Activation of MEK/ERK Signaling Promotes Adipogenesis by Enhancing Peroxisome Proliferator-activated Receptor γ (PPARγ) and C/EBPα Gene Expression during the Differentiation of 3T3-L1 Preadipocytes*

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We demonstrate that exposure of post-confluent 3T3-L1 preadipocytes to insulin, isobutylmethylxanthine ( MIX ), dexamethasone ( DEX ), and fetal bovine serum induces a rapid but transient activation of MEK1 as indicated by extensive phosphorylation of ERK1 and ERK2 during the initial 2 h of adipogenesis. Inhibition of this activity by treating the cells with a MEK1-specific inhibitor ( U0126 or PD98059 ) prior to the induction of differentiation significantly attenuated the expression of peroxisome proliferator-activated receptor ( PPAR ) γ , C/EBPα/enhancer-binding protein ( C/EBPα ) α, perilipin, and adipocyte-specific fatty acid-binding protein ( aP2 ). Treating the preadipocytes with troglitazone, a potent PPARγ ligand, could circumvent the inhibition of adipogenic gene expression by U0126. Fibroblast growth factor-2 ( FGF-2 ), in the presence of dexamethasone, isobutylmethylxanthine, and insulin, induces a prolonged activation of the MEK/ERK signaling pathway, which lasts for at least 12 h post-induction, and this activity is less sensitive to the MEK inhibitors. Consequently, preadipocytes treated with U0126 in the presence of fibroblast growth factor-2 ( FGF-2 ) express normal post-induction levels of MEK activity, and, in so doing, are capable of undergoing adipogenesis. We further show that activation of MEK1 significantly enhances the transactivation of the C/EBPα minimal promoter during the early phase of the differentiation process. Our results suggest that activation of the MEK/ERK signaling pathway during the initial 12 h of adipogenesis enhances the activity of factors that regulate both C/EBPα and PPARγ expression.

The differentiation of preadipocytes into mature insulin-responsive adipocytes involves exposure of a confluent, quiescent population of cells to a variety of effectors that activate a cascade of transcription factors commencing with CCAAT/enhancer-binding protein ( C/EBP ) α and C/EBPα, which ultimately induce the expression of C/EBPα and peroxisome proliferator-activated receptor ( PPAR ) γ (1, 2). Terminal differentiation involves the coordinated regulation of several programs of gene expression by C/EBPα and PPARγ, which include the induction of proteins responsible for insulin sensitivity. Using the 3T3-L1 model of adipogenesis, several investigators have demonstrated a role for cAMP in activating C/EBPα and for glucocorticoids in inducing C/EBPα as well as PPARγ (3–5). Insulin, acting through the IGF-1 receptor, is also required to ensure complete conversion of preadipocytes into adipocytes (6), but the precise role that it plays in the process is still unclear. The IGF-1 and insulin receptors are tyrosine kinases that can activate a series of signaling pathways in different cell types including the Ras-MAPK pathway. The p42 (ERK2) and p44 (ERK1) MAPKs are activated by phosphorylation on threonine and tyrosine residues by the dual specificity kinase MEK1, which induces their translocation into the nucleus where they activate or repress a variety of transcription factors involved in growth and differentiation (7). Several laboratories have investigated the role of p42/p44 MAPK in regulating adipogenesis, but the conclusions are somewhat controversial. Some studies claim that activation of MAPK by various effectors blocks adipogenesis (8–10), whereas others suggest that it promotes preadipocyte differentiation (11–14). It is quite possible that both claims are correct. The distinguishing factor might involve the precise time of MAPK activation during the initial stages of the differentiation process. For instance, effectors that activate the MEK/ERK pathway at late stages of adipogenesis are likely to block adipogenic gene expression due to a MAPK-dependent phosphorylation of PPARγ (15–19). Activation of the pathway early during adipogenesis prior to PPARγ expression might, on the other hand, promote differentiation by activating transcription factors operating to initiate PPARγ and C/EBPα expression.

The goal of these studies was to determine whether the MEK/ERK signaling pathway regulates expression and/or activity of the adipogenic transcription factors during the early phase of adipogenesis. The results demonstrate that exposure of 3T3-L1 preadipocytes to dexamethasone (DEX), isobutylmethylxanthine ( MIX ), insulin (INS), and fetal bovine serum (FBS) induces a robust, transient activation of the MEK/ERK pathway during the initial 1–2 h post-induction and that this activity is required for subsequent differentiation. Insulin, together with elevated cAMP levels ( MIX treatment ), appears to be the principal regulator of MEK activity, and promotes different
ferentiation by enhancing the expression of C/EBPα and PPARγ. Furthermore, FGF-2 is also capable of stimulating adipogenesis under conditions where MEK activity is limiting.

EXPERIMENTAL PROCEDURES

Cell Culture—3T3-L1 preadipocytes were cultured in growth medium (Dulbecco’s modified eagle medium (DMEM) containing 10% calf serum) until confluent and were then maintained in the same medium for an additional 2–3 days (5, 20, 21). Differentiation was induced at 2–3 days post-confluence by addition of 1 μM DEX, 0.5 mM MIX, 1.67 μM INS, and 10% FBS for 48 h, at which time the medium was replaced with DMEM containing 0.41 μM insulin and 10% FBS (22). For experiments performed with U0126 or PD98059, post-confluent 3T3-L1 preadipocytes were preincubated for 30 min or one hour, respectively, prior to induction of differentiation.

Reporter Plasmids, Transfections, and CAT Assays—The C/EBPα minimal promoter/CAT reporter plasmid corresponding to −270 to +133 bp of the 5’ upstream region of the C/EBPα gene was generated as described previously (23). A stable 3T3-L1 preadipocyte cell line was established by transfecting the C/EBPα/CAT reporter vector along with a plasmid containing a neomycin gene to facilitate selection with G418. A single colony of G418-resistant preadipocytes (#4–2 cells) was selected for expression of the reporter gene and ability to undergo differentiation in response to DEX, MIX, and insulin. Post-confluent #4–2 cells exposed to different combinations of DEX, MIX, insulin, and FBS were harvested at different times and lysed by freeze-thawing. CAT assays were performed in triplicate on the cell lysates as described previously (23), and CAT activity was expressed relative to equivalent amounts of protein in the assay.

Analysis of Protein—Cells were washed twice with cold phosphate-buffered saline and scraped in Western lysis buffer (300 μl/100 mm plate or 500 μl/100 mm plate) consisting of the following: 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 2% Nonidet P-40, 1 mM EDTA, pH 8.0, 20 mM sodium fluoride, 30 mM NaPPi, 0.2% SDS, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1 mM sodium vanadate, leupeptin, and aprotonin. Samples were incubated on ice with frequent vortexing for 15 min and centrifuged for 20 min at 14,000 rpm at 4°C. Protein content of each supernatant was quantified using a BCA kit (Pierce). Eighty micrograms of each supernatant sample of proteins was separated by electrophoresis through a 12% polyacrylamide gel and transferred to 0.45 μm Immobilon-P polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). Following transfer, membranes were blocked with milk and probed with the following primary antibodies: activated ERK1/ERK2 (New England Biolabs, Inc., Beverly, MA); C/EBPα, C/EBPβ, and PPARγ (Santa Cruz Biotechnology, Santa Cruz, CA); aP2/FABP (gift of Dr. D. Bernlohr, University of Minnesota, St. Paul, MN); perilipin (gift of Dr. A. Greenberg, Tufts University, Boston, MA). Specific proteins were identified by further incubation of corresponding membranes with horseradish peroxidase-conjugated secondary antibodies (Sigma) followed by treatment with enhanced chemiluminescence (Pierce) according to manufacturer’s instructions.

RESULTS

To understand the signaling events regulating the expression of the adipogenic transcription factors during the early phase of adipogenesis, we focused on the MEK/ERK pathway. To determine the precise time of activation of MEK1 activity during the differentiation of 3T3-L1 preadipocytes, confluent cells were exposed to DEX, MIX, insulin, and FBS. Fig. 1A demonstrates that these effectors induce a rapid (within 5 min) but short-lived activation of MEK1, as indicated by its ability to phosphorylate ERK1 and ERK2, which subsides to quiescent levels by 6 h post-stimulation; interestingly, there is a second burst of activity at 12 h. The immediate early activation of this signaling pathway is followed by progression of the cells through the G1 phase of the cell cycle (data not shown) and subsequent differentiation as indicated by the induction of C/EBPβ at 1–2 h. By 2 days post-induction, the cells have switched from growth to terminal differentiation as revealed by an increase in PPARγ, C/EBPα, and perilipin expression.

The data in Fig. 1A demonstrate that the adipogenic hormones, acting as both mitogens and inducers of terminal differentiation, stimulate a rapid, transient activation of the MEK/ERK signaling pathway. Because adipogenesis is induced by exposure of confluent preadipocytes to a combination of hormones, it was important to determine which of the effectors contributes to the activation of MEK1. In the experiment shown in Fig. 1B, total protein was harvested 10 min following treatment of cells with the indicated effectors in the presence of FBS and was subjected to Western blot analysis using an anti-activated ERK antibody. Lane 3 of this figure demonstrates that elevation of cAMP levels by MIX (i.e. activation of PKA) is a principal mediator of ERK2 activation, and pretreatment of the cells with the MEK1 inhibitor PD98059 attenuates the effect of MIX (lane 4). Insulin was a weak activator of ERK2 (lane 5) but was unable to activate ERK1; nevertheless, it did potentiate the phosphorylation of both of these kinases by MIX in the presence or absence of DEX (compare lanes 11 and 13 with lanes 3 and 7). Furthermore, this process was sensitive to treatment of the cells with PD98059 (lanes 12 and 14), suggesting that insulin and cAMP converge on pathways upstream of MEK1. Stimulation of the cells with FGF-2 in the presence of insulin, MIX, and DEX resulted in an even greater burst of MEK1 activity (lane 15), which was only slightly sensitive to PD98059 (lane 16).

Insulin Dose-dependent Activation of MEK Activity and Adipogenic Gene Expression—Several studies have demonstrated that insulin and/or insulin-like growth factor 1 functions as an inducer of adipogenesis in mouse preadipocytes (6, 14, 24–26). In fact, the data in Fig. 1B demonstrate that insulin in the
presence of MIX also induces MEK1 activity (lane 11). Consequently, we questioned whether there is a relationship between these effects of insulin on 3T3-L1 preadipocytes. Fig. 2 shows an insulin dose-dependent increase in C/EBPα, PPARγ, and perilipin gene expression on day 5 of differentiation, which is preceded by a corresponding insulin dose-dependent stimulation of MEK1 activity at 10 min post-induction. It is important to mention, however, that maximal ERK1/2 stimulation occurs at a higher dose of insulin (>64 nM) than that required for the stimulation of C/EBPα (especially the 42 kDa form) and PPARγ, which occurs around 44 nM. Furthermore, exposure of the cells to the MEK inhibitor U0126 completely blocks the ability of MEK1 to phosphorylate ERK1 and ERK2 and also attenuates the expression of C/EBPα, PPARγ, and perilipin expression.

The Inhibition of Adipogenic Gene Expression by the MEK Inhibitor U0126 Occurs in a Dose-dependent Manner and Can Be Reversed by Exposure of Cells to the PPARγ Ligand Troglitazone—To determine the optimum dose of U0126 required to inhibit both MEK1 activity and adipogenesis, confluent 3T3-L1 preadipocytes were exposed to increasing concentrations of the drug for 30 min prior to treatment with DEX, MIX, insulin, and FBS. The corresponding dose of U0126 was maintained in the culture medium during the initial 24 h of differentiation, and cells were harvested at either 15 min (MEK activity) or 5 days (adipogenic protein expression) later by changing the medium. Cells were harvested at 15 min (MEK activity) or at day 5 (adipogenic protein expression), and whole cell proteins were subjected to Western blot analysis using antibodies against phospho-ERK, C/EBPα, PPARγ, and perilipin.

treated with growth-inhibitory doses of U0126 (10 μM) could be induced to differentiate into adipocytes. To address this question, preadipocytes were treated with U0126 in the presence or absence of a potent PPARγ ligand troglitazone. Fig. 4 also demonstrates that treatment of the cells with the MEK1 inhibitor for increasing periods of time attenuates PPARγ, C/EBPα, and aP2 expression without significantly affecting C/EBPβ. In fact, an exposure time of 24 h is as effective at inhibiting adipogenesis as exposure times up to 120 h. These data are consistent with the notion that the MEK/ERK pathway is acting to regulate adipogenesis during the first few hours following hormonal induction (Fig. 1A). More importantly, Fig. 4 demonstrates that treatment of preadipocytes with troglitazone can rescue the U0126-associated block in adipogenic gene expression at all three exposure times in a manner that does not involve reactivation of clonal expansion by the PPARγ ligand (data not shown). These data suggest that adipogenesis can be induced in preadipocytes that are prevented from undergoing clonal expansion due to the presence of U0126. Furthermore, blocking MEK1 activity does not have any significant deleterious effect on the preadipocytes because they respond to troglitazone by inducing the normal level of C/EBPα and aP2 expression (Fig. 4, compare lanes 4, 8, and 12 with lanes 1, 5, and 9).

FGF-2 Induces Adipogenesis in the Presence of U0126—Our previous studies (30) in myogenic cells suggested that FGF-2 was a significantly more potent activator of the MEK/ERK signaling pathway than insulin or IGF-1. To investigate the role of FGF-2 in regulating MEK1 activity during adipogenesis, confluent preadipocytes were exposed to DEX, MIX, and insulin in the presence or absence of FGF-2 and/or PD98059. Fig. 5A demonstrates that FGF-2 induces a prolonged (>12 h) stimulation of MEK1 activity compared with the relatively short-lived (<2 h) activation in its absence (compare lane 19 with...
presence or absence of U0126 (10 µM) was assessed by exposure to DEX, MIX, insulin, and FBS in the presence or absence of U0126 (10 µM). When troglitazone was present it was added to the cultures along with the inducers, but U0126 was added 30 min prior to this time. Cells were exposed to U0126 for the indicated times. Total cellular protein was harvested at day 5 and subjected to Western blot analysis using antibodies against PPARγ, C/EBPα, C/EBPβ, and aP2.

Furthermore, it required several hours of exposure to the MEK inhibitor PD98059 to completely block the FGF-2-associated phosphorylation of ERK1/2. In fact, FGF-2 was capable of inducing a significant level of MEK1 activity in the presence of PD98059 during the initial 4–8 h post-induction. In light of this observation, we questioned whether FGF-2 might be capable of attenuating the inhibitory effect of U0126 on adipogenic gene expression by stimulating MEK1 activity. The experiment presented in Fig. 5B shows that exposure of preadipocytes to FGF-2 together with DEX, MIX, and insulin results in a significantly greater activation of MEK1 at 15 min post-induction than treatment with the adipogenic inducers alone (Fig. 5B, compare lane 5 with lane 3). The intense activation of MEK1 in the presence of FGF-2 could not be completely abrogated by the MEK1 inhibitor U0126; instead, it was attenuated to levels equivalent to those measured in the absence of FGF-2 (Fig. 5B, compare lane 6 with lane 3). Furthermore, this residual level of MEK1 activity was enough to facilitate the induction of PPARγ, C/EBPα, and perilipin expression in cells that were also exposed to U0126 (Fig. 5B, compare lane 6 with lane 4). It is important to mention that the preadipocytes were only exposed to FGF-2 for the initial 6 h of differentiation to activate MEK activity during this critical period. Prolonged exposure of cells to FGF-2 for times greater than 12–24 h inhibits adipogenesis by mechanisms that may also involve activation of the MEK/ERK pathway. To confirm the result shown in Fig. 5B and to determine the dose of FGF-2 required to induce adipogenesis under conditions where MEK1 activity is limiting, preadipocytes were stimulated to differentiate in the presence of an inhibitory dose of U0126 with increasing doses of FGF-2 for 6 h. The cells were then maintained in culture for 5 days. As observed previously, the level of MEK1 activity at 10 min post-induction in the presence of U0126 is very low (Fig. 5C, lane 3). Exposure of the preadipocytes to FGF-2 in the presence of U0126, however, restores this activity to normal post-induction levels and in so doing induces PPARγ, C/EBPα, and perilipin gene in an FGF-2 dose-dependent manner (Fig. 5C, lanes 3–9).

The MEK/ERK Pathway Regulates C/EBPα Gene Promoter Activity—It appears that inhibition of MEK1 activity by either U0126 or PD98059 (data not shown) significantly blocks adipogenic gene expression without affecting the induction of C/EBPβ expression during the early phase of preadipocyte differentiation. Studies by others and us (4, 5, 20) have previously demonstrated a role for C/EBPβ in initiating a cascade of transcriptional events that regulate terminal differentiation. We questioned, therefore, whether the MEK/ERK pathway potentiates the activity of C/EBPβ in inducing C/EBPα gene transcription. To address this question, we generated a stable 3T3-L1 cell line expressing a C/EBPα minimal promoter-CAT reporter gene (referred to as L1#4–2 cells). This minimal promoter corresponds to a fragment of genomic DNA consisting of −272 to +133 bp relative to the transcription start site of the C/EBPα gene. Previous studies have identified a C/EBP regulatory element at −170 to −195 and have also demonstrated that transcription from this minimal promoter requires C/EBPβ (23). The analyses presented in Fig. 6 demonstrate that expression of this reporter gene responds positively to effectors that enhance C/EBPβ in the L1#4–2 cells. Specifically, exposure of the cells to MIX and insulin or DEX, MIX, and insulin for different times over a 72 h period resulted in a peak of CAT activity at 36–60 h (Fig. 6A) with a corresponding increase in C/EBPβ expression (Fig. 6B). Exposure to FBS with or without insulin caused a negligible increase in CAT activity and C/EBPβ expression. The observation that the activity of the C/EBPα/CAT reporter gene is transient suggests that additional regulatory elements not found in the minimal promoter are required to maintain activity throughout the entire differentiation process. As predicted from the data in Fig. 5, FGF-2 significantly enhances C/EBPα promoter/reporter gene activity in 3T3-L1#4–2 cells following their exposure to MIX and insulin (Fig. 6C). Furthermore, treatment of the cells with PD98059 significantly attenuated the induction of the reporter gene by MIX and insulin in the presence or absence of FGF-2.

DISCUSSION

After several years of investigation it is becoming apparent that the differentiation of preadipocytes into mature fat cells is a complex process involving the interplay of many effectors both positive and negative that regulate a network of signaling pathways, which eventually converge on the adipogenic gene program. Some of the pathways operate to “fine tune” the functions of the mature adipocyte in response to changes in the overall physiologic status of the organism. For instance, insulin and β-adrenergic receptor activity antagonize each other to regulate lipolysis in response to the energy needs of the body. Some signaling events, however, function to regulate the expression and activity of the many transcription factors that orchestrate the differentiation process. This investigation provides evidence for a role of the MEK/ERK signaling pathway in regulating the expression of C/EBPα and PPARγ during adipogenesis. Specifically, exposure of 3T3-L1 preadipocytes to a mixture of adipogenic hormones consisting of DEX, MIX, insulin, and FBS activates MEK1 during the initial 1–2 h post-induction as indicated by its ability to phosphorylate ERK1 and ERK2. Inhibition of this activity by exposure of preadipocytes to the MEK1 inhibitors U0126 or PD98059 significantly attenuates expression of C/EBPα and PPARγ without affecting C/EBPβ expression. Furthermore, FGF-2 can induce adipogenic gene expression in preadipocytes exposed to U0126 by promoting the phosphorylation of ERK1/2. One mechanism by which the MEK/ERK pathway might activate C/EBPα expres-
The function is to enhance the ability of C/EBPα/H9252 to transactivate the C/EBPα/H9251 gene promoter.

Establishing a role for the MEK/ERK pathway in regulating adipogenesis has been difficult because many of the studies have arrived at completely opposite conclusions. For instance, several studies (15–19) have shown that stimulating ERK activity in adipocytes by exposure to mitogens attenuates adipogenic gene expression by mechanisms that likely involve phosphorylation and inactivation of PPARγ/H9253. Some studies (9) have shown that drugs or effectors that block adipogenesis are also potent inducers of ERK activity, and attenuation of this activity with the MEK inhibitors restores differentiation. Similarly, a recent study (31) provides evidence for a positive role for the retinoblastoma protein in facilitating adipogenesis by suppressing ERK activity. In contrast, several investigations including the present study have shown that some pro-adipogenic agents stimulate MEK/ERK activity, and, in some cases, attenuation of this activity with MEK inhibitors blocks adipogenesis (12, 13, 32). We suggest that stimulation of the MEK/ERK pathway by growth factors, such as FGF-2, counteracts the inhibitory effect of the MEK inhibitor U0126 on C/EBPα and PPARγ expression by maintaining pro-adipogenic levels of MEK activity during the initial 8 h of adipogenesis.

Fig. 5. FGF-2 counteracts the inhibitory effect of the MEK inhibitor U0126 on C/EBPα and PPARγ expression by maintaining pro-adipogenic levels of MEK activity during the initial 8 h of adipogenesis. A, proliferating 3T3-L1 preadipocytes (P) were grown to confluence, and 4 days later (0) the cells were pretreated with PD98059 (50 μM) or vehicle for 1.0 h prior to their induction to differentiate with DEX, MIX, insulin, and FBS. B and C, post-confluent 3T3-L1 preadipocytes were induced to differentiate with DEX, MIX, insulin, and FBS in the presence or absence of 1 nM FGF-2. Total cellular protein was harvested at the indicated times and subjected to Western blot analysis using the activated ERK antibody. B and C, post-confluent 3T3-L1 preadipocytes were induced to differentiate with DEX, MIX, insulin, and FBS in the presence or absence of 1 nM FGF-2. Total cellular protein was harvested at the indicated times and subjected to Western blot analysis using the activated ERK antibody. A, post-confluent 3T3-L1#4-2 cells containing an integrated C/EBPα minimal promoter/CAT reporter gene were exposed to various combinations of DEX, MIX, insulin, and FBS. Cells were harvested at the indicated times, and CAT activity was measured in cell extracts containing equal amounts of protein as described under “Experimental Procedures.” FBS; ○, insulin; ▲, DEX, MIX, and insulin; ●, MIX and insulin. B, a set of cultures corresponding to the same effector conditions were harvested at 40 h post-induction and subjected to Western blot analysis for expression of the different isoforms of C/EBPβ. C, post-confluent 3T3-L1#4-2 cells were exposed to DEX, MIX, insulin, and FBS in the presence or absence of FGF-2 and/or PD98059, and cell extracts were harvested at the indicated times for measurement of CAT activity. ○, DEX, MIX and insulin; ▲, DEX, MIX, insulin, and 50 μM PD98059; ●, FGF-2, DEX, MIX, and insulin; ▲, FGF-2, DEX, MIX, insulin, and 50 μM PD98059. Data are representative of three independent experiments.
ERK pathway can both promote and attenuate adipogenesis depending on its time of activation during the differentiation process. The data presented in Fig. 1 show that MEK activity is rapidly induced during the initial few hours following exposure of 3T3-L1 preadipocytes to insulin, MIX, and DEX. The peak of activity eventually subsides to pre-induction levels by 4–6 h, but there is a second reproducible burst of activity detected at 12 h. In addition, exposure of preadipocytes to FGF-2 induces an even greater induction of MEK activity, which is also confined to the initial 12 h of the differentiation process. In both cases, this time-restricted burst of MEK activity promotes differentiation by facilitating the expression of the principal adipogenic transcription factors, PPARγ and C/EBPα. It appears that activation of MEK1 at times following the induction of these factors will inhibit adipogenesis by attenuating their transcriptional activity.

The mechanisms that regulate the extent and duration of ERK activity might, therefore, play an important role in regulating adipogenic gene expression. The MAPK phosphatases (MKP-1 and MKP-2) that dephosphorylate and inactivate ERK1 and ERK2 are potential candidates for regulating ERK activity during adipogenesis. In fact, MKP-1 and MKP-2 are immediate early genes that are induced when quiescent cells are exposed to a variety of extracellular signals including mitogens (33, 34). It is conceivable, therefore, that insulin induces MKP-1/2 very rapidly during the early phase of adipogenesis so that ERK activity is confined to a limited but precise period of the differentiation process. In the case of FGF-2, induction of MKP-1 can be significantly delayed or attenuated, therefore, facilitating prolongation of ERK activity as observed in Fig. 5A.

What are the potential roles of the activated forms of ERK1 and ERK2 in promoting adipogenesis? Studies by others and us (4, 5, 20) have demonstrated a role for C/EBPβ, with or without C/EBPα, in initiating a cascade of transcriptional events that lead to expression of the many hundreds of proteins responsible for the mature fat cell phenotype. Consequently, factors that influence the ability of C/EBPβ to initiate this cascade of gene expression are possible targets of ERK1/2. In fact, the ERKs might phosphorylate C/EBPβ during the early phase of adipogenesis and enhance its ability to activate C/EBPα transcription. Consistent with this notion are studies in other systems demonstrating that MAPKs can enhance the transactivation properties of C/EBPβ via the phosphorylation of Thr-235 (35, 36).

Induction of adipogenesis also involves suppression of a host of negative effectors that act to maintain the preadipocyte in an undifferentiated state. It is conceivable, therefore, that the MEK/ERK pathway also operates to suppress the activity of these negative factors. In this regard, Lane and coworkers (37, 38) have recently shown that the Sp1 and AP-2 families of transcription factors repress C/EBPα transcription by blocking association of the C/EBPs with the C/EBP regulatory element within the promoter of the C/EBPα gene. The abundance of Sp1 and AP-2a decreases during 3T3-L1 differentiation; therefore, it is possible that the MEK/ERK pathway facilitates their down-regulation. In fact, these authors show that exposure of growth-arrested 3T3-L1 preadipocytes to agents that increase cAMP levels induces a rapid (within 2 to 4 h) and transient decrease in Sp1. They also show that the phosphorylated form of Sp1 decays more abruptly than the unphosphorylated form. Other studies (39–41) have shown that the activity of Sp1 is regulated by the ERK signaling pathway, which involves an ERK-associated phosphorylation of the transcription factor at a MAPK consensus site. It is possible, therefore, that elevation of cAMP in response to MIX along with insulin induces an ERK-associated modification of Sp1, which initiates its degradation, thus relieving repression on the C/EBPα promoter. This model of ERK regulation of C/EBPα promoter activity would also be consistent with the data presented in Fig. 6 because the 5′ region of the C/EBPα/CAT reporter gene contains the Sp1 elements previously shown by Lane and coworkers (37, 38) to repress C/EBPα transcription. In a similar manner, the MEK/ERK pathway could be suppressing other known repressors of adipogenesis. Notable among these negative effectors is Wnt-10b, which is secreted from preadipocytes and acts in an autocrine fashion to stimulate the Wnt/β-catenin signaling pathway. Activation of this pathway by Wnt-10b inhibits GSK3β activity, which leads to the translocation of β-catenin into the nucleus where it co-activates TCF/LEF transcription factors and, in so doing, inhibits expression of C/EBPα and PPARγ (42). Recent studies by MacDougall and coworkers (43) demonstrate that various components of the Wnt signaling pathway including Wnt-10b and the frizzled receptors (Fxl, 1, 2, and 5) are down-regulated during the initial 1–2 days of differentiation. Furthermore, suppression of Wnt-10b production appears to be mediated by a cAMP-associated inhibition of Wnt-10b mRNA expression. It is possible that the MEK/ERK signaling pathway is also involved in this process.

In conclusion, we suggest that the MEK/ERK signaling pathway can affect adipogenesis in different ways depending on its precise time of activation during the differentiation process. Future studies aimed at identifying the targets of the pathway should provide greater insight into the molecular mechanisms regulating the expression of PPARγ and C/EBPα during the differentiation of preadipocytes into adipocytes.

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Activation of MEK/ERK Signaling Promotes Adipogenesis by Enhancing Peroxisome Proliferator-activated Receptor γ (PPARγ) and C/EBPα Gene Expression during the Differentiation of 3T3-L1 Preadipocytes

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