Cloning and functional characterization of the 5′-flanking region of human methionine adenosyltransferase 1A gene

Zhaohui ZENG, Zong-Zhi HUANG, Changjin CHEN, Heping YANG, Zebin MAO and Shelly C. LU[1]

Division of Gastroenterology and Liver Diseases, Department of Medicine, USC Liver Disease Research Center, USC School of Medicine, 2011 Zonal Avenue, Los Angeles, CA 90033, U.S.A.

Methionine adenosyltransferase (MAT) is an essential cellular enzyme which catalyses the formation of *S*-adenosylmethionine, the principal methyl donor and precursor for polyamines. In mammals, two different genes, *MAT1A* and *MAT2A*, encode for liver-specific and non-liver-specific MAT respectively. We previously described a switch in the MAT expression from *MAT1A* to *MAT2A* in human liver cancer, which offered the cancerous cell a growth advantage. Loss of *MAT1A* expression was due to lack of gene transcription. To study regulation of the *MAT1A* gene, we have cloned and characterized a 1.9 kb 5′-flanking region of the human *MAT1A* gene. One transcriptional start site, located 25 nt downstream from a consensus TATA box, was identified by primer extension and RNase protection assays. The promoter contains several consensus binding sites for CAAT enhancer binding protein (C/EBP) and hepatocyte-enriched nuclear factor (HNF), transcriptional factors important in liver-specific gene expression. The human *MAT1A* promoter was able to efficiently drive luciferase expression in Chang cells, a human liver cell line, but not in HeLa cells. Sequential deletion analysis of the promoter revealed two DNA regions upstream of the translational start site, −705 to −839 bp and −1111 to −1483 bp, which are involved in positive and negative gene regulation, respectively. Specific protein binding to these regions was confirmed by electrophoretic-mobility-shift and DNase I footprinting assays. Similar to the situation with the rat *MAT1A*, glucocorticoid treatment also increased human *MAT1A* expression and promoter activity in a dose- and time-dependent manner.

Key words: Chang cells, glucocorticoid, luciferase expression, electrophoretic mobility shift, DNase I footprinting analysis

INTRODUCTION

Methionine adenosyltransferase (MAT) is a critical cellular enzyme which catalyses the formation of *S*-adenosylmethionine (SAM), the principal biological methyl donor and the ultimate source of the propylamine moiety used in polyamine biosynthesis [1,2]. In mammals, two different genes, *MAT1A* and *MAT2A*, encode for two homologous MAT catalytic subunits, α1 and α2 [3–5]. *MAT1A* is expressed only in liver and encodes the α1 subunit found in two native MAT isoenzymes, which are either a dimer (MAT III) or tetramer (MAT I) of this single subunit [5]. *MAT2A* encodes for a catalytic subunit (α2) found in a native MAT isoenzyme (MAT II) which is widely distributed [3–5]. *MAT2A* also predominates in the foetal liver and is progressively replaced by *MAT1A* during development [6,7]. In adults, increased expression of *MAT2A* is associated with rapid growth of the liver. We showed a switch in the gene expression from *MAT1A* to *MAT2A* in human liver cancer [8] and from 12 to 24 h after partial hepatectomy in the rat [9]. In liver cancer, absence of *MAT1A* expression is due to lack of gene transcription [8]. Using a cell-line model that differs only in the type of MAT expressed, we demonstrated that the type of MAT expressed by the cell significantly influences the rate of cell growth [10]. Specifically, *MAT1A* expression is associated with the lowest rate of cell growth, while the opposite is true of *MAT2A* expression [10]. Thus the switch in MAT expression in liver cancer plays an important pathogenetic role by facilitating liver cancer growth. To better understand gene regulation of human *MAT1A*, we cloned and characterized the 5′-flanking region of the human *MAT1A*, the gene that encodes the catalytic subunit of liver-specific MAT.

MATERIALS AND METHODS

Materials

Cell-culture media and foetal bovine serum were obtained from Gibco BRL Life Technologies (Grand Island, NY, U.S.A.). The Luciferase Assay System and the β-Galactosidase Enzyme Assay System were obtained from Promega (Madison, WI, U.S.A.). All restriction enzymes were obtained from either Promega or Gibco. Triamcinolone was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Source of normal human liver tissue

Normal liver tissue was resected from liver specimens of patients with metastatic colon or breast carcinoma. Written informed consent was obtained from each patient. Tissues were immediately frozen in liquid nitrogen for subsequent isolation of nuclear protein as described in [11].

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by University of Southern California School of Medicine's Human Research Review Committee.

Abbreviations used: MAT, methionine adenosyltransferase; SAM, *S*-adenosylmethionine; EMSA, electrophoretic-mobility-shift assay; C/EBP, CAAT enhancer binding protein; HNF, hepatocyte-enriched nuclear factor; AP-1, activator protein-1; IL-6, interleukin-6; GRE, glucocorticoid response element; CRE-BP, cAMP-response-element-binding protein; CMV, cytomegalovirus.

[1] To whom correspondence should be addressed (e-mail shellylu@hsc.usc.edu).

The sequence of the 1.9 kb 5′-flanking region of the human *MAT1A* gene has been deposited with the EMBL, GenBank® and DDJB Nucleotide Sequence Databases under the accession number AF110500.

Cloning of the 5′-flanking region of the human *MAT1A* gene

To clone the promoter region of the human *MAT1A* gene, the Genome Walker kit (ClonTech, Palo Alto, CA, U.S.A.) was used according to manufacturer's instructions. All oligonucleotide primers used in this study were synthesized by the Molecular Biology Core of the Liver Disease Research Center, USC School of Medicine. Human *MAT1A*-specific primary PCR primer sequence is 5′-GTCACACAAGCCATCCACCG-3′, which is the reverse of, and complementary to, +30 to 11 nt downstream of the translational start site of the human *MAT1A* gene [4]. The secondary PCR primer is 5′-TTCATCTTCTCACACTTCTC-CACTC-3′, which is the reverse of, and complementary to, −20 (upstream) to +5 nt (downstream) of the translational start site. PCR reactions were performed using a Perkin–Elmer DNA Thermal Cycler 480 with the following conditions: 94 °C for 25 s and 72 °C for 4 min × 7 cycles; 94 °C for 25 s and 67 °C for 4 min × 32 cycles, followed by a 4 min final extension at 67 °C. Secondary PCR was conducted using 1 μl of a 50-fold dilution of the primary PCR product as template, the same reaction composition and cycle parameters, with the exception that 20 thermocycles were performed instead of 32. PCR products were analysed on a 1.2 % (w/v) agarose/ethidium bromide gel. Using this technique, we were able to identify a single major PCR product from three of the five libraries (*Eco*RV, *Dra*I, *Ssp*I) provided in the Genome Walker kit. The largest PCR product was 1.9 kb which was obtained using the *Ssp*I library. This product was purified from the gel by QIAquick Gel Extraction kit (Qiagen, Valencia, CA, U.S.A.), cloned into pT-Adv (ClonTech) and sequenced in both directions using the automated ABI Prism dRhodamine Terminator Cycle Sequencer performed by the Sequencing and Genetic Analysis Core Facility, Department of Cell and Neurobiology, USC School of Medicine. The initial primers were universal primers for the pT-Adv vector, all subsequent primers were nested primers designed using the available sequence information and the MacVector software program. The nucleotide sequence was verified by multiple bidirectional sequencing reactions. Sequences were aligned and a consensus sequence generated using the ASSEMBLIGN software program.

Primer extension analysis

Primer extension analysis was performed as described in [12]. Two antisense oligonucleotide primers complementary to nt +46 to +75 and −38 to −9 relative to the translational start site of the human *MAT1A* [4] were end-labelled with [γ-^{32}P]ATP using T_4 polynucleotide kinase. A 2.5 μg portion of polyadenylated RNA from human liver isolated as described [13] (or yeast tRNA as a negative control) was annealed to 10^6 c.p.m. of the primer and extended with 200 units of Moloney-murine-leukaemia-virus reverse transcriptase (Superscript II; Life Technologies). The primer-extended products were analysed on 7 M urea/6 % polyacrylamide gels.

RNase protection assay

RNase protection assay was done according to instructions provided in the Multi-NPA™ manual (Ambion, Austin, TX, U.S.A.). The probe was synthesized by linear amplification, the primer was 5′-GTCACACAAGCCATCCACCG-3′, which is the reverse of, and complementary to, +30 to +11 nt downstream of the translational start site [4] and the template was a *Bpu*A1-digested fragment (362 bp) from the 1.9 kb 5′-flanking region.

Construction of 5′-deletion constructs

The 1.9 kb PCR product, representing the *MAT1A* 5′-flanking region, was cloned in the sense orientation upstream of the luciferase coding sequence of the pGL3-enhancer vector (Promega). The resulting plasmid, designated p*MAT1A*-Luc, is the construct that contains the longest 5′-flanking sequence (−1902 to +30) employed in the transfection assay. To prepare 5′-deletion constructs, p*MAT1A*-Luc was digested with *Acc*65I and one internal restriction enzyme. The internal restriction enzymes used for generating deletion constructs were *Nde*I (−1483 to +30), *Tth111*I (−1111 to +30), and *Pvu*II (−705 to +30). Between the *Pvu*II and *Tth111*I sites, we constructed another 5′-deletion (−839 to +30) using the Erase-a-Base System Kit (Promega) including an exonuclease III which only digests 5′-overhangs or blunt-end sites. The resulting DNA fragments containing the luciferase expression vector were blunt-ended by Klenow (Gibco) and self-ligated by T4 DNA ligase (ClonTech).

Analysis of promoter constructs in cell culture

Normal human liver cell line Chang (A.T.C.C. no.CCL-13) and human cervix epitheloid carcinoma cell line HeLa (A.T.C.C. no.CCL-2) were cultured according to instructions provided. We chose to study expression in Chang cells because *MAT1A* is expressed only in the liver. HeLa cells were included for comparison because the Chang cells obtained from A.T.C.C. may be contaminated with HeLa cells (see description of Chang cells in the A.T.C.C. catalogue). To study the relative transcriptional activities of the *MAT1A* promoter fragments, Chang and HeLa cells (1×10^6 cells in 4 ml of medium) were transiently transfected with 8 μg of *MAT1A* promoter luciferase gene construct, promoterless pGL3-enhancer vector (as negative control) or CMV-pGL3-enhancer-Luc construct (as positive control) and 2 μg of a β-galactosidase expression plasmid (as an internal standard of transfection efficiency) using the calcium phosphate precipitation method [14]. After 48 h, cells were harvested and lysed in 1 ml of reporter lysis buffer (Luciferase Assay System; Promega). The luciferase assay was performed on 20 μl of the cleared lysate and 100 μl of luciferase assay reagent using a TD-20/20 Luminometer (Promega). The β-galactosidase assay was done according to the supplier's instructions using 150 μl of the cell lysate. Protein concentration was determined using the Bio-Rad Protein Assay. The luciferase activity of each transfection was expressed as luciferase activity per β-galactosidase activity per protein concentration.

Effect of triamcinolone on *MAT1A* expression in Chang cells

Triamcinolone has been shown to increase the *MAT1A* expression in rats via an increase in transcriptional activity [15]. To see if triamcinolone exerts a similar effect on human *MAT1A* expression, Chang cells were treated with triamcinolone (0.01–1 μM) in the culture medium for 24 h or 0.1 μM for 6–48 h. At the end of the treatment, total RNA was extracted from Chang cells as described in [8] and the steady-state MAT1A mRNA level was determined using a specific MAT1A cDNA probe [8]. To ensure equal loading of RNA samples and transfer in each of the lanes prior to hybridization, membranes were rinsed with ethidium bromide and photographed. The same membranes were also rehybridized with ^{32}P-labelled β-actin or 18 S cDNA probes as described in [9,11]. Autoradiography and densitometry (Gel Documentation System; Scientific Technologies, Carlsbad, CA, U.S.A. and NIH Image 1.60 software program) were used to quantify relative RNA. Results of Northern-blot analysis were normalized to β-actin or 18 S probe.

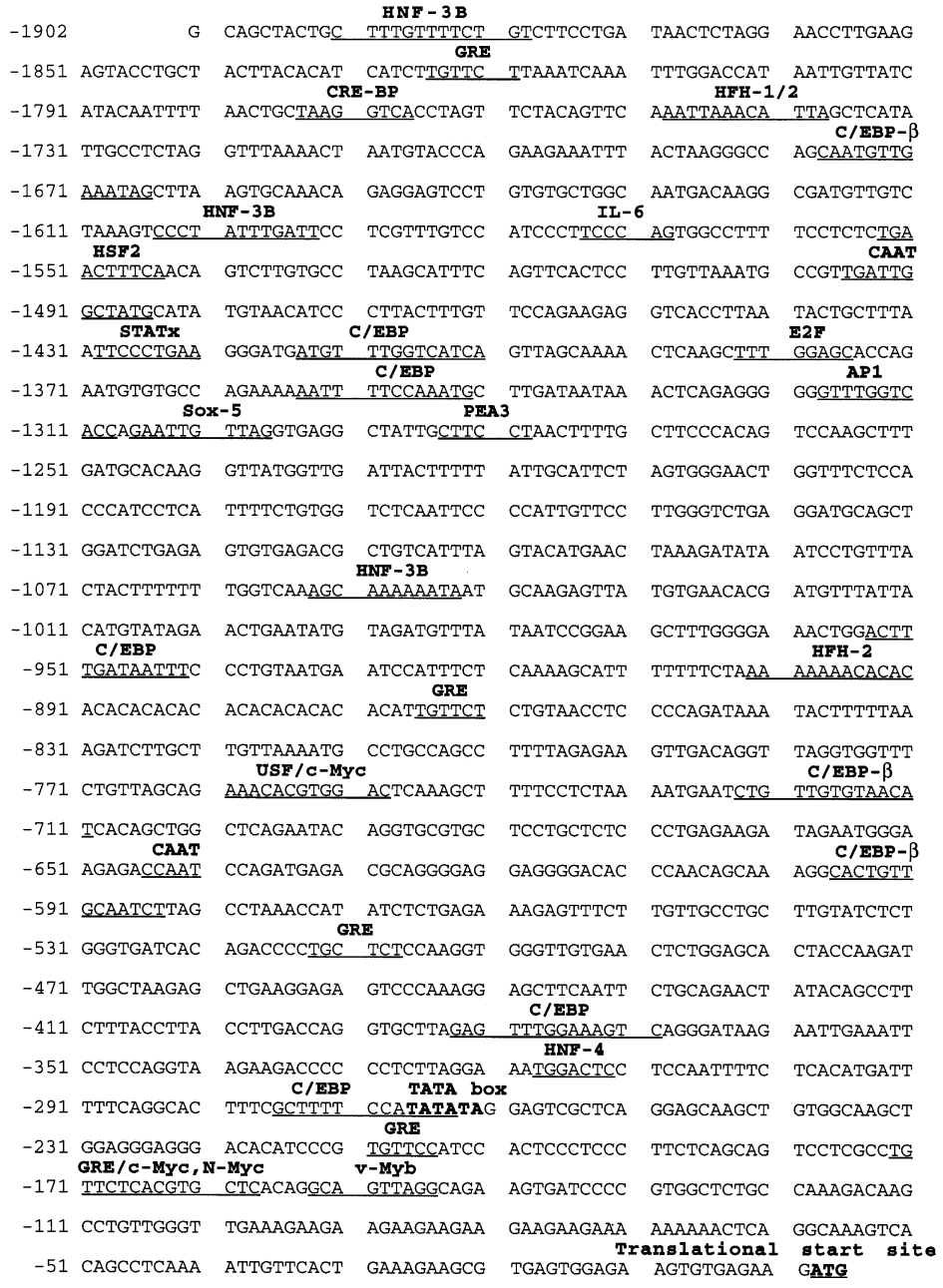

Figure 1 Nucleotide sequence of the 5′-flanking region of the human *MAT1A* gene

Sequence is numbered relative to the translational start site. The consensus TATA box is shown in **bold**. The putative regulatory elements are indicated in **bold** letters above the underlined sequences.

To examine the effect of triamcinolone on the human *MAT1A* promoter activity, Chang cells transfected with the human *MAT1A* promoter luciferase constructs were treated with triamcinolone (100 nM) or vehicle for 12, 24 or 44 h. For the 12 and 24 h treatments, cells were transfected for 28 h in total. Triamcinolone was added during the last 12 or 24 h. For the 44 h treatment, cells were transfected for 48 h in total. Luciferase activities driven by the human *MAT1A* promoter constructs in Chang cells were essentially the same in vehicle-treated controls regardless of the duration of transfection, and were pooled for analysis.

Electrophoretic-mobility-shift assay (EMSA)

EMSAs for positive and negative regulatory regions were done as described in [11,12]. A 15 μg portion of nuclear protein from normal human liver or Chang cells was pre-incubated with 2 μg of poly(dI-dC) in a buffer containing 10 mM Hepes (pH 7.6), 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 5 mM $MgCl_2$ and 10% (v/v) glycerol for 10 min on ice. ^{32}P-end-labelled double-stranded DNA fragments (positive fragment, −830 to −705; negative fragments, −1451 to −1309 and −1327 to −1225) were then added with or without a 50-fold excess of

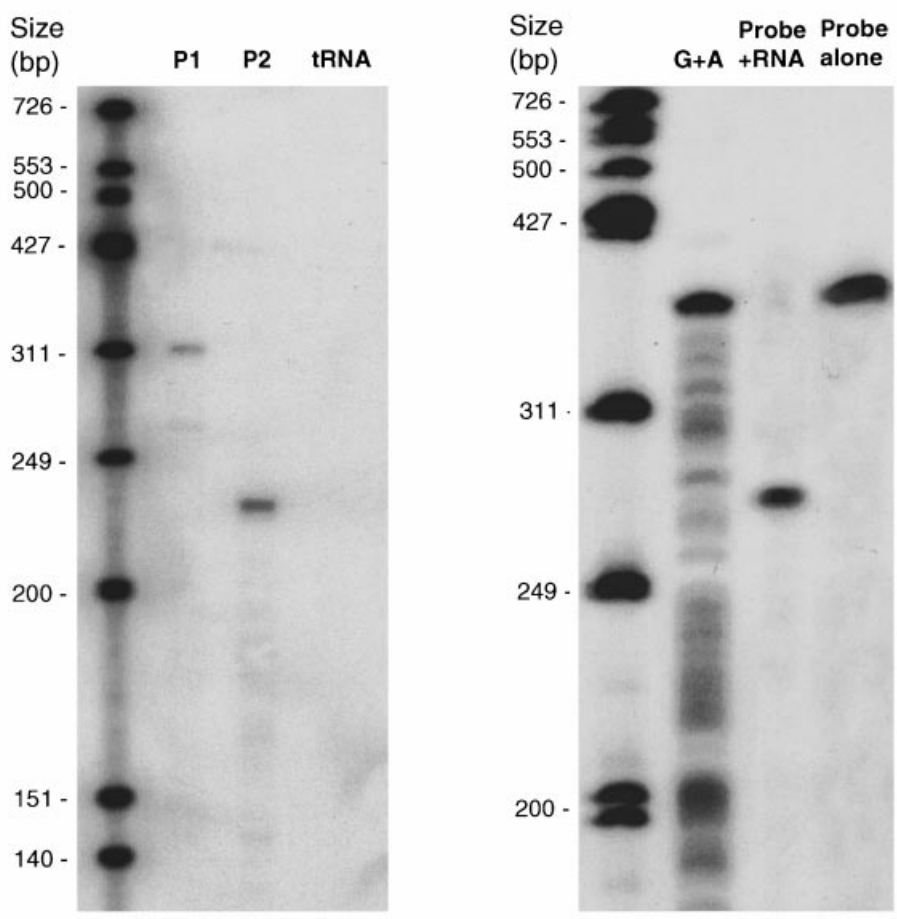

Figure 2 Determination of the transcriptional start site of the human *MAT1A* gene by primer-extension analysis (left panel) and RNase-protection assay (right panel)

In primer extension, two ^{32}P-end-labelled primers complementary to nt +46 to +75 (primer 1 or P1) and −38 to −9 (primer 2 or P2) of the human *MAT1A* cDNA were annealed to human liver polyadenylated RNA or yeast tRNA (primer 2 used) and extended with reverse transcriptase as described in the Methods section. In the RNase-protection assay, the primer was the reverse of, and complementary to, nt +30 to +11 of the human *MAT1A*. In the presence of RNA, a product of 268 nt was obtained. Size markers correspond to ϕX174 digested with *Hin*f1.

unlabelled specific or non-specific probe as competitor. Mixtures were incubated for 20 min on ice, loaded on a 4 % non-denaturing polyacrylamide gel and subjected to electrophoresis in 50 mM Tris/45 mM borate/0.5 mM EDTA, pH 8.0. Gels were dried and subjected to autoradiography.

DNase I footprinting analysis

Two ^{32}P-end-labelled fragments of the 5′-flanking region of human liver *MAT1A* gene were generated by digestion with restriction endonucleases. The fragment −976 to −504 was generated by digestion with *Msp*1 (−976) and *Sty*1 (−507), and the fragment −1483 to −1149 was generated by digestion with *Nde*1 (−1483) and *Sty*1 (−1152). Singly end-labelled fragments were generated by filling 5′-protruding ends with [α-^{32}P]dCTP (3000 Ci/mmol) or [α-^{32}P]dATP (3000 Ci/mmol) using the exo-Klenow enzyme. In addition, the fragment −1483 to −976 was labelled by [γ-^{32}P]ATP using T4 polynucleotide kinase and digested with *Sty*1 (−1152) for subsequent sequencing (−1483 to −1152). Labelled probes were purified by electrophoresis with 2 % agarose gel. Approx. 5×10^4 c.p.m. of end-labelled DNA fragments were incubated with 15–45 μg of nuclear protein from normal human liver. After 30 min incubation on ice, $CaCl_2$ and $MgCl_2$ were added to give a final concentration of 0.5 mM and 1 mM respectively. DNase I digestions were performed at room temperature for 1 min. Upon phenol extraction and ethanol precipitation, DNA fragments were resolved by electrophoresis in a denaturing 8 % acrylamide sequencing gel.

Statistical analysis

Data are given as mean ± S.E.M. Statistical analysis was performed using analysis of variance (ANOVA) followed by Fisher's test for multiple comparisons. Significance was defined as $P < 0.05$.

RESULTS

Cloning and sequencing of the 5′-flanking region of the human *MAT1A*

The sequence of the 1.9 kb product is shown in Figure 1. A canonical TATA box is located 263–268 nt upstream of the translational start site. Analysis of the transcription-factor-binding sites was done using Transcription Factor Search (Internet address: http://www.pdap1.trc.rwcp.or.jp/research/db/TFSEARCH.html). The 5′-flanking region of the human

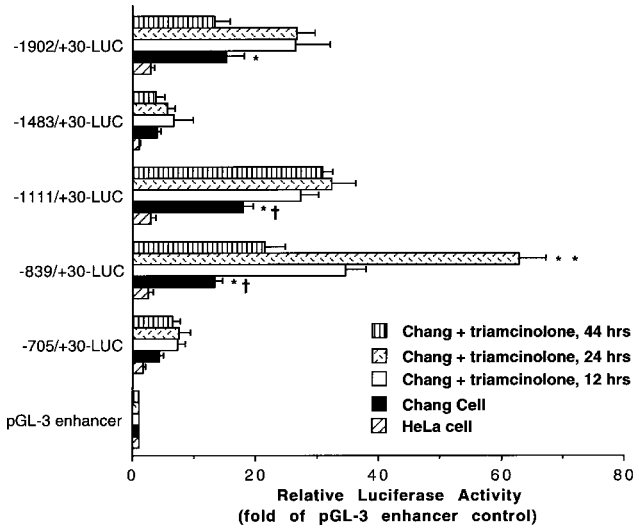

Figure 3 Transient transfection analysis of the human *MAT1A* promoter–luciferase constructs in Chang and HeLa cells

Progressive 5′-deletions of the *MAT1A* promoter extending from −1902 to +30 bp were generated and fused to the promoterless luciferase pGL-3 enhancer vector as described in the Methods section. Numbering is defined relative to the translational start site. Results represent mean ± S.E.M. from three to six independent experiments performed in triplicate. Triamcinolone treatment (100 nM) was for 12, 24 or 44 h. The luciferase activity of each transfection was expressed as luciferase activity/β-galactosidase activity/protein concentration. Data is expressed as relative luciferase activity to that of pGL-3 enhancer vector control, which is assigned a value of 1.0. $^{*}P < 0.05$ versus the pGL-3 enhancer control, −705/+30-LUC and −1483/+30-LUC constructs; †$P < 0.05$ versus triamcinolone treatments of different durations; $^{**}P < 0.05$ versus 12 and 44 h triamcinolone treatments (analysis of variance followed by Fisher's test).

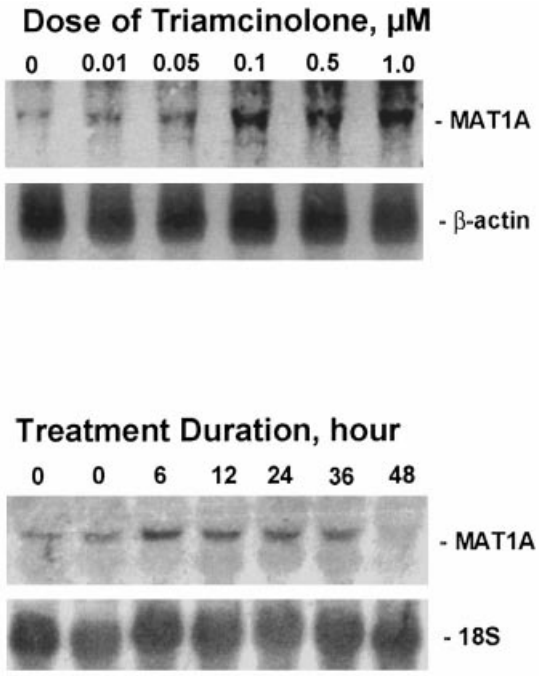

Figure 4 Effect of triamcinolone on steady-state *MAT1A* mRNA levels in Chang cells

Chang cells were treated with various doses of triamcinolone for 24 h (top panel) or with 0.1 μM at the time points shown (bottom panel). Total RNA (30 μg each lane) samples were extracted from these cells and analysed by Northern-blot hybridization with a ^{32}P-labelled *MAT1A* cDNA probe as described in the Methods section. The same membranes were then rehybridized with ^{32}P-labelled β-actin or 18 S cDNA probes. Representative Northern blots are shown.

MAT1A contains several consensus binding sites for CAAT enhancer binding protein (C/EBP) and hepatocyte-enriched nuclear factor (HNF), transcriptional factors important in liver-specific gene expression [16,17]. In addition, consensus binding sites for activator protein-1 (AP-1), CAAT, interleukin-6 (IL-6), glucocorticoid response element (GRE), cAMP response element binding protein (CRE-BP), E2F, PEA3, STATx, c-Myc and v-Myb are also present.

Transcriptional start site

Primer-extension analysis was first used to determine the transcriptional start site. Two antisense oligonucleotide primers complementary to +46 to +75 (primer 1) and −38 to −9 (primer 2) nt relative to the translational start site of the human *MAT1A* [4] were annealed to poly(A)$^+$ RNA from human liver and extended towards the 5′ end of the mRNA by reverse transcription. Figure 2 shows the primer extension reaction, which yielded a product of approx. 311 nt long using primer 1 and a product of approx. 230 nt long using primer 2. These products were not detected when the assay was carried out using tRNA. These results are consistent with the transcriptional start site being located approx. 25–27 nt downstream of the consensus TATA box or about 236–238 nt upstream of the translational start site. To further delineate and confirm the transcriptional start site, an RNase protection assay was carried out. Using a primer that is the reverse of, and complementary to, +30 to 11 nt downstream of the translational start site, a product of 268 nt was obtained (Figure 2). This confirms that the transcriptional start site is at 238 nt upstream of the translational start site.

Functional analysis of the 5′-flanking region of human *MAT1A*

To delineate sequences that drive the expression of the human *MAT1A*, five 5′-terminal nested deletion mutants ranging from −1902/+30 to −705/+30 were cloned into the promoterless luciferase reporter gene vector pGL3-enhancer. The promoterless construct pGL3-enhancer served as the background control. Luciferase activity was measured after transient transfection of Chang and HeLa cells with these constructs. Figure 3 shows that the human *MAT1A* promoter was able to efficiently drive luciferase expression in Chang cells but not in HeLa cells. In contrast, the CMV promoter was able to drive luciferase expression in both cell types (luciferase activity is 106 ± 21-fold and 544 ± 27-fold over pGL-3 enhancer control in Chang and HeLa cells respectively; results represent means ± S.E.M. from three to four independent experiments). In Chang cells, the construct −1111/+30 showed maximal promoter activity, the construct −839/+30 produced 80% of maximal activity, whereas the construct −705/+30 produced only 25% of maximal activity, indicating the presence of important elements between −705 and −839 that positively regulate promoter activity. The presence of negative regulatory elements is also suggested in the region of −1111 to −1483 as the construct −1483/+30 produced 22% of the maximal activity, while constructs −1111/+30 and −1902/+30 both produced maximal activity.

Effect of triamcinolone on *MAT1A* mRNA level and *MAT1A* promoter activity

The effect of triamcinolone on *MAT1A* mRNA expression in Chang cells is shown in Figure 4. A clear dose- and time-

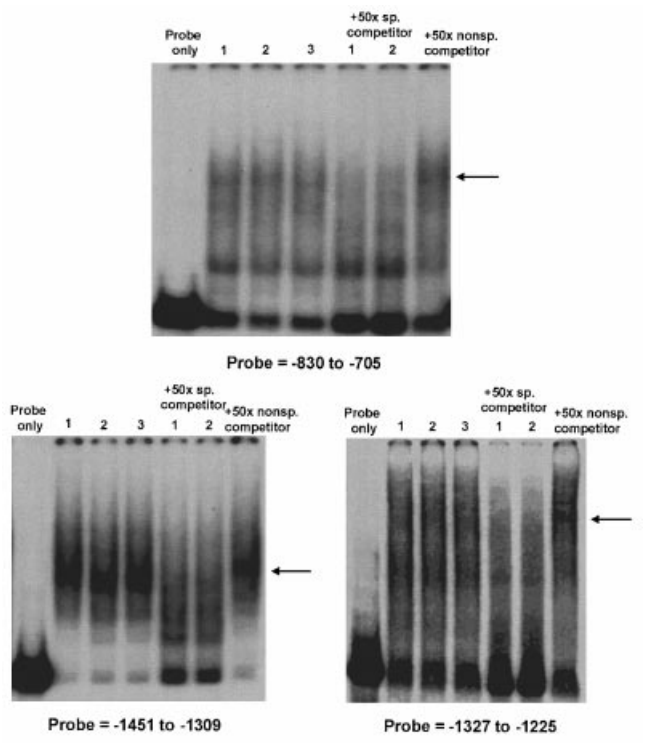

Figure 5 EMSAs for regions implicated in regulation of the human *MAT1A*

Nuclear protein extracts were obtained from normal human liver (triplicates) and EMSA was done as described in the Methods section using probes that span these regions. The arrows point to specific complexes which were competitively blocked by 50-fold excess of unlabelled specific probe. Similar results were obtained using nuclear protein extracts from Chang cells (results not shown).

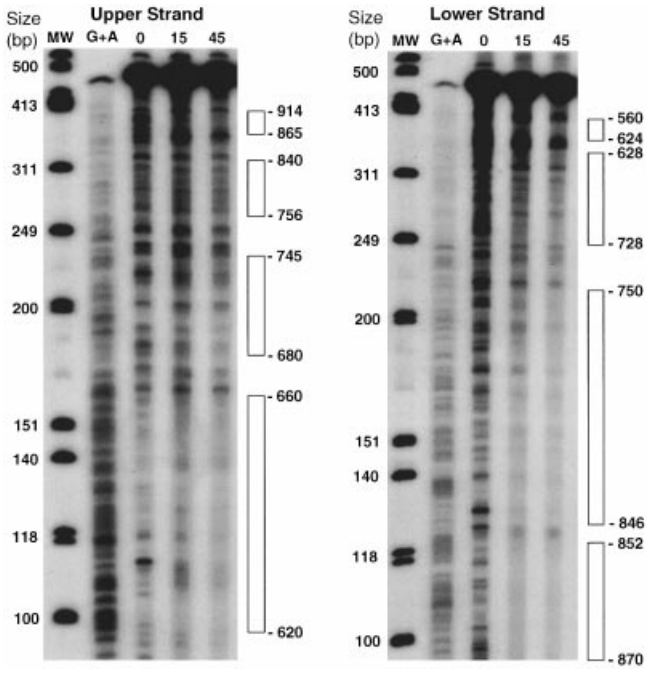

Figure 6 DNase I footprinting analysis of the −976 to −504 region of the human *MAT1A* promoter

DNA fragment containing the −976 to −504 region of the human *MAT1A* promoter was end-labelled on either strand and digested with DNase I in the absence (0) or presence of 15 or 45 μg of human liver nuclear protein extracts. Positions of the protected regions are indicated at the right of the Figures. Lanes G+A represent a Maxam–Gilbert sequencing reaction in the same fragments. Size markers correspond to ϕX174 digested with *Hin*f1.

dependent effect on the *MAT1A* expression is evident. After 24 h of treatment, 0.1 μM triamcinolone produced the maximal increase in the steady-state *MAT1A* mRNA level. A time course was then established using 0.1 μM triamcinolone. Maximal effect occurred as early as 6 h after treatment and returned towards baseline by 36 h. Consistent with this, triamcinolone exerted a significant increase in the luciferase expression driven by *MAT1A* constructs −1111/+30 and −839/+30 when the treatment was for 12 or 24 h. However, it exerted little effect on the luciferase expression if the treatment was for 44 h (Figure 3).

Analysis of regulatory regions using EMSA

On the basis of results of the deletion analysis, EMSAs were carried out using probes that span regions implicated in positive or negative regulation of the human *MAT1A*. The region −839 to −705 increased the promoter activity by 300%, whereas the region −1483 to −1111 reduced the promoter activity by 80%. Figure 5 shows that there is specific protein binding to these regions. The same results were obtained using nuclear proteins from normal human liver or Chang cells.

DNase I footprinting analysis of human *MAT1A* 5′-flanking region.

To further characterize the regulatory regions, DNase I footprinting analysis was carried out. Figure 6 shows that four nuclear-protein-dependent DNase I-protected areas are present

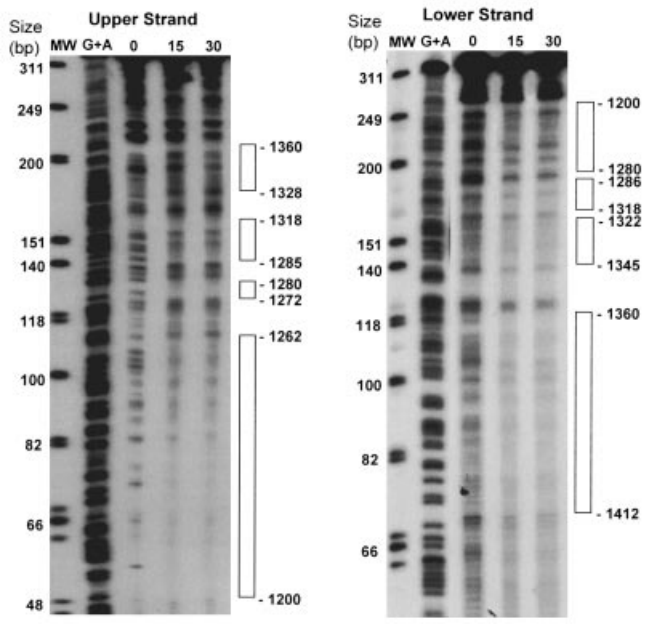

Figure 7 DNase I footprinting analysis of the −1483 to −1149 region of the human *MAT1A* promoter

DNA fragment containing the −1483 to −1149 region of the human *MAT1A* promoter was end-labeled on either strand and digested with DNase I in the absence (0) or presence of 15 or 30 μg of human liver nuclear protein extracts. Positions of the protected regions are indicated at the right of the Figures. Lanes G+A represent a Maxam–Gilbert sequencing reaction in the same fragments. Size markers correspond to ϕX174 digested with *Hin*f1.

in both strands in the region −976 to −504 of the human *MAT1A* promoter. Four nuclear-protein-dependent DNase I-protected areas are also present in both strands in the region −1483 to −1149 (Figure 7).

DISCUSSION

In mammals, two distinct genes encode for the enzyme MAT [3–5]. *MAT1A* is a liver-specific gene which is expressed in the liver shortly before birth and becomes the major form of *MAT* as the liver matures [7]. It is a marker for differentiated or mature liver phenotype. In contrast, *MAT2A* is expressed in all non-hepatic tissues as well as during periods of rapid liver growth [3,6–10]. Although the two *MAT* genes are highly homologous, the enzymes they encode have different kinetic profiles and regulatory properties [18]. Due to these differences, the type of MAT expressed by a cell can influence the cell's steady state SAM level, methylation status and growth rate [10].

Decreased activity of liver-specific MAT is well known in various liver diseases and may contribute to the pathogenesis of liver injury [1,18]. Both transcriptional and post-transcriptional regulation of liver-specific MAT have been described [1,15,18–25]. Most of the studies have focused on post-translational regulation of liver-specific MAT [1,18,22–25]. The liver-specific MAT contains several critical cysteine residues. Modifications of these residues can inactivate the enzyme by direct interference with the substrate-binding site(s) or by causing alteration in the oligomeric equilibrium (a decrease in the ratio of tetramer to dimer, which is less active at physiological substrate concentrations) [1,18,22–24]. We have also speculated recently that liver-specific MAT protein may be degraded more rapidly after treatment of rats with thioacetamide [25]. Less is known about transcriptional regulation. In rodents, glucocorticoid has been shown to increase *MAT1A* transcription and possibly mRNA stability [15,19]. On the other hand, prolonged hypoxia led to decreased *MAT1A* transcription and mRNA stability [21]. The only human condition where *MAT1A* gene transcription is altered is liver cancer, where *MAT1A* transcription is markedly reduced or absent [8]. Genomic cloning of human *MAT1A* was reported by Ubagai and colleagues [26]; however, the sequence of the promoter was not examined. To better understand transcriptional regulation of human *MAT1A*, we have cloned and characterized the 1.9 kb 5′-flanking region.

The sequence of 5′-flanking region of the human *MAT1A* shares little similarity to the 5′-flanking region of the rat or murine *MAT1A* [12,19] and no similarity to the 5′-flanking region of the human *MAT2A* [27]. Primer extension and RNase protection assays revealed a single transcriptional start site located 25 nt downstream of a putative TATA box or 238 nt upstream of the translational start site (Figure 2). This differs from the results of Alvarez and colleagues, which estimated the transcriptional start site to be approx. 90 nt upstream of the translational start site [4]. The explanation for this discrepancy is unclear. We confirmed the start site, however, using different primers and assays, and its proximity to the consensus TATA box further support the validity of our results. Although the sequence of the human *MAT1A* promoter shares little similarity with the rat *MAT1A* promoter, both contain several consensus binding sites for HNF, CAAT and GRE, and one or more binding sites for IL-6, PEA3 and AP-1 [12]. In addition, the human *MAT1A* promoter also contain several consensus binding sites for C/EBP and one or more sites for CRE-BP, E2F, STATx, c-Myc and v-Myb. HNF and C/EBP are transcriptional factors important in liver-specific gene expression [15,16]. Whether they are functionally active in mediating the liver-specific expression of *MAT1A* will require further study.

Transfection studies showed that the 5′-flanking sequence of the human *MAT1A* gene contained a functional promoter which was able to drive luciferase expression in Chang cells efficiently but not in HeLa cells. In Chang cells, the maximal activity was about 18-fold over that of vector control, whereas in HeLa cells the maximal activity was only 3-fold over that of vector control. This difference was not due to efficiency of transfection as luciferase activity, driven by the CMV promoter was actually 5-fold higher in HeLa cells. This suggests that the transfection efficiency was actually lower in Chang cells than in HeLa cells. Thus the difference in *MAT1A* promoter-driven luciferase expression in the two cell types is likely to be an underestimate of the true difference. The results also suggest cell-type specific transcriptional factors may be involved in mediating differential expression of human *MAT1A*. This contrasts with studies using the rat *MAT1A* promoter–luciferase chimaeric constructs, which exhibited activity in both liver-type cells and Chinese-Hamster cells [12]. Further studies are required to better understand these species differences.

Two regions in the human *MAT1A* promoter are important for the overall activity. One is between −705 and −1111 bp and another is between −1111 and −1483 bp, relative to the translational start site. The first region is involved in positive regulation, as luciferase expression increased more than 4-fold. The region between −705 and −839 bp is responsible for the bulk of the maximal activity. Specific protein binding to this region is demonstrated by EMSA. DNase I footprinting assay of the region from −976 to −504 revealed four protected areas in both strands, −620 to −660, −680 to −728, −750 to −840 and −852 to −870. Consensus binding sites in these regions include CAAT, C/EBP-β, USF, c-Myc and GRE. The region between −1111 to −1483 bp is involved in negative regulation as inclusion of this region decreased the luciferase activity by nearly 80%. Specific protein binding to the region from −1451 to −1225 bp is also demonstrated by EMSA. DNase I footprinting assay of the region from −1483 to −1149 revealed several protected areas in both strands, −1200 to −1262, −1286 to −1318, −1328 to −1345 and −1360 to −1412. Potential binding sites in these regions include PEA3, AP-1, Sox-5, C/EBP-β and E2F. The binding site for E2F, −1384 to −1377 (TTTGGAGC), differs by one nt from the consensus E2F-binding site (TTTSSCGC, where SS = C or G) [28]. E2F has been shown to act as a repressor for many genes that are important for cell cycle control [28]. Further work will be required to define the involvement of these *cis*-acting elements and transactivating/repressor proteins in the regulation of human *MAT1A* promoter. The negative effect of this region on luciferase activity was overcome by insertion of 420 bp upstream, suggesting the possible presence of enhancer elements in the region from −1902 to −1483. Further studies are required to confirm their presence.

Glucocorticoid induced a 3-fold increase in the rat *MAT1A* promoter activity [15]. The human *MAT1A* promoter also contains several consensus GRE-binding sites, and one of these (−847 to −842) was shown to be included in the DNase I-protected regions. Glucocorticoid exerted a strong dose- and time-dependent effect on the human *MAT1A* expression in Chang cells. Maximum effect was observed with 0.1 μM triamcinolone after 6 h treatment. *MAT1A* expression remained elevated until some time between 36 and 48 h after treatment. This was reflected in the luciferase expression driven by the human *MAT1A* promoter constructs. Triamcinolone treatment increased promoter activity in the 12 and 24 h-treated cells but

not the 44 h-treated cells. Triamcinolone exerted the largest increase in luciferase activity (4.5-fold) in the $-839/+30$ promoter construct. Three consensus GRE sites are present in this region, -514 to -509, -211 to -206 and -173 to -168. Additional work will be necessary to delineate the involvement of these sites. Triamcinolone exerted little effect with constructs $-1483/+30$ and $-1902/+30$. It appears that the presence of the strongly negative influence in the upstream region dominated any positive effect glucocorticoid may have. Alternatively, additional elements upstream of -1902 may be present that are important for the overall glucocorticoid effect.

In summary, we have cloned and analysed the 5′-flanking region of the human *MAT1A* gene. This will facilitate future studies examining transcriptional regulation of the human *MAT1A* gene.

This work was supported by National Institutes of Health grant DK-51719. Chang and HeLa cells were provided by the Cell Culture Core and oligonucleotide primers were generated by the Molecular Biology Core of the USC Liver Disease Research Center (DK48522). Sequencing was performed by the Sequencing and Genetic Analysis Core Facility, Department of Cell and Neurobiology, USC School of Medicine.

REFERENCES

1 Mato, J. M., Alvarez, L., Ortiz, P. and Pajares, M. A. (1997) Pharmacol. Ther. **73**, 265–280
2 Finkelstein, J. D. (1990) J. Nutr. Biochem. **1**, 228–237
3 Horikawa, S. and Tsukada, K. (1992) FEBS Lett. **312**, 37–41
4 Alvarez, L., Corrales, F., Martín-Duce, A. and Mato, J. M. (1993) Biochem. J. **293**, 481–486
5 Kotb, M., Mudd, S. H., Mato, J. M., Geller, A. M., Kredich, N. M., Chou, J. Y. and Cantoni, G. L. (1997) Trends Genet. **13**, 51–52
6 Horikawa, S., Ozasa, H., Ota, K. and Tsukada, K. (1993) FEBS Lett. **330**, 307–311
7 Gil, B., Casado, M., Pajares, M., Boscá, L., Mato, J. M., Martín-Sanz, P. and Alvarez, L. (1996) Hepatology **24**, 876–881
8 Cai, J., Sun, W. M., Hwang, J. J., Stain, S. and Lu, S. C. (1996) Hepatology **24**, 1090–1097
9 Huang, Z. Z., Mao, Z., Cai, J. and Lu, S. C. (1998) Am. J. Physiol. **275**, G14–G21
10 Cai, J., Mao, Z., Hwang, J. J. and Lu, S. C. (1998) Cancer Res. **58**, 1444–1450
11 Cai, J., Huang, Z. Z. and Lu, S. C. (1997) Biochem. J. **326**, 167–172
12 Alvarez, L., Sánchez-Góngora, E., Mingorance, J., Pajares, M. A. and Mato, J. M. (1997) J. Biol. Chem. **272**, 22875–22883
13 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
14 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., pp. 16.33–16.36, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
15 Gil, B., Pajares, M. A., Mato, J. M. and Alvarez, L. (1997) Endocrinology **138**, 1251–1258
16 Overdier, D. G., Porcella, A. and Costa, R. H. (1994) Mol. Cell. Biol. **14**, 2755–2766
17 Diehl, A. M., Johns, D. C., Yang, S., Lin, H., Yin, M., Matelis, L. A. and Lawrence, J. H. (1996) J. Biol. Chem. **271**, 7343–7350
18 Lu, S. C. (1998) Gastroenterology **114**, 403–407
19 Sakata, S. F., Shelly, L. L., Ruppert, S., Schutz, G. and Chou, J. Y. (1993) J. Biol. Chem. **268**, 13978–13986
20 Chawla, R. K. and Jones, D. P. (1994) Biochim. Biophys. Acta **1199**, 45–51
21 Avila, M., Carretero, M. V., Rodriguez, N. E. and Mato, J. M. (1998) Gastroenterology **114**, 364–371
22 Pajares, M. A., Duran, C., Corrales, F., Pliego, M. and Mato, J. M. (1992) J. Biol. Chem. **267**, 17598–17605
23 Avila, M. A., Mingorance, J., Martínez-Chantar, M. L., Casado, M., Martín-Sanz, P., Bascá, L. and Mato, J. M. (1997) Hepatology **25**, 391–396
24 Sánchez-Góngora, E., Ruiz, F., Mingorance, J., An, W., Corrales, F. J. and Mato, J. M. (1997) FASEB J. **11**, 1013–1019
25 Huang, Z., Mato, J. M., Kanel, G. and Lu, S. C. (1999) Hepatology **29**, 1471–1478
26 Ubagai, T., Lei, K. J., Huang, S., Mudd, S. H., Levy, H. L. and Chou, J. Y. (1995) J. Clin. Invest. **96**, 1943–1947
27 Mao, Z., Liu, S., Cai, J., Huang, Z. Z. and Lu, S. C. (1998) Biochem. Biophys. Res. Commun. **248**, 479–484
28 Helin, K. (1998) Curr. Opin. Genet. Dev. **8**, 28–35

Received 12 July 1999/18 November 1999; accepted 18 December 1999