

Regulation of Microsomal Triglyceride Transfer Protein Gene by Insulin in HepG2 Cells

Roles of MAPK^{erk} and MAPK^{p38}

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Microsomal triglyceride transfer protein (MTP) is rate limiting for the assembly and secretion of apolipoprotein B-containing lipoproteins. Elevated hepatic MTP mRNA level, presumably as a result of impaired insulin signaling, has been implicated in the pathophysiology of dyslipidemia associated with insulin resistance/type 2 diabetes. In this study, we showed that insulin decreases MTP mRNA level mainly through transcriptional regulation in HepG2 cells. We further characterized the corresponding signal transduction pathway, using chemical inhibitors and constitutively active and dominant negative forms of regulatory enzymes. We demonstrated that insulin inhibits MTP gene transcription through MAPK^{erk} cascade but not through the PI 3-kinase pathway. Activation of ras through farnesylation is not a prerequisite for the inhibition. In addition, cellular MAPK^{erk} and MAPK^{p38} activities play a counterbalancing role in regulating the MTP gene transcription. These complex regulations may represent a means to fine-tuning MTP gene transcription in response to a diverse set of environmental stimuli and may have important implications for the onset and development of diabetes-associated dyslipidemia. *Diabetes* 52:1073–1080, 2003

Diabetes is not only a disease characterized by elevated blood glucose but also a serious vascular disease with poor prognosis (1). Individuals with type 2 diabetes demonstrate an increased risk of cardiovascular abnormalities, which are closely associated with an increased level of plasma apolipoprotein B (apoB)-containing lipoproteins, i.e., VLDLs and LDLs (2,3). Metabolic labeling studies indicate that elevated plasma levels of lipoproteins in patients with diabetes are caused, at least in part, by an increased hepatic output of apoB-containing lipoproteins (1,4,5). A better understanding of how hepatic lipoprotein produc-

tion is regulated in patients with diabetes could potentially lead to the development of more effective strategic therapeutics to alleviate diabetic symptoms.

Recent advances have established the fundamental role of the microsomal triglyceride transfer protein (MTP) in the assembly of apoB-containing lipoproteins. MTP is an important enabler for the secretion of VLDLs by the liver, chylomicrons by the intestine (reviewed in ref. 6), and even LDLs by the heart (7). Functionally, MTP catalyzes the loading of lipids to the nascent apoB in the endoplasmic reticulum (ER). This stabilizes the newly synthesized apoB (8,9) and facilitates further processing, leading to its secretion. Reduction of MTP activity in animals by inhibitors (10) or gene knockouts (11–13) effectively lowers the plasma lipoprotein level, whereas enforced expression of hepatic MTP in mice (14) increases the plasma level of apoB-containing lipoproteins.

Emerging evidence has indicated that the pathophysiology of dyslipidemia observed under insulin resistance/type 2 diabetes is associated with an increased hepatic MTP mRNA level (15–18). This is presumably due to an impaired insulin-regulatory system as insulin is a negative regulator of the MTP gene (19,20). A recent study demonstrated that improving insulin sensitivity is associated with the normalization of the hepatic MTP expression and the reduction of VLDL secretion in insulin-resistant hamsters (19). Therefore, knowledge regarding how insulin regulates hepatic MTP gene transcription (21) would provide important insights toward the understanding of diabetes-associated dyslipidemia.

In this study, we elucidated the intracellular signaling pathways responsible for insulin-regulated MTP gene transcription, using chemical inhibitors and both dominant negative and constitutively active forms of regulatory enzymes in HepG2 cells. HepG2 cells were used for this study because they have lipoprotein production/regulation machineries and intact intracellular insulin signaling and are widely used to study lipoprotein assembly and secretion (22). Here we demonstrated that insulin inhibits MTP gene transcription through MAPK^{erk} signaling but not phosphatidylinositol 3-kinase (PI 3-K) signaling pathway. Farnesylated (active) ras proteins are not prerequisite for this inhibition. In addition, we described a mechanism for the fine-tuning of MTP transcription through a cross-talk between MEK1/2 and MAPK^{p38}, which may have significant implications for the onset and development of diabetes-associated dyslipidemia.

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apoB, apolipoprotein B; ER, endoplasmic reticulum; FBS, fetal bovine serum; LDL-R, LDL receptor; MAPK, mitogen-activated protein kinase; MTP, microsomal triglyceride transfer protein; PI 3-K, phosphatidylinositol 3-kinase; SREBP, sterol responsive element binding proteins.

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RESEARCH DESIGN AND METHODS

Materials. All chemical inhibitors were purchased from Calbiochem. Bovine insulin and DMSO were acquired from Sigma (St. Louis, MO). ECL Western blotting detection reagents were obtained from Amersham Pharmacia Biotech (Buckinghamshire, U.K.). Dual luciferase assay kit was obtained from Promega (Madison, WI). ERK1/2 antibody, akt antibody, and phospho-specific akt antibody were obtained from New England Biolabs. Phospho-specific ERK1/2 antibody, ras antibodies, and secondary antibody with horseradish peroxidase conjugate were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). HepG2 cells were obtained from American Type Culture Collection (Manassas, VA).

Construction of expression plasmids. The reporter construct pMTP-luc+ contains a 336-bp fragment encompassing position -250 to 86 of the human MTP promoter. This fragment was amplified from the human genomic library and tailored to contain *KpnI* and *BglIII* sites at the 5' and 3' ends. The fragment was digested by the restriction enzymes and inserted into a promoterless pGL3 luciferase vector (pGL3-Basic). The expression vectors encoding the dominant negative forms of the DN-ras (N17), the DN-raf-1 (K375M), the DN-MEK-1 (S218A/S222A), and the constitutively active forms of raf-1-BXB (BXB, 1→24-*KpnI*-302→648), MEK-1-ED (S218E/S222D), constitutively active myristoylated Akt (myristoylated, containing 12 amino acid NH₂-terminal sequence of the Lck gene, which carries the myristoylation/palmitoylation signal, followed by two Ala residues), and MKK-6-EE (S207E/T211E) (23) were generated by site-directed mutagenesis or deletion. All of the inserts of the plasmids were verified by sequencing.

Cell cultures. HepG2 cells were cultured at 37°C in growth medium (MEM containing 2 mmol/l nonessential amino acids, 2 mmol/l sodium pyruvate, 1.5 g/l sodium bicarbonate, 100 units/ml penicillin, 100 µg/ml streptomycin, 10% FBS, and supplemented with 0.3 mg/ml L-glutamine) in a humidified, 5% CO₂ incubator. The cells received fresh medium every 3 days.

Transfection and reporter assay. Cells were seeded at ~70% density in 12-well plates. A DNA/liposome mix containing 2 µg of LipofectAMINE 2000 (Invitrogen), 1 µg of pMTP-luc+, 0.1 µg of pRL-SV40, and 0.1–0.5 µg of expression plasmid (if necessary) DNA/well was used. The pRL-SV40 plasmid (Promega) coding for *Renilla* luciferase was co-transfected to serve as an internal control to normalize the transfection efficiency. Transfections were allowed to proceed for 6 h in the absence of serum, and the cells were recovered in medium supplemented with reduced serum level (2.5%) for 16 h. For inhibitor studies, HepG2 cells were first transfected with pMTP-luc+ for 6 h, treated for 30 min with 3× the effective level of inhibitors, and then incubated for 24 h with 1× the effective level of inhibitors in the presence or absence of 100 nmol/l insulin. In our experience, this protocol ensures an efficient blockade of a particular signaling molecule but avoids the potential cytotoxic effects of a prolonged treatment with high doses of inhibitors (data not shown). Finally, the cells were allowed to culture for 24 h and were lysed with passive lysis buffer (Promega), and luciferase activities were assayed using a dual luciferase reporter system (Promega) according to the manufacturer's instructions and were quantified in a Lumat LB 9507 luminometer (Berthold).

Western blot analysis. For Western blot analysis, cells were lysed in lysis buffer (20 mmol/l Tri-HCl [pH 7.4], 50 mmol/l NaCl, 1 mmol/l EDTA, 1 mmol/l EGTA, 1% Triton X-100, 25 mmol/l NaF, 1 mmol/l Na₃VO₄, 1 mmol/l phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, and 5 µg/ml aprotinin). Lysates were clarified by centrifuging at 10,000g for 5 min at 4°C, and protein concentrations were determined by Bradford assay (Bio-Rad). Immunoprecipitation was carried out where indicated. Protein samples were resolved by 10%/16.5% SDS-PAGE and transferred to polyvinylidene difluoride membrane (0.45-µm pore size, Immobilon-P, Millipore). Blocking, antibody incubation, and washes were conducted and immunoreactive bands were detected using the ECL reagents, according to the manufacturer's instructions. A representative blot from at least two separate experiments was shown.

Statistical analysis. Statistical analysis was performed using two-tailed Student's *t* test, and *P* < 0.05 was considered significant. Data are expressed as the means ± SD of triplicate samples, and the reproducibility was confirmed in three separate experiments.

RESULTS

Insulin decreases MTP mRNA level mainly through downregulating MTP gene transcription. We have previously shown that a physiological concentration of insulin (1 nmol/l) can cause a significant 30% decrease of the MTP mRNA level via insulin receptor signaling in HepG2 cells (20). Here we first determined whether this inhibition can be attributed to transcriptional regulation by measuring

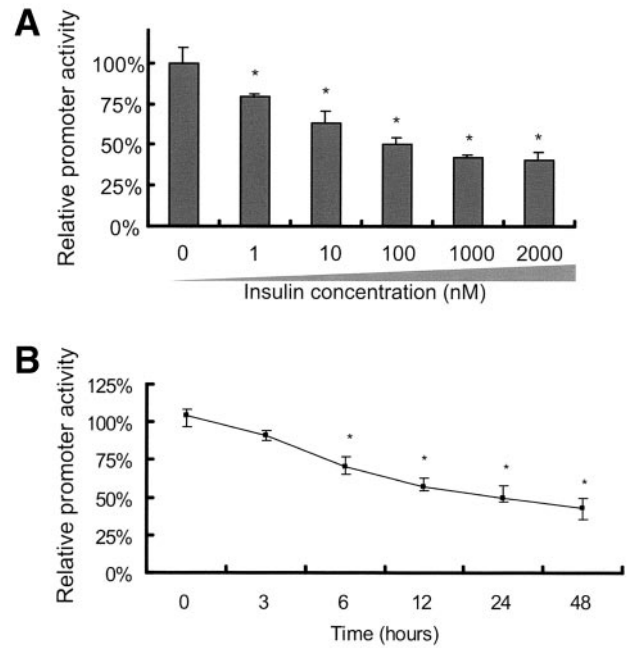


FIG. 1. The insulin-mediated inhibition on MTP promoter activity. HepG2 cells were transfected with pMTP-luc+, and reporter activity was measured. The MTP promoter activity obtained in the untreated control was considered as a reference (100%). **A:** Dose response. The transfected cells were cultured under bovine insulin (0–2 µmol/l) for 24 h. **B:** Time course. The transfected cells were cultured with or without 100 nmol/l insulin for 0–48 h. The relative promoter activities were presented as a ratio of the promoter activity in the presence of insulin to that in its absence. **P* < 0.05, significantly different from the reference value.

the dose-effect of insulin on MTP promoter activity. HepG2 cells were transfected with a human MTP promoter (-250 to 86)/luciferase reporter construct (pMTP-luc+) and then treated with 1–2,000 nmol/l insulin for 3–48 h. Our data indicated that insulin inhibited MTP promoter activity in a dose- and time-dependent manner. At concentrations of 1, 10, 100, and 1,000 nmol/l, insulin reduced MTP promoter activity by 25, 40, 50, and 65%, respectively (Fig. 1A). This is consistent with the insulin effect on MTP mRNA level, suggesting that insulin downregulates MTP mRNA level mainly through transcriptional regulation. Time-course study showed that the inhibition occurred within 3 h, became statistically significant (>25%; *P* < 0.05) after 6 h, persisted over time, and reached a maximum (60%) at 48 h after the initiation of insulin treatment (Fig. 1B).

The role of PI 3-K signaling axis in insulin-mediated inhibition of MTP gene transcription. The observation that insulin acutely inhibits apoB secretion in HepG2 cells through the PI 3-K signaling pathway (23,24) prompted us to examine whether PI 3-K signaling is required for insulin-mediated inhibition of MTP gene transcription. We used chemical inhibitors targeting molecules in the PI 3-K signaling axis. These inhibitors include wortmannin and LY294002 (inhibitors for PI 3-K) and rapamycin (inhibitor for p70S6K). As shown in Fig. 2A, whereas insulin treatment caused a significant 50% reduction of the MTP promoter activity, wortmannin, LY294002, and rapamycin did not cause any changes to the insulin effect. The efficacy of one of the inhibitors, LY294002, was confirmed by measuring the level of phosphorylated Akt (protein kinase B), an immediate downstream substrate of PI 3-K. As

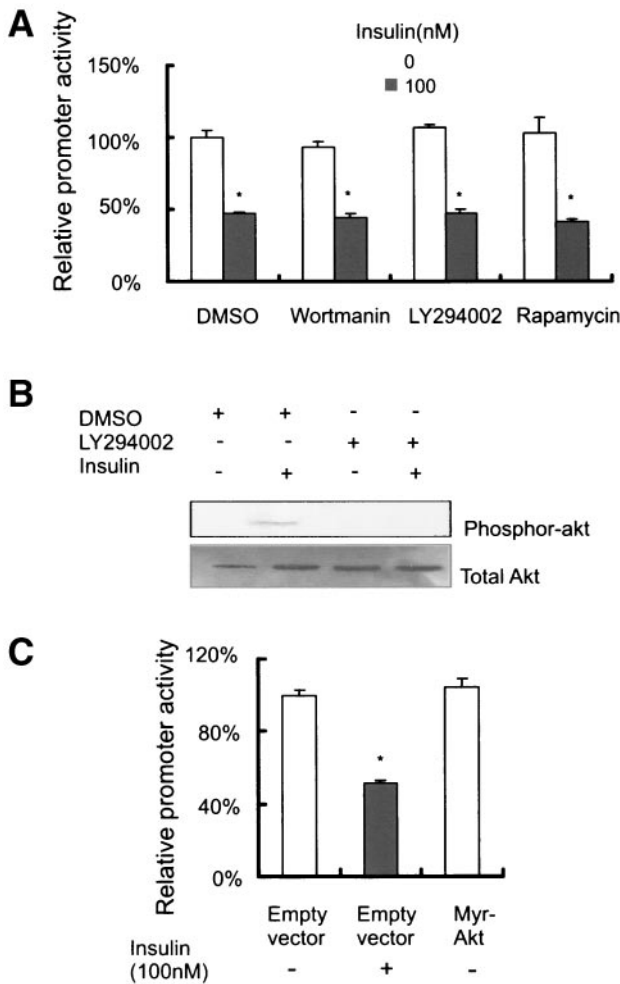


FIG. 2. Role of PI 3-K signaling axis on insulin-mediated inhibition. **A:** Effect of inhibitors of PI 3-K signaling axis. HepG2 cells were transfected with pMTP-luc+ and treated with (effective dosages) 8 $\mu\text{mol/l}$ LY294002, 17 $\mu\text{mol/l}$ wortmannin, or 2 $\mu\text{mol/l}$ rapamycin with or without 100 nmol/l insulin for 24 h. **B:** Effect of LY294002 on insulin-induced Akt phosphorylation. HepG2 cells were treated with 25 $\mu\text{mol/l}$ LY294002 for 1 h followed by stimulation of 100 nmol/l insulin for 15 min. Cell lysates were immunoblotted against anti-Akt or anti-phosphor-Akt antibody. **C:** Overexpression of myristoylated Akt on MTP promoter activity. HepG2 cells were co-transfected with pMTP-luc+ and expression vector encoding Myr-Akt as detailed in RESEARCH DESIGN AND METHODS. * $P < 0.01$, significantly different from the reference value.

shown in Fig. 2B, Western blot analysis confirmed that the insulin-induced phosphorylation of Akt was indeed inhibited by LY294002. Furthermore, an enforced expression of active (myristoylated) Akt also failed to imitate the inhibition offered by insulin (Fig. 2C). Taken together, these results indicated that the PI 3-K signaling axis is not involved in the insulin effect.

MEK1/2 activity is required for insulin-mediated inhibition. We next investigated whether MAPK^{erk} cascade, another signaling arm of insulin, is involved. HepG2 cells were treated with PD98059, a selective inhibitor of MEK1/2, before the administration of 100 nmol/l insulin. As shown in Fig. 3A, PD98059 abolished the insulin-mediated inhibition. Addition of LY294002 (a PI 3-K inhibitor) did not change the PD98059 effect, supporting a null role of PI 3-K on the insulin-mediated inhibition. In addition, U0126 (25), a structurally different inhibitor of MEK1/2, abolished insulin-mediated inhibition, whereas

U0124, an inactive derivative of U0126, did not produce any effect (Fig. 3B). Western blot analysis confirmed that U0126 indeed inhibited the insulin-induced phosphorylation of ERK1/2, an immediate downstream substrate of MEK1/2. In contrast, the inactive U0124 has no effect (Fig. 3C). The role of MEK was further studied by overexpressing both the dominant negative (DN) and the constitutively active (ED) mutants of MEK-1. Consistent with results obtained from chemical inhibitor studies, we showed that DN-MEK1 significantly alleviated the magnitude of insulin-mediated inhibition (from 50 to 25%). The constitutively active MEK1-ED caused a significant (~40%) inhibition of MTP promoter activity in the absence of insulin, and the addition of insulin did not significantly change the promoter activity. These results indicated that activation of MEK-1 is sufficient to uncouple the insulin effect on MTP promoter activity.

The MTP promoter activity is inversely correlated with the level of activated ERK1/2. To investigate further the relationship between MTP promoter activity and MEK1/2 activity, we examined the dose-response curve of U0126 (a MEK1/2 inhibitor). As shown in Fig. 4A, low concentrations of U0126 (up to 0.5 $\mu\text{mol/l}$) dose-dependently reduced insulin-mediated decrease in MTP promoter activity. At 0.5 $\mu\text{mol/l}$, U0126 completely abolished the insulin effect. Extrapolating this result with the complementary Western blot (Fig. 4B), it seems that 0.5 $\mu\text{mol/l}$ U0126 also completely abolished the insulin-stimulated increase of the activated (phosphorylated) ERK1/2. In fact, the level of activated ERK1/2 is identical to that of the untreated control. A further increase in U0126 concentrations (from 1 to 20 $\mu\text{mol/l}$) resulted in a gradient depletion of the residual amount of activated ERK1/2 (Fig. 4B) and a parallel gradient stimulation of MTP promoter activity (up to 30%). Pearson's correlation analysis ($R = -0.984$, $P = 0.0004$) indicated an inverse correlation between ERK1/2 level and the MTP promoter activity (Fig. 4C). Taken together, these results support the new idea that ERK plays an important role in the regulation of MTP gene transcription.

Insulin inhibits MTP gene transcription via Raf-1/MEK/ERK cascade. Having established a requirement of MEK and ERK, we examined the role of Raf-1, which is the most upstream member of the MAPK^{erk} cascade. We determined the effect of overexpressing a dominant negative (DN-raf-1) or a constitutively active (Raf-1-BXB) mutant of Raf-1. Our results (Fig. 5) indicated that an enforced expression of DN-raf-1 significantly reduced the insulin-mediated inhibition of the MTP promoter activity (from 50 to 23%). In contrast, cells transfected with constitutively active Raf-1-BXB plasmid exhibited a momentous 50% drop in MTP promoter activity, similar to that produced by insulin, and the addition of insulin did not significantly change the promoter activity. These results suggest that activation of Raf-1 can also uncouple the insulin effect on MTP promoter activity.

Ras is not required in the signaling. Ras is the classic upstream mediator of MAPK^{erk} cascade (rev. in 26). To test whether ras is required for signaling, we blocked the activation of ras by using two different kinds of farnesylation transferase inhibitors: FTase I I and FTase I III (27). Our results indicated that neither FTase I I nor FTase I III

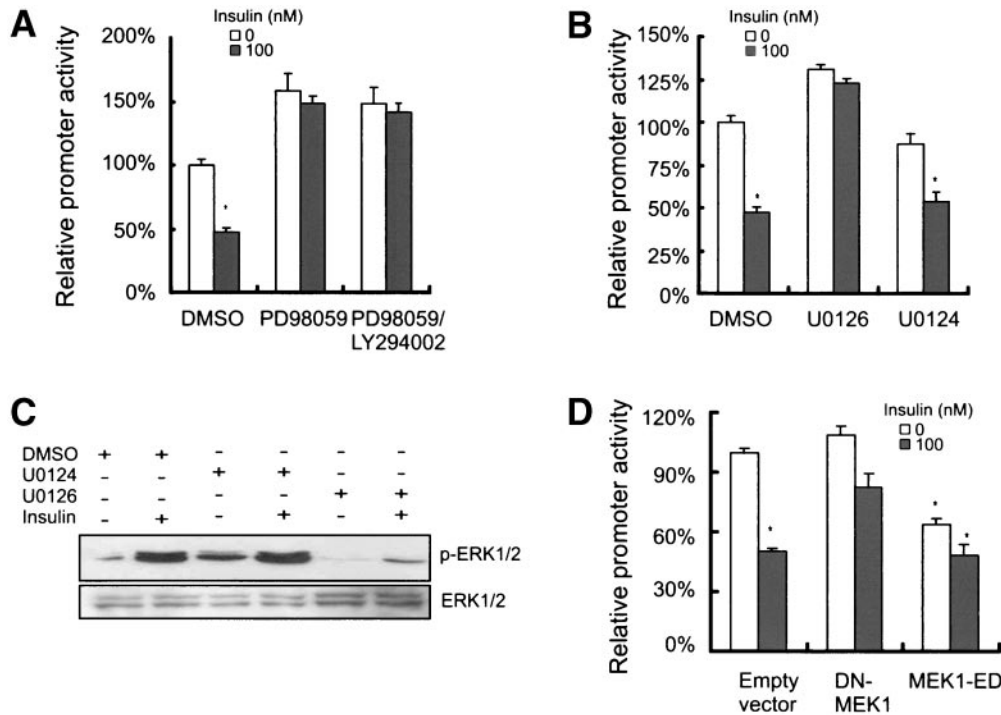


FIG. 3. Role of MAPK^{erk} cascade. **A** and **B**: Effect of MEK1/2 inhibitors on MTP promoter activity. HepG2 cells were transfected with pMTP-luc+ and treated with (effective dosages) 10 μ mol/l PD98059, 7 μ mol/l U0126, or 7 μ mol/l U0124 with or without 100 nmol/l insulin for 24 h. **C**: Effect of U0126 on insulin-induced ERK phosphorylation. HepG2 cells were treated with 20 μ mol/l U0126 or U0124 for 1 h followed by stimulation of 100 nmol/l insulin for 15 min. Cell lysates were immunoblotted against anti-ERK1/2 or anti-phosphor-ERK1/2 antibody. **D**: Enforced expression of DN-MEK-1 or MEK1-ED on MTP promoter activity with or without 100 nmol/l insulin. * $P < 0.01$, significantly different from the reference value.

had any effect on insulin-mediated inhibition of MTP promoter activity (Fig. 6A). The effectiveness of these inhibitors was demonstrated by an immunoblot method, in which we monitored the farnesylation status of ras. The unfarnesylated forms of ras proteins display reduced mobility in SDS-PAGE relative to their farnesylated versions (28); therefore, they can be separated by SDS-PAGE. As shown in Fig. 6B, in the unstimulated cells, both the unfarnesylated (U) and farnesylated (F) forms of ras were present. Insulin treatment increased the level of farnesylated ras, whereas FTase I I and FTase I III both inhibited the farnesylation of ras, reflecting the working of inhibitors. In addition, we showed that the overexpression of dominant negative ras protein did not have any effect on the insulin inhibition (Fig. 6C). Taken together, these results indicate that an activated ras protein is not a prerequisite in the insulin-dependent inhibition of MTP gene transcription.

Role of MAPK^{p38} signaling on insulin inhibition. A recent report has demonstrated the presence of cross-talk between the MAPK^{erk} and MAPK^{p38} cascades in liver cells (29). More specific, it was shown that MAPK^{p38} inhibits MEK1/2 (29,30). Having established that MAPK^{erk} cascade is the intracellular messenger for insulin, we therefore examined the potential role of MAPK^{p38} on insulin-mediated suppression of MTP promoter activity. HepG2 cells were treated with two specific MAPK^{p38} inhibitors, SB202190 and SB203580, before the addition of insulin. SB202474, an inactive derivative, was used as a negative control. As shown in Fig. 7A, none of the MAPK^{p38} inhibitors abrogated the insulin-mediated inhibition. To our surprise, SB202190 and SB203580 actually produced a 37 and 19% decrease of the MTP promoter activity, respec-

tively, in the absence of insulin challenge. Moreover, both SB202190 and SB203580 enhanced the magnitude of insulin inhibition (an extra ~ 28 and 22% decrease, respectively). These results indicated that MAPK^{p38} stimulates MTP promoter activity and sensitizes cells to the insulin effect. In contrast, the inactive form of inhibitor SB202474 did not exhibit these effects. In the complementary Western blot analysis (Fig. 7B), we showed that inhibition of MAPK^{p38} by SB202190 enhanced the activation/phosphorylation of ERK1/2, suggesting that MAPK^{p38} exerted its action through the MEK1/2 and ERK cascade.

Consistent with the results from inhibitor studies, stimulating MAPK^{p38} activity by the constitutively active MKK6-EE (29), in which the activating phosphorylation residues Ser207 and Thr211 were replaced by glutamic acids, significantly increased MTP promoter activity by 40% as compared with the empty vector control (Fig. 7C). The administration of insulin attenuated the MKK6-EE-stimulated increase. Taken together, our results further support a counterbalancing role of MAPK^{p38} and MAPK^{erk} in the regulation of MTP promoter activity.

DISCUSSION

We have previously shown that insulin downregulates MTP mRNA level via insulin receptor signaling (20,21). Results from this study indicated that insulin downregulates MTP mRNA level mainly through transcriptional regulation. As summarized in Fig. 8, the early cellular signals transduced from insulin are delivered via MAPK^{erk} cascade. Activation of ras proteins through farnesylation is not a prerequisite for the inhibitory event. PI 3-K, the major metabolic signaling cascade activated by insulin

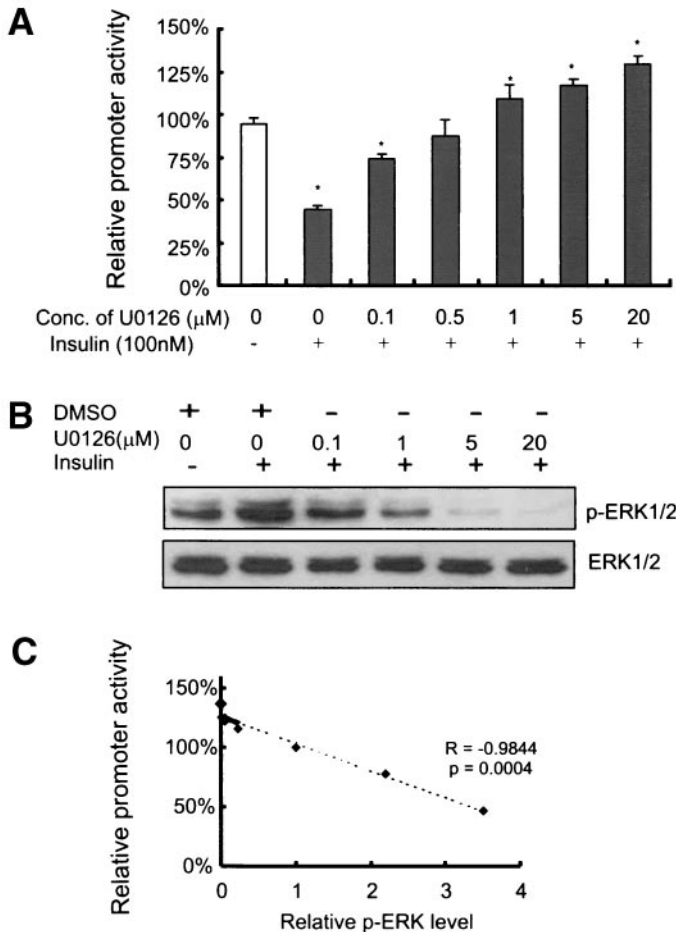


FIG. 4. Dose-dependent effect of U0126. **A:** Dose-dependent effect of U0126 on MTP promoter activity. HepG2 cells were transfected with pMTP-luc+ and treated with varying concentration of U0126 (0–20 $\mu\text{mol/l}$) plus 100 nmol/l insulin for 24 h. Pretreatment with the inhibitor was carried out in the same dosages as indicated. * $P < 0.05$, significantly different from the reference value. **B:** HepG2 cells were treated with varying dosages of U0126 for 1 h followed by stimulation of 100 nmol/l insulin for 15 min. Cell lysates were immunoblotted against anti-ERK1/2 or anti-phosphor-ERK1/2 antibody. **C:** Pearson's correlation analysis of MTP promoter activity and the relative phospho-ERK1/2 protein level. R represents Pearson's correlation coefficient.

(31), is not involved. We also demonstrated that cellular MEK1/2 and MAPK³⁸ activities play a counterbalancing role in suppressing and stimulating the MTP gene transcription, by which MAPK³⁸ inhibits MEK1/2.

This is the first report to show that MAPK^{erk} cascade is responsible for the regulation of MTP gene transcription by insulin. This conclusion is based on the following observations. First, we showed that two different classes of MEK1/2 inhibitors can block the insulin effect. The selectivity of these inhibitors has been described extensively, and they have negligible effect on other closely related MAPKs (25,29,32). Second, MTP promoter activity is inversely related to the level of the activated/phosphorylated ERK. Finally, to rule out the possibility that chemical inhibitors may have unwanted activities, we also confirmed our results using the dominant negative Raf/MEK1 as well as the constitutively active Raf/MEK1 enzymes. These results raise the possibility that MAPK^{erk} plays a direct role in lipoprotein production.

It is interesting to note that farnesylated (activated) ras

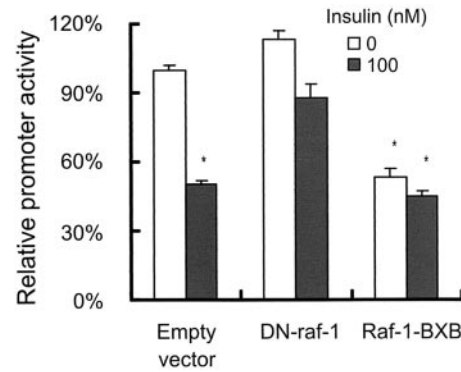


FIG. 5. The role of Raf-1. HepG2 cells were co-transfected with pMTP-luc+ and expression vector encoding DN-Raf-1 or Raf-BXB, followed by incubating with 0 or 100 nmol/l insulin for 24 h. * $P < 0.01$, significantly different from the reference value.

proteins are not required in the inhibitory event, suggesting an involvement of ras-independent MAPK^{erk} signaling. Recently, it has been demonstrated that although insulin significantly activates MAPK^{erk} and ras protein activities in both mitogenically active 3T3-L1 fibroblasts and metabolically active 3T3-L1 adipocytes, the latter does not require an active ras to activate MAPK^{erk} by insulin (33). HepG2 cells are also metabolically active and express a variety of liver-specific metabolic functions (22). Our data are consistent with the previous report and support a special role of ras-independent MAPK^{erk} in metabolic events.

Accumulating evidence (3,6,20,24) suggests that insulin regulates apoB lipoprotein metabolism through both acute and adaptive machineries. The acute machinery involves insulin-mediated activation of PI 3-K and subsequent localization of PI 3-K to ER to inhibit the secretion of apoB-containing lipoprotein particles and thereby promotes the intracellular degradation of apoB (23,24). This action occurs in cytosol, and the inhibition of apoB secretion can be observed within several minutes. The adaptive machinery involves insulin-mediated signal transduction to inhibit MTP gene transcription (6). Consequently, it causes reductions in MTP protein mass/enzyme activity and results in a higher rate of intracellular degradation of nascent apoB and a significant reduction in apoB secretion (6,8,34). Results from our study identified the MAPK^{erk} cascade (Raf-1/MEK/ERK) as the signaling bridge between insulin receptor and MTP gene transcription. Therefore, insulin-regulated apoB secretion comprises two functionally distinct signaling pathways, namely the PI 3-K and MAPK^{erk}, in which the former instantly directs and the latter potentiates the hypolipidemic effect of insulin. This also exemplifies the beauty of two separate insulin-signaling axes that coordinately control the apoB output from the liver in a highly organized temporal manner.

Besides regulating MTP gene transcription, MAPK^{erk} cascade plays an important role in the regulation of LDL receptor (LDL-R) gene expression, which is essential for the lipoprotein uptake by a number of cells. It is noteworthy that both insulin and MAPK^{erk} upregulate LDL-R gene transcription (35), whereas they both inhibit MTP gene transcription. The differential effect of MAPK^{erk} on these two genes suggests that distinct downstream mechanisms are involved. Consistent with this notion, recent evidence

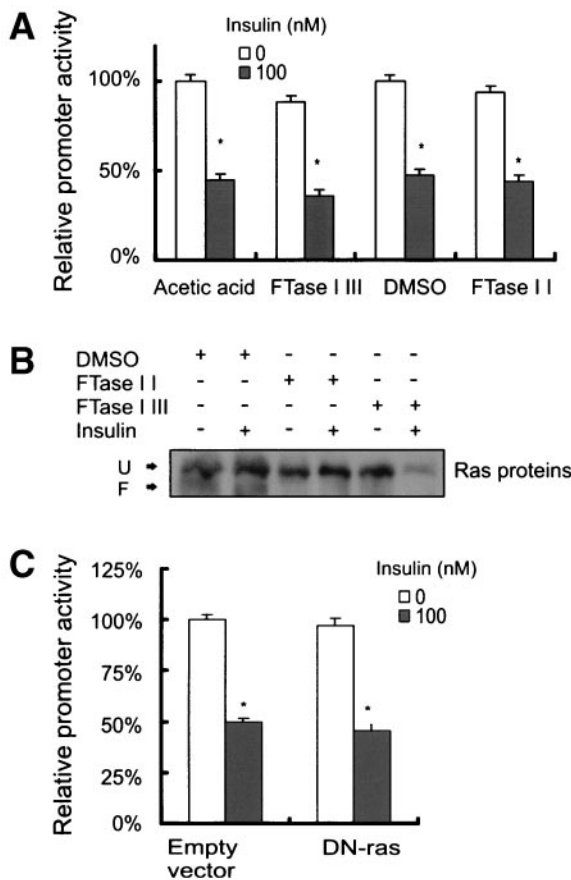


FIG. 6. The role of ras on insulin-mediated inhibition. **A:** Effect of farnesylation inhibitors on MTP promoter activity. HepG2 cells were transfected with pMTP-luc+ and treated with (effective dosages) 2 μ mol/l FTase I I or 200 nmol/l FTase I III with or without 100 nmol/l insulin for 24 h. DMSO and acetic acid are the vehicles for FTase I I and FTase I III, respectively. **B:** Effect of farnesylation inhibitors on the farnesylation status of ras. HepG2 cells were treated with 5 μ mol/l of FTase I I for 1 h followed by stimulation of 100 nmol/l insulin for 4 h. Cell lysates were immunoprecipitated with anti-ras antibody (clone Y13-238) and immunoblotted against anti-ras antibody (F132). **C:** Enforced expression of dominant negative forms of ras. HepG2 cells were co-transfected with pMTP-luc+ and expression vector encoding DN-ras, followed by incubating with 0 or 100 nmol/l insulin for 24 h. * $P < 0.01$, significantly different from the reference value.

(35,36) indicates that sterol responsive element binding proteins (SREBPs) are the downstream effectors of MAPK^{erk} to transactivate the LDL-R gene. It has previously been shown that the SREBPs are unlikely to mediate the insulin-dependent inhibition of MTP gene transcription. In contrast, SREBPs mediate the negative sterol-depletion response that suppresses MTP gene transcription (37).

In light of a recent report showing that stress-activated MAPK^{p38} negatively regulates LDL-R expression through modulating MAPK^{erk} cascade in HepG2 cells, a one-way communication has been established between MAPK^{p38} and MAPK^{erk}, in which MAPK^{p38} inhibits MAPK^{erk} (29,30). Here, we demonstrated that MAPK^{erk} and MAPK^{p38} have an antagonistic effect on MTP promoter activity. We showed that the MAPK^{p38} inhibitors SB202190/SB203580 enhanced the magnitude of insulin inhibition and significantly increased the level of activated ERK1/2. The specificity of these MAPK^{p38} inhibitors (38,39) have been described in several studies, and the possibility of them acting on other related MAPKs, such as ERK1/2 or JNKs

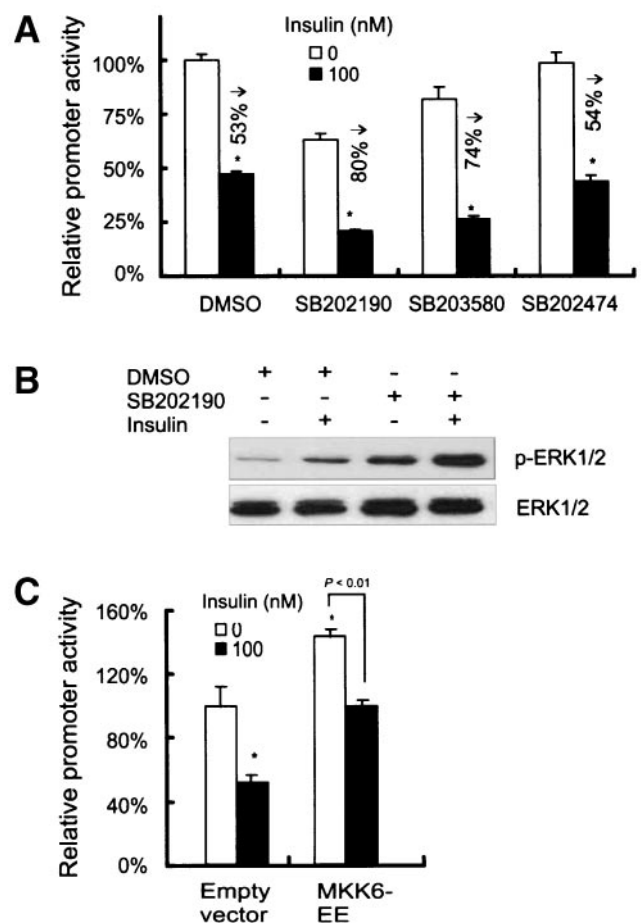


FIG. 7. The role of p38 MAPK on insulin-mediated inhibition. **A:** Effect of MAPK^{p38} inhibitors on MTP promoter activity. HepG2 cells were transfected with pMTP-luc+ and treated with (effective dosage) 7 μ mol/l SB202190, 7 μ mol/l SB203580, or 7 μ mol/l SB202474 with or without 100 nmol/l insulin for 24 h. Percentage of inhibition relative to the reference was shown. **B:** Effect of SB202190 on insulin-induced ERK phosphorylation. HepG2 cells were treated with 20 μ mol/l SB202190 for 1 h followed by stimulation of 100 nmol/l insulin for 15 min. Cell lysates were immunoblotted against anti-ERK1/2 or anti-phospho-ERK1/2 antibody. **C:** Enforced expression of constitutively active MKK-6. HepG2 cells were co-transfected with pMTP-luc+ and expression vector MKK6-EE, followed by incubating with or without 100 nmol/l insulin for 24 h. * $P < 0.01$, significantly different from the reference value.

(p46/54), is unlikely (29,32). Furthermore, we used an active MKK6 and showed that it can significantly enhance MTP promoter activity, which could be attenuated by the addition of insulin. The significance of these observations is twofold. First, it supports our finding that MAPK^{erk} cascade exerts an inhibitory effect on MTP gene transcription. This is because inhibition of MAPK^{p38} triggers activation of MEK1/2, which mimics a stimulation of ERK1/2. Second, such a cross-talk might represent an important mechanism for the fine-tuning of the transcription of the MTP gene in response to a diverse set of environmental stimuli, including growth factors and other stresses. Indeed, this notion gained support from a recent report showing that insulin-activated MAPK^{erk} could be attenuated by MAPK^{p38} (40).

At present, the role of MAPK^{erk} in diabetes is still being contested (rev. in 41). Several studies have shown that an elevated MAPK^{erk} is associated with the complications of insulin resistance/type 2 diabetes. MAPK^{erk} has been im-

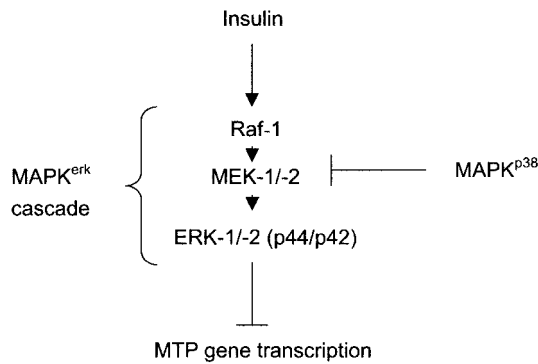


FIG. 8. A proposed signaling pathway for insulin-mediated regulation of MTP gene transcription in the liver. Activation of MAPK^{erk} by insulin leads to inhibition of MTP gene transcription, whereas activation of MAPK^{p38} attenuates the MAPK^{erk} effect. MAPK^{erk} is central to the regulation of MTP gene transcription. It integrates signals from insulin and MAPK^{p38} to fine-tune the transcription of the MTP gene. Pointed arrows denote stimulation; blunted arrows denote inhibition.

plicated in the apoptosis of the β -cell of islets (42) and the oxidized LDL-induced oxidative stress in glomerular injury (43). Both contribute to the aggravation of the symptoms of diabetes. However, results from us and others seem to suggest that the activation of MAPK^{erk} in the liver has a beneficial effect in the plasma lipid profiles, as the activation of MAPK^{erk} could induce LDL-R expression and suppress the hepatic MTP gene expression, both of which are essential to reduce the high level of plasma lipoproteins by facilitating apoB clearance and attenuating apoB secretion, respectively.

However, in animals with type 2 diabetes, the beneficial role of hepatic MAPK^{erk} seems to be compromised. In subjects with diabetes, the damaging effects of increased plasma glucose ultimately create osmotic and oxidative stresses to the hepatocytes and other cells (reviewed in ref. 41). These chronic stressors can activate MAPK^{p38} (reviewed in ref. 44), and the activated MAPK^{p38} inevitably inhibits MAPK^{erk} in the liver through the one-way cross-talk (38). As a result of the convergent interaction, the net effect of MAPK^{erk} on suppressing MTP gene transcription would be significantly reduced, leading to the upregulation of MTP mRNA. Therefore, we propose that the dysregulation of the MAPK^{erk}/MAPK^{p38} pathways contributes to an elevated hepatic MTP expression in diabetes. This may have important implications for the onset and development of diabetes-associated dyslipidemia.

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