Current Biology, Vol. 14, 2156–2161, December 14, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.11.002

Activation of cAMP Response Element-Mediated Gene Expression by Regulated Nuclear Transport of TORC Proteins

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Summary

The CREB family of proteins are critical mediators of gene expression in response to extracellular signals and are essential regulators of adaptive behavior and long-term memory formation [1–4]. The TORC proteins were recently described as potent CREB coactivators, but their role in regulation of CREB activity remained unknown [5, 6]. TORC proteins were found to be exported from the nucleus in a CRM1-dependent fashion. A high-throughput microscopy-based screen was developed to identify genes and pathways capable of inducing nuclear TORC accumulation. Expression of the catalytic subunit of PKA and the calcium channel TRPV6 relocalized TORC1 to the nucleus. Nuclear accumulation of the three human TORC proteins was induced by increasing intracellular cAMP or calcium levels. TORC1 and TORC2 translocation in response to calcium, but not cAMP, was mediated by calcineurin, and TORC1 was shown to be directly dephosphorylated by calcineurin. TORC function was shown to be essential for CRE-mediated gene expression induced by cAMP, calcium, or GPCR activation, and nuclear transport of TORC1 was sufficient to activate CRE-dependent transcription. *Drosophila* TORC was also shown to translocate in response to calcineurin activation in vivo. Thus, TORC nuclear translocation is an essential, conserved step in activation of cAMP-responsive genes.

Results and Discussion

Identification of Genes that Affect TORC Localization

Although transducers of regulated CREB (TORCs) are transcriptional coactivators, both an amino-terminal FLAG-TORC1 fusion protein as well as a carboxy-terminal TORC1-eGFP fusion protein were present predominantly in the cytoplasm of transfected HeLa cells (Figures 1A and 1C). Whereas TORC2- and TORC3-eGFP fusions were constitutively present in the nucleus in HeLa cells (see Figure S1, available with this paper's Supplemental Data online), they were cytoplasmic in HEK293 cells (Figures 1E and 1G). All of the human TORCs accumulated in the nucleus after treatment with leptomycin B (LMB), an inhibitor of CRM1-mediated nuclear export of proteins [7] (Figures 1B, 1D, 1F, and 1H), indicating that all TORCs were subject to active nuclear export.

A microscopy-based screen was developed to identify genes that induce nuclear accumulation of TORC1-eGFP. HeLa cells were cotransfected with TORC1-eGFP in combination with approximately 7680 individual full-length cDNA constructs [8], and the relative amounts of eGFP fluorescence in the cytoplasm and the nucleus were determined with an automated imaging system (Figure 1I). Translocation induced by LMB was detected in virtually all control wells. We utilized confocal microscopy to confirm TORC1-eGFP translocation in the highest scoring hits. Although most of the potential hits from this screen represented dying, rounded cells, two cDNAs encoding the murine transient receptor potential cation channel, subfamily V, member 6 (TRPV6) and a murine cyclic AMP (cAMP)-dependent protein kinase A catalytic subunit (cAMP-dependent protein kinase [PKA]) reproducibly resulted in TORC1-eGFP translocation. HeLa cells stably expressing TORC1-eGFP (HeLa::TORC1-eGFP cells) were transfected with either the TRPV6 or PKA cDNAs along with an alkaline phosphatase expression plasmid to mark transfected cells, and they were evaluated by confocal microscopy. Both TRPV6 and PKA resulted in virtually all the TORC1-eGFP moving to the nucleus in transfected cells (Figures 1J–1L).

TORC Translocation Is Regulated by cAMP

Translocation induced by PKA suggests that TORC localization would be regulated by cAMP levels. Indeed, TORC1-eGFP was efficiently shuttled to the nucleus after treatment of HeLa cells with either a combination of forskolin (an adenyl cyclase agonist) and 3-isobutyl-1-methylxanthine (IBMX; a phosphodiesterase inhibitor) or dibutyryl-cAMP (db-cAMP) alone (Figures 2A–2C). Similarly, TORC2-eGFP and TORC3-eGFP translocated to the nucleus in response to forskolin in stably expressing HEK293 cells (Figures 2D, 2E, 2G, and 2H), and both TORC2-eGFP and TORC3-eGFP accumulated in the nucleus in cells transfected with PKA (data not shown). Localization in the HEK293::TORC-eGFP cell lines was examined in response to isoproterenol, a β2-adrenergic receptor agonist, to determine if TORCs also respond to signaling through endogenous Gs-coupled receptors. Both TORC2-eGFP and TORC3-eGFP rapidly accumulated in the nucleus after exposure to isoproterenol (Figures 2F and 2I). The localization of endogenous TORC2 was examined in HEK293 cells by immunohistochemical staining after forskolin exposure to confirm that endogenous TORCs translocate in response to cAMP. Untreated cells displayed diffuse TORC2 staining throughout the cell with little clear nuclear staining (compare 2J and 2L, stained with Hoechst). In contrast, cells exposed to forskolin displayed predominantly nuclear TORC2 staining indistinguishable from that seen when the nuclei were stained with Hoechst (Figures 2K and 2M). In contrast to HeLa cells, the nuclear import of

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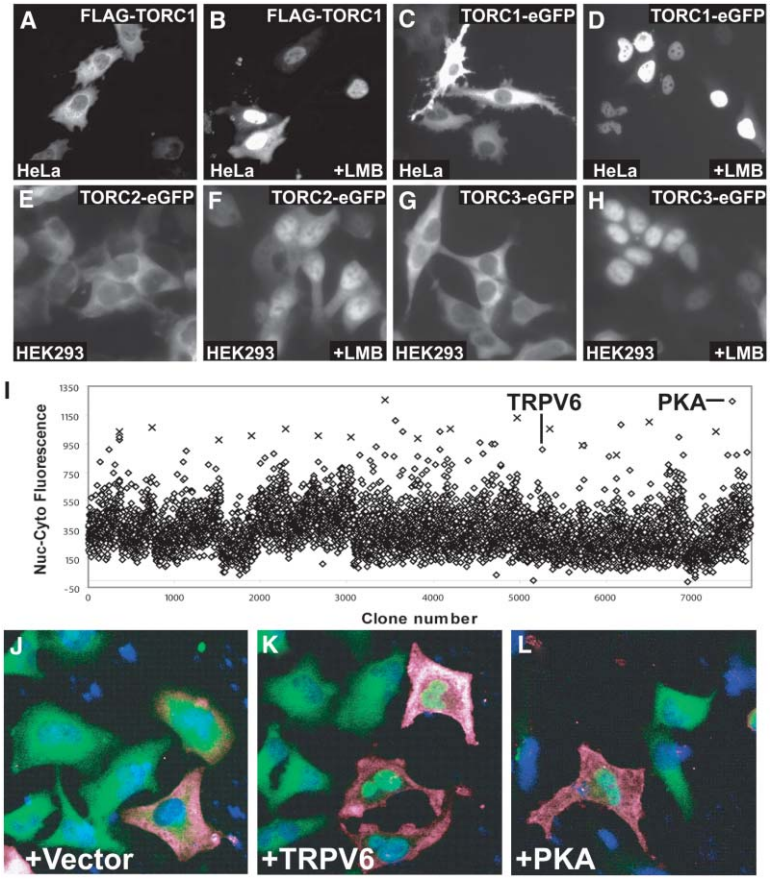

Figure 1. TORC Proteins Are Regulated by Subcellular Compartmentalization

HeLa cells were transfected with FLAG-TORC1 (A and B) or TORC1-eGFP (C and D). HEK293 cells were transfected with TORC2-eGFP (E and F) or TORC3-eGFP (G and H). Cells were either untreated or treated with LMB for 90 min. FLAG-tagged protein was visualized by immunofluorescence, and eGFP-tagged proteins were directly visualized by fluorescence in all experiments. The ratio of nuclear/cytoplasmic fluorescence of TORC1-eGFP after cotransfection with 7680 individual cDNAs is shown (I). Data for control wells treated with LMB are shown as X's, and those containing TRPV6 and PKA are indicated. Confocal localization of TORC1-eGFP transfected with empty vector (J), TRPV6 (K), or PKA (L) expression plasmids is shown. Alkaline phosphatase staining (pink) marks transfected cells, with Hoechst nuclear DNA staining in blue and TORC1-eGFP in green.

TORC1-eGFP was slow in HEK293 cells and required both LMB and forskolin/IBMX or isoproterenol treatment (Figure S3; data not shown), suggesting the need for both an import signal and blockade of export in this cell line to observe the majority of TORC1 to accumulate in the nucleus.

TORC Translocation Is Regulated by Calcineurin

TRPV6 is a calcium channel that is suspected to be a store-operated channel involved in capacitative calcium entry (CCE), which can induce calcium entry current when overexpressed [9]. CCE induces nuclear translocation of nuclear factor of activated T cells (NF-AT) through dephosphorylation of NF-AT by the calcium-dependent phosphatase calcineurin [10]. The localization of TORC1 was examined in response to ionomycin, a calcium ionophore, or cyclopiazonic acid (CPA), an inhibitor of the sarcoplasmic-endoplasmic reticulum calcium ATPase, to determine if TORCs were regulated in a manner similar to NF-AT. Both agents induce elevated intracellular calcium levels, and both potently induced nuclear accumulation of TORC1-eGFP (Figures 3A–3B; Figures S2A–S2C). Translocation in response to ionomycin was blocked by the immunophillin binding calcineurin inhibitors CsA and FK506 (Figures 3C and 3D) but not by rapamycin, an immunophillin binding compound that does not inhibit calcineurin (Figures S2D–S2F). Expression of a constitutively active calcineurin mutant was sufficient to induce nuclear accumulation of TORC1-eGFP (Figure 3E). The effect of CsA on TRPV6-induced translocation was also examined. Expression of TRPV6 efficiently induced nuclear translocation of TORC1-eGFP (Figures 3F and 3G). Treatment of TRPV6-overexpressing cells with CsA for 1 hr resulted in nearly complete transport of TORC1-eGFP back to the cytoplasm (Figure 3H), confirming that TORC translocation was specifically due to calcineurin activation and not due to other potentially toxic side-effects of TRPV6 overexpression.

It should be noted that transfection with an active calcineurin construct induced nuclear translocation of TORC2-eGFP but not TORC3-eGFP in HEK293 cells, yet nuclear translocation of all three human TORC-eGFP proteins in HEK293 cells was facilitated by ionomycin. Further, the ionomycin-induced translocation of TORC1-eGFP was completely blocked by CsA, whereas TORC2-eGFP was partially inhibited (Figure S3). The insensitivity of translocation to CsA by TORC2 and TORC3 may indicate that there are multiple calcium-induced events that can lead to translocation and that calcineurin activation is sufficient, but not necessary, for TORC2 translocation in response to calcium in HEK293 cells.

The effect of TRPV6 on expression of a calcineurin-dependent NF-AT-driven reporter gene was examined to confirm that TRPV6 was indeed an activator of calcineurin. TRPV6 transfection potently induced an NF-AT-dependent luciferase reporter gene in a CsA-sensitive manner (Figure 3I). These results indicate that TORC

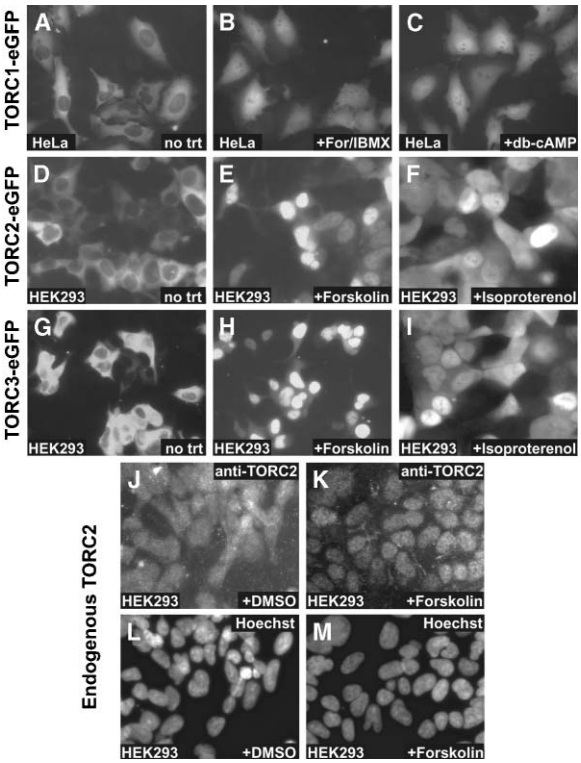

Figure 2. TORCs Translocate in Response to Elevated cAMP Levels

HeLa cells stably expressing TORC1-eGFP were exposed to either no treatment (A), forskolin and IBMX (B), or db-cAMP (C) for 2 hr. HEK293 cells stably expressing TORC2-eGFP (D–F) or TORC3-eGFP (G–I) were either untreated (D and G), treated with forskolin for 1 hr (E and H), or treated with isoproterenol for 1 hr (F and I). Endogenous TORC2 was visualized in HEK293 cells that were either treated with the vehicle DMSO (J and L) or with forskolin for 90 min (K and M). Anti-TORC2 staining is shown (J and K) with the corresponding Hoechst nuclear staining (L and M).

translocation is likely to occur concomitantly with that of NF-AT and that TORC translocation may be a novel target of the immunophillin binding immunosuppressants. Additionally, this is the first demonstration that TRPV6 expression is sufficient to activate calcineurin and ultimately NF-AT activity.

The role of calcineurin on nuclear transport of TORC1 induced by other stimuli was also examined. CsA did not block nuclear accumulation of any of the TORCs in response to increased intracellular cAMP levels induced by forskolin (Figures 3J and 3K; data not shown). TORC translocation was also induced by either ultraviolet (UV) irradiation of cells or the protein kinase C agonist phorbol 12-myristate-13-acetate (PMA) (Figures 3L–3O), and translocation by these stimuli was blocked by CsA treatment, demonstrating a requirement for calcineurin in these pathways. Thus, at least two independent pathways regulate TORC nuclear translocation: a calcineurin-dependent pathway and a cAMP-dependent calcineurin-independent pathway.

FLAG-TORC1 protein was examined for calcineurin-dependent post-translational modification to determine if TORC proteins were directly or indirectly modified by calcineurin. The FLAG-TORC1 was used for these studies because of the low abundance of endogenous TORC proteins and the complication of multiple size species of endogenous TORC1 proteins, possibly due to alternative splicing (see below). Coexpression of active calcineurin with FLAG-TORC1 in HeLa cells increases the electrophoretic mobility of FLAG-TORC1, and this mobility shift is blocked by exposure to CsA (Figure 3P), demonstrating that either active calcineurin itself or a protein activated by calcineurin modifies TORC1 in vivo. These observed mobility shifts were consistent with a calcineurin-dependent dephosphorylation of FLAG-TORC1. FLAG-TORC1 protein was purified from transfected cells and treated with alkaline phosphatase or purified calcineurin to determine if FLAG-TORC1 is directly dephosphorylated by calcineurin. Both calcineurin and alkaline phosphatase treatment increased the electrophoretic mobility of FLAG-TORC1, and both alkaline phosphatase and calcineurin significantly decreased reactivity of the FLAG-TORC1 protein with a phosphoserine-specific antibody (Figure 3Q), demonstrating that TORC1 is a phosphoprotein that is directly dephosphorylated by calcineurin in vitro.

The subcellular localization of the single ancestral *Drosophila* TORC (dTORC) was examined to determine whether regulated nuclear translocation is a general property of TORC proteins. An inducible eGFP-dTORC transgene was constructed and stably integrated into the *Drosophila* genome. Examination of intact salivary tissue from transgenic larvae demonstrated that dTORC was predominantly present in the cytoplasm in untreated cells and rapidly translocated to the nucleus upon ionomycin exposure (Figures 3R and 3S), with the majority of the dTORC shuttling to the nucleus 10 min after ionomycin exposure (see Movie 1). Cotreatment with CsA completely blocked dTORC translocation in response to ionomycin (Figure 3T), indicating that participation of calcineurin in the calcium-mediated cAMP response element binding protein (CREB) response is conserved between vertebrates and insects.

TORC Translocation Is Necessary and Sufficient for CRE Activation

Induction of a cAMP response element (CRE)-dependent luciferase reporter (CRE-luc) was compared in naive HeLa cells and HeLa cells stably expressing a TORC1-eGFP transgene to assess the affect of TORC1 localization on gene expression. Western blot analysis indicated that HeLa::TORC1-eGFP cells expressed significantly more TORC1 than naive cells (data not shown). Untreated naive HeLa cells and HeLa::TORC1-eGFP cells displayed similar levels of CRE-driven luciferase, whereas LMB treatment resulted in a significant induction only in the HeLa::TORC1-eGFP cells (Figure 4A). This observation indicates that nuclear accumulation of TORC1-eGFP in the nucleus was sufficient to activate CRE-driven gene expression. In addition, while ionomycin and PMA resulted in a modest level of activation in HeLa cells, these agents showed more than 10-fold larger increases in CRE-luciferase induction in HeLa::TORC1-eGFP cells. Induction by ionomycin was completely blocked by CsA. Thus, overexpression of TORC1 potently enhanced activation of CRE-driven gene ex-

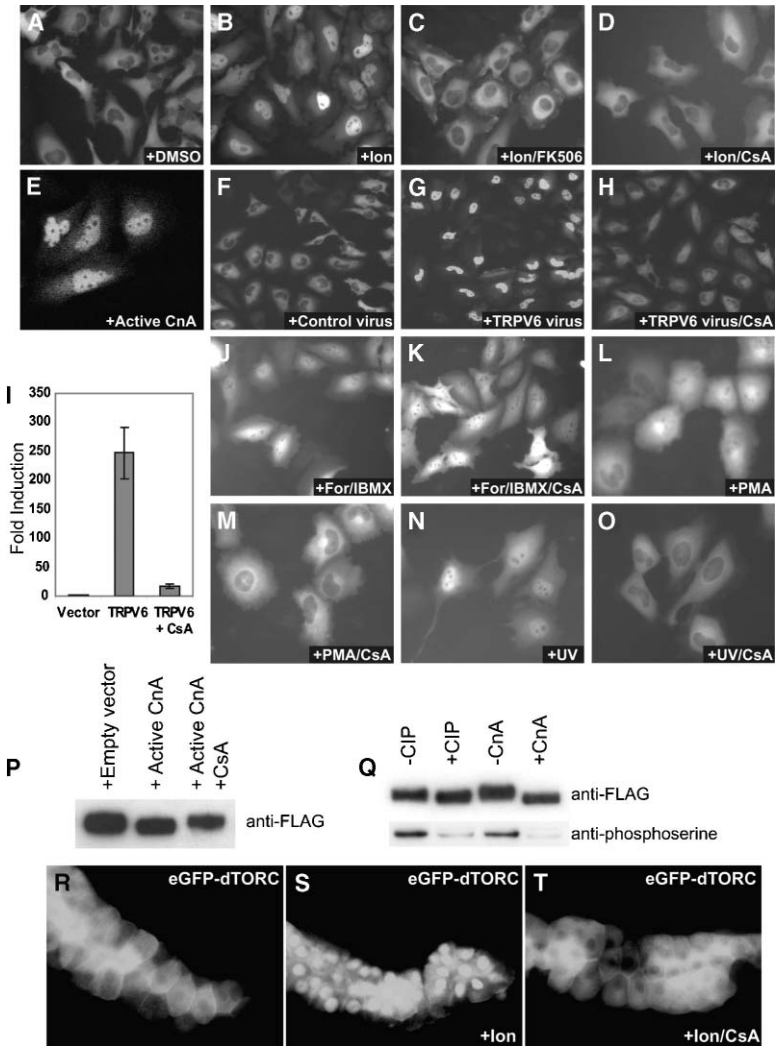

Figure 3. TORC1 Translocates in Response to Elevated Calcium Levels through Calcineurin Activation

HeLa cells stably expressing TORC1-eGFP were exposed to either DMSO (A) or ionomycin (B–D) for 1 hr. Exposure to FK506 (C) or CsA (D) for 1 hr completely blocked translocation in response to ionomycin. HeLa cells stably expressing TORC1-eGFP transiently transfected with a plasmid expressing a constitutively active form of calcineurin (E). HeLa::TORC1-eGFP cells were transduced with either the stop codon control virus (F) or a virus expressing TRPV6 (G and H) and were either untreated (F and G) or treated with CsA for 1 hr (H). HEK293 cells were transiently transfected with either empty vector or TRPV6 expression vector in combination with an NF-AT-dependent luciferase reporter in the presence or absence of CsA (I). Luciferase values are normalized to a cotransfected reporter to control for transfection efficiency in all experiments. HeLa::TORC1-eGFP cells were treated with forskolin/IBMX (J), forskolin/IBMX in combination with CsA (K), or PMA (L and M) or were exposed to 254 nM UV light (N and O). CsA blocked translocation by both PMA and UV (M and O). HeLa cells transfected with FLAG-TORC1 in combination with either the empty vector control or active calcineurin and were either untreated or treated with CsA, and the mobility of FLAG-TORC1 was examined by Western analysis of whole cell lysate (P). Purified FLAG-TORC1 protein was incubated in the presence or absence of either calf intestinal alkaline phosphatase (CIP) or calcineurin (CnA) before Western analysis (Q) with either anti-FLAG antibodies or antiphosphoserine antibodies. Salivary-gland tissue expressing dTORC-eGFP from *Drosophila* larvae was exposed to either the vehicle DMSO (R), 5 μM ionomycin (S), or 5 μM ionomycin with 10 μM CsA (T) for 1 hr.

pression in a calcineurin-dependent fashion. These observations suggest that the reported ability of CsA and FK506 to block induction of a subset of CREB-responsive genes in response to cAMP and membrane depolarization [11] is likely due to blockade of TORC function by these agents. Since the effect of LMB is significantly less than that observed with ionomycin, which also induces nuclear accumulation, it is likely that other signals or modifications are required in combination with nuclear localization to achieve maximal transcriptional activation.

Expression of both TORC1 and TORC2 was decreased by siRNAs in HeLa cells to determine if TORCs are essential for activation of CRE-dependent transcription. In contrast to HEK293 cells, which were reported to predominantly express only TORC2 [6], HeLa cells express comparable levels of both TORC1 and TORC2 mRNA and protein (data not shown; see below). Knockdown of either TORC1 or TORC2 alone was sufficient to modestly decrease CRE activation by forskolin/IBMX or ionomycin/PMA in HeLa cells (Figure 4B). In contrast, simultaneous blockade with TORC1 and TORC2 siRNAs strongly blocked induction by either stimulus. These effects were specific because the TORC siRNAs had little effect on induction of an NF-AT-dependent luciferase reporter by ionomycin and PMA. It is important to note that NF-AT is also activated by calcium in a calcineurin-dependent fashion; thus, the effect of TORC blockade is highly specific and downstream of calcineurin. The siRNAs were shown to block TORC1 and TORC2 protein production by Western blot analysis (Figure 4C). Decreased levels of TORC1 and TORC2 proteins had no effect on CREB1 phosphorylation by either stimulus, consistent with previous observations that TORCs activate CRE-driven expression independently of CREB phosphorlyation [6]. Although a single endogenous TORC2 species was observed in HeLa cells, at least two distinguishable isoforms of endogenous TORC1 are detected by Western analysis. These multiple TORC1 bands were not due to cross-reactivity of the antibodies to TORC2 or TORC3 because these isoforms were detected with multiple antibodies that recognize distinct epitopes of TORC1, and the abundance of these proteins was decreased specifically with TORC1 siRNA (data not shown).

The siRNA data above is complicated by the need to use multiple siRNAs and block multiple functionally redundant TORC proteins. A dominant-interfering TORC

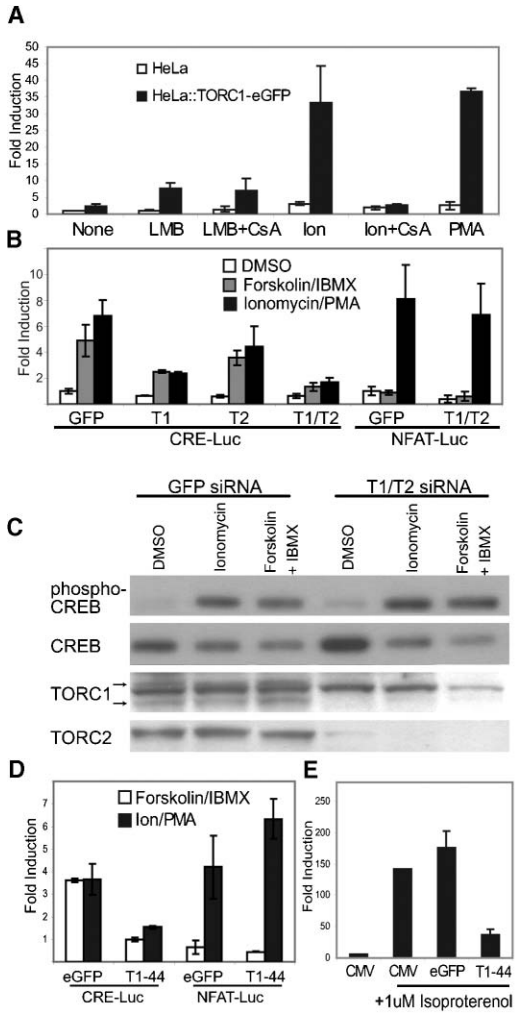

Figure 4. TORC Nuclear Translocation Is Necessary and Sufficient for CRE Activation

HeLa cells and HeLa cells stably expressing TORC1-eGFP were transfected with CRE-luc and stimulated with LMB, CsA, ionomycin, PMA, or the indicated combinations (A). HeLa cells were transiently transfected with either a CRE or NF-AT luciferase plasmid before transfection with siRNA versus GFP, TORC1 (T1), TORC2 (T2), or a combination of both TORC1 and TORC2 (T1/T2), and they were treated with either DMSO, forskolin and IBMX, or ionomycin with PMA (B). HeLa cells were transfected with either GFP or both TORC1- and TORC2-specific siRNAs before induction with DMSO, ionomycin, or forskolin and IBMX for 20 min and then underwent lysis and Western blot analysis with antibodies specific for the indicated proteins (C). TORC1-specific antibodies detect at least two distinct TORC1 species, indicated by arrows. HeLa cells were transfected with either the eGFP control or TORC1-44-eGFP expression vectors with either CRE luciferase or NF-AT luciferase before stimulation with forskolin and IBMX or ionomycin and PMA (D). HEK293 cells were transiently transfected with CRE-luc and β2-adrenergic receptor cDNA in combination with either the empty CMV vector, eGFP, or T1-44-eGFP and were exposed to isoproterenol for 18 hr (E).

protein that fuses the first 44 amino acids of TORC1, which constitutes the highly conserved CREB binding domain, to eGFP (T1-44eGFP) was designed to confirm the essential role of TORCs in CRE-driven transcription. This protein was shown to block activation by all three TORC proteins (data not shown). Expression of the T1-44eGFP fusion protein potently blocked CRE activation through either forskolin/IBMX or ionomycin/PMA but had no effect on activation of an NF-AT reporter by ionomycin/PMA (Figure 4D). Additionally, expression of T1-44eGFP prevented CRE activation through a transfected β2-adrenergic receptor by isoproterenol (Figure 4E). Thus, TORC function is both sufficient and essential for CRE-driven gene expression induced by cAMP, calcium agonists, and activation of Gs-coupled GPCRs.

Conclusions

The data presented in this paper demonstrate that TORC translocation is a second, essential switch required for activation of CRE-mediated gene expression by many, if not all, inducers of CREB. CREB activation by cAMP appears to require simultaneous nuclear translocation of both PKA and TORC proteins. This observation provides a novel model that explains the rapid and efficient activation of gene expression by cAMP. Previous studies have strongly implicated calcium-mediated CREB activation in behavior, and yet calcium-mediated CREB phosphorylation was shown in some instances to be insufficient for transcriptional activation [12]. Nuclear translocation of TORC in response to elevated calcium levels provides a mechanism to explain these observations.

Translocation of the TORCs resembles that of NF-AT in several ways. Both protein families are subject to nuclear export and regulated nuclear import, and these processes are directly regulated by calcineurin. It is interesting to note that NF-AT has been shown to bind and be regulated by the phosphoprotein binding protein 14-3-3 [13], and a recent study demonstrated that TORC1 and TORC3 copurify with 14-3-3 from HEK293 cells [14], suggesting that these proteins may share additional regulatory mechanisms. Additionally, the conservation of calcineurin-dependent translocation in human and *Drosophila* TORC proteins further suggests the importance of calcineurin in regulating CRE-dependent gene activation. Finally, because TORC1 translocation was shown to be sufficient to activate CREB-mediated transcription, and activation of CREB has been suggested to be a viable approach to enhancing memory and may be neuroprotective [15], the discovery of novel compounds that induce TORC nuclear translocation may provide new therapeutic agents for use as neuroprotective agents or memory enhancers.

Supplemental Data

Detailed Experimental Procedures, as well as several supplemental figures and a movie, are available online at http://www.current-biology.com/cgi/content/full/14/24/2156/DC1/.

Acknowledgments

We thank Eric Olson for providing the constitutively active calcineurin expression plasmid and Dalia Cohen and Fintan Steele for discussions on the manuscript. As this manuscript was under final review, an independent study also demonstrated that TORC2 activity and localization are regulated by cAMP and calcium signals [16].

Received: August 6, 2004
Revised: October 1, 2004
Accepted: October 27, 2004
Published online: November 4, 2004

References

1. Mayr, B., and Montminy, M. (2001). Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat. Rev. Mol. Cell Biol. *2*, 599–609.
2. Yin, J.C., Wallach, J.S., Del Vecchio, M., Wilder, E.L., Zhou, H., Quinn, W.G., and Tully, T. (1994). Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell *79*, 49–58.
3. West, A.E., Chen, W.G., Dalva, M.B., Dolmetsch, R.E., Kornhauser, J.M., Shaywitz, A.J., Takasu, M.A., Tao, X., and Greenberg, M.E. (2001). Calcium regulation of neuronal gene expression. Proc. Natl. Acad. Sci. USA *98*, 11024–11031.
4. Lonze, B.E., and Ginty, D.D. (2002). Function and regulation of CREB family transcription factors in the nervous system. Neuron *35*, 605–623.
5. Iourgenko, V., Zhang, W., Mickanin, C., Daly, I., Jiang, C., Hexham, J.M., Orth, A.P., Miraglia, L., Meltzer, J., Garza, D., et al. (2003). Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. Proc. Natl. Acad. Sci. USA *100*, 12147–12152.
6. Conkright, M.D., Canettieri, G., Screaton, R., Guzman, E., Miraglia, L., Hogenesch, J.B., and Montminy, M. (2003). TORCs: Transducers of regulated CREB activity. Mol. Cell *12*, 413–423.
7. Kudo, N., Matsumori, N., Taoka, H., Fujiwara, D., Schreiner, E.P., Wolff, B., Yoshida, M., and Horinouchi, S. (1999). Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. Proc. Natl. Acad. Sci. USA *96*, 9112–9117.
8. Strausberg, R.L., Feingold, E.A., Grouse, L.H., Derge, J.G., Klausner, R.D., Collins, F.S., Wagner, L., Shenmen, C.M., Schuler, G.D., Altschul, S.F., et al. (2002). Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc. Natl. Acad. Sci. USA *99*, 16899–16903.
9. Cui, J., Bian, J.S., Kagan, A., and McDonald, T.V. (2002). CaT1 contributes to the stores-operated calcium current in Jurkat T-lymphocytes. J. Biol. Chem. *277*, 47175–47183.
10. Hogan, P.G., Chen, L., Nardone, J., and Rao, A. (2003). Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev. *17*, 2205–2232.
11. Siemann, G., Blume, R., Grapentin, D., Oetjen, E., Schwaninger, M., and Knepel, W. (1999). Inhibition of cyclic AMP response element-binding protein/cyclic AMP response element-mediated transcription by the immunosuppressive drugs cyclosporin A and FK506 depends on the promoter context. Mol. Pharmacol. *55*, 1094–1100.
12. Brindle, P., Nakajima, T., and Montminy, M. (1995). Multiple protein kinase A-regulated events are required for transcriptional induction by cAMP. Proc. Natl. Acad. Sci. USA *92*, 10521–10525.
13. Chow, C.W., and Davis, R.J. (2000). Integration of calcium and cyclic AMP signaling pathways by 14-3-3. Mol. Cell. Biol. *20*, 702–712.
14. Jin, J., Smith, F.D., Stark, C., Wells, C.D., Fawcett, J.P., Kulkarni, S., Metalnikov, P., O'Donnell, P., Taylor, P., Taylor, L., et al. (2004). Proteomic, functional, and domain-based analysis of in vivo 14-3-3 binding proteins involved in cytoskeletal regulation and cellular organization. Curr. Biol. *14*, 1436–1450.
15. Tully, T., Bourtchouladze, R., Scott, R., and Tallman, J. (2003). Targeting the CREB pathway for memory enhancers. Nat. Rev. Drug Discov. *2*, 267–277.
16. Screaton, R.A., Conkright, M.D., Katoh, Y., Best, J.L., Canettieri, G., Jeffries, S., Guzman, E., Niessen, S., Yates, J.R., 3rd, Takemori, H., et al. (2004). The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. Cell *119*, 61–74.