S-Adenosylmethionine regulates *MAT1A* and *MAT2A* gene expression in cultured rat hepatocytes: a new role for S-adenosylmethionine in the maintenance of the differentiated status of the liver

ELENA R. GARCÍA-TREVIJANO,[1] M. UJUE LATASA,[1] M. VICTORIA CARRETERO, CARMEN BERASAIN, JOSÉ M. MATO,[2] AND MATÍAS A. AVILA

Unidad de Hepatología y Terapia Génica, Departamento de Medicina Interna, Facultad de Medicina, Universidad de Navarra, Pamplona, Spain

ABSTRACT **Methionine metabolism starts with the formation of S-adenosylmethionine (AdoMet), the most important biological methyl donor. This reaction is catalyzed by methionine adenosyltransferase (MAT). MAT is the product of two different genes: *MAT1A*, which is expressed only in the adult liver, and *MAT2A*, which is widely distributed, expressed in the fetal liver, and replaces *MAT1A* in hepatocarcinoma. In the liver, preservation of high expression of *MAT1A* and low expression of *MAT2A* is critical for the maintenance of a functional and differentiated organ. Here we describe that in cultured rat hepatocytes *MAT1A* expression progressively decreased, as described for other liver-specific genes, and *MAT2A* expression was induced. We find that this switch in gene expression was prevented by adding AdoMet to the culture medium. We also show that in cultured hepatocytes with decreased *MAT1A* expression AdoMet addition markedly increased *MAT1A* transcription in a dose-dependent fashion. This effect of AdoMet was mimicked by methionine, and blocked by 3-deazaadenosine and L-ethionine, but not D-ethionine, indicating that the effect was specific and mediated probably by a methylation reaction. These findings identify AdoMet as a key molecule that differentially regulates *MAT1A* and *MAT2A* expression and helps to maintain the differentiated status of the hepatocyte.—García-Trevijano, E. R., Ujue Latasa, M., Victoria Carretero, M., Berasain, C., Mato, J. M., and Avila, M. A. S-Adenosylmethionine regulates *MAT1A* and *MAT2A* gene expression in cultured rat hepatocytes: a new role for S-adenosylmethionine in the maintenance of the differentiated status of the liver. *FASEB J.* 14, 2511–2518 (2000)**

Key Words: biological methylation · hepatocarcinoma · methionine metabolism · gene expression

S-ADENOSYLMETHIONINE (ADOMET) IS the most important methyl donor in cellular metabolism (1, 2). In mammals AdoMet can be synthesized in all cells of the organism, however, it is in the liver where most of the dietary methionine is converted into AdoMet and where up to 85% of all methylation reactions take place (3, 4). The first step in methionine metabolism is catalyzed by methionine adenosyltransferase (MAT) (2–5). MAT is the product of two different genes, *MAT1A* and *MAT2A*, which code for two different enzymes, MATI/III and MATII respectively, with distinct kinetic and regulatory properties and that display a tissue-specific pattern of expression (2, 5–8). *MAT1A* is expressed only in the adult hepatocyte, whereas *MAT2A* shows a wider distribution and is responsible for AdoMet synthesis in extrahepatic tissues. In addition, *MAT2A* is expressed in the regenerating liver (9) and in the fetal hepatocyte, being replaced by *MAT1A* on maturation of the organ (10). Interestingly, in hepatocarcinoma (rat and human-derived liver cancer cell lines and hepatocellular carcinoma specimens resected from patients) a similar situation can be found, and the dedifferentiated cell recovers the fetal pattern expressing *MAT2A* in place of *MAT1A* (11). This switch in gene expression influences cellular AdoMet levels and appears to confer a growth advantage to the proliferating and transformed hepatic cell, probably through the differences in the MATI/III and MATII isozyme properties (12).

In addition to the neoplastic liver, impaired AdoMet synthesis has been reported in chronic liver disorders such as alcoholic and viral cirrhosis, which in many cases precede the development of hepatocarcinoma, and in response to several hepatotoxins (including ethanol, carbon tetrachloride, galactosamine, and acetaminophen) (reviewed in ref 2). The pathological relevance of impaired AdoMet production can be inferred from the protective

[1] Both authors made equal contribution to this work.

[2] Correspondence: Departamento de Medicina Interna, Edificio Los Castaños, Facultad de Medicina, Universidad de Navarra, 31008, Pamplona, Spain. E-mail: jmmato@unav.es

effects of exogenously administered AdoMet, as observed in experimental models of liver damage and in a recently reported clinical trial (2, 13). In addition, there is extensive evidence showing that liver carcinogenesis in rodents can be influenced by dietary restrictions in nutrients such as choline, methionine, folic acid, and vitamin B12, which compromise the cellular supply of methyl groups in the form of AdoMet (14). The link between AdoMet availability and the preservation of a functional and differentiated liver is further supported by the chemopreventive effects of AdoMet administration on the development of preneoplastic lesions and hepatocellular carcinoma in models of rat liver carcinogenesis (15, 16).

All these evidences suggest that the preservation of an adequate methyl donor availability, and thus AdoMet levels, is central to the homeostasis of the liver, of which the pattern of MAT isozyme gene expression is a hallmark. In an experimental model of rat hepatocytes in primary culture, we have addressed the influence of cellular AdoMet contents on *MAT1A* and *MAT2A* expression. A new role for AdoMet in the maintenance of the differentiated status of the hepatocyte through the differential regulation of both genes is proposed.

MATERIALS AND METHODS

Materials

AdoMet, in the stable form of sulfate-*p*-toluenesulfonate salt produced by Knoll (Milan, Italy) was provided by Europharma (Madrid, Spain). All other reagents were of the best quality commercially available and otherwise stated they were purchased from Sigma (St. Louis, Mo.).

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 (17). Cells were plated onto 60-mm collagen-coated culture dishes (type I collagen from rat tail; Collaborative Biomedical, 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 2-h incubation the culture medium was removed and cultures were refed the same medium with 2.5% 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 various treatments performed in this study.

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 *MAT1A* promoter-luciferase construct encompassing 1.4 kb of the 5′-flanking region of this gene (nucleotides −1405 to +65) (18), using the TFx50 reagent (Promega) according to the manufacturer's instructions. Five micrograms of the β-galactosidase expression vector pCH110 (Amersham, Little Chalfont, U.K.) were included as an internal standard of transfection efficiency. After 24 h, cells were harvested and luciferase and galactosidase activities were determined as described (18). Values reported are means of three independent experiments performed in duplicate.

Determination of AdoMet levels in cultured hepatocytes

After extensive washing with saline, cells were lysed and deproteinized with 0.4 M perchloric acid and centrifuged at 12000 *g* for 30 min at 4°C. Supernatants were analyzed by reverse-phase high-performance liquid chromatography as described previously (19).

RNA isolation and Northern blot analysis

Total hepatocyte RNA was isolated by the guanidinium thiocyanate method (20). Aliquots (15 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 (21). Rat cDNA probes for *MAT1A*, *MAT2A*, and albumin (22–24) were labeled with [α-^{32}P]dCTP (Amersham) 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 using the Molecular Analyst software (Bio-Rad, Hercules, Calif.).

Immunoblot analysis

For determination of MATI/III (the product of *MAT1A* gene) protein levels (6), hepatocytes were homogenized as described previously (21). Equal amounts of protein (20 μg) were subjected to 10% sodium dodecyl sulfate-polyacrilamide gel electrophoresis. Proteins were electrophoretically transferred to nitrocellulose membranes. Immunodetection of MATI/III was performed using a rabbit anti-rat MATI/III antiserum (25) and a horseradish peroxidase-conjugated secondary antibody. Blots were developed by enhanced chemoluminiscence according to manufacturer's instructions (Dupont, Boston, Mass.). To demonstrate that the observed effects were specific and that equal loading of the gels had been achieved, after immunodetection of MATI/III membranes were stripped and reprobed with an antibody specific for actin (Calbiochem-Novabiochem, Darmstadt, Germany).

Statistics

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

RESULTS

Rat hepatocytes were placed in primary culture and the levels of *MAT1A* expression were determined by Northern blotting at the onset of cultures ($t=0$) and after 12 h of incubation. In agreement with other

studies on the expression of liver-specific genes in cultured hepatocytes (26–28), a sharp reduction in *MAT1A* mRNA was observed (**Fig. 1*A***). However, if AdoMet was added to cultured hepatocytes at $t = 0$ and *MAT1A* mRNA was evaluated 12 h later a clear dose-dependent protection in the reduction of this mRNA was observed (Fig. 1*A*). AdoMet concentrations between 2 and 4 mM resulted in *MAT1A* mRNA levels similar to those found in recently isolated hepatocytes. This effect on *MAT1A* mRNA resulted in higher MATI/III protein levels, as determined by immunoblotting performed on cellular extracts from hepatocytes cultured for 18 h in the absence or presence of AdoMet (2 mM) (Fig. 1*B*). The effect of AdoMet on MATI/III protein was specific, because the levels of other cellular proteins such as actin were not affected by this treatment (Fig. 1*B*).

We next wanted to know at which level the AdoMet was acting on *MAT1A* expression. Steady-state mRNA levels are controlled through the interplay of various mechanisms. These mechanisms generally include the regulation of gene transcription and messenger stability. In an initial approach we tested whether AdoMet was able to reinduce *MAT1A* expression after its decay in culture. For this purpose, isolated hepatocytes were cultured for 12 h, then AdoMet (4 mM) was added to cells and incubation continued for another 12 h. *MAT1A* mRNA levels were measured at the onset of cultures (t=0), after the initial 12 h of culture, and after an additional 12 h of AdoMet treatment. When compared with control cultures (in the absence of AdoMet), a sharp induction of *MAT1A* mRNA levels was observed in response to AdoMet addition (**Fig. 2*A***). The accumulation of newly synthesized *MAT1A* mRNA suggested that the transcription of this gene could be stimulated in response to AdoMet. Further support for this hypothesis was obtained by treating cells with AdoMet in the presence of the transcriptional inhibitor actinomycin D. Under these conditions, the effect of AdoMet was completely blocked (Fig. 2*A*). Actinomycin D also prevented the effect of AdoMet on *MAT1A* expression when both agents were added to hepatocytes at the onset of cultures (t=0) (data not shown). Finally, direct evidence of AdoMet action on *MAT1A* gene promoter was obtained in transient transfection experiments using a construct encompassing the reporter gene luciferase under the control of rat *MAT1A* 5′-flanking region (18). Luciferase activity in lysates from hepatocytes transiently transfected with this construct was induced in a dose-dependent fashion in response to AdoMet treatment (Fig. 2*B*). In parallel experiments, treatment of hepatocytes with the glucocorticoid triamcinolone (1 μM) also resulted in the activation of *MAT1A* promoter and the induction of luciferase activity levels similar to those found with AdoMet treatment at 2 mM (data not shown). Taken together, these observations indicated that AdoMet was promoting *MAT1A* transcription.

It is known that AdoMet levels are reduced with time in isolated and cultured rat hepatocytes (17). We wanted to know whether in our culture conditions the exogenously added AdoMet could restore the intracellular pool of this compound. In agreement with previous studies on AdoMet uptake by liver or isolated hepatocytes (29–31), we observed that intracellular AdoMet levels were threefold higher than control levels after 5 min of incubation in the presence of 2 mM and eightfold higher with 4 mM of exogenously added AdoMet (81±24 pmol/mg of protein in control vs. 275±75 for 2 mM AdoMet and 675±102 for 4 mM AdoMet). Incubation for 5 min with 2 mM L-methionine also increased hepatocyte AdoMet concentration ~2.4-fold.

We were also interested in the molecular mechanisms through which AdoMet could mediate its effects on *MAT1A* expression. In this respect, we observed that L-methionine, the precursor of AdoMet together with ATP, was able to partially mimic the AdoMet effect on *MAT1A* mRNA levels after 12 h of treatment at a concentration of 2 mM. However, under the same conditions, the nonmetabolizable D-isomer of methionine elicited no response (**Fig. 3**), suggesting that methionine had to be metabolized to AdoMet to stimulate *MAT1A* expression and that dietary methionine may thus modulate the expression of this gene. Additional insight into the mechanism of AdoMet action was

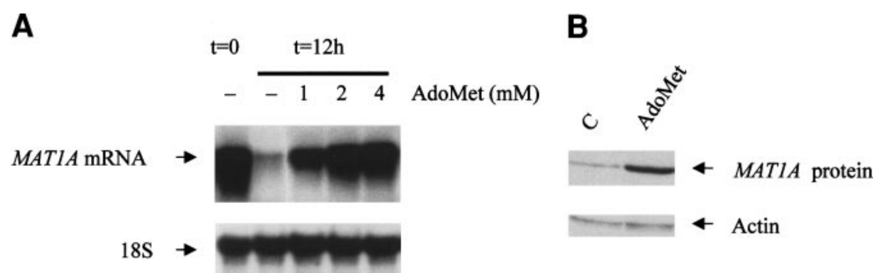

Figure 1. Effect of AdoMet on *MAT1A* expression in cultured rat hepatocytes. *A*) Rat hepatocytes were cultured as described in Materials and Methods. *MAT1A* mRNA levels were evaluated by Northern blotting at the onset of cultures (t=0) and after 12 h of incubation in the absence or presence of different concentrations of AdoMet. Blots were hybridized with an 18S rRNA cDNA probe to demonstrate equal loading. Representative blots are shown. *B*) MATI/III protein levels analyzed by Western blotting in rat hepatocytes cultured for 18 h in the absence or presence of 2 mM AdoMet (upper panel). Blots were also probed with an antibody for actin protein to demonstrate equal loading and the specificity of the effect of AdoMet (lower panel). Representative blots are shown.

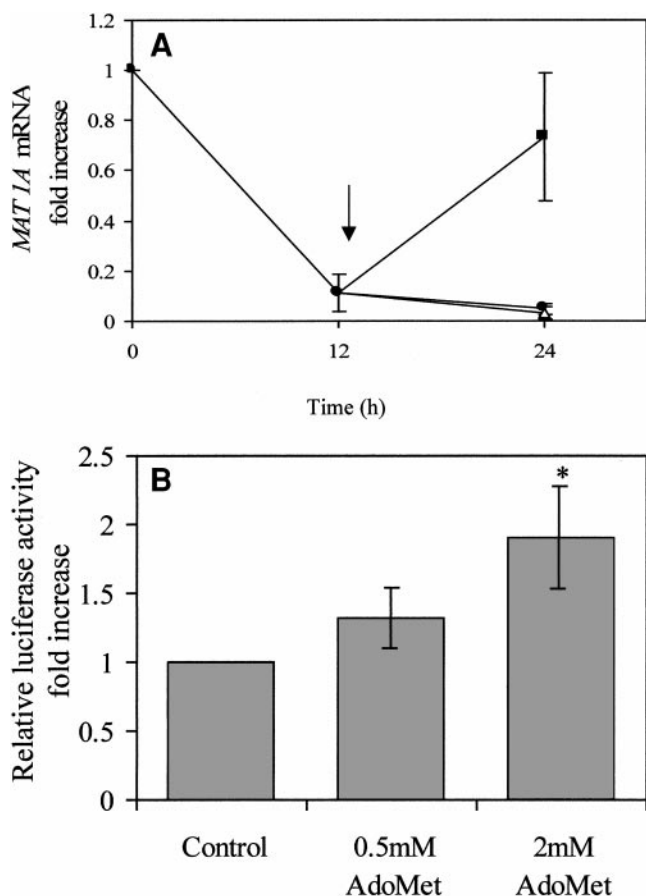

Figure 2. Effect of AdoMet on *MAT1A* transcription in cultured rat hepatocytes. *A*) Hepatocytes were cultured for 12 h and then 4 mM AdoMet was added to culture media (arrow) in the presence or absence of actinomycin D (5 μg/ml) and incubations continued for another 12 h. *MAT1A* mRNA levels were determined by Northern blotting in the absence of AdoMet (●), in AdoMet-treated cells (■) and in AdoMet plus actinomycin D-treated cells (Δ). Data are means ± SE. *B*) Hepatocytes were transfected with a construct harboring 1.4 kb of *MAT1A* promoter coupled to a luciferase reporter gene as described in Materials and Methods. Luciferase activity was measured in cell lysates of control and AdoMet-treated hepatocytes after 24 h of incubation. Luciferase activities, normalized for β-galactosidase expression, are expressed as fold increase with respect to untreated controls. *$P<0.05$ with respect to control values. Data are means ± SE

obtained with two other experimental approaches. First, when AdoMet treatment was performed in the presence of 10 μM of the adenosine analog 3-deaza-adenosine (C^3-Ado) (32), the induction of *MAT1A* expression was substantially impaired (Fig. 3). C^3-Ado is an inhibitor of S-adenosylhomocysteine hydrolase and leads to a significant increase in the intracellular levels of S-adenosylhomocysteine (AdoHcy), additionally it can be converted into the more stable 3-deaza-derivative of AdoHcy (S-3-deaza-adenosylhomocysteine, C^3-AdoHcy) (32). AdoHcy and C^3-AdoHcy are strong inhibitors of methylation reactions (2, 32). Second, under similar conditions the simultaneous addition of AdoMet and L-ethionine (2 mM), the ethyl analog of methionine, resulted in the impairment of AdoMet action (Fig. 3). If instead of L-ethionine cells were treated with D-ethionine, AdoMet induction of *MAT1A* expression was not affected (data not shown). Ethionine is also a substrate for MATI/III, which converts it into S-adenosylethionine, a molecule that is only slowly metabolized further and thus accumulates in the cell and inhibits AdoMet actions (33, 34).

The overall transcriptional activity of cultured hepatocytes was not stimulated by AdoMet treatment in a nonspecific fashion. This was confirmed when AdoMet effects on another liver-specific gene such as albumin were tested. In this case, and as previously reported, albumin expression decayed with time in culture; however, AdoMet treatment was not able to prevent it, whereas under the same conditions the down-regulation of *MAT1A* mRNA was almost completely reversed (**Fig. 4*A, C***). As mentioned in the introduction the progressive decay in the expression of liver-specific genes such as *MAT1A* and *albumin* observed in cultured hepatocytes is often accompanied by the activation of other genes that are not normally expressed in this organ. Some of these genes, such as the uncoupling protein 2 (UCP2) and the enzyme telomerase, have been proposed to provide the hepatic cell with metabolic traits that favor a proliferative phenotype (28, 35, 36). This is supported by the fact that such genes are also activated in transformed hepatic cells (28, 36). These observations led us to examine the expression of *MAT2A* in our model of cultured hepatocytes. Interestingly,

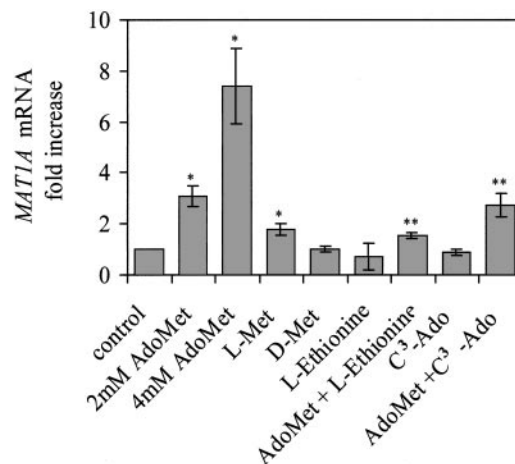

Figure 3. Mechanism of AdoMet regulation of *MAT1A* expression. Hepatocytes were treated with 2 and 4 mM AdoMet; 2 mM L-methionine; 2 mM D-methionine; 2 mM L-ethionine; 2 mM AdoMet plus 2 mM L-ethionine; 10 μM C^3-Ado; 10 μM C^3-Ado plus 4 mM AdoMet for 12 h and *MAT1A* mRNA levels were determined by Northern blotting. *Differences were statistically significant ($P<0.05$) with respect to control levels (control: without AdoMet). **Differences between 2 mM AdoMet vs. 2 mM AdoMet plus L-ethionine, and 4 mM AdoMet vs. 4 mM AdoMet plus C^3-Ado were statistically significant ($P<0.05$). Data are means ± SE.

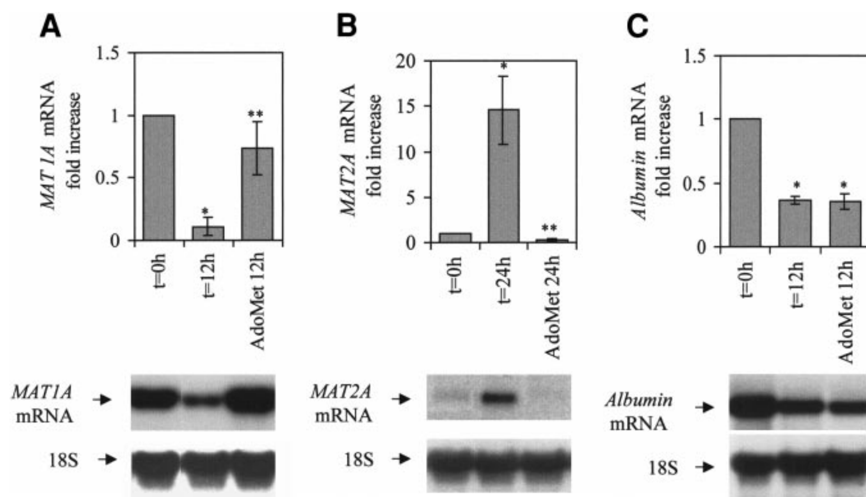

Figure 4. *MAT1A, albumin,* and *MAT2A* mRNAs in cultured rat hepatocytes: effect of AdoMet treatment. *A*) *MAT1A* mRNA levels were reduced after 12 h in culture (t=12 h). This was prevented by AdoMet (AdoMet 12 h). *B*) *MAT2A* expression was induced with time in culture (t=24 h), this induction was prevented in the presence of AdoMet (AdoMet t=24 h). Blots were hybridized with a probe for 18S rRNA to check for equal loading. *C*) *Albumin* gene expression was examined in cultured hepatocytes in the absence or presence of AdoMet. *Differences were statistically significant ($P<0.05$) with respect to controls at $t = 0$. **Statistically significant differences ($P<0.05$) between AdoMet treated and untreated hepatocyte cultures. Representative blots are shown. Data are means ± SE.

MAT2A expression was already elevated 12 h after hepatocytes were plated (data not shown), and levels increased significantly after 24 h in culture (Fig. 4*B*). More remarkably, when cells were cultured for 24 h in the presence of AdoMet (4 mM), the induction of *MAT2A* expression was completely prevented (Fig. 4*B*).

DISCUSSION

As we mentioned, an adequate supply of one-carbon units to the liver is essential for the preservation of its differentiated functions. When the flow of one-carbon units is impaired, because severe dietary restrictions or liver damage, many of the tissue-specific functions of this organ are compromised and can be subsequently lost in a transition to a preneoplastic condition. An early biochemical event in these situations is the reduced availability of AdoMet, which may be because of a shortage of its precursor (as in methionine-choline-deficient diets) (14), impaired synthesis because of the inactivation of MATI/III or impaired *MAT1A* expression, or the overall derangement of the methionine cycle (2, 7, 11). The beneficial effects of AdoMet administration in such situations, which include the prevention of the neoplastic conversion of the liver, further support the importance of this metabolic pathway and a role for this compound in the maintenance of liver functions (2, 13, 15, 16, 29). However, the molecular mechanisms of these effects of AdoMet are not fully understood.

The preservation of *MAT1A* expression, and thus MATI/III activity, is a fundamental trait of the healthy and differentiated hepatocyte. Loss of *MAT1A* expression accompanies the malignant transformation of the liver, and its replacement by the product of *MAT2A* gene appears to confer a growth advantage to the transformed cell (12). In this context, we wanted to know whether cellular AdoMet levels could influence the expression of the methionine-metabolizing gene *MAT1A*. For this purpose, we turned to an experimental model of rat hepatocytes in primary culture in which this hypothesis could be directly addressed. It has been shown that cultured hepatocytes tend to lose the expression of liver-specific genes as a function of time in culture, reflecting a certain degree of de-differentiation (26, 27). In agreement with these notions, we observe a progressive reduction in *MAT1A* mRNA steady-state levels with culture time. When AdoMet was added to hepatocytes at the onset of cultures, the decrease in *MAT1A* mRNA levels was prevented in a dose-dependent manner. The effect of AdoMet was very potent and at certain concentrations *MAT1A* mRNA expression returned to levels found in recently isolated hepatocytes. This effect on *MAT1A* mRNA was also observed at the protein level and proved to be specific because the amount of other cellular proteins, such as actin, was not affected.

In our culture conditions, we have observed a dose-dependent increase in intracellular AdoMet on addition of this compound to the medium. Because of the low membrane permeability of AdoMet, high extracellular concentrations in the culture media had to be used to increase significantly the intracellular AdoMet content. The increase of intracellular AdoMet content observed under these conditions is likely to occur *in vivo* after feeding a methionine reach diet. It should be noted that cellular uptake of exogenous AdoMet is a controversial issue and that conflicting data can be found in the literature (30, 31, 37). However, even in those studies, in which it was concluded that AdoMet was not found to be taken up by cells, when concentrations >200 μM were used, intracellular levels of AdoMet were increased (31). Thus, it has been proposed that by

increasing the extracellular concentrations of AdoMet above certain levels, the intracellular pool of this compound can be modulated (37), and such was our purpose in the present experiments.

Gene expression regulation in mammalian cells can be achieved at various levels. The two main processes involved are the modulation of gene transcription and the control of mRNA stability. Under these premises, we next addressed the mechanisms that could mediate the effects of AdoMet on *MAT1A* mRNA levels. Our observations indicate that AdoMet effects on *MAT1A* mRNA levels took place mainly at the transcriptional level. Several lines of evidence support this notion. *1*) AdoMet effects on *MAT1A* mRNA levels were suppressed in the presence of the transcriptional inhibitor actinomycin D when both agents were added together at the onset of cultures; *2*) AdoMet was able to reinduce *MAT1A* expression after 12 h of decay in culture, reaching levels close to those found in recently isolated hepatocytes; *3*) the reinduction of *MAT1A* expression was completely blocked by actinomycin D; and *4*) AdoMet was able to dose-dependently stimulate reporter gene expression in transient transfection experiments in which the rat *MAT1A* promoter coupled to the luciferase gene was introduced in cultured hepatocytes. The magnitude of this effect, at 2 mM AdoMet, was similar to the response elicited by saturating concentrations of glucocorticoids, which we have previously described as potent regulators of *MAT1A* gene expression in liver parenchymal cells (38). Although the identity of the promoter elements and binding factors responsible for AdoMet effect have not been identified yet, this observation strongly supports the activation of *MAT1A* gene transcription by AdoMet.

The precise mechanisms through which AdoMet stimulated *MAT1A* transcription are still not known; nevertheless, we have performed a series of experiments that suggest the likely involvement of a methylation reaction. We observed that AdoMet effect on *MAT1A* mRNA levels were blunted when cells were treated in the presence of the adenosine analog C^3-Ado. This compound raises intracellular concentrations of AdoHcy and C^3-AdoHcy, potent competitive inhibitors of transmethylation reactions (2, 3, 32). Similarly, L-ethionine also interfered with the effects of AdoMet on *MAT1A* mRNA. L-Ethionine is adenylated at the expense of ATP to produce S-adenosylethionine, a compound that is only slowly metabolized further (33, 34). S-Adenosylethionine accumulates in the cell and competes with AdoMet in transmethylaton reactions (39). The effect of L-ethionine was mediated through its conversion into S-adenosylethionine, because the nonmetabolizable isomer, D-ethionine, at the same concentration did not interfere with AdoMet induction of *MAT1A* expression (data not shown). Taken together, these observations suggest that a methyl transfer reaction participates in the mechanism of action of AdoMet. In addition, these data also support the idea that AdoMet would be acting intracellularly, where AdoHcy induced by C^3-Ado, and S-adenosylethionine are synthesized and accumulated. AdoMet effects were partially mimicked by L-methionine treatment of cultured hepatocytes. This effect is probably mediated through the conversion of this amino acid to AdoMet, because D-methionine, which is not a substrate of MATI/III, did not affect *MAT1A* expression. In addition, these observations may contribute to explain the observed elevation in rat liver MATI/III activity in response to increased dietary methionine through the enhancement in *MAT1A* expression (40, 41).

As mentioned before, and as we observe in the case of *MAT1A*, hepatocytes isolated from healthy livers tend to lose the expression of liver-specific genes with time in culture (26, 27). Concomitantly, it has been realized that other genes that are not normally expressed in the differentiated liver, but that are activated in hepatocarcinoma cells, become induced (28, 35, 36). Such genes are thought to endow the hepatic cell with metabolic traits compatible with a proliferate phenotype (28, 35). Thus, in this regard it has been proposed that the cultured hepatocyte reproduces to a certain extent some of the genetic changes that occur during the neoplastic conversion of the liver. Our present observation of *MAT2A* expression induction in cultured hepatic cells fits well within this hypothesis. Indeed, *MAT2A* expression is induced when normal hepatocytes proliferate after partial hepatectomy (9) and because of malignant transformation (11, 12, 42). Nevertheless, what we find more interesting is the inhibition of *MAT2A* expression by AdoMet treatment. Thus, in response to this agent we observe the simultaneous, although opposed, regulation of *MAT1A* and *MAT2A* expression in the same cell, whereas the decay in albumin gene expression was not affected.

Although it is possible that AdoMet only prevents the change in *MAT1A/MAT2A* gene expression, without affecting the expression of other genes, these effects may be relevant to the preservation of the differentiated status of the hepatocyte regarding methionine metabolism. Our observations demonstrate the exquisite specificity of AdoMet effects on ‘hepatocyte gene expression. Additionally, these data strongly suggest that the maintenance of certain AdoMet levels can be crucial in preventing changes in gene expression that may contribute to the development of a preneoplastic phenotype. In addition, our results may contribute to an understanding of the mechanisms underlying the hepatocarcinogenic effects of experimental models in which liver AdoMet levels are persistently reduced, such as me-

thionine-choline-deficient diets and the administration of agents such as ethionine and diethylnitrosamine (14, 16, 39, 43). These novel actions of AdoMet may also be behind the preventive effect of this compound in the development of chemically induced hepatocarcinoma, a model in which AdoMet administration results in growth inhibition and the remodeling of preneoplastic lesions (15, 16), and in the inhibition of cellular growth in AdoMet-treated human hepatocarcinoma cells in culture (12).

In summary, our present results lead us to propose a novel role for AdoMet in the specific regulation of liver gene expression. These novel actions of AdoMet may open a new scenario in the biological roles played by this molecule, which now could be regarded also as an hepatotrophic agent involved in the preservation of the differentiated status of the hepatocyte, at least regarding one-carbon metabolism. FJ

This work was supported by grants SAF 98/0132, SAF 99/0038 from CICYT (Spain), Europharma, and Knoll to J. M. M, grant 923/98 from Gobierno de Navarra to M. A. A. M., and grant AA12677-01A1 from the National Institutes of Health to M. A. A. and J. M. M. U. L. is a fellow of Fundación Renal Íñigo Álvarez de Toledo; E. R. G. T. and M. V. C. are fellows of the Universidad de Navarra. We thank Estefanía Fenández for technical help.

REFERENCES

1. Cantoni, G. L. (1975) Biochemical methylations: selected aspects. *Annu. Rev. Biochem.* **44,** 435–441
2. Mato, J. M., Alvarez, L., Ortiz, P., and Pajares, M. A. (1997) S-Adenosylmethionine synthesis: molecular mechanisms and clinical implications. *Pharmacol. Ther.* **73,** 265–280
3. Finkelstein, J. D. (1990) Methionine metabolism in mammals. *J. Nutr. Biochem.* **1,** 228–236
4. Mudd, S. H., and Poole, J. R. (1975) Labile methyl balances for normal humans on various dietary regimens. *Metabolism* **24,** 721–735
5. Kotb, M., and Geller, A. M. (1993) Methionine adenosyltransferase: structure and function. *Pharmacol. Ther.* **59,** 125–143
6. Kotb, M., Mudd, S. H., Mato, J. M., Geller, A. M., Kredich, N. M., Chou, J. Y., and Cantoni, G. L. (1997) Consensus nomenclature for the mammalian methionine adenosyltransferase genes and gene products. *Trends Genet.* **13,** 51–52
7. Torres, L., Avila, M. A., Carretero, M. V., Latasa, M. U., Caballería, J., López-Rodas, G., Boukaba, A., Lu, S. C., Franco, L., and Mato, J. M. (2000) Liver-specific methionine adenosyltransferase *MAT1A* gene expression is associated with a specific pattern of promoter methylation and histone acetylation: implications for *MAT1A* silencing during transformation. *FASEB J.* **14,** 95–102
8. Torres, L., López-Rodas, G., Latasa, M. U., Carretero, M. V., Boukaba, A., Rodríguez, J. L., Franco, L., Mato, J. M., and Avila, M. A. (2000) DNA methylation and histone acetylation of rat methionine adenosyltransferase 1 A and 2 A genes is tissue-specific. *Int. J. Biochem. Cell Biol.* In press
9. Huang, Z. Z., Mao, Z., Cai, J., and Lu, S. C. (1998) Changes in methionine adenosyltrasnferase during liver regeneration in the rat. *Am. J. Physiol.* **275,** G14–G21
10. Gil, B., Casado, M., Pajares, M. A., Boscá, L., Mato, J. M., Martín-Sanz, P., and Alvarez, L. (1996) Differential expression pattern of S-adenosylmethionine synthetase isoenzymes during rat liver. *Hepatology* **24,** 876–881
11. Cai, J., Sun, W., Hwang, J., Stain, S. C., and Lu, S. C. (1996) Changes in S-adenosylmethionine synthetase in human liver cancer: molecular characterization and significance. *Hepatology* **24,** 1090–1097
12. Cai, J., Mao, Z., Hwang, J., and Lu, S. C. (1998) Differential expression of methionine adenosyltransferase genes influences the rate of growth of human hepatocellular carcinoma cells. *Cancer Res.* **58,** 1444–1450
13. Mato, J. M., Cámara, J., Fernández de Paz, J., Caballería, L., Coll, S., Caballero, A., García-Buey, L., Beltrán, J., Benita, V., Caballería, J., Solà, R., Moreno-Otero, R., Barrao, F., Martín-Duce, A., Correa, J. A., Parés, A., Barrao, E., García-Magaz, I., Puerta, J. L., Moreno, J., Boissard, G., Ortiz, P., and Rodés, J. (1999) S-Adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. *J. Hepatol.* **30,** 1081–1089
14. Newberne, P. M., and Rogers, A. E. (1986) Labile methyl groups and the promotion of cancer. *Annu. Rev. Nutr.* **6,** 407–432
15. Garcea, R., Daino, L., Pascale, R., Simile, M. M., Puddu, M., Frassetto, S., Cozzolino, P., Seddaiu, M. A., Gaspa, L., and Feo, F. (1989) Inhibition of promotion and persistent nodule growth by S-adenosyl-L-methionine in rat liver carcinogenesis: role of remodeling and apoptosis. *Cancer Res.* **49,** 1850–1856
16. Pascale, R. M., Marras, V., Simile, M. M., Daino, L., Pinna, G., Bennati, S., Carta, M., Seddaiu, M. A., Massarelli, G., and Feo, F. (1992) Chemoprevention of rat liver carcinogenesis by S-adenosyl-L-methionine: a long-term study. *Cancer Res.* **52,** 4979–4986
17. Avila, M. A., Carretero, M. V., Rodriguez, E. N., and Mato, J. M. (1998) Regulation by hypoxia of methionine adenosyltransferase activity and gene expression in rat hepatocytes. *Gastroenterology* **114,** 364–371
18. Alvarez, L., Sánchez-Góngora, E., Mingorance, J., Pajares, M. A., and Mato, J. M. (1997) Characterization of rat liver-specific methionine adenosyltransferase gene promoter. *J. Biol. Chem.* **272,** 22875–22883
19. Henning, S. M., McKeer, W., and Swendseid, M. E. (1989) Hepatic content of S-adenosylmethionine and glutathione in rats receiving treatments modulating methyl donor availability. *J. Nutr.* **119,** 1478–1482
20. Chomczynski, P., and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159
21. Avila, M. A., Velasco, J. A., Cho, C., Lupu, R., Wen, D., and Notario, V. (1995) Hyperactive autocrine loop mediated by a NDF-related factor in neoplastic hamster embryo fibroblasts expressing an activated *cph* oncogene. *Oncogene* **10,** 963–971
22. Alvarez, L., Asunción, M., Corrales, F., Pajares, M. A., and Mato, J. M. (1991) Analysis of the 5′ noncoding region of the rat liver S-adenosylmethionine synthetase mRNA and comparison of the Mr deduced from the cDNA sequence and the purified enzyme. *FEBS Lett.* **290,** 142–146
23. Horikawa, S., Sasuga, J., Shimizu, K., Ozasa, K., and Tsukada, K. (1990) Molecular cloning and nucleotide sequence of cDNA encoding the rat kidney S-adenosylmethionine synthetase. *J. Biol. Chem.* **265,** 13683–13686
24. Sargent, T. D., Wu, J. R., Sala-Trepat, J. M., Wallace, R. B., Reyes, A. A., and Bonner, J. (1979) The rat serum albumin gene: analysis of cloned sequences. *Proc. Natl. Acad. Sci. U. S. A.* **76,** 3256–3260
25. Ruiz, F., Corrales, F. J., Miqueo, C., and Mato, J. M. (1998) Nitric oxide inactivates rat hepatic methionine adenosyltransferase in vivo by S-nitrosylation. *Hepatology* **28,** 1051–1057
26. Caron, J. M. (1990) Induction of albumin gene transcription in hepatocytes by extracellular matrix proteins. *Mol. Cell. Biol.* **10,** 1239–1243
27. Schuetz, E. G., Li, D., Omiecinski, C. J., Muller-Eberhard, U., Kleinman, H. K., Elswick, B., and Guzelian, P. S. (1988) Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. *J. Cell. Physiol.* **134,** 309–323
28. Nozawa, K., Kurumiya, Y., Yamamoto, A., Isobe, Y., Suzuki, M., and Yoshida, S. (1999) Up-regulation of telomerase in primary cultured rat hepatocytes. *J. Biochem.* **126,** 361–367
29. Chawla, R. K., Watson, W. H., Eastin, C. E., Lee, E. Y., Schmidt, J., and McClain, C. J. (1998) S-Adenosylmethionine deficiency and TNF-alpha in lipopolysaccharide-induced hepatic injury. *Am. J. Physiol.* **275,** G125–G129
30. Engstrom, M. A., and Benevenga, N. J. (1987) Rates of oxidation of the methionine and S-adenosylmethionine methyl carbons in isolated rat hepatocytes. *J. Nutr.* **117,** 1820–1826

31. Bontemps, F., and Van den Berghe, G. (1997) Metabolism of exogenous S-adenosylmethionine in isolated rat hepatocyte suspensions: methylation of plasma-membrane phospholipids without intracellular uptake. *Biochem. J.* **327,** 383–389
32. Chiang, P. K. (1998) Biological effects of inhibitors of S-adenosylhomocysteine hydrolase. *Pharmacol. Ther.* **77,** 115–134
33. Brada, Z., Bulba, S., and Tershakovec, G. R. (1981) The dose-related metabolism of L-ethionine in acute experiments with rats. *Res. Commun. Pathol. Pharmacol.* **31,** 357–374
34. Brada, Z., Hrsta, I., and Bulba, S. (1988) Formation of S-adenosylethionine in liver of rats chronically fed with DL-ethionine. *Cancer Res.* **48,** 4464–4468
35. Kimura, K., Jung, B. D., Kanehira, K., Irie, Y., Cañas, X., and Saito, M. (1999) Induction of uncoupling protein (UCP) 2 in primary cultured hepatocytes. *FEBS Lett.* **457,** 75–79
36. Carretero, M. V., Torres, L., Latasa, M. U., García-Trevijano, E. R., Prieto, J., Mato, J. M., and Avila, M. A. (1998) Transformed but not normal hepatocytes express UCP2. *FEBS Lett.* **439,** 55–58
37. Watson, W. H., Zhao, Y., and Chawla, R. K. (1999) S-Adenosylmethionine attenuates the lipopolysaccharide-induced expression of the gene for tumour necrosis factor α. *Biochem. J.* **342,** 21–25
38. Gil, B., Pajares, M. A., Mato, J. M., and Alvarez, L. (1997) Glucocorticoid regulation of hepatic S-adenosylmethionine synthetase gene expression. *Endocrionology* 138, 1251–1258.
39. Hoffman, J. L. (1994) Bioactivation by S-adenosylation, S-methylation, or N-methylation. In *Advances in Pharmacology* (Thomas August, J., Anders, M. W., and Murad, F., eds) pp. 449–477, Academic, San Diego
40. Finkelstein, J. D., and Martin, J. J. (1986) Methionine metabolism in mammals. *J. Biol. Chem.* **261,** 1582–1587
41. Matsumoto, C., Suma, Y., and Tsukada, K. (1984) Changes in the activities of S-adenosylmethionine synthetase isozymes from rat liver with dietary methionine. *J. Biochem.* **95,** 287–290
42. Horikawa, S., Kobayasi, Y., Sugiyama, T., Terashima, H., Wada, K., and Tsukada, K. (1993) Expression of non-hepatictype S-adenosylmethionine synthetase isozyme in rat hepatomas induced by 3′-mathyl-4-dimethylaminoazobenzene. *FEBS Lett.* **334,** 69–71
43. Counts, J. L., Sarmiento, J. I., Harbison, M. L., Downing, J. C., McClain, R. M., Goodman, J. I. (1996) Cell proliferation and global methylation status changes in mouse liver after phenobarbital and/or choline-devoid, methionine-deficient diet administration. *Carcinogenesis* **17,** 1251–1257

Received for publication March 30, 2000.
Revised for publication May 25, 2000.