Calcineurin Mediates the Calcium-dependent Inhibition of Adipocyte Differentiation in 3T3-L1 Cells

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Recent studies have revealed that the calcium-dependent serine/threonine phosphatase calcineurin mediates the effects of intracellular calcium in many different cell types. In this study we investigated the role of calcineurin in the regulation of adipocyte differentiation. We found that the specific calcineurin inhibitors cyclosporin A and FK506 overcame the antidioprogenic effect of calcium ionophore on the differentiation of 3T3-L1 preadipocytes. This finding suggests that calcineurin is responsible for mediating the previously documented Ca\(^{2+}\)-dependent inhibition of adipogenesis. We further demonstrate that the expression of a constitutively active calcineurin mutant potently inhibits the ability of 3T3-L1 cells to undergo adipocyte differentiation by preventing expression of the proadipogenic traits commitment markers, peroxisome proliferator-activated receptor \(\gamma\) (PPAR\(\gamma\)) and CCAAT/enhancer-binding protein \(\alpha\) (C/EBP\(\alpha\)). This calcineurin-mediated block in adipocyte differentiation is rescued by ectopic expression of PPAR\(\gamma\). Finally, we demonstrate that inhibition of endogenous calcineurin activity with either FK506 or a specific calcineurin inhibitory peptide enhances differentiation of 3T3-L1 cells in response to suboptimal adipogenic stimuli, suggesting that endogenous calcineurin activity normally sets a signaling threshold that antagonizes efficient adipocyte differentiation. Collectively, these data indicate that calcineurin acts as a Ca\(^{2+}\)-dependent molecular switch that negatively regulates commitment to adipocyte differentiation by preventing the expression of critical proadipogenic transcription factors.

Adipocytes are highly specialized cells that play a key role in energy homeostasis by regulating the storage and release of energy in response to changing nutritional needs (1). In addition to their role in energy balance, adipocytes also perform important endocrine functions by secreting a variety of factors that regulate such processes as food intake, insulin responsiveness, reproduction, vascular remodeling, and the immune response (2). Although adipocytes clearly play an important physiological role, the excessive accumulation of adipose tissue can result in obesity, which is known to be a significant risk factor for a number of other disease states including insulin resistance, type-2 diabetes, hypertensions, cardiovascular disease and cancer (3). Obesity can arise from either an increase in individual adipocyte cell size or from an increase in total adipocyte cell number as a result of increased de novo adipocyte differentiation (1). Accordingly, the molecular mechanisms that govern the regulation of adipocyte growth and differentiation are of considerable scientific interest and have been the subject of much investigation (1, 4, 5).

Considerable progress in our understanding of adipocyte biology has come from the study of the 3T3-L1 preadipocyte cell line (6), which under the appropriate in vitro culture conditions can be efficiently induced to undergo terminal differentiation into morphologically distinct, triglyceride-laden, mature adipocytes. Adipocyte differentiation is induced in 3T3-L1 preadipocytes by treatment of confluent, growth-arrested cells with the adipogenic hormones methylisobutylxanthine (Mix),\(^1\) dexamethasone (Dex), and insulin, collectively known as MDI. A large body of accumulated data has revealed that this process of adipocyte differentiation proceeds via a highly orchestrated and coordinated cascade of transcription factors, including members of the CCAAT/enhancer-binding protein (C/EBP) family and peroxisome proliferator-activated receptor \(\gamma\) (PPAR\(\gamma\)) (1, 4, 5). MDI-treated, growth-arrested 3T3-L1 preadipocytes synchronously enter the cell cycle and initially express the early transcription factors C/EBP\(\beta\) and C/EBP\(\delta\) (7, 8). C/EBP\(\beta\) and C/EBP\(\delta\) then elicit the expression of the proadipogenic transcription factors PPAR\(\gamma\) (7), which in turn induces the expression of C/EBP\(\alpha\) (9). Together, PPAR\(\gamma\) and C/EBP\(\alpha\) then are believed to play a dual role in adipogenesis by first inducing withdrawal from the cell cycle and then directing the expression of adipocyte-specific genes that ultimately result in the acquisition of the mature adipocyte cell fate (10–13).

This process of adipocyte differentiation is influenced by a variety of different extrinsic factors and intracellular signaling pathways (4, 14). Of particular interest to the current study are the effects of intracellular calcium on adipocyte differentiation. A number of reports have demonstrated that increases in intracellular calcium concentration ([Ca\(^{2+}\)]) during the early phase of human and 3T3-L1 preadipocyte differentiation act to potently inhibit adipogenesis (15–17). However, the effects of calcium on this process may be complex, because increases in [Ca\(^{2+}\)], in human preadipocytes during the later stages of dif-

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\(1\) The abbreviations used are: Mix, methylisobutylxanthine; Dex, dexamethasone; MDI, methylisobutylxanthine, dexamethasone and insulin; C/EBP, CCAAT/enhancer-binding protein; PPAR\(\gamma\), peroxisome proliferator-activated receptor \(\gamma\); NFAT, nuclear factor of activated T cells; [Ca\(^{2+}\)], intracellular calcium concentration; CsA, cyclosporin A; CNmut, constitutively activated calcineurin mutant; MSCV, murine stem cell virus; IRES, internal ribosomal entry sequence; pEGFP, permitted enhanced green fluorescent protein; Ab, antibody; LTR, long terminal repeat.

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EXPERIMENTAL PROCEDURES

Cell Culture and Adipocyte Differentiation—3T3-L1 preadipocytes (ATCC) were cultured in a growth medium of Dulbecco’s modified Eagle’s medium with high glucose (Invitrogen) supplemented with 10% (v/v) fetal calf serum (Hyclone), 100 units/ml penicillin G, and 100 μg/ml streptomycin (Invitrogen). To induce adipocyte differentiation, cells were grown until 2 days postconfluence (day 0) and then treated for 2 days with growth medium plus MDI (0.5 mM methylisobutylxanthine, 1 μM dexamethasone, and 10 μg/ml insulin, all from Sigma). The cells were re-fed with growth medium that contained 10 μg/ml insulin at day 2 and every 2 days thereafter with growth medium alone. After 10 days, cells were fixed with formalin and stained with the lipophilic dye Oil Red O (Sigma). Stained cells were either photographed or counterstained with Giemsa and visualized by bright field microscopy. Where indicated, cells were treated additionally with 2 μM ionomycin, 5 ng/ml FK506, 1 μg/ml CsA (all from Calbiochem), or vehicle control (ethanol).

Retroviral Expression Constructs—The retroviral expression vector pMSCV-CNNmut was generated by insertion of a XhoI-EcoRI fragment that contained the previously described CNmut (21) into pMSCV-GFP downstream of the viral long terminal repeat and upstream of the IRES-GFP cassette. The pMSCV-H2K retroviral expression vector was created by replacing GFP in the pMSCV-GFP retroviral expression vector with a PCR-amplified truncated murine major histocompatibility class I H-2Kk cDNA from pMACS Kk.II (Miltenyi Biotec). pMSCV-PARP-1 was created by introducing the full-length, cDNA-encoding murine PARP-1 (a gift from J. Reddy, Northwestern University) into pMSCV-H2K. pMSCV-VIVIT-GFP was constructed by inserting an oligonucleotide that encoded a previously described (32) calcineurin-inhibitory peptide (MAGHPHPVITGPHE) into eGFP-N5 (Clontech) and then introducing the resulting VIVIT-GFP fusion sequence into pMSCV-H2K.

Retrovirus Production and Infection of 3T3-L1 Cells—Retroviral expression vectors were cotransfected with pSVS-G (Clontech) into the GP293 panretic packaging cell line (Clontech) by using LipoAMINE Plus (Invitrogen). The medium was replaced after 24 h, and viral supernatants were harvested at 2 days post-transfection and stored at –80 °C. For infections, 5 × 10^4 3T3-L1 cells were plated per well of a 6-well plate. The next day, the medium was replaced with 2 ml of viral supernatant that contained 8 μg/ml polybrene (Sigma), and plates were centrifuged at 2000 rpm for 1.5 h at room temperature. After removal of the viral supernatant, cells were expanded in growth medium for subsequent analysis. For double infections, previously infected cells were replated after 3 days and infected with the second virus as described previously.

Flow Cytometric Analysis—On day 3 after infection, cells were analyzed for GFP fluorescence or were stained additionally with a phycocyanin-coupled anti-mouse H-2Kk Ab (56–7.5; BD Biosciences). 10,000 events were analyzed using a FACS Caliber flow cytometer and CELLQuest software (BD Biosciences).

Immunoblot and Northern Blot Analysis—Protein extracts prepared from cells harvested at the indicated times postdifferentiation were resolved by SDS-PAGE and subjected to immunoblot analysis with the relevant Abs. All Abs (PPARγ (H-100), C/EBPδ (14AA), C/EBPβ (H-7), and C/EBPα (C-20)) were purchased from Santa Cruz Biotechnology. For Northern blot analysis, total RNA was isolated from cells by using Trizol (Invitrogen) on the indicated day after differentiation was induced. RNA samples (10 μg) were separated by using 1.2% agarose, 2.2 M formaldehyde gel electrophoresis and transferred to Hybond-N mem-

RESULTS

Calcineurin Is Required for the Ca2+ -dependent Inhibition of Adipocyte Differentiation—To investigate the role of calcineurin in the regulation of adipocyte differentiation, we first examined whether the specific calcineurin inhibitors FK506 and CsA were able to attenuate the previously reported inhibitory effect of calcium ionophore on the differentiation of the 3T3-L1 preadipocyte cell line (15). As shown in Fig. 1, 2 day postconfluent plates of 3T3-L1 cells that were treated with MDI efficiently differentiated into morphologically distinct, fat-laden adipocytes with accumulated cytoplasmic triglycerides that stained red with Oil Red O. Notably, the presence of either FK506 or CsA did not affect the ability of MDI to induce 3T3-L1 cells to undergo adipocyte differentiation. This fact indicates that calcineurin is not required for MDI-induced adipocyte differentiation, as was originally proposed by Ho et al. (23). Consistent with a previous report (15), treatment of 3T3-L1 cells with the calcium ionophore ionomycin potently blocked their differentiation. Significantly, we found that this inhibitory effect of calcium ionophore was abrogated in the presence of either FK506 or CsA (Fig. 1). These data therefore indicate that calcineurin activity is required to mediate the inhibitory effects of calcium ionophore on the differentiation of 3T3-L1 preadipocytes into mature adipocytes and suggest that calcineurin is likely to negatively regulate adipocyte differentiation.

A Calcium-independent, Constitutively Active Form of Calcineurin Inhibits Adipocyte Differentiation in 3T3-L1 Cells—To
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Calcineurin inhibits adipocyte differentiation in 3T3-L1 cells. MSCV-CNmut retroviruses. The percentage of GFP expressing 3T3-L1 cells infected with either MSCV-GFP or MSCV-CNmut retroviruses were induced to undergo differentiation as described under “Experimental Procedures.” Whole cell extracts were prepared at the indicated time points and analyzed by SDS-PAGE followed by immunoblotting with the indicated Ab: PPARγ (A, upper panel), C/EBPα (A, lower panel), C/EBPβ (B, upper panel), C/EBPδ (B, lower panel).

Further investigate the effects of calcineurin on adipogenesis, we used an efficient retroviral gene delivery system to introduce a previously characterized (21) calcium-independent, constitutively active calcineurin mutant (CNmut) into 3T3-L1 cells. The cDNA that encoded CNmut was introduced into the MSCV-GFP retroviral vector under the control of the MSCV promoter and upstream of an IRES-GFP expression cassette, thereby allowing the expression of both CNmut and GFP from a single bicistronic mRNA (Fig. 2A). Using these vectors, we were routinely able to generate a high-titer retrovirus capable of stably infecting >95% of 3T3-L1 cells (Fig. 2B). As shown in Fig. 2C, 3T3-L1 preadipocytes infected with the control MSCV-GFP retrovirus and treated with MDI efficiently differentiated into mature Oil Red O-staining adipocytes. In contrast, we found that cells infected with the MSCV-CNmut retrovirus and stimulated with MDI did not undergo the characteristic morphological changes associated with adipocyte differentiation and did not stain red with Oil Red O. As expected, we were able to rescue adipogenesis in these CNmut-expressing cells by treatment with FK506 (data not shown). To confirm the inhibitory effect of CNmut on adipocyte differentiation, we next examined the time course of expression of the late adipocyte-specific marker gene, aP2. As shown in Fig. 2D, expression of aP2 mRNA was readily detectable in control MSCV-GFP-infected cells after treatment with MDI, whereas this transcript was not detectable in MDI-induced CNmut-expressing cells. Taken together, these results indicate that sustained calcineurin activity in 3T3-L1 preadipocytes inhibits adipocyte differentiation.

Sustained Calcineurin Activity Does Not Affect the Induction of PPARγ and C/EBPα—The transcription factors PPARγ and C/EBPα are known to be necessary and sufficient for adipocyte differentiation (10, 11, 24, 25). To investigate the molecular mechanism that underlies the inhibitory effect of calcineurin on adipocyte differentiation, we next examined the effects of CNmut on the expression of PPARγ and C/EBPα. Thus, cell extracts prepared from 3T3-L1 cells infected with either MSCV-GFP or MSCV-CNmut that had been induced to undergo adipocyte differentiation by treatment with MDI were analyzed for expression of PPARγ and C/EBPα by immunoblot analysis. As shown in Fig. 3A, the expression of both PPARγ and C/EBPα was readily detectable in cells infected with MSCV-GFP. In contrast, we did not observe any appreciable expression of either PPARγ or C/EBPα in CNmut-expressing cells (Fig. 3A). Thus, calcineurin activity appears to inhibit adipocyte differentiation by preventing the expression of the proadipogenic transcription factors PPARγ and C/EBPα.

Sustained Calcineurin Activity Inhibits the Expression of the Proadipogenic Transcription Factors PPARγ and C/EBPα—The transcription factors PPARγ and C/EBPα are known to be necessary and sufficient for adipocyte differentiation (11, 24, 25). To investigate the molecular mechanism that underlies the inhibitory effect of calcineurin on adipocyte differentiation, we next examined the effects of CNmut on the expression of PPARγ and C/EBPα. Thus, cell extracts prepared from 3T3-L1 cells infected with either MSCV-GFP or MSCV-CNmut that had been induced to undergo adipocyte differentiation by treatment with MDI were analyzed for expression of PPARγ and C/EBPα by immunoblot analysis. As shown in Fig. 3A, the expression of both PPARγ and C/EBPα was readily detectable in cells infected with MSCV-GFP. In contrast, we did not observe any appreciable expression of either PPARγ or C/EBPα in CNmut-expressing cells (Fig. 3A). Thus, calcineurin activity appears to inhibit adipocyte differentiation by preventing the expression of the proadipogenic transcription factors PPARγ and C/EBPα.

Sustained Calcineurin Activity Does Not Affect the Induction of C/EBPβ and C/EBPδ—The expression of PPARγ and C/EBPα is thought to be regulated during adipocyte differentiation by the transcription factors C/EBPβ and C/EBPδ (7, 8). We therefore examined whether calcineurin activity blocks the induction of PPARγ and C/EBPα by interfering with the expression of C/EBPβ and C/EBPδ. As shown in Fig. 3B, exposure of MSCV-GFP-infected control cells to differentiation-inducing conditions resulted in the expression of both C/EBPβ and
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Calcineurin activity might merely nonspecifically perturb 3T3-L1 cellular physiology by creating a cellular environment incompatible with cellular differentiation. To distinguish between these possibilities, we tested whether ectopic expression of PPARγ was able to bypass the block in adipogenesis and rescue adipocyte differentiation in CNmut-expressing cells. Thus, 3T3-L1 cells were infected sequentially first with MSCV-CNmut and then with either MSCV-PPARγ or MSCV-H2K as a control. The MSCV-PPARγ retroviral vector directs the expression of both PPARγ1 and the murine major histocompatibility class I molecule, H2Kα from a single bicistronic mRNA (Fig. 4A). As a result, successful infection with this virus can be monitored readily by fluorescence-activated cell sorter analysis with a fluorescence-conjugated anti-H2Kα mAb. Using this sequential infection protocol, we were able to doubly infect >95% of cells with MSCV-CNmut and either MSCV-H2K or MSCV-PPARγ (Fig. 4B). As expected, cells infected with both MSCV-CNmut and MSCV-H2K failed to undergo adipocyte differentiation under standard differentiation conditions (Fig. 4C). In contrast, cells that co-expressed both CNmut and PPARγ1 were found to efficiently undergo the characteristic morphological changes associated with adipocyte differentiation and stained positive for Oil Red O (Fig. 4C). Fluorescent microscopic analysis of these cells revealed that they still expressed the CNmut-IRES-GFP transgene, ruling out the trivial possibility that PPARγ1 rescued adipogenesis by inhibiting expression of CNmut (data not shown). Furthermore, we observed that expression of PPARγ1 overcomes the inhibitory effects of ionomycin on adipogenesis (Fig. 4D). The efficient rescue of adipocyte differentiation in both CNmut-expressing and ionomycin-treated cells by ectopic expression of PPARγ1 suggests that calcineurin principally inhibits adipogenesis by preventing the expression of the proadipogenic transcription factor PPARγ.

Inhibition of Endogenous Calcineurin Activity Enhances Adipocyte Differentiation—Having demonstrated that the sustained activation of calcineurin either by treatment with calcium ionophore or ectopic expression of CNmut potently inhibits adipogenesis, we wanted to investigate the potential role of calcineurin during the normal process of in vitro adipocyte differentiation. For these experiments, we took advantage of our observation that treatment of 3T3-L1 preadipocytes with suboptimal adipogenic stimuli (Mix and Dex without insulin) resulted in only modest adipocyte differentiation that occurred primarily in isolated patches of cells. As seen in Fig. 5A, stimulation of 3T3-L1 cells with decreasing concentrations of Mix and Dex resulted in a dose-dependent decrease in adipocyte differentiation. However, when 3T3-L1 cells were differentially infected under these suboptimal conditions in the presence of either FK506 or CsA, we observed enhanced differentiation with a significant increase in both the number and the size of these adipogenic cell clusters (Fig. 5A and data not shown). To further implicate endogenous calcineurin in the regulation of adipocyte differentiation, we took advantage of a previously characterized specific peptide inhibitor of calcineurin, VIVIT-GFP, which has been shown to specifically inhibit the ability of calcineurin to activate NFAT proteins (22). As predicted, expression of VIVIT-GFP in 3T3-L1 cells was able to overcome the inhibitory effects of ionomycin on adipocyte differentiation (Fig. 5B). Consistent with the effect of FK506 (Fig. 5A), we found that inhibition of endogenous calcineurin activity with VIVIT-GFP dramatically enhanced adipocyte differentiation in response to suboptimal adipogenic stimuli (Fig. 5C). Taken together, these results demonstrate that endogenous calcineurin activity acts to antagonize the normal process of adipogenesis and is likely to set a signaling threshold required for efficient adipocyte differentiation.

**DISCUSSION**

In the current study, we provide multiple lines of evidence to indicate that the Ca2+ -calmodulin-regulated phosphatase calcineurin acts to negatively regulate adipocyte differentiation. We demonstrate that the activation of the calcineurin signaling pathway inhibits adipogenesis by preventing the expression of the proadipogenic transcription factors PPARγ and C/EBPα.
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Fig. 5. Inhibition of endogenous calcineurin activity enhances the adipocyte differentiation of 3T3-L1 preadipocytes in response to suboptimal adipogenic stimuli. A, 2-day, postconfluent, 3T3-L1 preadipocytes were incubated in a growth medium that contained 2-fold serial dilutions of 1 μM Dex and 0.5 mM Mix (1x MD) for 2 days in the presence or absence of FK506 and thereafter in a growth medium that contained either FK506 or vehicle control. After 10 days, cells were stained with Oil Red O. B, 3T3-L1 preadipocytes infected with either MSCV-VIVIT-GFP or control MSCV-H2K virus were induced to undergo adipocyte differentiation with MDI in the presence or absence of FK506 and thereafter in a growth medium that contained either FK506 or vehicle control. After 10 days, cells were stained with Oil Red O. C, 3T3-L1 preadipocytes infected with either MSCV-VIVIT-GFP or control MSCV-H2K virus were grown for 2 days postconfluence and then induced to undergo adipocyte differentiation by incubation in growth medium that contained 2-fold serial dilutions of 1 μM Dex and 0.5 mM Mix (1x MD) for 2 days and maintained thereafter in growth medium alone. After 10 days, cells were stained with Oil Red O.

Conversely, we found that inhibition of endogenous calcineurin activity markedly enhances adipocyte differentiation in 3T3-L1 cells in response to suboptimal adipogenic stimuli. Together our findings suggest that the level of activation of the endogenous calcineurin signaling pathway in preadipocytes is unlikely to play an important role in setting the signaling threshold required for commitment to the terminal phase of adipocyte differentiation. Given that calcineurin activity is exquisitely sensitive to changes in [Ca2+]i, (27), the role of calcineurin in the regulation of adipocyte differentiation is likely to be especially important in the response to environmental cues such as prostaglandin F2α, that modify the level of intracellular Ca2+. Prostaglandin F2α has been shown previously to inhibit adipogenesis by a Ca2+-dependent mechanism (16). On the basis of our findings, we propose that calcineurin is an intrinsic negative regulatory component of the adipogenic signaling pathway that acts as a Ca2+-dependent molecular switch to inhibit adipocyte differentiation in response to exogenous agents that elevate [Ca2+]i.

It is important to note that our primary conclusion that calcineurin acts to negatively regulate adipocyte differentiation conflicts with a previous study by Ho et al. (23). On the basis of their identification of an NFAT binding site in a proximal promoter element of the adipocyte-specific aP2 gene and their observation that CsA inhibits MDI-induced 3T3-L1 differentiation, these authors proposed a positive role for the calcineurin/NFAT signaling pathway in the regulation of adipogenesis. However, a previous study by Yeh et al. (28) found that neither CsA nor FK506 inhibited the differentiation of 3T3-L1 cells. In fact, they found that a molar excess of FK506 was able to overcome the antiadipogenic effects of the structurally related drug rapamycin. Because both FK506 and rapamycin are known to mediate their biological activities by binding to a common intracellular receptor, FK506-binding protein (29), the ability of FK506 to overcome the antiadipogenic effects of rapamycin indicates that functional FK506-FK506 binding protein complexes are unable to block adipogenesis. This observation and our current data argue strongly against a positive role for calcineurin in adipocyte differentiation, as originally suggested by Ho et al. (23). Instead, our data provide multiple independent lines of evidence that demonstrate a novel inhibitory function for calcineurin in the regulation of adipocyte differentiation. At present, the reason behind the discrepancy between our data and those of Ho et al. is not clear but may be related to either the method of drug delivery or specific cell culture conditions.

Our finding that sustained calcineurin activity inhibits adipocyte differentiation by preventing the expression of PPARγ and C/EBPα, but not C/EBPβ and C/EBPδ, suggests a number of potential mechanisms by which calcineurin may inhibit adipogenesis. First, calcineurin may interfere directly with the activity of C/EBPα and C/EBPδ. Although C/EBPβ is thought to be regulated during 3T3-L1 differentiation by a phosphorylation-dependent mechanism (30), no evidence currently exists to suggest that C/EBPβ is a direct substrate of calcineurin. In addition, it appears that calcineurin does not affect the expression of known inhibitors of C/EBPβ activity such as LIP (Fig. 3B), an inhibitory C/EBPβ isoform that arises by alternative translational initiation (26), or the expression of CHOP-10 (data not shown), which is believed to represent an endogenous dominant-negative inhibitor of the C/EBP family (31). Second, calcineurin may inhibit adipocyte differentiation by affecting a parallel pathway to C/EBPβ and C/EBPδ that is also required for the efficient expression of PPARγ and C/EBPα. In this regard, activation of the mitogen-activated protein kinase signaling pathway has been shown to inhibit adipocyte differentiation (32, 33). This effect is controversial, however, because other studies have suggested a positive role for this pathway in adipogenesis (34, 35). In addition, the AMP- response element-binding protein/activating transcription factor-2 (ATF-2) and p38 kinase signaling pathways have both been shown to be required for PPARγ expression and efficient adipocyte differentiation in 3T3-L1 cells (36–38). Interestingly, calcineurin has been shown to affect the activity of each of these signaling pathways in a number of distinct cell types (39–41), making each pathway a potential target for the antiadipogenic activity of calcineurin. Third, calcineurin may inhibit adipocyte differentiation by activating a pathway that directly represses expression of PPARγ and C/EBPα. Indeed, undifferentiated 3T3-L1 preadipocytes are known to express a number of proteins that have been shown to potently inhibit adipogenesis by preventing the expression of PPARγ. The expression of these proteins, which include PREF-1 (42), Wnt-10b (43), and the transcription factors GATA-2 and GATA-3 (44), must be down-regulated to ensure successful adipocyte differentiation. Interestingly, calcineurin has recently been implicated in up-regulating the expression of GATA-2 in muscle cell precursors, where it is believed to play a role in the promotion of muscle cell
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The role of calcineurin in the regulation of adipocyte differentiation may be secondary to a transforming effect of NFATc1 in these cells (Fig. 5, A and B). This notion is further supported by our observation that ectopic expression of a constitutively active NFATc1 mutant also inhibits the MDI-induced differentiation of 3T3-L1 cells by preventing the expression of PPARγ and C/EBPα, although this may be secondary to a transforming effect of NFATc1 in these cells. Experiments are currently under way to further delineate the role of calcineurin in the regulation of adipocyte differentiation.

Collectively, our findings identify calcineurin as a Ca2+-dependent negative regulator of adipocyte differentiation and provide evidence that the level of endogenous calcineurin activity in preadipocytes plays an important role in determining the efficiency of adipogenesis. Because it is now clear that an increase in adipogenesis can contribute to increased adipose tissue mass and the development of obesity (1), our data suggest a potential in vivo role for calcineurin in the regulation of obesity and its associated diseases. Indeed, our observation that inhibition of endogenous calcineurin activity leads to enhanced adipogenesis may explain the increased obesity, hyperlipidemia, and type-2 diabetes that have been reported in patients treated with the immunosuppressive drugs CsA and FK506 (49–51). On the basis of our results, further investigation of the in vivo role of calcineurin in the regulation of obesity is clearly warranted.

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