pathway, MEF2 may regulate the expression of genes that are critical for survival of newly differentiated neurons. Consistent with this possibility is our finding that mutations of MEF2 that cause a loss of MEF2-dependent transcription also lead to a loss of calcium-dependent neuronal survival.

These findings extend the scope of the cellular responses that are controlled by MEF2 proteins. Although neuronal survival has been thought to occur largely through direct posttranslational modifications of components of the cell death machinery, our results suggest that transcription-dependent events regulated by MEF2 proteins also have a critical role in mediating the survival of neurons. The observation that MEF2 activity is regulated by calcium raises the possibility that MEF2s may mediate other calcium-dependent signaling events in addition to calcium-dependent neuronal survival. It is possible that, like the cyclic adenosine monophosphate response element binding protein (CREB), a well-characterized mediator of calcium-dependent transcriptional responses (*23–28*), MEF2s may regulate calcium-dependent changes in transcription that affect synaptic function and thereby mediate adaptive neuronal responses.

References and Notes

1. A. E. Chambers, S. Kotecha, N. Towers, T. J. Mohun, *EMBO J.* **11**, 4981 (1992).
2. A. E. Chambers *et al.*, *Genes Dev.* **8**, 1324 (1994).
3. D. G. Edmondson, G. E. Lyons, J. F. Martin, E. N. Olson, *Development* **120**, 1251 (1994).
4. M. H. Lin, B. A. Bour, S. M. Abmayr, R. V. Storti, *Dev. Biol.* **182**, 240 (1997).
5. Q. Lin, J. Schwarz, C. Bucana, E. N. Olson, *Science* **276**, 1404 (1997).
6. B. Lilly *et al.*, *Science* **267**, 688 (1995).
7. G. Ranganayakulu *et al.*, *Dev. Biol.* **171**, 169 (1995).
8. J. D. Molkentin, B. L. Black, J. F. Martin, E. N. Olson, *Cell* **83**, 1125 (1995).
9. S. Kaushal, J. W. Schneider, B. Nadal-Ginard, V. Mahdavi, *Science* **266**, 1236 (1994).
10. G. E. Lyons, B. K. Micales, J. Schwarz, J. F. Martin, E. N. Olson, *J. Neurosci.* **15**, 5727 (1995).
11. H. Ikeshima, S. Imai, K. Shimoda, J. Hata, T. Takano, *Neurosci. Lett.* **200**, 117 (1995).
12. D. Leifer *et al.*, *Proc Natl Acad Sci U.S.A.* **90**, 1546 (1993).
13. T. H. Han and R. Prywes, *Mol. Cell. Biol.* **15**, 2907 (1995).
14. Z. Mao and M. E. Greenberg, unpublished data.
15. E. E. Geisert Jr. and A. Frankfurter, *Neurosci. Lett.* **102**, 137 (1989).
16. S. A. Bayer and J. Altman, *Exp. Neurol.* **107**, 48 (1990).
17. J. D. Molkentin, B. L. Black, J. F. Martin, E. N. Olson, *Mol. Cell. Biol.* **16**, 2627 (1996).
18. X. Lin, S. Shah, R. F. Bulleit, *Brain Res. Mol. Brain Res.* **42**, 307 (1996).
19. J. L. Franklin and E. M. Johnson Jr., *Trends Neurosci.* **15**, 501 (1992).
20. J. L. Franklin and E. M. Johnson Jr., *Ann. N.Y. Acad. Sci.* **747**, 195 (1994).
21. T. M. Miller, M. G. Tansey, E. M. Johnson Jr., D. J. Creedon, *J. Biol. Chem.* **272**, 9847 (1997).
22. J. Han, Y. Jiang, Z. Li, V. V. Kravchenko, R. J. Ulevitch, *Nature* **386**, 296 (1997).
23. K. Deisseroth, H. Bito, R. W. Tsien, *Neuron* **16**, 89 (1996).
24. H. Bito, K. Deisseroth, R. W. Tsien, *Cell* **87**, 1203 (1996).
25. H. Bito, K. Deisseroth, R. W. Tsien, *Curr. Opin. Neurobiol.* **7**, 419 (1997).
26. M. Sheng, M. A. Thompson, M. E. Greenberg, *Science* **252**, 1427 (1991).
27. L. Santella and E. Carafoli, *FASEB J.* **11**, 1091 (1997).
28. M. R. Montminy *et al.*, *J. Neurosci.* **6**, 1171 (1986).
29. F. Saudou, S. Finkbeiner, D. Devys, M. E. Greenberg, *Cell* **95**, 55 (1998).
30. H. Dudek *et al.*, *Science* **275**, 661 (1997).
31. Z. Xia, M. Dickens, J. Raingeaud, R. J. Davis, M. E. Greenberg, *Science* **270**, 1326 (1995).
32. Granule neurons from P6 rat cerebellum were cultured in vitro for 5 days in full medium (serum + KCl) and transfected with the indicated constructs along with cytomegalovirus (CMV)–β-Gal. Sixteen hours after transfection, cells were washed and placed in medium containing 25 mM KCl but no serum (Fig. 2, A to E), 5 mM KCl (Fig. 2F), or serum-containing medium (Fig. 2C). After 12 hours, cells were fixed and stained with an antibody to β-Gal and Hoechst dye. For kinetics studies, cells were fixed at various time points after transfection. Cells were scored in a blinded fashion as apoptotic or healthy based on their nucleic and cell body–neurite morphology.
33. Y. T. Yu *et al.*, *Genes Dev.* **6**, 1783 (1992).

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Apoptosis of T Cells Mediated by Ca^{2+}-Induced Release of the Transcription Factor MEF2

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T cell receptor (TCR)–induced apoptosis of thymocytes is mediated by calcium-dependent expression of the steroid receptors Nur77 and Nor1. Nur77 expression is controlled by the transcription factor myocyte enhancer factor 2 (MEF2), but how MEF2 is activated by calcium signaling is still obscure. Cabin1, a calcineurin inhibitor, was found to regulate MEF2. MEF2 was normally sequestered by Cabin1 in a transcriptionally inactive state. TCR engagement led to an increase in intracellular calcium concentration and the dissociation of MEF2 from Cabin1, as a result of competitive binding of activated calmodulin to Cabin1. The interplay between Cabin1, MEF2, and calmodulin defines a distinct signaling pathway from the TCR to the Nur77 promoter during T cell apoptosis.

Apoptosis of T lymphocytes can be induced by multiple signaling pathways. Whereas the Fas and tumor necrosis factor–α pathways are involved in the elimination of activated peripheral T cells, a distinct pathway emanating from the TCR is responsible for thymic negative selection (*1, 2*). Orphan steroid receptors including Nur77 and Nor1 have been identified as crucial mediators of TCR-induced apoptosis (*3–5*). TCR-mediated Nur77 expression requires an increase in intracellular calcium concentration (*6*). Two Ca^{2+}-regulated DNA elements in the Nur77 promoter were identified as consensus binding sites for MEF2 (*6*). These observations implicated MEF2, originally discovered as a transcription factor for muscle-specific gene expression (*7–12*), as a Ca^{2+}-dependent transcription factor for Nur77 expression.

The protein phosphatase calcineurin has

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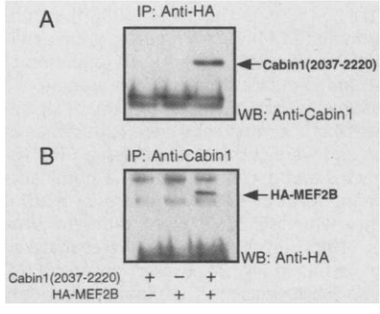

Fig. 1. The COOH-terminal fragment of Cabin1 [Cabin1(2037–2220)] interacts with MEF2B in a Ca^{2+}-sensitive manner. (**A** and **B**) Coimmunoprecipitation of Cabin1(2037–2220) and MEF2B. Jurkat T cells transfected with pSGCabin1(2037–2220) and pSGHA-MEF2B were incubated for 24 hours. The cells were lysed, followed by immunoprecipitation using either anti-Cabin1 or HA mAb. The precipitated protein was subjected to protein immunoblot with appropriate antibodies.

an endogenous inhibitor named Cabin1 (*13*), or Cain in rat (*14*). We applied the yeast two-hybrid system with a COOH-terminal 502–amino acid fragment as bait to identify Cabin1 binding proteins (*13*). One positive clone was isolated upon screening 10^6 cDNA clones from a human lymphocyte cDNA library. Sequence analysis of the Cabin1 interacting clone revealed that it encoded full-length MEF2B (*8, 9*).

The interaction between Cabin1 and MEF2B was confirmed by coimmunoprecipitation. When a 184–amino acid COOH-terminal fragment of Cabin1 [Cabin1(2037–2220)] and hemagglutinin (HA)–tagged MEF2B were coexpressed in Jurkat T cells, Cabin1(2037–2220) immunoprecipitated with a monoclonal antibody (mAb) to HA (Fig. 1A) and HA-MEF2B was precipitated with polyclonal antibody to Cabin1 (anti-Cabin1) (Fig. 1B). We then determined the minimal structural domains in both Cabin1 and MEF2 that are required for their interaction. The minimal Cabin1 interacting domain in MEF2B was determined by coimmunoprecipitation. A series of COOH-terminally truncated MEF2B mutants were generated and expressed in Jurkat T cells together with Cabin1(2037–2220). Although most of the transactivation domain of MEF2B was dispensable, the MADS/MEF2 box (DNA binding domain)

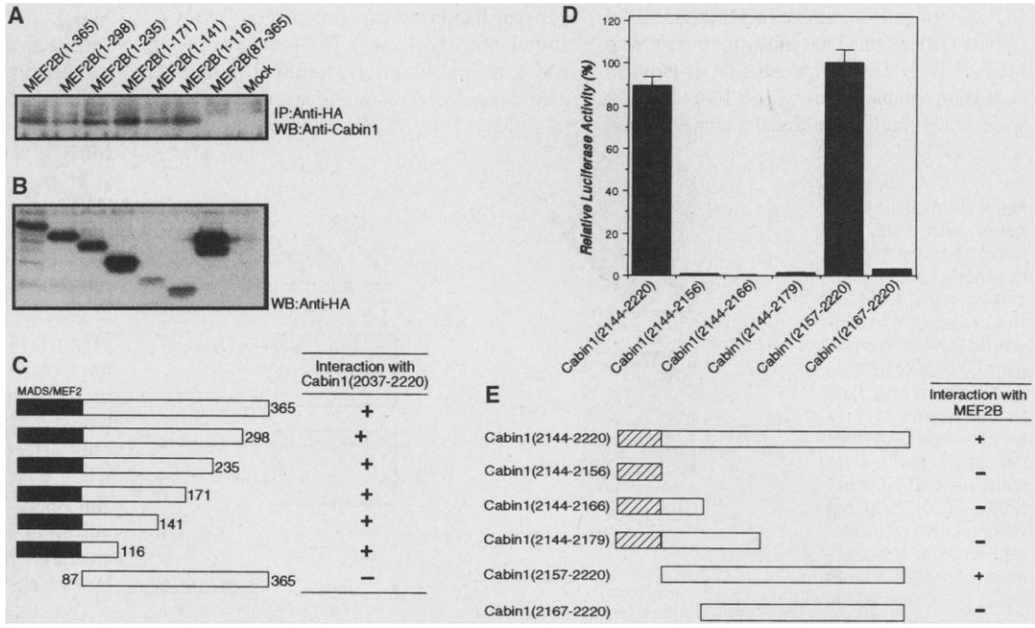

Fig. 2. Mapping of the Cabin1-MEF2 interacting domains. (**A** to **C**) Mapping of minimal Cabin1 binding domain in MEF2B. In (A), MEF2B-truncation mutants and Cabin1(2037–2220) were coimmunoprecipitated. DO11.10 cells were transfected with various MEF2B truncation mutants tagged with the HA epitope at the NH_2-termini along with pSGCabin1(2037–2220). Cell lysates were immunoprecipitated with HA mAb and probed with anti-Cabin1 by protein immunoblot. Expression of MEF2B truncation mutants is shown in (B). Cell lysates containing equal amounts of total proteins were assessed by protein immunoblot to determine the expression level of MEF2B using HA mAb. In (C), MEF2B truncation mutants are shown schematically. The MADS/MEF2 box is highlighted by filled bars; the transactivation domain of MEF2B is indicated by hollow bars. (**D** and **E**) Mapping of the MEF2B binding domain in Cabin1. The interaction between Cabin1–14 truncation mutants and MEF2B (D) was determined using the mammalian two-hybrid assay. DO11.10 cells were transfected with various Cabin1(2144–2220) truncation mutants fused to the Gal4 DNA binding domain along with pVPMEF2B. Luciferase activity was normalized to β-galactosidase activity. A schematic representation of Cabin1(2144–2220) and various truncation mutants (E) shows the minimal calcineurin-binding domain (2144–2157), highlighted by hatched bars (*13*).

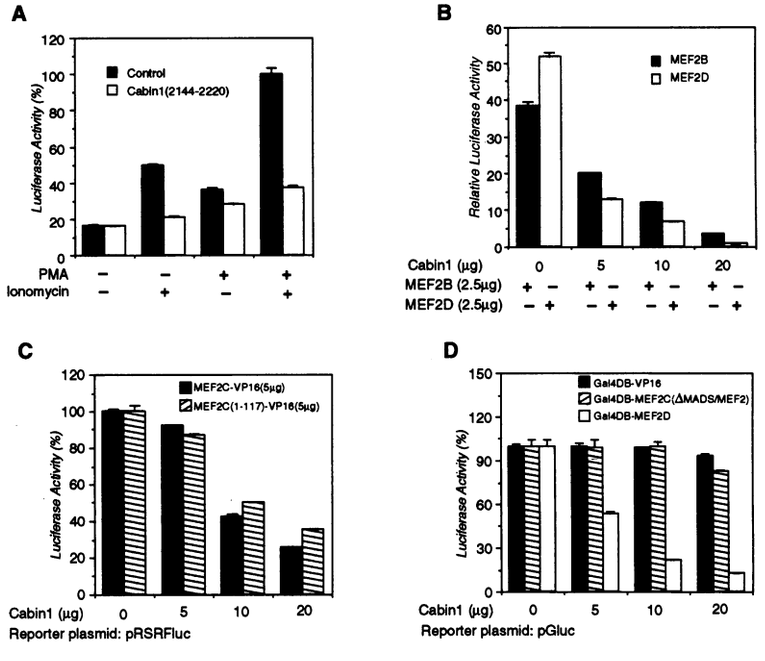

Fig. 3. Cabin1 inhibits MEF2 transcriptional activity. (**A**) Overexpression of Cabin1(2037–2220) blocks MEF2 transcriptional activity in response to PMA and ionomycin. (**B**) Full-length Cabin1 inhibits transcription mediated by MEF2B and MEF2D. pSGMEF2B or pSGMEF2D was transfected along with varying amounts of full-length Cabin1 expression plasmid into DO11.10 cells. Transcriptional activity of MEF2 was measured by luciferase reporter gene activity under the control of two tandem MEF2-binding sites. (**C** and **D**) Inhibition of MEF2 transcriptional activity by Cabin1 is mediated through the MADS/MEF2 domain. In (C), full-length Cabin1 is shown to inhibit the DNA-binding activity of MEF2. DO11.10 cells were transfected with the indicated amounts of MEF2(1–117)-VP16, MEF2C-VP16, and pSG-Cabin1 along with pRSRFluc reporter plasmid under the control of MEF2-binding sites. In (D), transcription mediated by the transactivation domain of MEF2 is shown to be resistant to Cabin1. DO11.10 cells were transfected with Gal4-MEF2C(MADS/MEF2) (10 μg), Gal4-MEF2D (10 μg), and pSG-Cabin1 (10 μg) along with pGluc reporter plasmid under the control of Gal4 binding sites. Luciferase activity was normalized against β-galactosidase activity.

was essential for interaction between MEF2B and Cabin1 (Fig. 2, A to C). Because the MADS/MEF2 box is highly conserved among the four known isoforms of MEF2 (A through D), we suspected that all isoforms of MEF2 could bind to Cabin1, as was subsequently confirmed (*15*). The mammalian two-hybrid system was used to map the minimal Cabin1 domain required for MEF2B interaction with Cabin1 fragments fused to the Gal4 DNA binding domain and MEF2B fused to the VP16 activation domain (*16*). Cabin1(2144–2220), a COOH-terminal 77–amino acid fragment of Cabin1 (*13*), retained the ability to interact with MEF2B (Fig. 2E). NH_2- and COOH-terminal truncation mutants of Cabin1(2144–2220) were tested, and the minimal Cabin1 domain sufficient for mediating MEF2B interaction was the COOH-terminal 64 amino acids, Cabin1(2157–2220) (Fig. 2E).

We next determined whether the interaction between Cabin1 and MEF2 affected MEF2 transcriptional activity. The effect of Cabin1 on activity of a MEF2-driven luciferase reporter gene (*6*) was measured in DO11.10 upon stimulation with PMA, ionomycin, or both. Both PMA and ionomycin individually activate the MEF2 reporter gene, with ionomycin inducing higher MEF2-dependent luciferase expression (Fig. 3A). Costimulation of cells with PMA and ionomycin had a synergistic effect, resulting in optimal expression of the reporter gene. Coexpression of Cabin1(2144–2220) inhibited PMA- and ionomycin-stimulated reporter gene expression (Fig. 3A). Cabin1 also inhibited reporter gene expression driven by expressed MEF2B and MEF2D in a concentration-dependent manner (Fig. 3B).

Given the interaction of Cabin1 with the MADS/MEF2 box of MEF2 isoforms (Fig. 2), its effect on MEF2 transcriptional activity was probably dependent on this domain. When MEF2C(1–117)-VP16 or full-length MEF2C fused to VP16 was cotransfected with the MEF2-luciferase reporter gene (pRSR-FLuc), full-length Cabin1 inhibited reporter gene activation in a concentration-dependent manner (Fig. 3C). In contrast, when the transactivation domain of MEF2C fused to the Gal4 binding domain [Gal4DB-MEF2C(ΔMADS/MEF2)] was used to drive transcription of a Gal4-driven reporter gene, expression of Cabin1 had no effect on the reporter gene expression (Fig. 3D). When the chimeric protein between Gal4 DBD and full-length MEF2D (Gal4DB-MEF2D) was expressed, the reporter gene activation regained sensitivity to Cabin1, which suggests that Cabin1 does not interfere with MEF2 transcriptional activity by blocking its DNA binding. This is consistent with the observation that TCR signaling only affected MEF2 transcriptional activity without changing its DNA binding activity, and MEF2 is likely to be bound to DNA in quiescent T cells (*6, 17*). The precise mechanism of transcriptional repression of MEF2 by Cabin1 remains to be elucidated.

We then investigated how MEF2 is released from Cabin1 and activated in response to Ca^{2+} signal in vivo. The Cabin1-MEF2 interaction may be subject to regulation by calcium and calmodulin (CaM); the minimal MEF2 binding domain of Cabin1 shares the same structural features as CaM binding motifs from a number of proteins—including MyoD, CaM-dependent kinase II, and calcineurin—with a basic region followed by a long α helix (*18–20*). We first assessed whether Cabin1 binds to CaM by use of CaM-sepharose to find CaM binding proteins in T cell extract. Endogenous Cabin1 was detected by protein immunoblot (*21*). The binding of Cabin1 to CaM sepharose occurred in a Ca^{2+}-dependent manner (Fig. 4A). Purified recombinant GST-Cabin1(2037–2220) fusion protein also bound to CaM in a Ca^{2+}-dependent manner (Fig. 4B). Thus, Cabin1 is a direct CaM binding protein.

To determine whether binding of CaM to Cabin1 could displace MEF2, we incubated [^{35}S]MEF2D with Cabin1(2037–2220) and CaM (*22*). GST-Cabin1(2037–2220) bound MEF2D in the presence of EGTA, Ca^{2+} alone, or CaM alone (Fig. 4C). Ca^{2+} plus CaM, however, competed with MEF2D for Cabin1 binding, indicating that activated CaM could displace MEF2D from Cabin1 in vitro. Consistent with these observations, endogenous Cabin1 coimmunoprecipitated with

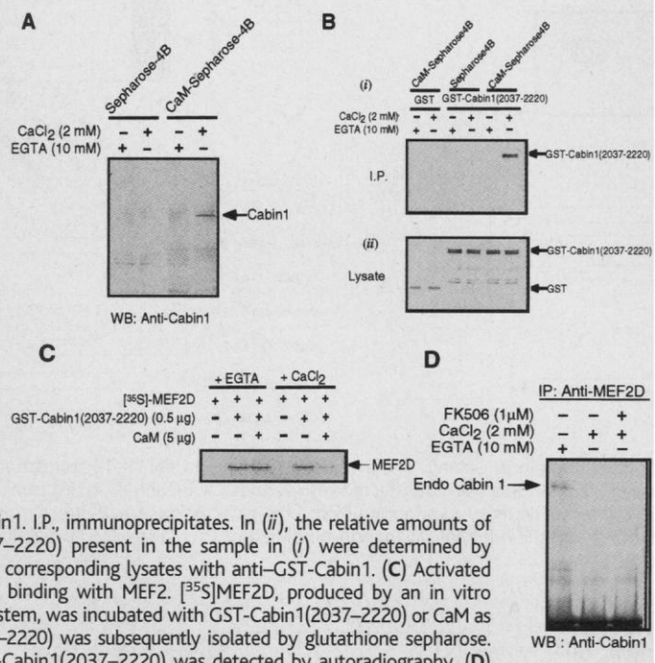

Fig. 4. Calmodulin competes with MEF2 for Cabin1 binding. (**A**) Endogenous Cabin1 associates with CaM in the presence of calcium. (**B**) Purified recombinant GST-Cabin1(2037–2220) can directly bind to CaM-sepharose in a calcium-dependent manner. In (*i*), purified recombinant GST-Cabin1 (2037–2220) (500 ng) was incubated with CaM-sepharose in the presence of either 2 mM $CaCl_2$ or 10 mM EGTA. Proteins bound to CaM-sepharose were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with anti–GST-Cabin1. I.P., immunoprecipitates. In (*ii*), the relative amounts of GST and GST-Cabin1(2037–2220) present in the sample in (*i*) were determined by protein immunoblot of the corresponding lysates with anti–GST-Cabin1. (**C**) Activated CaM competes for Cabin1 binding with MEF2. [^{35}S]MEF2D, produced by an in vitro transcription-translation system, was incubated with GST-Cabin1(2037–2220) or CaM as indicated. GST-Cabin1(2037–2220) was subsequently isolated by glutathione sepharose. [^{35}S]MEF2D bound to GST-Cabin1(2037–2220) was detected by autoradiography. (**D**) Calcium inhibits the interaction between endogenous Cabin 1 and MEF2D in an FK506-resistant manner. Immunoprecipitation of MEF2D was from DO11.10 cell lysates containing EGTA, calcium, and calcium plus FK506, respectively. The immunoprecipitates were subjected to SDS-PAGE and protein immunoblot analysis using anti-Cabin1.

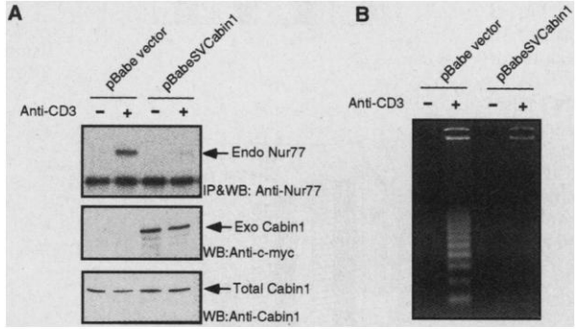

Fig. 5. Nur77 and T cell apoptosis is regulated by both calcineurin and Cabin1. (**A**) Cabin1 overexpression blocks the induction of endogenous Nur77. DO11.10 cells stably transfected with pBabe-SVCabin1 or pBabe (10^7 cells per lane) were stimulated with CD3 mAb for 3 hours. Cell lysates were immunoprecipitated and probed with anti-Nur77. Cell lysates containing the same amounts of total proteins were also subjected to protein immunoblot analysis using anti–c-myc (middle panel) and anti-Cabin1 (bottom panel). (**B**) Cabin1 blocks TCR-mediated apoptosis. DO11.10 cells stably transfected with pBabe or pBabe-SVCabin1 (2×10^6 cells per lane) were stimulated with CD3 mAb for 8 hours. Cytosolic DNA was resolved in 1.2% agarose gel and visualized with ethidium bromide.

endogenous MEF2D, the most abundant isoform of MEF2 in T cells, only in the presence of EGTA (Fig. 4D). The presence of calcium abrogates the binding of MEF2D to Cabin1. The antagonism by calcium is not sensitive to FK506, suggesting that the calcium effect was not mediated by calcineurin.

Calcineurin participates in the activation of MEF2, because Ca^{2+}-dependent MEF2 activation is sensitive to the calcineurin inhibitor cyclosporin A (*6*). To dissect the roles of the calcineurin binding domain and the MEF2 binding domain of Cabin1 in the regulation of MEF2, we generated Cabin1(2157–2220; ΔCNBD) and Cabin1(2157–2220; ΔMEFBD), in which these respective domains were individually deleted (*23*). The Ca^{2+}-dependent luciferase activation of a Nur77 promoter-driven reporter gene was inhibited by overexpression of Cabin1(2157–2220) as well as the two deletion mutants, suggesting that both calcineurin and Cabin1 are involved in the regulation of MEF2. In contrast, the interleukin-2 (IL-2) reporter gene activation by PMA and ionomycin was sensitive to Cabin1(2157–2220) and Cabin1(2157–2220; ΔMEFBD), but was resistant to the mutant in which the calcineurin binding domain was removed (*23*). This result is consistent with the dependence of the IL-2 promoter on calcineurin but not MEF2. Thus, MEF2 is subject to regulation by Cabin1 independent of the effect of Cabin 1 on calcineurin activity.

Because both the calcineurin and MEF2 binding domains in Cabin1 serve to down-regulate MEF2 activity, overexpression of Cabin1 should inhibit TCR-mediated Nur77 expression and apoptosis. Thus, we transfected DO11.10 cells directly with the retroviral vector pBabeSVCabin1 encoding the full-length Cabin1 containing an NH_2-terminal c-Myc tag under the control of the SV40 promoter and selected for stably transfected cells using puromycin (*24*). Expression of transfected Cabin1 was confirmed by protein immunoblot (Fig. 5A, middle panel). When the total amount of Cabin1 was detected using anti-Cabin1, overexpression resulted in a doubling of Cabin1 (Fig. 5A, bottom panel). We then examined the effect of overexpression of Cabin1 on endogenous Nur77 expression by protein immunoblot. Nur77 expression was induced by treatment of DO11.10 cells with CD3 mAb (*6*), and overexpression of Cabin1 almost completely inhibited both Nur77 expression (Fig. 5A) and TCR-mediated apoptosis, as judged by nuclear chromosomal DNA fragmentation (Fig. 5B).

The MEF2 family of transcription factors are involved in diverse cellular processes, including muscle and neuronal cell differentiation (*25*). Because Cabin1 binds to MEF2 and sequesters MEF2 in a transcriptionally inactive state, it is a good candidate for an endogenous inhibitor that maintains the quiescence of MEF2 in unstimulated cells. The dissociation of the Cabin1-MEF2 complex by calmodulin provides a calcium switch for MEF2 activation and represents a novel mechanism of transcriptional activation by calcium. Although it remains to be elucidated how calcineurin modulates MEF2, it has been shown that activated nuclear factor AT is involved in enhancing the transcriptional activity of MEF2 in response to calcineurin (*26*). Thus, calcium signaling bifurcates, one mediated through CaM, Cabin1, and MEF2 and the other via CaM, calcineurin, and MEF2. The binding of Cabin1 to MEF2 exerts one level of control over MEF2 activity. Dissociation of MEF2 from Cabin1 via competition by activated CaM defines a novel signaling pathway involved in Nur77 expression and thymocyte apoptosis. Given that Cabin1 is ubiquitously expressed (*13*) and that it interacts with all known isoforms of MEF2, this mode of regulation of MEF2 by Cabin1 and CaM may be operative in other physiological processes involving MEF2 (*25*).

References and Notes

1. A. Winoto, *Curr. Opin. Immunol.* **9**, 365 (1997).
2. B. Wong and Y. Choi, *ibid.*, p. 358.
3. J. D. Woronicz, B. Calnan, V. Ngo, A. Winoto, *Nature* **367**, 277 (1994).
4. Z. G. Liu, S. W. Smith, K. A. McLaughlin, L. M. Schwartz, B. A. Osborne, *ibid.*, p. 281.
5. L. E. Cheng, F. K. Chan, D. Cado, A. Winoto, *EMBO J.* **16**, 1865 (1997).
6. J. D. Woronicz *et al.*, *Mol. Cell. Biol.* **15**, 6364 (1995).
7. L. A. Gossett, D. J. Kelvin, E. A. Sternberg, E. N. Olson, *ibid.* **9**, 5022 (1989).
8. R. Pollock and R. Treisman, *Genes Dev.* **5**, 2327 (1991).
9. Y. T. Yu *et al.*, *ibid.* **6**, 1783 (1992).
10. D. Leifer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1546 (1993).
11. J. F. Martin, J. J. Schwarz, E. N. Olson, *ibid.*, p. 5282.
12. J. F. Martin *et al.*, *Mol. Cell. Biol.* **14**, 1647 (1994).
13. L. Sun *et al.*, *Immunity* **8**, 703 (1998).
14. M. M. Lai, P. E. Burnett, H. Wolosker, S. Blackshaw, S. H. Snyder, *J. Biol. Chem.* **273**, 18325 (1998).
15. H.-D. Youn and J. O. Liu, unpublished data.
16. H.-L. Hsu, I. Wadman, R. Baer, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3181 (1994).
17. H.-D. Youn and J. O. Liu, unpublished data.
18. Web figure 1 can be found at www.sciencemag.org/feature/data/1039959.shl.
19. M. Ikura *et al.*, *Science* **256**, 632 (1992).
20. B. Corneliussen *et al.*, *Nature* **368**, 760 (1994).
21. DO11.10 cells were lysed in buffer B [20 mM tris-HCl (pH 7.4), 0.1 M NaCl, 1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride (PMSF)] with either 2 mM $CaCl_2$ or 10 mM EGTA, incubated with CaM-sepharose (Pharmacia) for 4 hours, and washed four times with buffer B. Recombinant GST-Cabin1(2037–2220) (500 ng) was incubated in buffer B in the presence of either 2 mM $CaCl_2$ or 10 mM EGTA for 1 hour, followed by incubation with CaM-sepharose for 1 hour. The beads were washed four times with Buffer B. Proteins bound to beads were resolved by SDS–polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose membrane, and probed using anti–GST-Cabin1 polyclonal antibodies, which recognize both GST and Cabin 1 polypeptides.
22. MEF2D protein was labeled with [^{35}S]methionine using an in vitro transcription/translation system (Novagen). Translated MEF2D (50 μl per lane) was diluted with 1 ml of tris buffer [50 mM tris (pH 7.4), 100 mM NaCl, 0.5% NP-40, and 1 mM PMSF] in the presence of either 2 mM $CaCl_2$ or 10 mM EGTA. 0.5 μg of GST-Cabin1(2037–2220) was added alone or in combination with 5 μg of CaM to MEF2D-translated products. Reaction mixtures were precipitated using glutathione beads after a 2-hour incubation, separated by10% SDS-PAGE, and developed by autoradiography.
23. Web figure 2 can be found at www.sciencemag.org/feature/data/1039959.shl.
24. The pBabeSVCabin1 expression vector was constructed by subcloning the Sal I fragment from pSG-Cabin1, which contains an SV40 promoter and a polyadenylation site flanking the full-length Cabin1 cDNA, into the Sal I site of the pBabe vector [W. S. Pear, G. P. Nolan, M. L. Scott, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8392 (1993)]. DO11.10 cells were transfected with pBabeSVCabin1 and incubated for 24 hours. Transfected cells were treated with puromycin (2 μg/ml) for another 24 hours and dead cells were removed by Ficoll gradient centrifugation. Puromycin-resistant cells were selected by diluting (1:10) transfected cells into puromycin-containing RPMI medium every 3 days until all control DO11.10 cells were dead. The expression of exogenous Cabin1 was confirmed by protein immunoblot using a c-Myc mAb. DNA fragmentation assay was performed as described [K. S. Sellins and J. J. Cohen, *J. Immunol.* **139**, 3199 (1987)].
25. B. L. Black and E. N. Olsen, *Annu. Rev. Cell Dev. Biol.* **14**, 167 (1998).
26. H.-D. Youn, T. Chatila, J. O. Liu, unpublished data.

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References and Notes

[10] **MEF2C, a MADS/MEF2-Family Transcription Factor Expressed in a Laminar Distribution in Cerebral Cortex**

Dana Leifer; Dimitri Krainc; Yie-Teh Yu; John McDermott; Roger E. Breitbart; John Heng; Rachael L. Neve; Barry Kosofsky; Bernardo Nadal-Ginard; Stuart A. Lipton

Proceedings of the National Academy of Sciences of the United States of America, Vol. 90, No. 4. (Feb. 15, 1993), pp. 1546-1550.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%2819930215%2990%3A4%3C1546%3AMAMTFE%3E2.0.CO%3B2-X

[11] **Myocyte Enhancer Factor (MEF) 2C: A Tissue-Restricted Member of the MEF-2 Family of Transcription Factors**

James F. Martin; John J. Schwarz; Eric N. Olson

Proceedings of the National Academy of Sciences of the United States of America, Vol. 90, No. 11. (Jun. 1, 1993), pp. 5282-5286.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%2819930601%2990%3A11%3C5282%3AMEF%282A%3E2.0.CO%3B2-D

[16] **Formation of in vivo Complexes Between the TAL1 and E2A Polypeptides of Leukemic T Cells**

Hai-Ling Hsu; Isobel Wadman; Richard Baer

Proceedings of the National Academy of Sciences of the United States of America, Vol. 91, No. 8. (Apr. 12, 1994), pp. 3181-3185.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%2819940412%2991%3A8%3C3181%3AFOIVCB%3E2.0.CO%3B2-L

LINKED CITATIONS

[19] **Solution Structure of a Calmodulin-Target Peptide Complex by Multidimensional NMR**
Mitsuhiko Ikura; G. Marius Clore; Angela M. Gronenborn; Guang Zhu; Claude B. Klee; Ad Bax
Science, New Series, Vol. 256, No. 5057. (May 1, 1992), pp. 632-638.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819920501%293%3A256%3A5057%3C632%3ASSOACP%3E2.0.CO%3B2-W

[24] **Production of High-Titer Helper-Free Retroviruses by Transient Transfection**
Warren S. Pear; Garry P. Nolan; Martin L. Scott; David Baltimore
Proceedings of the National Academy of Sciences of the United States of America, Vol. 90, No. 18. (Sep. 15, 1993), pp. 8392-8396.
Stable URL:
http://links.jstor.org/sici?sici=0027-8424%2819930915%2990%3A18%3C8392%3APOHHRB%3E2.0.CO%3B2-P