Changes in methionine adenosyltransferase during liver regeneration in the rat

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Huang, Zong-Zhi, Zebin Mao, Jiaxin Cai, and Shelly C. Lu. Changes in methionine adenosyltransferase during liver regeneration in the rat. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G14–G21, 1998.—Liver-specific and non-liver-specific methionine adenosyltransferase (MAT) are products of two genes (MAT1A and MAT2A, respectively) that catalyze the formation of S-adenosylmethionine (SAM), the principal methyl donor. We previously showed that MAT2A expression was associated with more rapid cell growth. Here we examined changes in hepatic MAT gene expression and related consequences after two-thirds partial hepatectomy (PH) in rats. The mRNA levels of both MAT forms increased from 3 to 6 h, but the MAT1A level then fell below baseline from 12 to 24 h, whereas the MAT2A level remained elevated up to 4 days after PH. The increase in the MAT2A mRNA level was due to increased gene transcription and mRNA stabilization. The change in the MAT1A mRNA level was posttranscriptional and did not require de novo protein synthesis. Changes in MAT activity were consistent with an increased amount of MAT isozymes. SAM levels, the ratio of SAM to S-adenosylhomocysteine (SAH), and DNA methylation fell from 6 to 24 h, whereas SAH levels increased slightly at 12 and 24 h after PH. Both increased SAM utilization and MAT2A gene expression likely contributed to the fall in SAM.

MAT1A; MAT2A; S-adenosylmethionine; S-adenosylhomocysteine; deoxyribonucleic acid methylation

METHIONINE adenosyltransferase (MAT) catalyzes the formation of S-adenosylmethionine (SAM), the principal biological methyl and propylamino donor, and is essential to normal cell function (30, 36). Mammalian MAT exists as three different isoforms, designated as α (or MATI), β (or MATII), and γ (or MATIII) (19, 20, 23). The α and β forms are believed to be confined to the liver and composed of four and two identical subunits from a product of the same gene, MAT1A (see Ref. 23 for a consensus nomenclature of the mammalian MAT and gene products). On the other hand, the γ form is a product of a separate gene (MAT2A) and is widely distributed (19, 20, 23). The γ form (MATIII) predominates in the fetal liver and is progressively replaced by the liver-specific isoforms during development (14, 18).

Different isoforms of MAT differ in kinetic and regulatory properties (3, 28, 32, 33, 36, 37). In terms of kinetic properties, the parameters varied in different studies, depending on how pure the enzymes were and what method was used to purify these enzymes. The general consensus is that the Michaelis constant (Km) for methionine is lowest for the γ form (~4–10 µM), intermediate for the α form (23 µM–1 mM), and highest for the β form (215 µM–7 mM), with different studies reporting different absolute values (3, 28, 32, 33, 36, 37). The activity of MAT is also modulated by SAM, the product of the reaction it catalyzes. SAM strongly inhibits the γ form (IC50 = 60 µM), whereas it minimally inhibits the α form (IC50 = 400 µM) and stimulates the β form (up to 8-fold at 500 µM SAM concentration) (37).

We reported recently in various human-derived liver cancer cell lines and hepatoma tissues resected from patients a switch in the gene expression from MAT1A to MAT2A (5). Consistent with the known differences in the kinetic parameters of different MAT isoforms for methionine, MAT activity of cancerous liver cells was much higher at lower and physiologically relevant methionine concentrations (50–80 µM) than MAT activity of cultured normal rat and human hepatocytes, which exhibited much higher MAT activity at high (5 mM) methionine concentrations (5, 12). We also showed that the type of MAT expressed by the cell strongly influences the rate of cell growth and DNA synthesis (4). In a human hepatocellular carcinoma cell line model we created, which differs only in the type of MAT expressed by using transfection and antisense strategies, we found expression of MAT2A to be associated with more rapid cell growth and DNA synthesis, and the converse was true of expression of MAT1A (4). These observations led us to speculate whether the expression of MAT changes during liver regeneration after two-thirds partial hepatectomy (PH), a model in which >90% of the remaining cells reenter the cell cycle, initiate DNA synthesis within 12–16 h, and achieve complete restoration of the original liver mass within 7–10 days (26). In this study we examined changes in MAT gene expression, MAT activity, and the consequence of these changes during the course of liver regeneration after PH.

MATERIALS AND METHODS

Materials

SAM, S-adenosylhomocysteine (SAH), cycloheximide (CHX), 5-methylcytosine (5-Mc), cytosine, CTP, GTP, and HEPES were purchased from Sigma Chemical (St. Louis, MO). Actinomycin D and α-amanitin were purchased from Boehringer Mannheim (Indianapolis, IN). [32P]dCTP (3,000 Ci/mmol), [32P]dUTP (3,000 Ci/mmol), and L-[methyl-3H]-methionine (214 mCi/mmol) were purchased from NEN-DuPont (Boston, MA). Total RNA isolation kit was obtained from Promega (Madison, WI). All other reagents were of analytic grade and were obtained from commercial sources.
Animal Experiments

Male Sprague-Dawley rats (Harlan Laboratory Animals, San Diego, CA), weighing 200-220 g, were housed with a 12:12-h light-dark cycle and fed Purina rodent chow (Ralston Purina, St. Louis, MO) and water ad libitum. All animals received humane care in compliance with the National Research Council’s criteria for humane care as outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication no. 86–23, revised 1985). The protocol for PH was approved by our vivarium.

PH was performed according to methods described by Higgins and Anderson (16). After a 3- to 7-day equilibration period, animals underwent PH after being anesthetized with an intraperitoneal injection of ketamine (20 mg/200 g body wt) and xylazine (0.2 mg/200 g body wt) in 0.2-ml volume in the morning between 9 and 10 AM. The animals were allowed to recover with free access to water and food after the surgical procedure.

At various time points after PH, animals were killed and pieces of liver were snap frozen in liquid nitrogen for subsequent measurement of SAM, SAH, and %5-mC levels, MAT activity, MAT1A and MAT2A steady-state mRNA levels, and gene transcriptional rates.

To determine the effect of RNA or protein synthesis inhibition on changes in MAT expression during liver regeneration, the protocol of Kren et al. (25) was followed with slight modifications. Animals were subjected to PH or sham operation, and at the completion of the surgery they received either α-amanitin (50 µg/100 g body wt) or for 24 h with 10 µM leptomycin B, an inhibitor of the nuclear factor of activated T cells (NF-ATc). At various time points after PH, animals were killed and pieces of liver were snap frozen in liquid nitrogen for subsequent measurement of SAM, SAH, and %5-mC levels, MAT activity, MAT1A and MAT2A steady-state mRNA levels, and gene transcriptional rates.

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MAT Activity

MAT activity was measured in liver cytosol as described previously (5, 9). Livers were homogenized in 4 vol of 10 mM Tris–HCl (pH 7.5) containing 0.3 M sucrose, 0.1% β-mercaptoethanol, 1 mM benzamidine, and 0.1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 12,000 × g for 20 min, and the supernatant was subsequently centrifuged at 100,000 × g for 65 min to obtain liver cytosol. The reaction mixture contained 80 mM TES (pH 7.4), 50 mM KCl, 40 mM MgCl2, 5 mM ATP, 10 mM diithiothreitol, 0.5 mM EDTA, 50 µM or 5 mM methionine, and 0.3–0.5 µCi L-[methyl-3H]methionine. Liver cytosol containing 250–400 µg of protein as determined by the method of Bradford (1) was then added to the reaction mixture (final volume 100–160 µl) for 40 min at 37°C. At the end of the incubation, the mixture was applied to a phosphocellulose paper disk (HA 0.45 µm, Millipore) and placed on a filtering system for washing. Adequacy of washing was first determined, and no radioactivity was recovered after washing with 5 ml of ice-cold distilled water. The disk was added to 10 ml of ScintiVerse E for scintillation counting (Beckman model LS6000TA Liquid Scintillation Counter). A 4°C blank was included for different methionine concentrations and subtracted from 37°C values. The activity of MAT was temperature dependent and varied linearly relative to time (between 0 and 50 min) and to the amount of protein extracted (between 125 and 500 µg). MAT activity is reported in nanomoles of SAM formed per milligram protein per 40 min.

Extraction of Nucleic Acids

RNA was isolated from frozen liver specimens according to the method of Chomczynski and Sacchi (8). RNA concentration was determined spectrophotometrically before use, and the integrity was checked by electrophoresis with subsequent ethidium bromide staining.

Northern Hybridization Analysis

Northern hybridization analysis was performed on total RNA using standard procedures as described previously, using specific MAT1A and MAT2A cDNA probes (5, 35). Both probes were labeled with [32P]dCTP using a random-primer kit (Primer-It II Kit; Stratagene, La Jolla, CA). To ensure equal loading of RNA samples and transfer in each of the lanes, before hybridization, membranes were rinsed with ethidium bromide and photographed, and the same membranes were then rehybridized with [3P]-labeled 18S cDNA probe comprised of a 303-bp fragment corresponding to nucleotides 1003–1305 of the published rat 18S sequence (38) or [32P]-labeled rat asialoglycoprotein receptor (ASGPR) cDNA probe comprised of a 408-bp fragment corresponding to nucleotides 44–451 of the published rat ASGPR sequence (27). Both 18S and ASGPR gene expression have been shown to remain unchanged during liver regeneration (11, 25).

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Nuclear Run-On Transcription Assay

Isolation of nuclei and nuclear run-on transcription assay were done as described recently (5). Briefly, 2 × 107 nuclei (determined using a hemacytometer) were resuspended in 50 µl nuclei-freezing buffer (50 mM Tris–HCl, pH 8.0, 50% [vol/vol] glycerol, 5 mM MgCl2, 0.1 mM EDTA), snap frozen with liquid nitrogen, and kept at −80°C. After nuclear run-on assay using isolated nuclei from above, identical amounts of labeled nuclear RNAs (1 × 106 counts/min) were hybridized overnight with plasmid DNAs (10 µg each), consisting of MAT1A cDNA in pBluescript II SK (+) or MAT2A cDNA in pUC18, rat 18S cDNA (1 µg), or plasmid vector alone with a slot-blot apparatus (Bio-Dot SF; Bio-Rad, Hercules, CA) as described previously (5). After hybridization, filters were washed and subjected to autoradiography and densitometry as previously described. Results of the nuclear run-on assay were normalized to 18S or ASGPR.

DNA Methylation

Hepatic 5-mC DNA content was measured as described (10). DNA was extracted from livers and hydrolyzed with 88% formic acid at 180°C for 25 min. The DNA hydrolysate was evaporated under nitrogen and resuspended in an equal volume of 100 mM HCl. The DNA bases released by hydrolysis were analyzed by HPLC (series 410 LC pump, Perkin Elmer) with a LC-90 ultraviolet (UV) detector and a LC-100 integrator (Perkin Elmer). Samples (100–200 µg) were injected into a Partisil SCX 10-µm column (25 × 0.44 cm ID; Whatman, Fairfield, NJ) at room temperature, at a flow rate of 2.5 ml/min, and cytosine and 5-mC were eluted with 0.02 M ammonium phosphate-HCl buffer (pH 2.3). The DNA bases were identified by spiking with standards with detection at 280 nm at a sensitivity scale of 0.01 on the UV detector. The amount of 5-mC and cytosine was calculated by comparing the area of the peaks with known standards. The percentage
of methylation was calculated by the ratio of nanomoles 5-mC to nanomoles 5-mC plus nanomoles cytosine multiplied by 100.

SAM and SAH Measurement

SAM and SAH levels were measured as described previously, with slight modifications (13, 24). Liver specimens were homogenized in PBS, and an aliquot was saved for protein determination by the method of Bradford (1). The homogenate was pelleted at 1,000 g for 5 min (Beckman glycoprotein receptor centrifuge). The pellet was resuspended in 0.5 M perchloric acid (PCA) and centrifuged at 1,000 g for 15 min, and the aqueous layer was quantitatively removed and neutralized with 3 M KOH. SAM and SAH were determined in the neutralized PCA extracts by HPLC (series 410 LC pump, Perkin Elmer) with a LC-90 UV detector and a LC-100 integrator (Perkin Elmer) using a Partisil SCX 10-µm column (25 × 0.44 cm ID; Whatman). SAM and SAH were eluted isocratically at 1 ml/min with 0.05 M NH₄H₂PO₄ containing 2% acetonitrile (vol/vol) and adjusted to pH 2.6 with 2 M H₃PO₄. SAM and SAH were identified by measuring absorbance at 254 nm at a sensitivity scale of 0.01. The amount of SAM and SAH in each sample was calculated from standard curves of SAM and SAH prepared at the same time as the samples. The identity of SAM and SAH peaks was also confirmed by spiking the sample with known standards. The retention times for SAM and SAH were 12 and 18 min, respectively. SAM and SAH levels are reported as nanomoles per milligram protein, and the ratio of SAM to SAH was determined for each sample.

Statistical Analysis

For comparison between different time points, ANOVA was performed. For changes in mRNA levels and gene transcription rates, ratios of MAT1A or MAT2A to 18S or ASGPR densitometric values were compared. Significance was defined as P < 0.05.

RESULTS

Changes in Hepatic MAT mRNA Levels After PH

Changes in the steady-state MAT mRNA levels were measured by Northern blot analysis using specific MAT1A and MAT2A cDNA probes. The MAT1A mRNA level increased by 3 h after PH, peaked at 6 h (near doubling), and then fell below baseline from 12 to 24 h after PH (~50% of baseline at 24 h) followed by recovery at 72 h after PH (Fig. 1). MAT2A mRNA is not detectable in the normal adult liver on Northern blot analysis under our experimental conditions, but its presence can be detected after PH. However, instead of a rapid fall at 12 h after PH as observed with the MAT1A mRNA level, the MAT2A mRNA level remained elevated up to 4 days after PH (Fig. 2). Table 1 summarizes the changes in these mRNA levels after normalization to a housekeeping gene (18S). Similar

Table 1. Changes in steady-state MAT1A and MAT2A mRNA levels and gene transcription rates after PH in the rat

<table>
<thead>
<tr>
<th>Time After PH</th>
<th>% of 0 h</th>
<th>mRNA level</th>
<th>Gene transcription rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 h</td>
<td>153 ± 15*</td>
<td>174 ± 29*</td>
<td>111 ± 8 231 ± 32*</td>
</tr>
<tr>
<td>6 h</td>
<td>191 ± 19*</td>
<td>410 ± 80*</td>
<td>119 ± 26 177 ± 21*</td>
</tr>
<tr>
<td>12 h</td>
<td>59 ± 13*</td>
<td>363 ± 87*</td>
<td>119 ± 26 177 ± 21*</td>
</tr>
<tr>
<td>24 h</td>
<td>48 ± 4*</td>
<td>302 ± 55*</td>
<td>119 ± 26 177 ± 21*</td>
</tr>
<tr>
<td>3 days</td>
<td>80 ± 15</td>
<td>338 ± 67*</td>
<td>119 ± 26 177 ± 21*</td>
</tr>
<tr>
<td>4 days</td>
<td>70 ± 16</td>
<td>183 ± 47</td>
<td>119 ± 26 177 ± 21*</td>
</tr>
<tr>
<td>7 days</td>
<td>77 ± 4</td>
<td>117 ± 21</td>
<td>119 ± 26 177 ± 21*</td>
</tr>
</tbody>
</table>

Results are mean % of 0 h ± SE from 4 to 7 regenerating livers for mRNA levels and 3 regenerating livers for nuclear run-on experiments for each time point. PH, partial hepatectomy; MAT, methionine adenosyltransferase. mRNA levels were measured by Northern blot analysis and gene transcriptional rates were measured by nuclear run-on assay as described in MATERIALS AND METHODS. *P < 0.05 vs. 0 h by ANOVA followed by Fisher’s test.
results were obtained if the housekeeping gene used was ASGPR (see Figs. 4 and 5).

Mechanism of Change in MAT Gene Expression After PH

Gene transcription rates. To examine if the changes in MAT mRNA levels during liver regeneration are due to changes in transcriptional activity of the MAT genes, a nuclear run-on assay was performed. As shown in Fig. 3, the transcriptional activity of MAT2A was increased at 3 and 12 h after PH and returned to baseline at 7 days after PH. However, the transcriptional activity of MAT1A remained unchanged during the course of liver regeneration (Fig. 3). Table 1 summarizes the changes in MAT gene transcription rates after normalization to the housekeeping gene 18S.

Effect of RNA synthesis inhibition. Figure 4 summarizes the effect of RNA synthesis inhibition on MAT gene expression after PH. To control for the effect of the agents used, sham-operated controls were included. In the case of MAT1A, RNA synthesis inhibition had no effect on the change in the mRNA level during the first 12 h after PH (Fig. 4A). The MAT1A mRNA level remained essentially unchanged up to 12 h in the sham-operated group that received actinomycin D and α-amanitin (AA). At 12 h post-PH, both PH groups had lower MAT1A mRNA levels than the sham group. With regard to MAT2A, blocking RNA synthesis completely prevented the rise in the mRNA level (Fig. 4B). However, whereas the MAT2A mRNA level slowly declined in the sham plus AA group (half-life 8 h when the data were fitted by linear regression), it remained essentially unchanged in the PH plus AA group. This suggests the stability of MAT2A mRNA was increased.

Effect of protein synthesis inhibition. Figure 5 summarizes the effect of protein synthesis inhibition on MAT gene expression after PH. In the case of MAT1A, blocking protein synthesis with CHX also had no effect on the increase in the mRNA level during the first 6 h after PH (Fig. 5A). The effect of CHX on MAT2A mRNA level is more complicated. Even the sham-operated animals exhibited a doubling in the MAT2A mRNA level, similar to the PH group treated with CHX. However, at 6 h after PH, the PH group that received CHX was not able to increase the MAT2A mRNA level to the same magnitude as the PH controls (Fig. 5B).

Changes in Hepatic MAT Activity After PH

MAT activity was measured using two different methionine concentrations, 50 µM and 5 mM, because MATII, the gene product of MAT2A, has K_m for methionine in the 4- to 10-µM range, whereas MATI and MATIII, gene products of MAT1A, have K_m values for methionine in the millimolar range (3, 28, 32, 33, 37).
sented MAT2A gene product, increased at 3 h, peaked at 12 h, and returned to baseline at 4 days after PH (Table 2). MAT activity at 5 mM methionine concentration, representing MAT1A gene products, also increased at 3 and 6 h after PH but returned to baseline thereafter (Table 2).

Changes in Hepatic Levels of SAM, SAH, and DNA Methylation After PH

Hepatic SAM levels fell soon after PH (by 6 h) to 46 and 34% of baseline at 12 and 24 h after PH, respectively, and recovered thereafter (Fig. 6A). Hepatic SAH levels increased at 12 and 24 h after PH (Fig. 6B) so that the resultant SAM-to-SAH ratio fell dramatically at 12 and 24 h after PH (Fig. 6C). Because both an increase in SAH level and a fall in the ratio of SAM to SAH are known to inhibit transmethylation reactions (30), we also examined changes in DNA methylation during the course of regeneration. As expected, DNA methylation fell significantly at 6, 12, and 24 h after PH (Fig. 7).

**DISCUSSION**

MAT is the only enzyme that catalyzes the formation of SAM, the principal biological methyl donor and a precursor to polyamine synthesis (30). We showed that expression of MAT2A is associated with more rapid cell growth in the liver (4, 5). In addition, others have shown that expression of MAT2A and its gene product predominated in the fetal liver and were progressively replaced by MAT1A and its gene products during development (14, 18). We were therefore interested in investigating changes in MAT gene expression during liver regeneration.

There are two previous reports on changes in MAT during liver regeneration (17, 31). In an early report before cloning of the MAT genes, Okada and colleagues (31) measured MAT activity using 25 µM methionine and reported that liver MAT activities and their sensitivity to dimethylsulfoxide, an agent known to stimulate liver-specific MAT isoforms without affecting the non-liver-specific MAT isoform, remained unchanged in the regenerating rat liver 24 h after PH. However, the absolute MAT activities at 25 µM methionine did increase in that report, perhaps not reaching statistical significance (31). More recently, Horikawa and colleagues (17) found the mRNA levels of both MAT1A and MAT2A were elevated from 4 to 8 h after PH and returned to baseline by 48 h after PH. The mechanism(s) or the consequence of these changes, namely MAT activity, SAM levels, and SAM-to-SAH ratios, were not examined (17). Our results are in agreement with those of Horikawa and colleagues with respect to changes in MAT2A mRNA levels after PH. We also found an early increase in the MAT2A mRNA level but the level remained elevated up to 4 days after PH. With respect to changes in MAT1A mRNA levels, we also

<table>
<thead>
<tr>
<th>Time After PH</th>
<th>MAT Activity, nmoi SAM formed·mg protein·1·40 min·1</th>
<th>Methionine, 50 µM</th>
<th>Methionine, 5 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>0.077 ± 0.003</td>
<td>1.19 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>3 h</td>
<td>0.118 ± 0.019*</td>
<td>1.98 ± 0.25*</td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>0.138 ± 0.012*</td>
<td>2.09 ± 0.15*</td>
<td></td>
</tr>
<tr>
<td>12 h</td>
<td>0.237 ± 0.018*</td>
<td>1.56 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>0.163 ± 0.010*</td>
<td>1.32 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td>0.129 ± 0.019*</td>
<td>1.29 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td>0.062 ± 0.002</td>
<td>1.08 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>0.058 ± 0.004</td>
<td>1.10 ± 0.17</td>
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</table>

Results are means ± SE from 6 to 13 regenerating livers for each time point. MAT, methionine adenyltransferase. Liver cytosol was obtained after various time points after PH, and MAT activity was determined under 50 µM or 5 mM methionine concentrations as described in MATERIALS AND METHODS. *P < 0.05 vs. 0 h by ANOVA followed by Fisher’s test.
found an early increase from 3 to 6 h after PH but by 12 h after PH the level fell below baseline and remained low until 3 days after PH. It is unclear whether the differences in our results are due to differences in the strain and age of the animals used and/or different time points examined.

The mechanism(s) for the changes in the two MAT mRNA levels are quite different. In the case of MAT1A, RNA or protein synthesis inhibition did not alter the change in the mRNA level after PH. This confirms the nuclear run-on results and demonstrates that the mechanism for the change in MAT1A mRNA levels after PH is posttranscriptional and mediated by factors that do not require de novo synthesis. This is in agreement with the findings of Kren et al. (25) that a posttranscriptional mechanism accounted for 17 of 19 genes whose mRNA level increased during the course of liver regeneration. The MAT1A mRNA level remained unchanged for 12 h after treatment with actinomycin D and α-amanitin in the sham-operated group. Either the MAT1A mRNA has a very long half-life, or a labile short-lived factor is required for mRNA degradation. Interestingly Gil et al. (15) reported similar findings using a rat hepatoma cell line, H35. Thus cells treated with actinomycin D exhibited much slower rates of MAT1A mRNA decay (15). With regard to MAT2A, there are two mechanisms that accounted for the increase in the mRNA level after PH. One is increased transcription and the other is increased mRNA stability. Inhibiting protein synthesis led to a doubling in the MAT2A mRNA level even in the sham-operated group. This has been shown for many other mRNA species where inhibition of protein translation stabilized the message (34). However, de novo protein synthesis is required to achieve the magnitude of increase in MAT2A mRNA levels normally observed at 6 h after PH. One possibility is that a labile transactivating factor is involved in increasing the MAT2A gene transcription. Much more work will be required to completely elucidate all of these mechanisms.

In the absence of specific MAT antibodies, we used MAT activity at 50 μM and 5 mM methionine concentrations to reflect changes in the activity of non-liver-specific and liver-specific MAT isozymes, respectively. Consistent with the known differences in the $K_m$ values for methionine of different MAT isozymes, MAT activity at 50 μM was much higher in cells that express only MAT2A, whereas MAT activity at 5 mM was much higher in cells that express mainly MAT1A (5). MAT activity at both methionine concentrations increased at 3 and 6 h after PH. MAT activity at 50 μM peaked at 12 h after PH and remained elevated until 4 days after PH. This most likely reflects increased non-liver-specific MAT isozyme activity as it occurred in parallel with changes in MAT2A mRNA level. Although part of

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**Fig. 6.** Hepatic S-adenosylmethionine (SAM, A) and S-adenosylhomocysteine (SAH, B) levels and SAM-to-SAH ratios (C) at various times after PH. Results represent means ± SE from 3 regenerating livers for each time point. SAM and SAH levels were measured by HPLC as described in MATERIALS AND METHODS, and ratio of SAM to SAH was determined for each sample. *P < 0.05 vs. 0 h by ANOVA followed by Fisher’s t-test.

**Fig. 7.** Hepatic DNA methylation at various times after PH. Results represent means ± SE from 3 regenerating livers for each time point. 5-mC, 5-methylcytosine. DNA methylation was determined by percent of total cytosine methylated as described in MATERIALS AND METHODS. *P < 0.05 vs. 0 h by ANOVA followed by Fisher’s t-test.
the increase in MAT activity at 50 µM at 3 and 6 h may be contributed by increased activities of liver-specific MAT isozymes, the peak of the MAT activity at 50 µM was at 12 h, a time point when MAT1A mRNA level and MAT activity at 5 mM fell below or returned to baseline. MAT activity at 5 mM remained at baseline from 12 to 24 h after PH, although the MAT1A mRNA level fell below baseline at the same time points. Possible explanations for the discordance in mRNA level and protein activity include differences in translational efficiency, protein stability, and protein activity itself. The latter is especially plausible given that the activity of the liver-specific MAT isoforms but not the non-liver-specific MAT isofrom is modulated by the intracellular thiol-disulfide status (9, 32, 33). Oxidized glutathione inhibited the liver-specific MAT isoforms, which was reversed by GSH (33). A 30% fall in liver GSH correlated with a 60% fall in MAT activity (9). We reported that at 12 and 24 h after PH, hepatic GSH levels doubled (21). It is possible that higher GSH levels at these times during liver regeneration helped to maintain liver-specific MAT activity despite a fall in the steady-state MAT1A mRNA level. Finally, increased activity of the non-liver-specific MAT isozyme at 12 and 24 h after PH may have also contributed to maintain normal MAT activity at 5 mM.

The hepatic SAM level fell early in the course of liver regeneration and reached its lowest level (~40% of baseline) around 12 to 24 h after PH. At these same time points the hepatic SAH level increased, resulting in a dramatic fall in the SAM-to-SAH ratios. One explanation for the fall in the SAM level is increased utilization for polyamine synthesis, which is known to be increased in the first 24–48 h after PH (29). Because the fall in SAM level coincided with increased expression of MAT2A (at 12 h after PH MAT2A expression predominated in the regenerating liver) it is speculative whether this change in MAT gene expression also contributed to the fall in SAM levels. Two major differences between liver-specific and non-liver-specific MATs are the kinetic parameters and the regulation by SAM. Non-liver-specific MAT has a K_m for methionine that is much lower (~10 µM) than that of the liver-specific MATs (mM range) and is near the intracellular methionine concentration (~50 µM) (12). Thus non-liver-specific MAT is likely to be more active under physiological conditions. Non-liver-specific MAT is strongly inhibited by SAM with an IC_50 of 60 µM (32), which is close to the normal intracellular SAM concentration (2, 6). At the same SAM concentration, the liver-specific MATs are either unaffected (MATI) or stimulated (MATII) (32). In tissues that express only the non-liver-specific MAT isoform, the rate of SAM synthesis is near maximal and the SAM level is relatively unaffected by fluctuations in methionine concentration because of the negative feedback. This is in contrast to tissues that express mostly liver-specific MAT, where the rate of SAM synthesis increases with increasing methionine availability (12). Thus, in the regenerating liver, as the proportion of total MAT activity contributed by non-liver-specific MAT increases, the SAM level may fall due to feedback inhibition by SAM. Consistent with this, the SAM level in HuH-7 cells was highest in cells that express only MAT1A, followed by cells that express both MAT1A and MAT2A, and lowest in cells that express only MAT2A (4). Thus it is possible that both increased SAM utilization and MAT2A gene expression contributed to the fall in SAM.

SAH, a product of all transmethylation reactions, is in turn a potent competitive inhibitor of transmethylation reactions (30). SAH is hydrolyzed to adenosine and homocysteine in a reaction catalyzed by SAH hydrolase. However, this hydrolysis reaction is readily reversible and strongly favors SAH synthesis (30). Under normal physiological conditions, enzymatic removal of adenosine (to inosine and AMP) and homocysteine (to cysteine or remethylated to methionine) prevents accumulation of SAH (12, 30). Any condition that leads to increased adenosine and/or homocysteine levels would favor accumulation of SAH. Additional studies will be required to address the mechanism(s) for the increase in SAH level at 12 and 24 h after PH.

Transmethylation reactions are modulated by SAH levels and SAM-to-SAHR ratios (12, 30). Consistent with this, DNA methylation changed in parallel with SAM-to-SAH ratios in the regenerating liver. DNA methylation is related to mammalian gene activities, somatic inheritance, and cellular differentiation. Activation of some genes has been ascribed to the demethylation of critical mCpG loci, and silencing of some genes may be related to the methylation of specific CpG loci (7). Recently, Kren and colleagues (25) reported that c-myc, p53, H-ras, and ornithine decarboxylase, four genes whose mRNA levels increased after PH due to increased mRNA stability, also had decreased genomic methylation. They speculated whether the genomic methylation status in these genes was responsible for changes in the mRNA stability as had been shown previously for other genes (22). Thus the changes observed in DNA methylation after PH may play an important role in modulating changes in gene expression during liver regeneration.

In summary, the mRNA levels of MAT1A and MAT2A undergo dramatic changes after PH by different mechanisms. At 12 h after PH, MAT2A expression predominated in the regenerating liver, which coincided with a fall in SAM level, SAM-to-SAH ratio, and DNA methylation. These changes may help to meet the increased demand of SAM utilization and to modulate the expression of various genes during liver regeneration through changes in DNA methylation.

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