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Hepatocyte growth factor induces *MAT2A* expression and histone acetylation in rat hepatocytes: role in liver regeneration

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ABSTRACT

Methionine adenosyltransferase (MAT) catalyzes the synthesis of S-adenosylmethionine (AdoMet). MAT is the product of two genes: *MAT1A* and *MAT2A*. *MAT1A* is expressed in adult liver, whereas *MAT2A* is expressed in proliferating fetal hepatocytes and during liver regeneration. Furthermore, *MAT2A* replaces *MAT1A* upon neoplastic transformation of the liver; this action has been shown to affect cellular AdoMet levels and cell growth. The mechanisms behind *MAT2A* induction during the physiological proliferative response of the hepatocyte are not known. In a model of rat hepatocytes in culture, we demonstrate that *MAT2A* transcription is induced time- and dose-dependently by hepatocyte growth factor (HGF), a key factor in liver regeneration. In addition we show for the first time that HGF stimulates the acetylation of histones (H4) associated with *MAT2A* promoter. HGF effects were blocked in the presence of the tyrosine kinase inhibitor genistein. These effects of HGF may be responsible for the induction of *MAT2A* expression and acetylation of histones associated with *MAT2A* promoter that we also demonstrate here in a model of liver regeneration. Finally, we identify AdoMet as an inhibitor of *MAT2A* response to HGF, which suggests that this metabolite may participate in the signaling process that leads to termination of liver regeneration.

Key words: *S*-adenosylmethionine • methylthioadenosine • gene expression • methionine metabolism

S-adenosylmethionine (AdoMet) is the principal biological methyl and propylamino donor in cellular metabolism (1–3). AdoMet is synthesized by methionine adenosyltransferase (MAT) from methionine and ATP. Mammals have three MAT isoforms: MAT I, MAT II,and MAT III (4). MAT I and MAT III are a tetramer and dimer, respectively, of the same α1 subunit encoded by the *MAT1A* gene, whereas MAT II is formed by the α2 catalytic subunit, which is the product of *MAT2A* gene and a regulatory β subunit (3, 5). The products of *MAT1A* and *MAT2A* genes have distinct kinetic and regulatory properties, which have been shown to influence the intracellular levels of AdoMet (3, 6). The expression of both *MAT1A* and *MAT2A* genes is tightly controlled; *MAT1A* is expressed in the adult and differentiated hepatocyte as part of a metabolic phenotype, whereas *MAT2A* is widely expressed outside the liver parenchyma and, to a very low extent, in the adult hepatocyte (3). However, when the hepatocyte proliferates, as during liver regeneration and the fetal stages of liver development, *MAT2A* expression is induced (7, 8). Similarly, in human and rat hepatocarcinoma (HCC) the transcription of *MAT1A* is silenced by being replaced by *MAT2A* expression (9–11). In a model of human HCC cells in culture in which the type of MAT expressed was manipulated by transfection and antisense strategies, it was found that expression of *MAT2A* was associated with reduced AdoMet levels and more rapid cell growth and DNA synthesis, whereas the opposite was found for *MAT1A* (6). Moreover, the addition of AdoMet to cultured HCC cells and AdoMet administration in rodent HHC models reduced cell growth and neoplastic progression (6, 12). There is also a relationship between AdoMet levels in the hepatocyte and the type of MAT gene expressed. In this regard, we have recently shown in cultured rat hepatocytes that elevated AdoMet levels favors the maintenance of a differentiated and metabolic phenotype, in which *MAT1A* is expressed and *MAT2A* is silenced, whereas reduced (lower than normal) AdoMet content leads to the opposite situation (13).

As mentioned above, liver regeneration after two-thirds partial hepatectomy (PH) is among the pathophysiological conditions in which the expression of *MAT2A* is induced in the hepatocyte (7). In this situation, most (90%) of the remaining liver parenchyma enters the cell cycle and initiates DNA synthesis, and after 7–10 days the original liver mass is restored (14, 15). Induction of *MAT2A* gene transcription has been proposed to influence AdoMet levels in the regenerating hepatocyte, which in turn would affect transmethylation reactions, such as DNA methylation, and gene expression (7). Little is known, however, about the signals and mechanisms that trigger the induction of *MAT2A* expression in the proliferating normal hepatocyte. It is widely accepted that chromatin structure is crucial in regulating gene expression in eukaryotes (16). In this regard, the interplay of remodeling complexes and covalent histone modifications seems to be essential for the access of DNA binding factors (17, 18). Histone acetylation is the best-characterized posttranslational modification, and it has long been associated with the transcriptional state of many genes (19–21), but the unambiguous correlation between transcriptional activity and histone hyperacetylation has only been proven in the past few years (for review, see ref. 20). It is obvious that the acetylation state of histones over a given DNA sequence depends on the relative activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs), which in turn may depend on both the specific recruitment of these enzymes and the control of their activity. The possibility of specific targeting of these two types of enzymes to promoters has been discussed recently (19), and it is also known that HATs (22) and HDACs (23) may be controlled by phosphorylation and by interaction with other proteins (20). It has been proposed that the level of selective histone acetylation may depend on signal

transduction pathways (24), but little is known about the possible signal cascades for which HATs and/or HDACs are the endpoints. In this context we have addressed the molecular mechanisms behind *MAT2A* induction during hepatocyte proliferation.

MATERIALS AND METHODS

AdoMet, in the stable form of sulfate-*p*-toluenesulfonate salt produced by Knoll Farmaceutici, Milan, Italy, was provided by Europharma (Madrid, Spain). Human recombinant HGF was from Calbiochem. All other reagents, unless otherwise stated, were purchased from Sigma (St. Louis, Mo.).

Animal experiments

Male Wistar rats (200 g), were fed *ad libitum* a standard laboratory diet and received humane care according to our institution's guidelines for the use of laboratory animals. Two-thirds PH was performed as previously described (7). At various times after PH, animals were killed and liver samples were snap-frozen in liquid nitrogen for subsequent measurement of *MAT2A* mRNA levels and nuclei isolation for nucleosome immunofractionation as described below.

Nuclei isolation and immunofractionation of nucleosomes

Nuclei from male Wistar rat kidney and liver tissues were prepared essentially as described previously (11, 25). Cultured hepatocytes (10^7 cells per condition) were preincubated or not with genistein (30 minutes, 10 µg/mL) and then treated or left untreated with 50 ng/mL of HGF for 1 h, washed twice with saline, scraped, and lysed in 1 mL of the buffer described below (microccocal digestion buffer) plus 1% SDS. For microccocal nuclease digestion, nuclei were resuspended in digestion buffer (0.25 M sucrose, 10 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM sodium butyrate, 2 µL per mL of buffer of mammalian protease inhibitor cocktail (Sigma), 50 mM Tris-HCl, pH 7.5) at a concentration of 5 mg DNA per mL. Nuclei from liver and isolated hepatocytes were digested with microccocal nuclease (Worthington) at 15 u per mg DNA and nuclei from kidney with 20 u per mg DNA for 10 min at 37ºC. The digestion was stopped by adding EDTA to a final concentration of 5 mM, and the samples were cooled on ice and centrifuged at 12,000 x g for 10 min. The supernatant was saved and the pellet was resuspended in lysis buffer (0.25 mM EDTA, 10 mM sodium butyrate, 2 µL per mL of buffer of mammalian protease inhibitor cocktail (Sigma), 50 mM Tris-HCl, pH 7.5) incubated 10 min on ice and recentrifuged as above. The first and second supernatants were pooled and analyzed on agarose gels to determine the extent of the microccocal digestion. Mononucleosomes were further purified by sucrose gradient centrifugation. The pooled supernatants were loaded on a linear sucrose gradient (5%–30%) in fractionation buffer (50 mM NaCl, 10 mM sodium butyrate, 1 mM EDTA, 2µL per mL of buffer of mammalian protease inhibitor cocktail (Sigma), 10 mM Tris-HCl, pH 7.5) and spun at 40,000 rpm for 18 h in a Beckman SW41 rotor. After centrifuging, the tubes were fractionated, and fractions corresponding to the mononucleosomal peak, determined by absorbance at 260 nm, were pooled and used in the immunofractionation experiments.

Immunofractionation of nucleosomes

Immunofractionation of mononucleosomes obtained by microccocal nuclease digestion of nuclei were performed essentially as previously described (11, 25). Polyclonal antisera to hyperacetylated H4 was raised by immunization with a synthetic peptide corresponding to residues 2–19 of histone H4 and acetylated in the ε-amino groups of lysines 5, 8, 12, and 16 as described (11). IgG (500 μg) was dissolved in fractionation buffer (50 mM NaCl, 10 mM sodium butyrate, 1 mM EDTA, 2 μL per mL of buffer of mammalian protease inhibitor cocktail (Sigma), 10 mM Tris-HCl, pH 7.5) and were incubated with 100 mg of protein A Sepharose prewashed with fractionation buffer for 3 h at 4ºC under gentle rotation. The protein A Sepharose bound-antibody was mixed with 2 mg of soluble chromatin in a final volume of 3 mL of fractionation buffer and incubated overnight with gentle rotation at 4ºC. The immunocomplexes were collected by centrifugation (6500 x g, 1 min) on a 0.65 μm Ultrafree filter unit (Millipore), and the filtered-through fraction was removed and stored on ice. Protein A Sepharose beads were washed eight times with 500 μL of fractionation buffer as before, and filtrates were pooled together (unbound fraction). Antibody-bound fraction was eluted from protein A Sepharose by addition of 400 μL of 1.5% SDS in fractionation buffer and rotated for 15 min at room temperature. After centrifugation as before, the eluate was saved and protein A Sepharose was reincubated for another 15-min period with 400 μL of 0.5% SDS in fractionation buffer. The two eluates (bound fraction) were pooled. DNA from all chromatin fractions (input, unbound, and bound) was purified and quantitated by fluorescence with PicoGreen dye (Molecular Probes).

Slot-blot and hybridization analysis

DNA samples were denatured in 0.5 M NaOH, 1.5 M NaCl for 10 min at 37ºC followed by 1 min at 95ºC and then kept on ice. DNA (400 ng) from each fraction was loaded through a slot blot manifold (BioRad) on a Biodyne B membrane (Pall) prewetted in 2X SSC. The filters were immersed in denaturation solution for 5 min, neutralized in 1.5 M NaCl, 1 mM EDTA, 0.5 M Tris-HCl, pH 7.5 for 30 s, and the membranes were dried by baking at 80ºC for 30 min. Hybridizations were carried out by using a 570 bp probe derived from rat *MAT2A* promoter (nucleotides –10 to –580) (26). The probe was labeled by random priming. Filters were prehybridized for 2 h and hybrydized overnight by using Quick Hyb solution (Stratagene) at 65ºC in the presence of 200 μg of heat-denatured salmon sperm DNA. Filters were rinsed in 2X SSC, 0.1% SDS and washed sequentially twice with 2X SSC, 0.1% SDS at 65ºC for 5 min, once with 2X SSC, 0.1% SDS at 65ºC for 30 min, and once with 0.2X SSC, 0.1% SDS at 65ºC for 20 min. The radioactivity present in the filters was initially measured with an InstantImager (Packard), and the filters were then autoradiographed.

Isolation and culture of rat hepatocytes

Liver cells were isolated from male Wistar rats (200–250 g) by collagenase (Gibco-BRL, Paisley, U.K.) perfusion as described previously (27). Cells were plated onto 60-mm collagen-coated culture dishes (type I collagen from rat tail, Collaborative Biomedical Products, Bedford, Mass.) at a density of 3×10^6 cells per dish. Cultures were maintained in MEM medium (Gibco-BRL) supplemented with 10% fetal calf serum (Gibco-BRL), nonessential amino acids (Gibco-

BRL), 2 mM glutamine, 50 mM penicillin, and 50 mg/mL streptomycin sulfate (Gibco-BRL). After a 2-h incubation, the culture medium was removed and cultures were refed the same medium with 0.5% fetal calf serum. Cell viability was measured by trypan blue exclusion, and no significant differences were observed at any time between controls and any of the different treatments performed in this study.

Assay of DNA synthesis

Hepatocytes were plated at a density of 3×10^4 cells/well in a 96-well flat-bottomed plate in MEM supplemented with nonessential amino acids (Gibco-BRL) and 5% fetal calf serum. After 6 h in culture, medium was replaced for fresh medium, and cells were cultured in the absence of serum for 18 h. At this point HGF (50 ng/mL) was added to the culture medium. A 6-h pulse of [^{3}H]thymidine (25 Ci/mmol, Amersham Pharmacia Biotech, Little Chalfont, U.K.) (1 µCi/well) was begun 18 h after HGF addition. Cells were harvested, and thymidine incorporation was determined in a scintillation counter (Topcount, Meredin, Conn.).

Transient transfection of rat hepatocytes in culture

Hepatocytes were seeded as described above in collagen-coated multiwell dishes (5×10^5 cells per 30–mm well) 12 h before transfections. Cells were transfected with 5 µg of a human *MAT2A* promoter-luciferase construct in the pGL3-basic vector (Promega, Madison, Wis.), encompassing 1.3 kb of the 5'-flanking region of this gene (nucleotides – 1329 to + 60) (28), using the TFx50 reagent (Promega) according to the manufacturer's instructions. β-galactosidase expression vector pCH110 (5 µg; Amersham Pharmacia Biotech, Little Chalfont, U.K.) were included as an internal standard of transfection efficiency. After 24 h, cells were treated with HGF (50 ng/mL for 3 h) and harvested. Luciferase and galactosidase activities were determined as described (29). Control transfections and HGF treatments were performed with an empty pGL-3-basic vector. Values reported are means of three independent experiments performed in duplicate.

RNA isolation and Northern blot analysis

Total hepatocyte and liver tissue RNA was isolated by the guanidinium thiocyanate method (30). Aliquots (30 µg) of total RNA were size-fractionated by electrophoresis in a 1% agarose gel under denaturing conditions. RNAs were then blotted and fixed to Nytran membranes (Schleicher & Schuell, Keene, N.H.). Prehybridization and hybridization were performed as described previously (31). A rat cDNA probe for *MAT2A* was generated by RT-PCR by using primers designed according to the reported cDNA sequence (32). The probe was labeled with [α-^{32}P]dCTP (Amersham Pharmacia Biotech, Little Chalfont, U.K.) by random priming. Hybridization with a probe for 18S rRNA was performed as a loading control. Membranes were then exposed to X-ray films, and signals were quantitated by using the Molecular Analyst software (Bio-Rad, Hercules, Calif.).

Statistics

Unless otherwise stated, the data are the means ± SE of at least three independent experiments. Statistical significance was estimated with Student's *t*-test. A P value of < 0.05 was considered significant.

RESULTS

As previously mentioned, actively transcribing genes are associated with hyperacetylated histones, and this modification appears to play a mechanistic role in achieving gene expression. In this regard, we wanted to know whether there were differences in the acetylation degree of histones associated with *MAT2A* promoter between expressing and nonexpressing tissues, such as kidney and liver. For this purpose, mononuclosomes obtained from both tissues were immunoprecipitated with an antibody specific to hyperacetylated histone H4, as described in Materials and Methods and as we reported previously (11, 25). DNA was extracted from the input, unbound, and bound fractions; equal amounts from each fraction were immobilized onto nylon membranes by slot-blot and hybridized to a 570 bp probe derived from the 5'-flanking region of rat *MAT2A* gene, close to the transcription initiation site. The intensity of signal from the antibody-bound slot relative to the intensity from the input slot gives the enrichment generated by the antibody selection. Our data show a strong enrichment (almost sixfold) in the bound fraction compared with the input fraction in kidney, where the gene is actively transcribed (Fig. 1A). However, no preferential selection by the antibody was observed in the liver, indicating the absence of hyperacetylation in this region of *MAT2A* in the tissue where the gene is not expressed (Fig. 1A). It is convenient to remark that, because we immunoprecipitate a population of mononucleosomes and the DNA probe covers 570 bp, fold of enrichment corresponds to a minimum value and the enrichment may be higher *in vivo.* As mentioned above, *MAT2A* is expressed in hepatocytes when these cells proliferate during the regenerative response of the liver after PH. We subsequently analyzed the steady-state *MAT2A* mRNA levels in this experimental model. In agreement with previous reports, *MAT2A* mRNA was not detectable in the normal adult liver on Northern blot analysis, but its levels rapidly increased at 3 h after PH (Fig. 1B), remained elevated by 6 h and started to decline by 12 h (Fig. 1B). The acetylation status of histones associated with *MAT2A* promoter in the regenerating liver was analyzed as described above at various times after PH. Figure 1B shows how the degree of acetylation of histones (H4) associated with *MAT2A* promoter was increased in a time-dependent fashion after PH, paralleling the induction of *MAT2A* expression.

We were interested in identifying the factor(s) that could mediate the observed induction of *MAT2A* transcription in the early phases of the hepatic regenerative response. Among the known factors responsible for the rapid changes in gene expression after PH, HGF plays a central role (33, 34). To test whether HGF could induce *MAT2A* expression in the hepatocyte, we turned to an experimental model of rat hepatocytes in primary culture. In this experimental setting we observed a dose- and time-dependent induction of *MAT2A* mRNA by HGF treatment (Fig. 2A and B). The mRNA levels of other genes commonly expressed in the hepatocyte, such as *MAT1A*, was not affected by HGF treatment (Fig. 2A), indicating that the observed effect on *MAT2A* induction was specific. HGF binds and mediates its cellular effects through the interaction with a cell surface receptor endowed with tyrosine activity, which is the product of the *c-met* proto-oncogene ($p190^{MET}$) (35–37). Inhibition of $p190^{MET}$ tyrosine kinase by genistein, a tyrosine phosphorylation inhibitor, has been shown to impair HGF signaling (38, 39). As

expected, hepatocytes treated with genistein (10 μg/mL) prior to HGF stimulation displayed significantly reduced levels of *MAT2A* mRNA, compared with cells treated with HGF in the absence of this flavonoid (Fig. 2C). To further characterize the mechanism of HGF induction of *MAT2A* expression in cultured hepatocytes, these cells were transfected with a construction harboring 1.3 kb of the human *MAT2A* 5' region (28) upstream of a luciferase reporter gene, and the effect of HGF on reporter gene expression was tested. As shown in Figure 2D, HGF treatment of transfected cells resulted in almost 2.5-fold increase in luciferase activity. HGF had no effect in cells transfected with the empty pGL-3-basic vector (data not shown). This effect of HGF further confirms *MAT2A* as a new target for this growth factor in the hepatocyte and suggests that transcriptional activation is likely involved in the elevation of *MAT2A* steady-state mRNA levels in response to HGF.

Enhancement of histone acetylation has emerged as a common mechanism associated with the induction of gene expression (20, 40). Here we have shown that histones associated with *MAT2A* promoter become hyperacetylated as the expression of this gene is induced early in the liver regenerative response (Fig. 1B). Thus, it was interesting to know whether the activation of *MAT2A* by HGF in cultured hepatocytes was accompanied by changes in the acetylation of histones associated with its promoter. To assess this point, mononucleosomes isolated from control and HGF-treated hepatocytes (1 h treatment with 50 ng/mL HGF) were immunoprecipitated as described above with the antibody specific to hyperacetylated histone H4. DNA was extracted from the various fractions, slot-blotted, and hybridized with the *MAT2A* promoter probe as before. As shown in Figure 3, HGF treatment resulted in a significant induction (almost threefold enrichment) in the degree of acetylation of histones associated with *MAT2A* promoter with respect to untreated controls. Blockage of HGF receptor tyrosine kinase activity with genistein also inhibited the stimulatory effect of this growth factor on histone acetylation at the level of *MAT2A* promoter (Fig. 3).

In isolated rat hepatocytes, we have previously shown that, with time in culture, the expression of *MAT2A* was progressively induced, whereas that of the liver-specific gene *MAT1A* was reduced dramatically, probably reflecting the degree of dedifferentiation of cultured hepatocytes and the transition from a metabolic to a proliferative state (13). The addition of AdoMet to the culture medium prevented such changes in *MAT1A* and *MAT2A* expression, thus preserving the differentiated phenotype of the hepatocyte, at least regarding MAT gene expression (13). In view of these effects, it was important to know whether *MAT2A* induction by HGF in cultured rat hepatocytes could be modulated by AdoMet. For this purpose hepatocytes were preincubated with different concentrations of AdoMet for 30 min and then treated with 50 ng/mL of HGF for 3 h. As shown in Figure 4A, AdoMet addition resulted in the dose-dependent inhibition of *MAT2A* induction by HGF. This effect of AdoMet was not mediated through the impairment of p190^{MET} activation by HGF. The tyrosine kinase activity of this receptor, determined as the autophosphorylation of its β subunit in response to HGF, was not affected by the presence of AdoMet in the culture medium (data not shown). However, the effect of AdoMet could be related to its conversion into 5'-methylthio-adenosine (MTA), a metabolite of AdoMet in the polyamine biosynthetic pathway (41, 42). This compound effectively blocked *MAT2A* induction by HGF in cultured hepatocytes, when it was added to the medium (500 μM) 30 min before growth factor treatment (Fig. 4B). The effect of MTA on *MAT2A* expression was specific, because its addition did not affect the expression of other genes such as albumin (not shown) or

MAT1A (Fig. 4B). We have also evaluated the effect of AdoMet on the mitogenic response of cultured rat hepatocytes to HGF. As shown in Figure 4C, AdoMet could partially inhibit HGF-stimulated [^{3}H]thymidine incorporation into DNA.

DISCUSSION

As previously mentioned, in the adult and quiescent hepatocyte AdoMet is synthesized by MAT I/III, the product of *MAT1A* gene. However, when hepatocytes proliferate, as occurs during liver regeneration, malignant transformation, or during the fetal period, transcription of *MAT2A* is activated resulting in the expression of MAT II, the form of MAT normally expressed outside the liver (3, 7). Evidence has been reported that shows that this switch in MAT gene expression provides the cell with a proliferative advantage. This advantage may stem from the different regulatory and kinetic properties of MAT I/III and MAT II, which impact on the intracellular AdoMet levels (6). However, nothing was known about the mechanisms that govern *MAT2A* expression during the physiological proliferative response of the hepatocyte. The aim of this study was to identify the factors and mechanisms behind *MAT2A* induction under such condition.

For these purposes, we first examined the acetylation status of histones (H4) associated with *MAT2A* promoter in the adult rat liver, where the gene is not expressed, and in an expressing tissue such as kidney. In agreement with the expression profile of this gene, H4 molecules were hypoacetylated in the liver, whereas the opposite situation was found in kidney. Next, it was important to know whether in the proliferating liver there were changes in the acetylation levels of histones associated with *MAT2A* promoter. Our data showed that *MAT2A* induction in the proliferating liver was indeed accompanied by a clear enhancement in the acetylation levels of H4 molecules in its promoter region. This modification was time-dependent and matched *MAT2A* expression kinetics.

These dynamic changes in *MAT2A* gene expression and promoter-associated histone H4 acetylation suggested that this gene could be a target for the growth factors that mediate the rapid changes in gene expression after PH. HGF is probably the most relevant factor responsible for the onset of hepatocellular proliferation and the induction of some early-responsive genes under this condition (14, 15). These notions led us to assess the effect of HGF on *MAT2A* expression in cultured rat hepatocytes. In this experimental system we observed that *MAT2A* was induced by HGF treatment in a dose- and time-dependent fashion, and that HGF could transactivate *MAT2A* promoter in transient transfection experiments by using a promoter-reporter construct. Furthermore, HGF induced the hyperacetylation of histone H4 associated with *MAT2A* promoter. This effect and the subsequent increase in *MAT2A* mRNA levels were dependent on the tyrosine kinase activity of the HGF receptor. Taken together, these observations reveal *MAT2A* as a novel HGF-regulated gene in the hepatocyte. Epidermal growth factor (EGF), another growth factor involved in hepatocyte proliferation (14, 15), also induced *MAT2A* expression in hepatocytes (Avila et al., unpublished results).

Recent advances in the field of histone acetylation have shown that this posttranslational modification does not only correlate with gene activity, but it plays an active role in the regulation of gene transcription (20, 43). Through the interplay between HATs and HDACs, the level of histone acetylation is set. Several transcriptional activators and coactivators possess

HAT activity, and the same is true for transcriptional repressors and HDAC activity (43). It has been shown that steroid hormones and vitamins A and D can induce hyperacetylation of histones at the promoters of target genes *in vivo* through the recruitment of p300/CBP HAT activity (44). Also, evidence indicates that the enzymatic activity of HATs is regulated by proliferation signals (45, 46). Furthermore, although this work was in progress, the ability of EGF to promote the phosphorylation and acetylation of histone H3 associated with *c-fos* promoter was reported (47). In this context, HGF-induced hyperacetylation of H4 molecules associated with *MAT2A* promoter could play a role in the induction of *MAT2A* expression by making this region of the chromatin accessible to transcription factors. It has been reported that acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomes, at least *in vitro* (48). This finding is, to our knowledge, the first description of such effect of HGF and one of the first reports, together with EGF (47), on the induction of histone acetylation at the level of a target gene by a growth factor. Whether HGF also promotes histone H4 phosphorylation or histone H3 modifications (phosphorylation/acetylation) remains to be determined.

The process of liver regeneration involves many complex mechanisms that are not completely understood. A major area of research in this field is the identification of the signals that control the enormous hepatocyte proliferative potential, both in its triggering and termination. Although HGF is a potent mitogen for cultured hepatocytes, injection of HGF in normal rats through the portal vein results in a poor response in terms of DNA synthesis (for review, 14 and 15). Thus hepatocytes need to be primed in order to become competent for replication and to respond to growth factors. This priming event has been attributed to the cytokines tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) (14, 15). The involvement of TNF-α and IL-6-induced reactive oxygen and nitrogen species has been suggested (49–51). On the other end, namely the repression of the hepatocyte proliferative activity and termination of the regenerative process, the question remains as to the factors and signals implicated. Transforming growth factor-β1 (TGF-β1) is a known inhibitor of proliferation in hepatocyte cultures (52), however liver regeneration proceeds to completion in transgenic mice with enhanced expression of TGF-β1 in the liver (53). Changes in the hepatocellular levels of key metabolites may contribute, together with the above-mentioned factors, to the orchestration of the regenerative process. We propose that changes in AdoMet levels in the hepatocyte could play a role in this scenario. We have previously observed that, in cultured hepatocytes that display AdoMet levels below normal concentrations (27), replenishment of the intracellular pool of this metabolite can be achieved through its addition to the culture medium (13). Our present observations show that, in the presence of exogenously added AdoMet, the induction of *MAT2A* expression in response to HGF was impaired. Hence, at least regarding this novel target of HGF, the cellular contents of AdoMet could indeed modulate hepatocyte responsiveness to this growth factor. Regarding the mechanism of action of AdoMet, its effect could be mediated through its chemical degradation or enzymatic conversion into MTA (54), because the addition of this compound also inhibited HGF effect. This observation rules out the involvement of a methylation reaction in AdoMet effect, given the fact that MTA is not a methyl donor compound. Nevertheless, the detailed mechanism of action of AdoMet is not known yet. In support of our hypothesis, which is summarized in Figure 5, it is a fact that levels of AdoMet are dramatically reduced in the liver shortly after PH, which coincides with the onset of DNA synthesis and the induction of early-responsive genes (7). Reactive oxygen and nitrogen species induced by TNF-α and IL-6 shortly after PH may contribute to AdoMet downregulation through the inhibition of MAT I/III activity (55–57). In this context, the basal expression of

MAT2A in the hepatocyte, although very low, may play a role during the early moments of liver regeneration by preventing a complete depletion of AdoMet levels, because MAT II is not inhibited by free radicals (55–57). The hepatic contents of MTA are also downregulated during liver regeneration (58, 59). In addition, AdoMet and MTA administration to rats undergoing PH inhibited DNA synthesis (59, 60). This finding agrees with our present observation of the inhibition by AdoMet of HGF-induced DNA synthesis in cultured hepatocytes.

In summary, we have identified *MAT2A* as novel target gene for HGF in cultured hepatocytes, which suggests that this growth factor could be responsible for the induction of this gene in the liver after PH. In addition we show for the first time that HGF can induce localized changes in chromatin structure through the enhancement of histone H4 acetylation associated with a target gene. This observation supports the more general hypothesis of the involvement of such posttranslational modifications of histones in the induction of gene expression by extracellular signals (24, 47). Finally we propose that AdoMet or MTA could be one of those long-sought key factors/metabolites that modulate the hepatocyte response to growth factors during liver regeneration, at least regarding methionine metabolism.

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Fig. 1

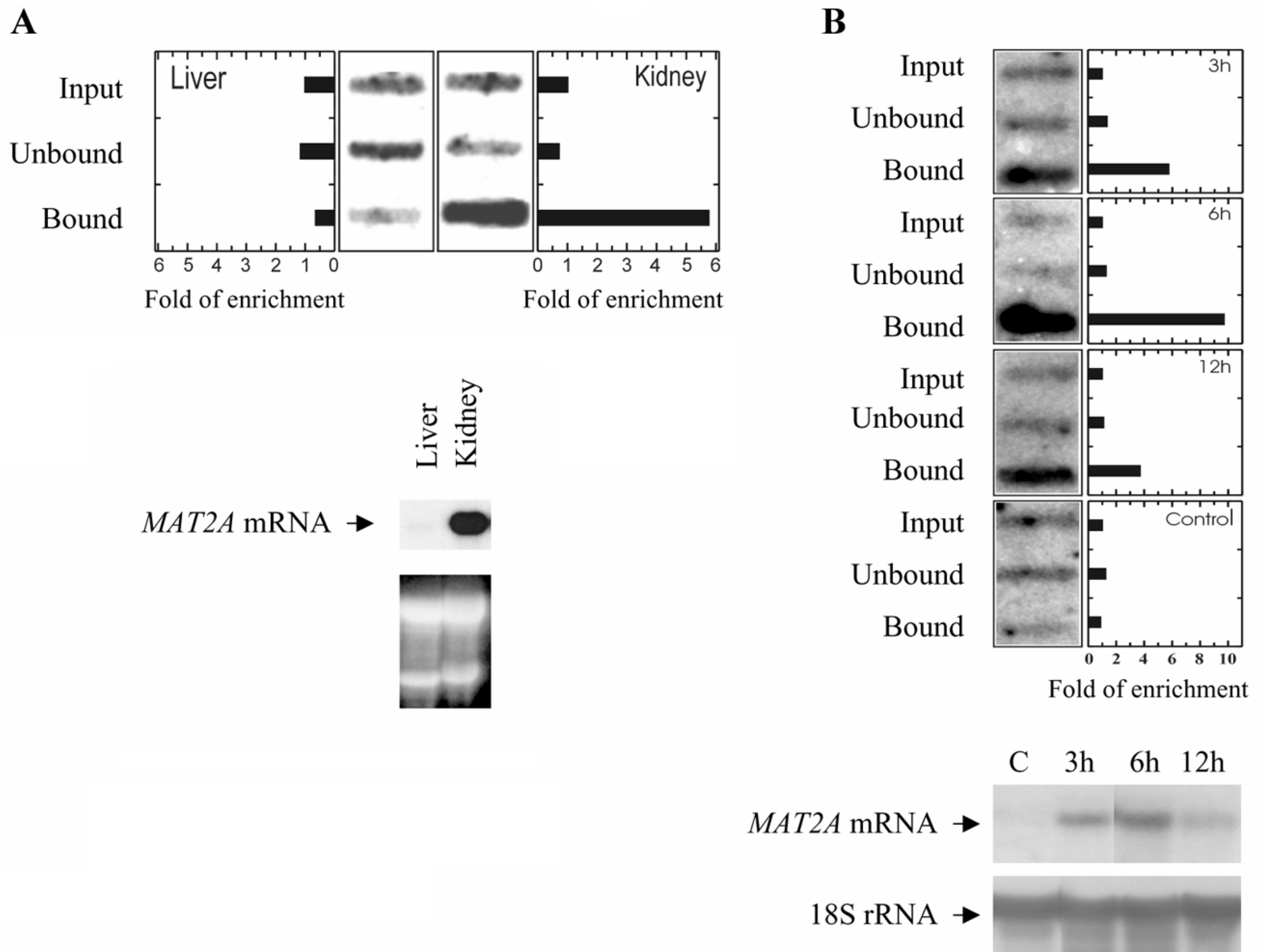

Figure 1. Acetylation of histones (H4) associated with *MAT2A* promoter in rat liver and kidney, and in rat liver after PH **(A)** Mononucleosomes from rat liver and kidney were prepared and immunoprecipitated with an antibody specific for hyperacetylated histone H4, as described in Materials and Methods. DNA was extracted from input, unbound, and bound fractions; equal amounts were loaded and analyzed by slot-blot hybridization with a probe for *MAT2A* promoter. Quantitation of the radioactivity incorporated in each slot is also shown. Representative autoradiograms are shown. Lower panel shows *MAT2A* expression in rat liver and kidney as assayed by Northern blotting with a rat cDNA probe. Ethidium bromide staining of the gel is shown as loading control. **(B)** Mononucleosomes from rat liver were obtained at different time points after PH and were subsequently immunoprecipitated as described. DNA from the different fractions was extracted, slot-blotted, and hybridized with a probe derived from *MAT2A* promoter. The control panel corresponds to the immunoprecipitation of mononucleosomes from the liver of a sham-operated rat 6 h after the intervention. Quantitation of the radioactivity incorporated in each slot is also shown. Representative autoradiograms are shown. Lower panel shows *MAT2A* expression, assayed by Northern blotting, in rat liver after PH at the same time points in which histone acetylation status was measured. 18S rRNA levels were determined as loading control.

Fig. 2

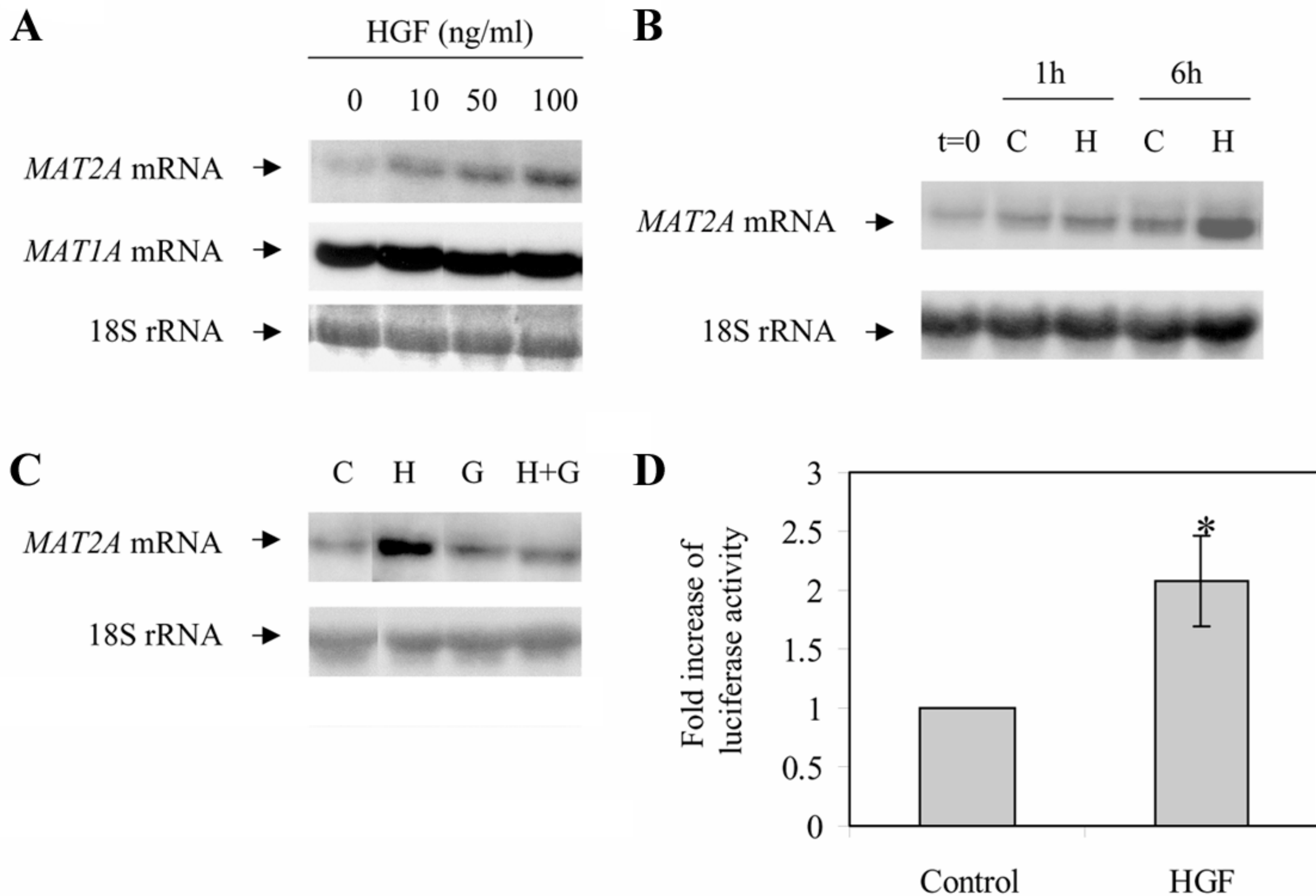

Figure 2. Induction of MAT2A expression by HGF in cultured rat hepatocytes **(A)** Hepatocytes in culture were treated for 3 h with increasing concentrations of HGF and *MAT2A* and *MAT1A* mRNA levels were determined by Northern blotting. Hybridization with a probe for the 18S rRNA was performed as loading control. This is a representative blot of three experiments performed in duplicate. **(B)** Time-dependent induction of *MAT2A* expression in cultured hepatocytes treated with HGF (100 ng/mL) as analyzed by Northern blotting (C, controls; H, HGF). Hybridization with a probe for 18S rRNA was performed as loading control. This is a representative blot of three experiments performed in duplicate. **(C)** Pretreatment of hepatocytes with genistein (10 µg/mL for 30 min) (G) impairs *MAT2A* gene induction by HGF treatment (100 ng/mL for 3 h) (H). *MAT2A* expression was analyzed by Northern blotting, and 18S rRNA levels were determined as loading control. This is a representative blot of three experiments performed in duplicate. **(D)** Effect of HGF on *MAT2A* promoter activity. Hepatocytes were transfected with a *MAT2A* promoter-luciferase reporter construct as described in Materials and Methods. Transfected cells were treated for 3 h with HGF (50 ng/mL). Luciferase activities, normalized for β-galactosidase expression, are expressed as fold increase over respective controls. *$P < 0.05$ respect control values. Data are means ± SE.

Fig. 3

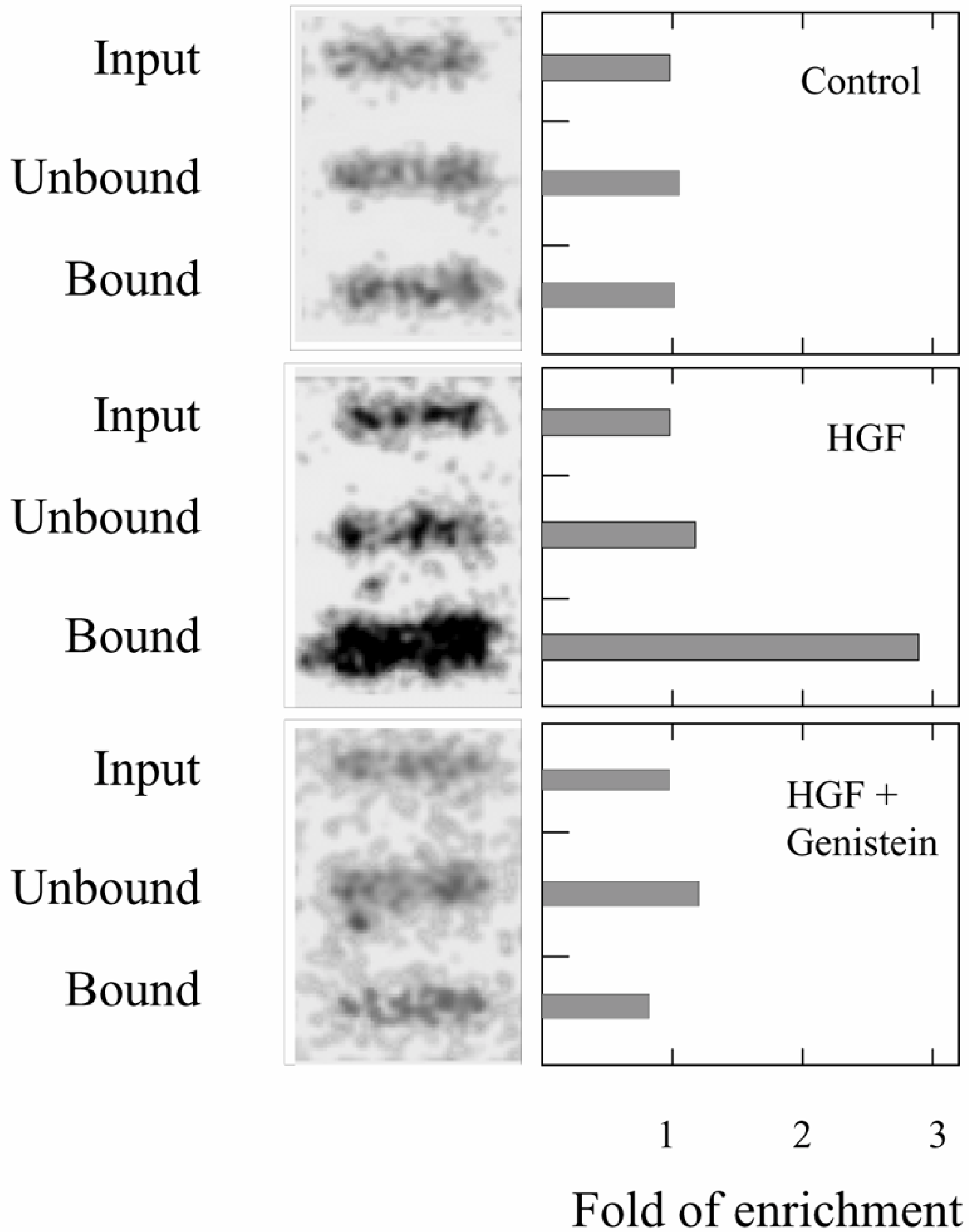

Figure 3. HGF induces the acetylation of histone H4 associated with *MAT2A* promoter in cultured rat hepatocytes
Cells were preincubated or not with genistein (10 µg/mL) for 30 min and then treated for 1 h with 50 ng/mL of HGF. Mononucleosomes were prepared and immunoprecipitated as described in Materials and Methods. DNA was isolated from the different fractions, slot-blotted, and hybridized with a probe derived from *MAT2A* promoter. Quantitation of the radioactivity incorporated in each slot is also shown. Representative autoradiograms are shown.

Fig. 4

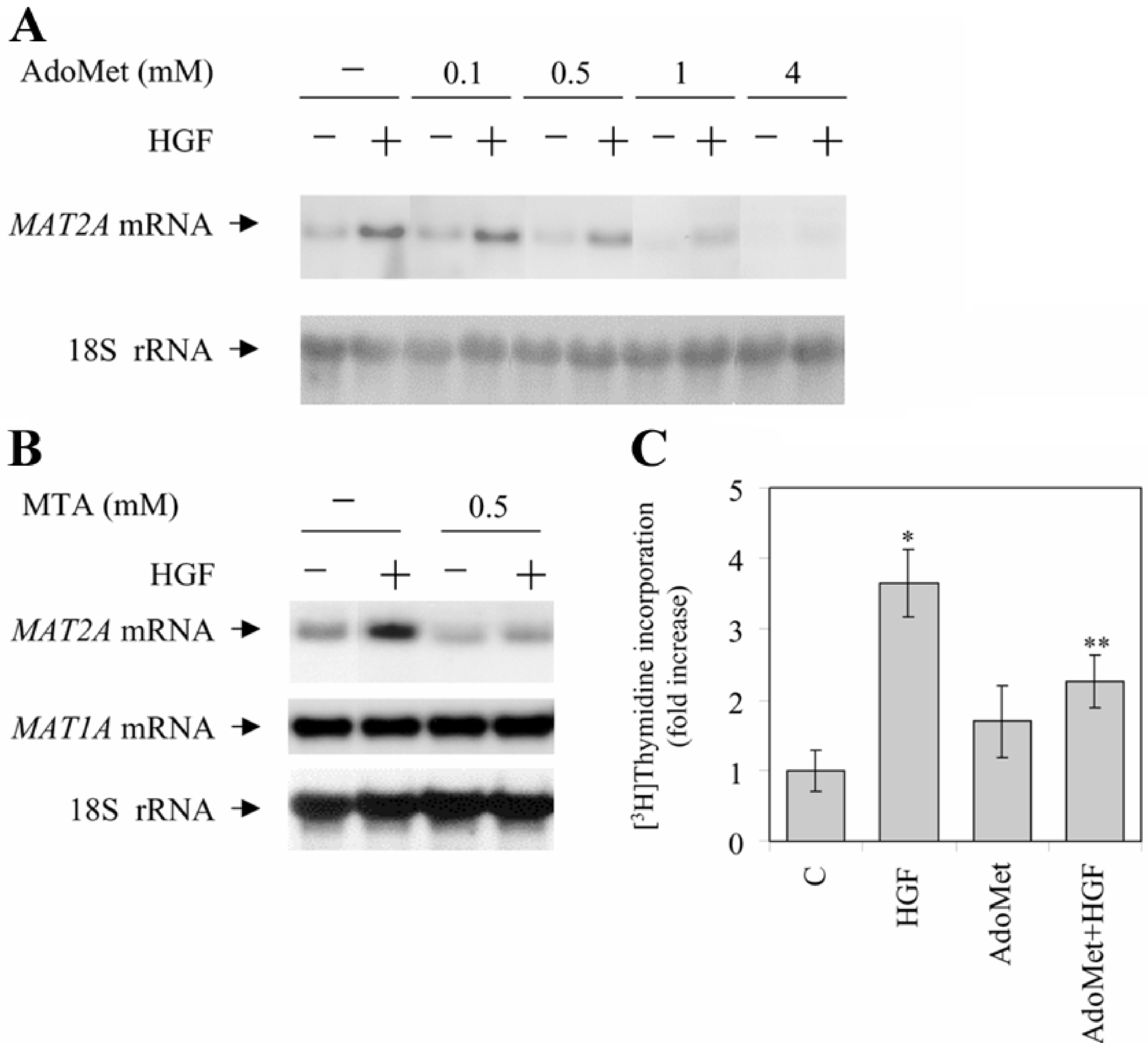

Figure 4. AdoMet and MTA inhibit the induction of MAT2A expression by HGF in cultured rat hepatocytes
Cells were pretreated for 30 min with increasing concentrations of AdoMet **(A)** or 500 µM of MTA **(B)**, then HGF (50 ng/mL) was added to the cultures and incubation continued for another 3 h. *MAT2A* and *MAT1A* expression were analyzed by Northern blotting. Hybridization with a probe for 18S rRNA was performed as loading control. Representative blots of three experiments performed in duplicate are shown. **(C)** DNA synthesis, measured as [^{3}H]thymidine incorporation, in cultured rat hepatocytes in response to HGF (50 ng/mL) treatment in the presence or absence of AdoMet (4 mM). Experiments were carried out as described in Materials and Methods. Data are expressed as fold increase over control and are means ± SE of three experiments performed in triplicate. *$P < 0.05$ respect control (C) value, **$P < 0.05$ respect HGF value.

Fig. 5

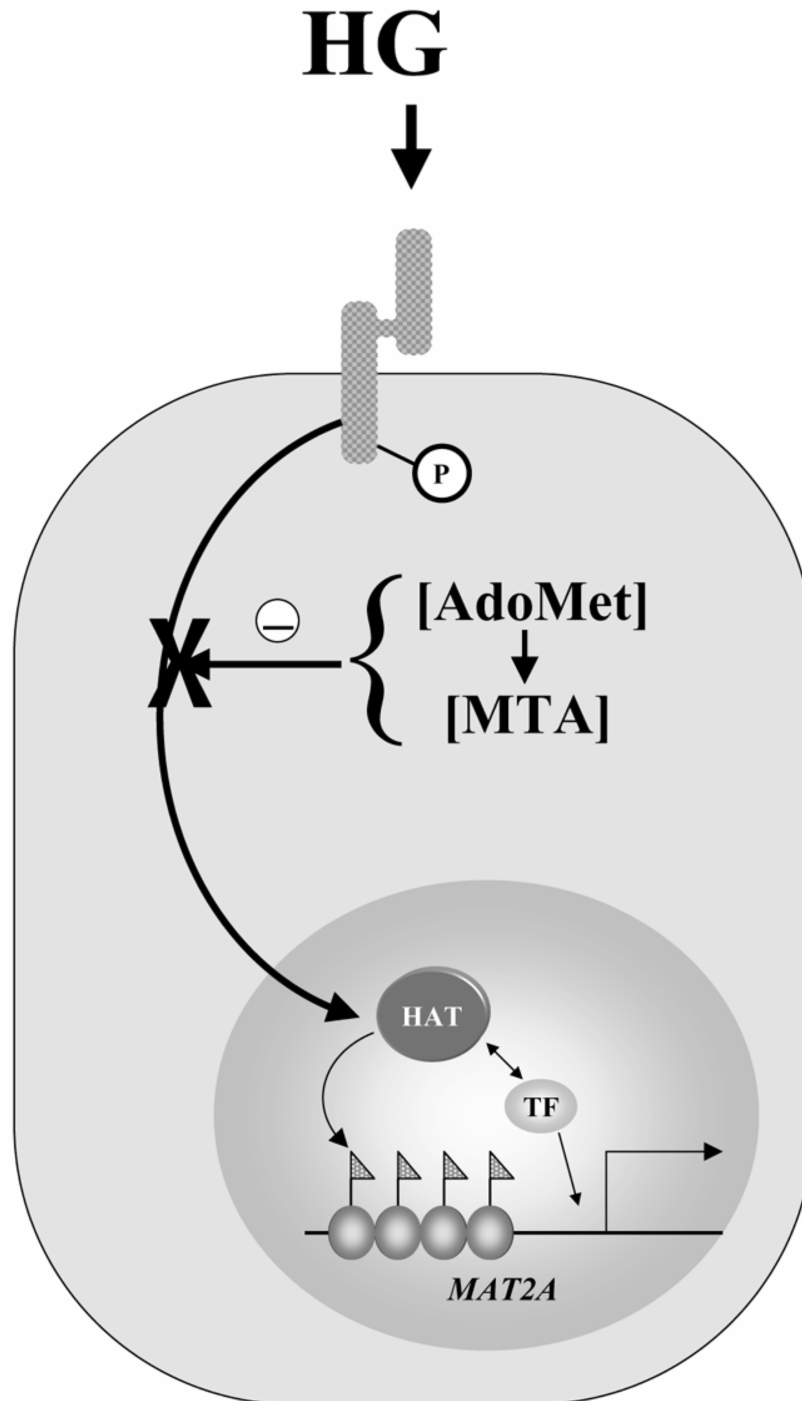

Figure 5. Model of HGF induction of *MAT2A* gene expression in rat hepatocytes and its modulation by AdoMet/MTA levels Intracellular signals generated at p190^{MET} receptor after HGF binding result in the recruitment of HAT complexes to *MAT2A* promoter. Such changes in chromatin structure at the level of the target gene would favor the interaction of transcription factors (TF) and promoter transactivation. The induction of *MAT2A* expression in the hepatic parenchymal cell by HGF would be conditioned by AdoMet and MTA contents. AdoMet and MTA levels in the liver are dramatically reduced early after PH, when HGF levels rise and *MAT2A* expression is activated. Subsequently, as intracellular AdoMet and MTA concentrations recover to normal levels the hepatocyte would be rendered refractory to HGF, at least regarding the induction of *MAT2A* expression. We would like to propose that fluctuations in the concentrations of these metabolites could be part of the priming events and terminating signals that modulate the liver regenerative process.