a control NFAT site from the IL-4 promoter demonstrated significant binding (data not shown). While it is reasonable to conclude that the MKP-1 minimal promoter is regulated in an NFAT-independent manner, it is formally possible that one or more relevant NFAT sites lie upstream of the area analyzed.

Implications for p38 MAPK and Cardiac Hypertrophy—A large number of reports have demonstrated that the MKK3/6-p38 MAPK signaling branch is sufficient to initiate the hypertrophic program in cultured cardiomyocytes. For example, transfection of cultured cardiomyocytes with a MKK6-encoding expression vector or infection with a MKK6-encoding adenovirus produced a profound hypertrophy response in vitro (5–7). In addition, Zhang et al. (33) generated transgenic mice expressing an activated form of TAK1 (MAP kinase kinase signaling factor), which resulted in p38 MAPK activation and hyperpolarization of an activated form of TAK1 (MAPK kinase kinase signaling branch) in cultured cardiomyocytes.

The observation that p38 MAPK activation can promote apoptosis in cultured cardiomyocytes (6), while calcineurin signaling was shown to be cardioprotective (34). These relationships are of particular interest, since calcineurin (antiapoptotic) promotes p38 MAPK inactivation. Accordingly, sustained p38 MAPK activation in the heart might be predicted to promote decompensation and cardiomyopathy, consistent with the phenotype of Tak1 transgenic mice (33). In this respect, p38 MAPK activation might represent a deleterious signaling pathway in the heart and is a desirable target for pharmacologic inhibition.

REFERENCES
1. van Zwieten, P. A. (2000) Cardiacoex. Res. 45, 82–91
2. Molkentin, J. D. (2000) Circ. Res. 87, 731–738
3. Sugden, P. H., and Clerk, A. (1998) Circ. Res. 82, 345–352
4. Clerk, A., Michael, A., and Sugden, P. H. (1998) J. Cell Biol. 142, 523–535
5. Zechner, D., Theraulaz, D. J., Hanford, D. F., McMahon, P. M., and Glombotski, C. C. (1997) J. Cell Biol. 138, 115–127
6. Wang, Y., Huanf, S., Sah, V. P., Ross, J., Heller-Brown, J., Han, J., and Chien, K. R. (1998) J. Biol. Chem. 273, 2161–2166
7. Nomoto, S., Sheng, Z., and Lin, A. (1998) Mol. Cell. Biol. 18, 3518–3526
8. Liang, F., and Gardner, D. G. (1999) J. Clin. Invest. 104, 1603–1612
9. Liang, F., Lu, S., and Gardner, D. G. (2000) Hypertension 35, 188–192
10. Choukroun, G., Hajjar, R., Kyriakis, J. M., Bonventre, J. V., Rosenzweig, A., Robbins, J. A., and Molkentin, J. D. (2000) J. Biol. Chem. 275, 331–341
11. Molkentin, J. D., Lu, J. R., Antos, C. L., Markham, B., Richardson, J., Robbins, J. Grant, S. R., and Olsen, E. (1998) Cell 93, 215–228
12. Taigen, T., De Windt, L. J., Lim, H. W., and Molkentin, J. D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1196–1201
13. De Windt, L. J., Lim, H. W., Haq, S., Force, T., and Molkentin, J. D. (2000) J. Biol. Chem. 275, 13571–13579
14. O’Keefe, J. S., Tamura, J., Kincaid, R. L., Tocci, M. J., and O’Neill, E. A. (1992) Nature 357, 692–694
15. Hoej, T., Sun, Y. L., Williamson, K., and Xu, X. (1995) Immunity 2, 461–472
16. Masadu, E. S., Naito, Y., Tokumitsu, H., Campbell, D., Saito, F., Hannum, C., Arai, K., and Arai, N. (1995) Mol. Cell. Biol. 15, 2697–2706
17. Nagasu, T., Metz, R., Chen, L., Kiyotaki, M., and Arai, N. (1995) J. Biol. Chem. 270, 651–653
18. Chajry, N., Martin, P. M., Cochet, C., and Berthois, Y. (1996) Eur. J. Biochem. 235, 97–102
19. Parwareswaran, N., Namib, P., Hall, C. S., Brooks, D. P., and Spilman, W. S. (2000) J. Biol. Chem. 275, 331–338
20. Franklin, C. C, and Kraft, A. S. (1997) J. Biol. Chem. 272, 16917–16923
21. Hutter, D., Chen, F., Barnes, J., and Liu, Y. (2000) J. Biol. Chem. 275, 155–163
22. Chu, Y., Soltak, P. K., Khosravi-Far, B., Der, C. J., and Kelly, K. (1996) J. Biol. Chem. 271, 6497–6501
23. Li, C., Hu, Y., Mayr, M., and Xu, Q. (1999) J. Biol. Chem. 274, 25273–25280
24. Dorham, P. L., and Russo, A. P. (2000) Mol. Endocrinol. 14, 1570–1582
29. Sommer, A., Burkhardt, H., Keyse, S. M., and Luscher, B. (2000) FEBS Lett. 474, 146–150
30. Sussman, M. A., Welch, S., Gude, N., Kheury, P. R., Daniele, S. R., Kirkpatrick, D., Walsh, R. A., Price, R. L., Lim, H. W., and Molkentin, J. D. (1999) Am. J. Pathol. 155, 2101–2113
31. Xia, Y., McMillin, J. B., Lewis, A., Moore, M., Zhu, W. G., Williams, R. S., and Kellems, R. E. (2000) J. Biol. Chem. 275, 1855–1863
32. Bueno, O. F., De Windt, L. J., Lim, H. W., Tymitz, K. M., Witt, S. A., Kimball, T. R., and Molkentin, J. D. (2001) Circ. Res. 86, 98–106
33. Zhang, D., Gaussin, V., Taffet, G. E., Belaguli, N. S., Yamada, M., Schwartz, R. J., Michael, L. H., Overbeek, P. A., and Schneider, M. D. (2000) Nat. Med. 6, 556–563
34. De Windt, L. J., Lim, H. W., Taigen, T., Wencker, D., Condorelli, G., Dorn, G. W. II., Kita, N., and Molkentin, J. D. (2000) Circ. Res. 86, 255–263
