Role of MAPK Phosphatase-1 (MKP-1) in Adipocyte Differentiation*

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Both time-dependent modulation of intracellular signaling molecules and sequential induction of transcriptional regulators are essential for the differentiation of preadipocytes into adipocytes. We have now shown that the activity, but not the abundance, of p42/p44 mitogen-activated protein kinase (MAPK) is down-regulated during adipocyte differentiation. This decrease in p42/p44 MAPK activity does not appear to be a direct effect of hormonal inducers of differentiation but rather represents a characteristic event of adipocyte differentiation that is achieved through a persistent change in intracellular signaling. Although the phosphorylation or abundance of MEK, an upstream kinase for p42/p44 MAPK, was not altered during differentiation, the abundance of MAPK phosphatase-1 (MKP-1), a negative regulator of p42/p44 MAPK, was increased with a time course similar to that of the down-regulation of p42/p44 MAPK activity. Ectopic expression of MKP-1 in preadipocytes reduced and depletion of endogenous MKP-1 in mature adipocytes increased the activity of p42/p44 MAPK. Prevention of the up-regulation of MKP-1 abundance in preadipocytes by expression of Mkp-1 antisense RNA resulted in persistence of p42/p44 MAPK activation and blocked differentiation, effects that were reversed by the MEK inhibitor PD98059. These results suggest that MKP-1 plays an essential role in adipocyte differentiation through down-regulation of p42/p44 MAPK activity.

The amount of adipose tissue, which is altered in various physiological and pathological conditions, is an important determinant of energy homeostasis in living animals (1). Given that mature adipocytes do not undergo cell division, the number of adipocytes in vivo is thought to increase as a result of the proliferation of preadipocytes and their subsequent differentiation into mature adipocytes. Studies with cultured cells, such as mouse 3T3-L1 and 3T3-F442A cell lines, have shed light on the mechanisms of growth and differentiation of preadipocytes (2, 3). Differentiation of these cells occurs in two discrete steps. The cells first undergo several rounds of mitosis, known as clonal expansion, after which they become quiescent again, express adipocyte-specific proteins, and acquire biochemical and morphological characteristics of mature adipocytes (2, 3). Both the proliferation and differentiation of preadipocytes are characterized by marked changes in the pattern of gene expression that are achieved by the sequential induction of transcription factors. Exposure of preadipocytes to appropriate hormonal inducers of differentiation thus results in early and transient expression of the β and δ isoforms of the CCAAT/enhancer-binding protein (C/EBP), which in turn appear to contribute to the proliferation of these cells as well as to the subsequent increase in the expression of C/EBPα and peroxisome proliferator-activated receptor γ (PPARγ) (4, 5). The latter two proteins then mediate the activation of a variety of adipocyte-specific genes (2–4).

Both extracellular and intracellular signaling molecules also play key roles in the differentiation of preadipocytes. Down-regulation of Pref-1 (6) and Wnt-10 (7), secreted proteins that are abundant in preadipocytes, is thus important for differentiation of these cells. Fibroblast growth factor-10, which is expressed transiently during adipocyte differentiation, is also required to maintain the expression of C/EBPβ until a specific stage of differentiation (8). With regard to intracellular signaling molecules, phosphoinositide 3-kinase, which is specifically activated at a late phase of differentiation, contributes to the expression of proteins that characterize mature adipocytes (9). A preferential inhibitor of p38 mitogen-activated protein kinase (MAPK) prevents the differentiation of preadipocytes (10), and overexpression of MAPK kinase 6, an activator of p38 MAPK, triggers the spontaneous differentiation of these cells (11). Introduction of an active form of the protein kinase Akt also induces spontaneous differentiation of preadipocytes (12). Although these various observations indicate the importance of specific intracellular signaling in the differentiation of preadipocytes, the mechanisms by which such signaling is regulated remain largely unknown.

We now show that the activity of the p42 and p44 isoforms of MAPK is down-regulated during differentiation of preadipocytes. We provide evidence that the abundance of MAPK phosphatase (MKP-1) increases during the differentiation of these cells and that this protein plays an essential role in the differentiation program by down-regulating the activity of p42/p44 MAPK.

* This work was supported by grants from Yamanouchi Foundation for Research on Metabolic Disorders and the Suzunori Memorial Foundation (to H. S.), funds from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to M. K. and W. O.), and a grant for the Cooperative Link of Unique Science and Technology for Economy Revitalization from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to M. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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‡ The abbreviations used are: C/EBP, CCAAT/enhancer-binding protein; PPARγ, peroxisome proliferator-activated receptor γ; MAPK, mitogen-activated protein kinase; MKP, MAPK phosphatase; PBS, fetal bovine serum; IBMX, isobutylmethylxanthine; DMEM, Dulbecco’s modified Eagle’s medium.

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

Received for publication, July 1, 2004
Published, JBC Papers in Press, July 20, 2004, DOI 10.1074/jbc.M407353200

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EXPERIMENTAL PROCEDURES

Antibodies, Reagents, and Kinase Assay—Antibodies to phosphorylated ERK1/2, Akt (Ser473), MAPK (Thr183/Tyr185/Tyr187), and p38 MAPK, phosphorylated (Thr180, Tyr182) p38 MAPK, MEK, phosphorylated (Thr160, Tyr162) p38 MAPK, p38 MAPK, CREB, and to PPARγ were obtained from Cell Signaling Technologies (Beverly, MA). Antibodies to C/EBPα, C/EBPβ, MKP-1, MKP-2, and to MKP-3 were from Santa Cruz Biotechnology (Santa Cruz, CA), and antibodies to Akt were as described previously (13). PD98059 was obtained from Calbiochem. Antibodies to p21 (14) and troglitazone were kindly provided by D. Bernlohr (University of Minnesota, Minneapolis) and Sankyo Pharmaceutical Co. (Tokyo, Japan), respectively. The kinase activity of immunoprecipitates prepared with antibodies to p42/p44 MAPK (εc292) was assayed with myelin basic protein as substrate as described previously (15).

Cell Culture and Staining—3T3-L1 and 3T3-F442A preadipocytes were maintained as described previously (9). The differentiation of 3T3-L1 preadipocytes into adipocytes was induced by incubation of confluent cells cultured in the presence of 10% fetal bovine serum (FBS) first for 2 days with insulin (5 μg/ml), 0.25 mM dexamethasone, and 0.5 mM isobutylmethylxanthine (IBMX) and then with insulin (5 μg/ml) alone for an additional 2 days. Alternatively, the cells were cultured in the presence of 10% FBS and treated with IBMX (0.5 mM) for only 2 days. Differentiation of 3T3-F442A preadipocytes was induced by exposing confluent cells to troglitazone (10 μM) in the presence of 10% FBS for 2 days. After incubation with these reagents, the 3T3-L1 and 3T3-F442A cells were returned to normal culture medium, which was replenished every other day. Oil red O staining (8) and staining for β-galactosidase activity (15) were performed as described.

Fractionation of Adipose Tissue—For preparation of stromal-vascular and mature adipocyte fractions of epididymal adipose tissue removed from 10-week-old C57BL/6 mice, the tissue was washed with and minced in Dulbecco’s modified Eagle’s medium (DMEM) and then incubated on a shaking platform for 20 min at 37°C with the same medium containing collagenase (0.5 mg/ml). The mixture was then passed through a nylon filter (pore size, 250 μm) to remove undigested material, and the filtrate was centrifuged for 10 min at 200 × g at 4°C. Floating cells (the mature adipocyte fraction) and the pellet (the stromal-vascular fraction) were recovered, washed three times with DMEM, and then lysed and subjected to immunoblot analysis.

Expression Plasmids and Adenoviral Vectors—A mammalian expression vector encoding MKP-1 (pcDNA3.1/MKP-1) was constructed by subcloning a cDNA for the human MKP-1 ortholog (CL-100) (16), kindly provided by S. Keyse (Molecular Pharmacology Unit, Ninewells Hospital, Dundee, UK), into pcDNA3.1 (Invitrogen). To construct an adenoviral vector that encodes Mkp-1 antisense RNA, we subcloned a mouse Mkp-1 cDNA containing both the entire coding region and 157 bp of the 5’-untranslated region (17), kindly provided by N. Tanos (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), in the antisense orientation into pAdCMV; the resulting construct was introduced into 293 cells together with DNA-TPC by transfection with the use of an adenovirus expression kit (Takara, Tokyo, Japan) as described previously (9). An adenoviral vector encoding β-galactosidase was kindly provided by I. Saito (Tokyo University, Japan). For adenovirus-mediated gene transfer into 3T3-L1 preadipocytes, cells cultured to subconfluence were infected with viruses at a multiplicity of infection of 10 plaque-forming units/cell. Two days after infection, differentiation was induced as described above. Alternatively, 3T3-L1 adipocytes at 6 days after the induction of differentiation were infected with viruses at a multiplicity of infection of 30 plaque-forming units/cell; the cells were subjected to immunoblot analysis 2 days after infection.

Electroporation—For transfection of 3T3-L1 preadipocytes with pcDNA3.1/MKP-1 or with an expression vector for β-galactosidase (SRE/LacZ), the cells were detached from culture dishes by incubation with phosphate-buffered saline containing 0.25% trypsin and collagenase (0.5 mg/ml). Approximately 5 × 10^6 cells were mixed with 5 μg of plasmid in the solution provided with a cell line Nucleofector kit V (Amaxa, Cologne, Germany), and the plasmid was then introduced into the cells by electroporation with the use of a Nucleofector (Amaxa) instrument according to the T-20 program.

RESULTS

Down-regulation of p42/p44 MAPK Activity during Adipocyte Differentiation—3T3-L1 preadipocytes, a well characterized model for studying adipocyte differentiation, were induced to differentiate by incubation with insulin, dexamethasone, and IBMX for 2 days and then with insulin alone for an additional 2 days. At various times before, during, and after such treatment, the cells were harvested, and the activity of p42/p44 MAPK was assayed in immunoprecipitates prepared with antibodies that recognize both p42 and p44 isoforms of this kinase. The substantial p42/p44 MAPK activity that was apparent in preadipocytes before exposure to the inducers of differentiation had decreased by 4 days after the onset of differentiation induction (Fig. 1a). At 8 days postinduction, when >90% of the cells had acquired morphological and biochemical characteristics of mature adipocytes (data not shown), the activity of p42/p44 MAPK was <20% of that observed in the untreated cells. Immunoblot analysis of cell lysates with antibodies specific for phosphorylated p42/p44 MAPK revealed that the extent of phosphorylation of the isoforms, which reflects their kinase activity, decreased in parallel with the kinase activity (Fig. 1a); the abundance of the kinases was not affected by the induction of differentiation, consistent with previous observations (10). In contrast, phosphorylation of p38 MAPK was not affected by the inducers of differentiation but was increased by treatment of the cells with anisomycin, an activator of this isozyme (Fig. 1b).

Insulin activates p42/p44 MAPK in various cell types. However, the activity of p42/p44 MAPK in 3T3-L1 preadipocytes not exposed to insulin or the other inducers of differentiation was higher than that in cells at relatively late stages of differentiation induced by these agents (Fig. 1a). Induction of the differentiation of 3T3-L1 preadipocytes by exposure to dexamethasone and IBMX alone (without insulin) for 2 days resulted in the accumulation of lipid droplets in ~60% of the cells within 8 days after the onset of treatment (data not shown). Down-regulation of the phosphorylation of p42/p44 MAPK was also observed during the differentiation process (Fig. 1c). Moreover,
when 3T3-F442A cells, a preadipocyte cell line closely related to 3T3-L1 cells (2), were induced to differentiate by incubation for 2 days with troglitazone, a synthetic agonist for PPARγ (18), phosphorylation of p42/p44 MAPK was decreased with a time course similar to that observed during the differentiation of 3T3-L1 cells (Fig. 1d). These results suggested that the down-regulation of p42/p44 MAPK activity during the differentiation of preadipocytes is dependent neither on the specific hormonal inducers nor on the specific cell line but rather reflects an intrinsic modification of intracellular signaling characteristic of the differentiation program.

To verify this hypothesis, we deprived either 3T3-L1 preadipocytes or fully differentiated adipocytes (8 days after induction) of serum for 16 h, stimulated the cells by reexposure to serum for 10 min, and then assayed cell lysates for the phosphorylation of p42/p44 MAPK and of Akt, both of which are known to be activated by serum. Serum elicited a marked increase in the extent of phosphorylation of both p42/p44 MAPK and of Akt, both of which are known to be activated by serum. Serum elicited a marked increase in the extent of phosphorylation of both p42/p44 MAPK and of Akt, both of which are known to be activated by serum. Serum elicited a marked increase in the extent of phosphorylation of both p42/p44 MAPK and of Akt, both of which are known to be activated by serum. Serum elicited a marked increase in the extent of phosphorylation of both p42/p44 MAPK and of Akt, both of which are known to be activated by serum. Serum elicited a marked increase in the extent of phosphorylation of both p42/p44 MAPK and of Akt, both of which are known to be activated by serum.

Fig. 2. Serum-induced activation of various signaling molecules and abundance of various proteins in preadipocytes and mature adipocytes. a, 3T3-L1 preadipocytes that had not been induced to differentiate (time 0) or 3T3-L1 mature adipocytes 8 days after the induction of differentiation with insulin, IBMX, and dexamethasone, and IBMX were deprived of serum for 16 h and then incubated for 10 min in the absence or presence of 10% FBS. Cell lysates were then subjected to immunoblot analysis with antibodies to the indicated proteins. b, total cell lysates prepared from the stromal-vascular (SV) fraction or the mature adipocyte fraction of mouse epididymal adipose tissue were subjected to immunoblot analysis with antibodies to the indicated proteins. All data are representative of at least three independent experiments.

Up-regulation of MKP-1 during Adipocyte Differentiation—The activity of p42/p44 MAPK is regulated both by the upstream kinase MEK, which phosphorylates both threonine and tyrosine residues of p42/p44 MAPK, and by a dual specificity phosphatase, which dephosphorylates the residues of p42/p44 MAPK phosphorylated by MEK (19). Although phosphorylated MEK was readily detected in 3T3-L1 preadipocytes that had been stimulated with serum for 10 min, it was detected in cells treated with inducers of differentiation only at low levels revealed by prolonged exposure of immunoblots (Fig. 3a). However, neither the amount of phosphorylated MEK nor that of total MEK changed during differentiation. Moreover, the extent of serum-induced phosphorylation of MEK was similar in nondifferentiated preadipocytes and in differentiated adipocytes.
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MKP-1 Expression Determines p42/p44 MAPK Activity during Adipocyte Differentiation—We next investigated whether the increase in the abundance of MKP-1 is related to the down-regulation of p42/p44 MAPK activity during differentiation of preadipocytes. For these experiments, we used electroporation to introduce exogenous genes into 3T3-L1 preadipocytes. Transfection with an expression vector for β-galactosidase revealed that >70% of the cells expressed the exogenous gene (Fig. 4a), indicative of a high efficiency of transfection with this method. The amount of MKP-1 in 3T3-L1 preadipocytes transfected with a vector for this protein was similar to that in nontransfected differentiated adipocytes (Fig. 4b). Transfection with the MKP-1 vector did not affect the abundance of other proteins such as CREB, a transcription factor involved in adipocyte differentiation (2). The serum-induced phosphorylation of p42/p44 MAPK, however, was greatly diminished in preadipocytes transfected with the MKP-1 vector compared with that apparent in cells transfected with the corresponding empty vector (Fig. 4c), consistent with the notion that the change in the abundance of MKP-1 is a major determinant of that in the activity of p42/p44 MAPK during differentiation of 3T3-L1 cells.

To establish more directly a causal link between MKP-1 expression and p42/p44 MAPK activity during adipocyte differentiation, we next examined the effect of depletion of endogenous MKP-1 on p42/p44 MAPK activity. Given that transfection of fibroblasts with a vector encoding Mkp-1 antisense RNA was shown to prevent the expression of Mkp-1 (20), we constructed an adenoviral vector (AxCAMkp-1-1AS) that contains Mkp-1 cDNA in the antisense orientation. As expected, infection of 3T3-L1 differentiated adipocytes with AxCAMkp-1-1AS, but not with a control adenovirus encoding β-galactosidase (AxCA LacZ), resulted in a marked reduction in the amount of MKP-1 without an effect on that of CREB (Fig. 4d). Conversely, the extent of serum-induced phosphorylation of p42/p44 MAPK was greater in the differentiated cells infected with AxCAMkp-1AS than in those infected with the control adenovirus (Fig. 4e). These results thus support the conclusion that the increase in the abundance of MKP-1 is a major contributor to the decrease in p42/p44 MAPK activity during adipocyte differentiation.

Up-regulation of MKP-1 Expression Is Required for Adipocyte Differentiation—We next investigated the physiological importance of the up-regulation of MKP-1 expression during adipocyte differentiation. 3T3-L1 preadipocytes were infected with AxCAMkp-1AS or with the control virus AxCA LacZ and were subsequently exposed to hormonal inducers of differentiation (insulin, dexamethasone, and IBMX). Eight days after exposure to the inducers of differentiation, the abundance of MKP-1 in cells infected with AxCAMkp-1AS was ~10% that in cells infected with the control virus (Fig. 5a). Consistent with this observation, the differentiation-associated down-regulation of p42/p44 MAPK activity apparent in the cells infected with the control virus did not occur in the cells infected with AxCAMkp-1AS (Fig. 5b). Furthermore, although the cells infected with
AxCAlacZ exhibited substantial accumulation of lipid droplets 8 days after exposure to the inducers of differentiation, lipid accumulation was markedly inhibited in the cells infected with AxCAMkp-1AS (Fig. 5c). The induction of PPARγ, C/EBPα, and aP2 was also greatly reduced in cells infected with AxCAMkp-1AS compared with that apparent in cells infected with the control virus (Fig. 5d). The abundance of CREB was not affected by expression of Mkp-1 antisense RNA (Fig. 5, a and d), and the up-regulation of C/EBPβ expression, which is maximal 2 days after exposure to the hormonal inducers of differentiation, was similar in cells infected with AxCAMkp-1AS and in those infected with AxCAlacZ (Fig. 5e). These results indicate that the induction of MKP-1 expression is essential for adipocyte differentiation and that MKP-1 contributes to this process at a step after the induction of C/EBPβ.

3T3-L1 cells that had been infected with AxCAMkp-1AS and exposed to inducers of differentiation in the presence of PD98059, a pharmacological inhibitor of MEK, manifested a reduced level of p42/p44 MAPK phosphorylation similar to that apparent in cells that had been infected with the control virus before induction of differentiation (Fig. 5b). Such treatment with PD98059 also prevented the inhibitory effect of MKP-1 depletion on adipocyte differentiation, as monitored by the accumulation of lipid droplets (Fig. 5c) and by the up-regulation of adipocyte marker proteins including PPARγ, C/EBPα, and aP2 (Fig. 5d). These results indicate that the inhibitory effect of AxCAMkp-1AS on adipocyte differentiation is attributable to the sustained activation of p42/p44 MAPK.

**DISCUSSION**

We have shown that the activity of p42/p44 MAPK decreases during the differentiation of cultured preadipocytes, consistent with previous observations (10). Furthermore, our results suggest that this down-regulation of p42/p44 MAPK activity is intrinsic to the differentiation program, reflecting a change in the intracellular signaling machinery, and is independent of the specific inducers of differentiation. The extent of the serum-induced phosphorylation of p42/p44 MAPK was thus greater in nondifferentiated preadipocytes than in mature adipocytes, even though the abundance of p42/p44 MAPK did not differ between the two states. An insulin-induced increase in the activity of p42/p44 MAPK apparent several hours after exposure of 3T3-L1 cells to insulin, dexamethasone, and IBMX has been shown to enhance the induction of PPARγ and C/EBPα expression during differentiation (21). This finding is consistent with the fact that insulin increases the proportion of differentiated cells but is not absolutely required for differentiation. Signaling by MEK and p42/p44 MAPK has also been shown to contribute to the mitotic clonal expansion that occurs within several days after the induction of differentiation of 3T3-L1 cells (22, 23). In contrast, incubation of preadipocytes with growth factors that induce a prolonged activation of p42/p44 MAPK prevents the cells from differentiating into adipocytes (24–26), and such inhibitory effects are attenuated by the MEK inhibitor PD98059 (24). Together, these various observations suggest that, although p42/p44 MAPK signaling is impor-
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tant for early steps of the differentiation program, sustained activation of this signaling inhibits adipocyte differentiation. The down-regulation of p42/p44 MAPK activity that occurs during the differentiation of preadipocytes thus appears to be essential for completion of the differentiation process.

The MKP-1 gene is an immediate early gene whose expression is induced rapidly by a variety of extracellular stimuli. Agents used to induce the differentiation of 3T3-L1 cells, including insulin (27), glucocorticoids (28), and cyclic AMP analogs (29), have thus been shown to increase expression of the MKP-1 gene. However, the induction of MKP-1 gene expression by such stimuli is usually transient (19, 27–29). Indeed, we found that the abundance of MKP-1 was increased within several hours after exposure of 3T3-L1 preadipocytes to insulin, dexamethasone, and IBMX but had returned to basal levels by 16 h.2 The pronounced increase in the amounts of both MKP-1 mRNA and protein apparent 4 days after exposure of 3T3-L1 cells to these inducers of differentiation as well as the persistence of the increased level of MKP-1 gene expression in differentiated adipocytes are thus likely attributable to an alteration of the transcripational machinery programmed during differentiation and not to a direct effect of the differentiation inducers per se. Given that the time courses of the expression of C/ebpα2 and Pparγ were similar to that of MKP-1 expression, one or both of these transcription factors may play a role in transcriptional activation of the MKP-1 gene. In this regard, the promoter of the mouse MKP-1 gene contains a sequence (TGGTGGATA, nucleotides −1155 to −1147) to which both C/EBPα and C/EBPβ are able to bind. Furthermore, the induction of MKP-1 expression normally observed during liver regeneration does not occur in mice that lack the C/EBPβ gene (30). The mechanism by which expression of the MKP-1 gene is up-regulated during the differentiation of preadipocytes remains to be established, however.

The down-regulation of the activity and phosphorylation of p42/p44 MAPK appeared related to the up-regulation of MKP-1 expression during the differentiation both of 3T3-L1 preadipocytes in culture as well as of cells in adipose tissue derived from animals. Furthermore, ectopic expression of MKP-1 in preadipocytes at a level similar to that of the endogenous protein in mature adipocytes resulted in a marked decrease in the extent of p42/p44 MAPK phosphorylation, and conversely, depletion of endogenous MKP-1 in mature adipocytes triggered an increase in the level of p42/p44 MAPK phosphorylation, suggesting that MKP-1 is a major regulator of p42/p44 MAPK activity during the differentiation of adipocytes. Prevention of the up-regulation of MKP-1 expression by transfection of preadipocytes with a vector for MKP-1 antisense RNA also greatly inhibited adipogenesis in cells exposed to insulin, dexamethasone, and IBMX. The phosphorylation of p42/p44 MAPK was not down-regulated even 8 days after exposure of 3T3-L1 preadipocytes expressing MKP-1 antisense RNA to the inducers of differentiation. Moreover, inhibition of p42/p44 MAPK activity by treatment with PD98059 restored the ability of cells expressing MKP-1 antisense RNA to undergo differentiation into adipocytes. The sustained activation of p42/p44 MAPK in these cells thus appeared to be the cause and not a result of the prevention of differentiation by MKP-1 depletion. Although MKP-1 dephosphorylates and inactivates kinases other than p42/p44 MAPK (19), the restorative effect of PD98059 indicates the importance of p42/p44 MAPK as a target of MKP-1 during adipocyte differentiation. Whereas the induction of PPARγ and C/EBPα expression during adipocyte differentiation was markedly attenuated by depletion of MKP-1, that of C/EBPβ expression was unaffected. These results are consistent with our observation that the activity of p42/p44 MAPK began to decrease after the onset of the increase in C/EBPβ expression during differentiation. The up-regulation of MKP-1 expression and the down-regulation of p42/p44 MAPK activity therefore likely contribute to adipocyte differentiation at a step after the induction of C/EBPβ expression. The activity of many members of the nuclear receptor family, including that of PPARγ, is affected by their phosphorylation status. Phosphorylation of PPARγ/2 on Ser112 (equivalent to Ser2 of PPARγ1) is catalyzed by p42/p44 MAPK and inhibits its transactivation activity (31, 32). Depletion of MKP-1 in 3T3-L1 preadipocytes thus likely promotes the phosphorylation of PPARγ by p42/p44 MAPK and thereby prevents the cells from acquiring the characteristics of mature adipocytes. PPARγ appears capable of inducing the expression both of itself and of C/EBPα (33, 34). The prevention of the increase in the abundance of PPARγ and of C/EBPα by expression of MKP-1 antisense RNA might thus be attributable to inhibition of the transactivation activity of PPARγ. We have found that growth factor-induced inhibition of transactivation activity of PPARγ was prevented either by MKP-1 overexpression or by PD98059,2 which is consistent with our hypothesis that MKP-1 can regulate the transactivation activity of PPARγ.

In summary, we have shown that the down-regulation of p42/p44 MAPK activity is a characteristic feature of and is crucial for adipocyte differentiation and that this biochemical change is attributable to an increase in the abundance of MKP-1. The abundance of MKP-1 is regulated by a variety of stimuli at both the transcriptional and post-transcriptional levels (19). Given that the putative MAPK phosphorylation sites in PPARγ influence both the size of fat cells and whole body insulin sensitivity in living animals (35), MKP-1 signaling in adipose tissue may provide a target for the development of new treatments for obesity and diabetes mellitus.

Acknowledgments—We thank D. Bernlohr, S. Keyse, N. Tonko, and I. Saito for antibodies to p42, cDNA for the human MKP-1 ortholog (CL-100), cDNA for mouse Mkph-1, and the adenoviral vector for β-galactosidase, respectively. We also thank Y. Yamauchi and M. Idei for technical assistance.

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and Masato Kasuga

J. Biol. Chem. 2004, 279:39951-39957.
doi: 10.1074/jbc.M407353200 originally published online July 20, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M407353200

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