Molecular Cloning, Characterization, and Regulation of the Human Mitochondrial Serine Hydroxymethyltransferase Gene*

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The human mitochondrial serine hydroxymethyltransferase (mSHMT) gene was isolated, sequenced, and characterized. The 4.5-kilobase gene contains 10 introns and 11 exons, with all splice junctions conforming to the GT/AG rule. The 5′ promoter region contains consensus motifs for several regulatory proteins including PEA-3, Sp-1, AP-2, and a CCCTCCC motif common to many genes expressed in liver. Consensus TATA or CAAT sequence motifs are not present, and primer extension and 5′-rapid amplification of cDNA ends studies suggest that transcription initiation occurs at multiple sites. The mitochondrial leader sequence region of the deduced mRNA contains two potential ATG start sites, which are encoded by separate exons. The intervening 891-base pair intron contains consensus promoter elements suggesting that mSHMT may be transcribed from alternate promoters. 5′-Rapid amplification of cDNA ends analysis demonstrated that the first ATG is transcribed in human MCF-7 cells. However, transcription of Chinese hamster ovary cells deficient in mSHMT activity with the human mSHMT gene lacking exon 1 overcame the cell’s glycine auxotrophy and restored intracellular glycine concentrations to that observed in wild-type cells, showing that exon 1 is not essential for mSHMT localization or activity and that translation initiation from the second ATG is sufficient for mSHMT import into the mitochondria. Mitochondrial SHMT mRNA levels in MCF-7 cells did not vary during the cell cycle and were not affected by the absence of glycine, serine, folate, thymidylate, or purines from the media.

Folates function as a family of cofactors by carrying one-carbon units that are required for the synthesis of glycine, thymidylate, purines, methionine, and numerous methylation reactions in mammalian cells. Serine is the major source of one-carbon units that are generated in a reaction catalyzed by the enzyme serine hydroxymethyltransferase (SHMT).1 Alternately, one-carbon units can also be generated from glycine in cells that contain a glycine cleavage activity. SHMT is a pyridoxal phosphate-dependent enzyme that catalyzes the reversible interconversion of serine and H4PteGlu to glycine and 5,10-CH2-H4PteGlu. SHMT is present in both the mitochondria (mSHMT) and the cytoplasm (cSHMT) in mammalian cells. The human SHMT cDNAs encoding the two isozymes have been isolated and the genes localized to chromosomes 12q31 and 17p11.2, respectively (1). Currently, the metabolic role of the individual SHMT isozymes is not clearly understood. Chinese hamster ovary cells lacking mSHMT activity are auxotrophic for glycine, suggesting that the mitochondria are the primary site of glycine synthesis, whereas the enzymes responsible for thymidylate, purine, and methionine synthesis are present in the cytoplasm (2). The central role of SHMT isozymes in producing one-carbon-substituted folate cofactors has suggested that the regulation of these enzymes may influence cell growth and proliferation and that they may be targets for the development of antineoplastic agents.

Total SHMT activity and the concentration and metabolism of serine and glycine varies among tissues, reflecting the different roles of these amino acids in different organs (3). This effect is most pronounced in the brain, where it has been demonstrated that there is a direct correlation between SHMT activity and glycine concentration in different regions of rat brain (4). SHMT activity may also be developmentally regulated as SHMT-specific activity is 2-fold higher in the the optical lobe of the rhesus monkey neonate and adult compared with the fetus (5). There is evidence that SHMT is hormonally regulated as SHMT activity is elevated in the uterus after injection of 17β-estradiol to ovariectomized rats, with a 6-fold acceleration of [3,14C]serine incorporation into purines, whereas testosterone increases the specific activity of SHMT in the prostate (6). SHMT may also be controlled by nutrient availability as SHMT activity is increased 50% in folate-deficient versus folate-supplemented chickens (7). Total SHMT enzyme activity has also been demonstrated to be increased in tumor cells (8).

Several studies have suggested that mSHMT is primarily responsible for glycine synthesis in the cell (2, 9). Revertants of mutant Chinese hamster ovary cells (CHO) that lack mSHMT activity showed a correlation between SHMT levels, intracellular glycine concentrations, and protein synthesis rates (2). While SHMT enzyme activity and one-carbon flux display both developmental, nutritional, and tissue-specific regulation, the metabolic significance of these changes has been difficult to interpret as most of these studies have been performed with crude homogenates that did not distinguish between mitochondria and cytoplasm.

WTT2, wild-type Chinese hamster ovary cells; dFBS, dialyzed fetal bovine serum; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcriptase-polymerase chain reaction.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U23143. To whom correspondence should be addressed. Tel.: 510-642-5202; Fax: 510-642-0535; E-mail: bandie@violet.berkeley.edu.

1 The abbreviations used are: SHMT, serine hydroxymethyltransferase; mSHMT, mitochondrial serine hydroxymethyltransferase; cSHMT, cytoplasmic serine hydroxymethyltransferase; kb, kilobase; bp, base pair; nt, nucleotide; H4PteGlu, tetrahydrofolate; 5,10-CH2-H4PteGlu, methylenetetrahydrofolate; CHO, Chinese hamster ovary cells; GlyA, Chinese hamster ovary cells deficient in mSHMT activity; 5,10-CH2-H4PteGlu, methylenetetrahydrofolate; CHO, Chinese hamster ovary cells; GlyA, Chinese hamster ovary cells deficient in mSHMT activity;
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Mitochondrial and cytoplasmic SHMT activities. In order to better understand the differential metabolic roles of the SHMT isoforms, we have cloned and structurally characterized the human mSHMT gene and have commenced studies to determine the factors responsible for regulating its endogenous expression.

**EXPERIMENTAL PROCEDURES**

**Materials**—PteGlu, reduced folates, amino acids, nucleotides, nucleotides, alcohol dehydrogenase, pyruvic acid, and NADH were obtained from Sigma. [3H]dCTP (60 Ci/mmol) and [2-3H]glycine (45.8 Ci/mmol) were obtained from Dupont NEN. Restriction and modification enzymes were obtained from Boehringer Mannheim, Promega, or New England Biolabs. Taq polymerase was from Perkin-Elmer. DEME, a modification of a minimum essential medium lacking serine, glycine, folic acid, thymidine, and hypoxanthine, was purchased from JHR Biosciences. All other materials were of high quality and obtained from various commercial vendors.

**Cell Culture**—MCF-7 human breast cancer cells (HTB22) were obtained from the American Type Culture Collection; wild-type CHO cells (WTT2) were obtained from Dr. Sharon Krag, Johns Hopkins University; GlyA, a CHO cell mutant lacking mSHMT activity, was obtained from Dr. Larry Thompson, Lawrence Livermore Labs. Cells were determined to be free of mycoplasma contamination by fluorescent DNA determined to be free of mycoplasma contamination by fluorescent DNA by the manufacturer's instruction. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (Hyclone Inc.) in a 5%CO2 atmosphere. Wild-type CHO and MCF-7 cells were maintained in DMEM supplemented with 10% fetal bovine serum (Hyclone Inc.) and 20 µM folic acid, whereas culture media for GlyA were supplemented with 200 µM glycine. For some experiments, the folic bovine serum was dialyzed (dFBS) against 10 volumes of phosphate-buffered saline for 24 h with buffer changes every 4 h to deplete serum glycine and folate. Media were also supplemented with combinations of glycine (200 µM), hypoxanthine (20 µM), thymidine (20 µM), and serine (200 µM) for studies concerning effects of nutrient availability on mSHMT mRNA levels in MCF-7 cells. MCF-7 cells were synchronized using Lovastatin for studies concerning effect of nutrient availability on mSHMT mRNA levels in MCF-7 cells. MCF-7 cells were synchronized using Lovastatin for studies concerning effect of nutrient availability on mSHMT mRNA levels in MCF-7 cells. MCF-7 cells were synchronized using Lovastatin for studies concerning effect of nutrient availability on mSHMT mRNA levels in MCF-7 cells. MCF-7 cells were synchronized using Lovastatin for studies concerning effect of nutrient availability on mSHMT mRNA levels in MCF-7 cells. MCF-7 cells were synchronized using Lovastatin for studies concerning effect of nutrient availability on mSHMT mRNA levels in MCF-7 cells. 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**Exon sequences are shown in uppercase and intron sequences in lowercase. All intron/exon boundaries follow the GT/AG rule. Intron sizes were determined directly by DNA sequencing.**

| Exon | cDNA Location | Junction | Intron | Size | Junction |
|------|---------------|----------|--------|------|----------|
| 1    | 1–33          | gtaagatagg | 1      | 891  | cctgccctcag  |
| 2    | 34–231        | gccggactggg | 2      | 475  | accucccccg  |
| 3    | 232–311       | gttgactggg | 3      | 152  | cccacccag  |
| 4    | 312–512       | gttgataggg | 4      | 297  | cacttccag  |
| 5    | 513–594       | cttgcgtcctc | 5     | 160  | gttgtgctcag  |
| 6    | 595–717       | aacccctcag | 6      | 128  | accctgcccg  |
| 7    | 718–857       | gccggtctcct | 7     | 333  | tctccacccag  |
| 8    | 858–1023      | gttggggctgg | 8     | 217  | tcaatcctccag  |
| 9    | 1024–1123     | gtaagccagc | 9      | 86   | cccacctcag  |
| 10   | 1124–1387     | gtaagctcgc | 10    | 123  | cttctccctag  |

cSHMT contamination. Protein concentrations were determined by the method of Lowry as described previously using bovine serum albumin as a standard (15).

Amino Acid Analysis—Intracellular free amino acids were isolated from cultured cells by a modification of the procedure described previously (16). Cells were cultured in 100-mm plates containing 10 ml of α-minimum essential medium/10% FBS lacking glycine for 36 h. The media was removed by aspiration, and the cells were washed five times with 10 ml of phosphate-buffered saline. The cells were harvested using a cell scraper after the addition of 300 μl of 5% trichloroacetic acid. Cell extracts were transferred to microcentrifuge tubes, vortexed for 30 s, and centrifuged at 10,000 × g for 10 min. The trichloroacetic acid was removed from the supernatant by extracting three times with an equal volume of water-saturated diethylether. The aqueous solution containing the free amino acids was vacuum-dried. The free amino acids were quantified following o-phthaldialdehyde derivatization at the Cornell Biotechnology Analytical/Synthesis Facility. Intracellular serine and glycine concentrations were determined by DNA sequencing.

**RESULTS**

Isolation and Organization of the mSHMT Gene—A λgt11 Fix II library was screened as described under “Experimental Procedures,” and five recombinants were obtained, two of which were found to be identical by restriction enzyme mapping and Southern analysis. The identical recombinants were restriction mapped, and the gene was localized to a 4.0-kb HindIII-NotI fragment and a 1.0-kb HindIII-HindIII fragment. Both fragments were sequenced. The 1-kb fragment contained exon 1 and 314 nucleotides of 5′-flanking sequence. The 4.0-kb fragment contained the remainder of the coding region of the mSHMT gene but lacked the terminal 121 nucleotides of the 3′-untranslated region present in the cDNA. The introns were sequenced, and the mSHMT gene nucleotide sequence has been deposited in the EMBL/Genbank Data Libraries (accession number U23143). Sequence analysis and restriction mapping of the remaining three clones suggested that they were not the mSHMT or cSHMT genes and are currently being investigated.

The coding sequence of the gene was in agreement with the previously published cDNA sequence (1) with two exceptions. Codon 281 in the cDNA contains the nucleotide C in the number 1 position, whereas the gene contains the nucleotide T resulting in a Phe to Leu change. Both the human and rabbit cSHMT enzymes contain a Leu in this position, whereas the rabbit mitochondrial protein contains a Phe at this position. The third position of codon 293 coding for Leu in the cDNA contains a T, whereas the gene contains the nucleotide G in this position; however, both codons code for Leu.

The gene contains 10 introns and 11 exons spanning about 4.5 kb (Table I). The entire nucleotide sequence was determined, and all intron/exon splice junctions conform to the gt-ag rule (17). All introns are relatively small ranging from 86 to 891 bp. The 3′ splice sites do not show any preference for thymidine at the −4 position as has been found for mitochondrial aspartate aminotransferase and other nuclear-encoded mitochondrial proteins (18). The gene contains a 2-fold higher occurrence of type 2 intron splice junctions than the average for mammalian genes (19), with 50% type 0 introns, 20% type 1 introns, and 30% type 2 introns.

Analysis of the Mitochondrial Leader Sequence—Previous studies have suggested that mSHMT is located in the mitochondrial matrix (20), and the primary sequence of the rabbit liver mSHMT has been determined by amino acid sequencing (21). Amino-terminal sequencing of the rabbit liver mSHMT enzyme did not yield a start methionine suggesting either that the mitochondrial import prescence had been cleaved or the enzyme may have been subjected to proteolysis at its amino terminus during purification (21). Fig. 1 shows the amino-terminal residues of the human mSHMT primary sequence, deduced from the nucleotide sequence obtained by 5′-RACE analysis, aligned with the rabbit liver mSHMT protein sequence. Amino-terminal residues 1–8 in the rabbit liver primary sequence align to residues 30–37 in the human mSHMT (Fig. 1), suggesting that residues 1–29 in the human mSHMT primary sequence represent a mitochondrial import prescence. The prescence is rich in the amino acids Arg, Leu, and Ser which are favorable for mitochondrial import. Analysis of the mSHMT prescence suggests that it can form an amphipathic α-helix with the positively charged Arg residues and hydrophobic residues residing on opposite sides of the helix, typical of classical mitochondrial import presences (22). The translational initiation site contains a near consensus translation initiation sequence (23), with a G in the −3 and −6 positions. However, the mitochondrial leader peptide contains an internal methionine prescence at position 22. The two methionine codons are separated by an 891-bp intron that could potentially serve as an alternate promoter as seen in the rat glucokinase gene (24). The second AUG codon is also contained within a near consensus translation initiation sequence, with
the nucleotide A in the −3 position and G in the +4 position and −6 positions.

To determine if intron 1 serves as an alternate promoter, 5′-RACE products were generated from human MCF-7 cell mRNA. FiReen RACE products were sequenced, and all included the initiation codon present in exon 1. If intron 1 served as an alternate promoter in these cells, primers complementary to the 3′-untranslated region of intron 1 and to internal cDNA sequences would be expected to generate PCR products using MCF-7 cell cDNA as a template. However, no such amplification products were detected. Intron 1 does not serve as a promoter region in human MCF-7 cells. However, these studies do not preclude the possibility that this region may serve as a promoter in other cell types.

**Transfection of GlyA Cells with the mSHMT Gene**—To determine if translation initiation could occur from the second ATG in the mSHMT mRNA and result in the synthesis of a functional mSHMT protein, GlyA cells were transfected with the human mSHMT gene lacking exon 1 and under the control of the cytomegalovirus immediate early enhancer/promoter in the mammalian expression vector pCep4 (Invitrogen). Five stable transfectants (GlyA-human-mSHMT) were obtained by selection in media containing hygromycin, and colonies were maintained for over 12 months in the selection media. Fig. 2 shows RT-PCR analysis of total RNA isolated from WTT2, GlyA, and GlyA-human-mSHMT cells using primers designed to amplify 300 bp of the human mSHMT cDNA as described under “Experimental Procedures.” A fragment of the expected size was amplified from GlyA-human-mSHMT, WTT2, and GlyA cDNA. This suggests that CHO mSHMT mRNA contains sufficient nucleotide identity to the human mRNA to permit amplification by the human-specific primers and that the human gene is correctly spliced in CHO cells. All five stable GlyA-human-mSHMT transfectants were enriched with mSHMT mRNA compared with WTT2 cells as determined by RT-PCR. The GlyA-human-mSHMT transfectants contained 10–20 amol of human mSHMT mRNA/mg of total RNA. In comparison, MCF-7 cells contain 1 attomole mSHMT mRNA/mg total RNA.

Expression of the mSHMT gene lacking exon 1 in GlyA cells eliminated the glycine auxotrophy in all transfectants. All CHO cell lines were characterized by measuring SHMT activity and by analyzing intracellular amino acid concentrations (Table II) as described under “Experimental Procedures.” Intracellular serine is elevated 9-fold in GlyA cells compared with WTT2 cells, whereas glycine is nearly undetectable in GlyA cells, consistent with the absence of mSHMT activity. GlyA-human-mSHMT glycine levels were elevated about 10-fold compared with GlyA cells and were similar to WTT2 cells, whereas intracellular serine levels in the transfectants were decreased 50%. These changes in intracellular amino acid concentrations occurred despite minor increases in mSHMT activity Table II). WTT2 cells displayed 2- and 50-fold higher total and mitochondrial SHMT activities, respectively, compared with GlyA cells when assayed using the [3H]glycine exchange assay. Expression of the partial human mSHMT gene lacking exon 1 in GlyA cells resulted in less than a 10% increase in total SHMT activity but nearly a 3-fold increase in mSHMT activity. These results suggest that expression of the human mSHMT gene lacking exon 1 in GlyA cells is sufficient for mSHMT expression and mitochondrial import. These results also suggest that only a 2–3-fold increase in mSHMT activity is required to alleviate the glycine auxotrophy of GlyA and that mSHMT activity can be reduced greater than 90% without compromising intracellular glycine concentrations. It is also apparent from whole cell SHMT activity measurements that expression of the partial human mSHMT gene did not result in measurable increased SHMT activity in the cytoplasm. Expression of mSHMT in the cytoplasm would not be expected to overcome the glycine auxotrophy as overexpression of cSHMT in GlyA cells does not overcome the glycine requirement.

**Analysis of the Promoter Regions**—The transcriptional initiation sites of the mSHMT gene were determined by primer extension analysis of total cellular RNA isolated from MCF-7 cells. Multiple transcription initiation sites of equal intensities were observed (Fig. 3) which is consistent with the absence of TATA or CAAT-like sequences (Fig. 4). The 5′ promoter region of the mSHMT gene contains consensus DNA recognition sequence for Sp-1 (−290 to −284), AP-2 (−249 to −241), HC3 (−150 to −144), and PEA3 (−239 to −245). A zeste-white sequence (−184 to −190) is present and may account for some of the observed changes in mSHMT expression during development. Additionally, a CCCTCCC motif (−128) is present that is common to a number of genes that are expressed predominantly in the liver (25).

The sequence 5′ to the second translational initiation site
The expression of many genes involved in DNA synthesis, including those encoding folate-dependent enzymes such as dihydrofolate reductase and thymidylate synthase, is enhanced upon entry into the S phase of the mammalian cell cycle. Both dihydrofolate reductase and thymidylate synthase contain a cis-acting element that binds the transcription factor E2F, and E2F binding is sufficient for growth-regulated promoter activity at the G1/S phase boundary (26). MCF-7 mSHMT mRNA levels were measured by RT-PCR through the cell cycle to determine if the mSHMT gene is co-regulated with other genes involved in DNA synthesis. Cells were synchronized with Lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Lovastatin blocks the cell cycle reversibly in G1, and the block can be released by mevalonic acid, the product of the reductase reaction. Lovasta
tin is preferable to other cell cycle blocking agents as DNA synthesis is inhibited over 96% and cells remain synchronized for at least three cycles after release of the block. The percentage of cells in S-phase was determined by [3H]thymidine incorporation and by histone H4 expression as described (10). No variations in mitochondrial SHMT or control glyceraldehyde-3-phosphate dehydrogenase mRNA levels were observed (Fig. 5) suggesting that mSHMT mRNA levels are not cell cycle-regulated.

**Nutrient Control of SHMT Expression in MCF-7 Cells**—A previous study showed that SHMT activity in chicks was increased in folate deficiency (7), suggesting that folate status and perhaps other nutrients may play a role in mSHMT expression. The hydroxymethyl group of serine is incorporated into purines, methionine, and thymidylate, and the availability of these products of one-carbon metabolism may regulate mSHMT expression. MCF-7 cells were cultured in DMEM, 10% dFBS with and without purines, thymidine, methionine, glycine, or serine as described under “Experimental Procedures.” The omission of purines, thymidine, methionine, glycine, serine, or combinations thereof did not change SHMT mRNA levels after maintenance in this media for 72 h (data not shown) as determined by competitive RT-PCR. Likewise, mSHMT mRNA levels were unchanged in folate-deficient MCF-7 cells passaged for over 7 weeks in media lacking folic acid. These results suggest that mSHMT gene transcription is not influenced by nutrient status associated with folate-mediated one-carbon metabolism in MCF-7 cells.

**DISCUSSION**

The differential metabolic role of the two SHMT isozymes in folate-dependent anabolic pathways is not understood, but mSHMT activity has been suggested to be the primary source of intracellular glycine (2, 9). In order to understand the differential metabolic roles of the SHMT isozymes, we have cloned the mSHMT gene and initiated studies to determine the factors and mechanisms that cells use to regulate the endogenous expression of the gene. Recent studies have demonstrated that elimination of mitochondrial folate pools in CHO cells results in a glycine auxotrophy (27, 28) and that CHO cell glycine auxotrophs lacking mSHMT activity have an elevated intracellular serine concentration but are not able to accumulate intracellular glycine (9). These data suggest that mSHMT is primarily responsible for glycine synthesis and that cSHMT is not effective in catalyzing the formation of glycine from serine even in the presence of elevated serine concentrations (9). However, the possibility that the cell lines had additional mutations that may have been responsible for the observed metabolic disturbances could not be eliminated. Our results confirm that GlyA cells also contain very low levels of intracellular glycine despite the high accumulation of intracellular serine and the presence of cSHMT activity. In addition, we have shown that increasing the mSHMT activity in GlyA cells overcomes the glycine auxotrophy, thereby demonstrating that mSHMT deficiency alone is responsible for the glycine auxotrophy associated with GlyA cells.

Expression of the partial human mSHMT gene in GlyA cells resulted in only modest increases in mSHMT activity, representing less than 5% of total WTT2 cell mSHMT activity, despite expression of high levels of human mSHMT mRNA in the GlyA-human-mSHMT cells. We conclude that exon 1 of the mSHMT gene is not essential for mSHMT activity or mitochondrial import. These data also suggest that mSHMT activity can be inhibited greater than 95% in CHO cells without inducing a

![Fig. 3. Transcriptional start sites determined by primer extension analysis.](image)

**TABLE II**

| Cell line                      | Serine | Glycine | Serine | Glycine |
|--------------------------------|--------|---------|--------|---------|
|                                | Valine | Valine  | Valine | Valine  |
| WTT2                           | 0.3 ± 0.1 | 2.7 ± 0.3 | 0.11 ± 0.3 | 2.2 ± 0.5 |
| GlyA                           | 2.7 ± 0.2 | 0.2 ± 0.1 | 13.5 ± 0.2 | 7.6 ± 0.2 |
| GlyA-human-mSHMT-1             | 1.3 ± 0.1 | 2.5 ± 0.2 | 0.52 ± 0.2 | 1.2 ± 0.4 |
| GlyA-human-mSHMT-2             | 1.8 ± 0.1 | 2.8 ± 0.3 | 0.64 ± 0.3 | 1.2 ± 0.4 |

*H* exchanged, dpm/h/mg × 10^-3
glycine auxotrophy or affecting intracellular glycine levels, although serine levels were still elevated.

It has been suggested that the mitochondrial and cytoplasmic SHMT isozymes function cooperatively in shuttling one-carbon units between the cytoplasm and mitochondria analogous to the shuttling of reducing equivalents that occurs in the malate-aspartate shuttle (29). Studies of the regulatory regions of the aspartate aminotransferase genes demonstrated that the mitochondrial and cytoplasmic isozymes do not share common promoter elements (30), and the two genes are regulated independently. Previous studies have demonstrated that the relative ratio of SHMT in the cytoplasm and mitochondria varies among cell and tissues, suggesting that the SHMT genes are also regulated independently. While mSHMT is found in all cell types, it is enriched in certain tissues, including the kidney and liver. Analysis of the human mSHMT 5’ promoter region suggests that it is a housekeeping gene with multiple transcriptional initiation sites, but it also contains a CCCTCCC element that is present in many genes that are expressed predominantly in the liver (24) including the phenylalanine hydroxylase gene. It is also of interest that the 5’ promoter region contains a zeste-white element that may be responsible for the observed developmental variations in SHMT activity and serine metabolism (3). We have also demonstrated that mSHMT message levels do not change throughout the cell cycle in MCF-7 cells, consistent with similar observation for the yeast mSHMT message levels (31). Additionally, no evidence for changes in mSHMT mRNA levels in MCF-7 cells was observed during folate, purine, thymidine, or methionine deprivation. Continuing studies will elucidate the contribution of the mSHMT promoter elements to mSHMT expression and their influence on glycine and folic acid metabolism.

Fig. 4. Analysis of the two translational initiation regions present in the 5’ region of the mSHMT gene. The first nucleotide of the first ATG codon (in exon 1) is numbered as +1. Consensus regulatory sequences are shown in boxes, and transcriptional initiation sites are indicated by arrowheads.

Fig. 5. Determination of human mSHMT mRNA levels in MCF-7 cells throughout the cell cycle. mSHMT mRNA levels were determined over a period of 36 h in synchronized MCF-7 cells following release of the Lovastatin block (time 0) by the addition of mevalonic acid. The upper band represents the 500-bp mimic internal standard, whereas the lower band represents the mSHMT mRNA.
not result in measurable increases in SHMT activity in the cytoplasm. The low level of mSHMT activity resulting from the expression of the human mSHMT gene lacking exon 1 in the GlyA transfectants is most probably due to either inefficient translation from the translation initiation site located in exon 2 or rapid turnover of the mSHMT protein in the cytoplasm prior to mitochondrial import due to its shortened import presequence. In light of these observations, we are currently investigating the stability of the mSHMT protein in the cytoplasm and the amino acid residues that are essential for mitochondrial import, and we are determining whether the putative promoter elements present in intron 1 are capable of expressing reporter genes in human cells.

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