Organization and Expression of the Mouse MTH1 Gene for Preventing Transversion Mutation

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Hisato Igarashi‡, Teruhisa Tsuzuki‡, Tetsuya Kakuma‡, Yohei Tominaga‡, and Mutuo Sekiguchi‡†¶

From the ‡Department of Biochemistry, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-82, Japan, and the §Department of Biology, Fukuoka Dental College, Fukuoka 814-01, Japan

An enzyme, 8-oxo-7,8-dihydrodeoxyguanosine triphosphatase (8-oxo-dGTPase), is present in various organisms and plays an important role in the control of spontaneous mutagenesis. The enzyme hydrolyzes 8-oxo-dGTP, an oxidized form of dGTP, to 8-oxo-dGMP, thereby preventing the occurrence of A:T to C:G transversion, caused by misincorporation. We isolated the mouse genomic sequence encoding the enzyme and elucidated its structure. The gene, named MTH1 for mutT homologue 1, is composed of at least five exons and spans approximately 9 kilobase pairs. A genomic region containing the pseudogene was also isolated. The promoter region for the gene is GC-rich, contains many AP-1 and AP-2 recognition sequences, and lacks a typical TATA box. Primer extension and S1 mapping analyses revealed the existence of multiple transcription initiation sites, among which a major site was defined as +1. The putative promoter region was placed upstream of the chloramphenicol acetyltransferase reporter gene, and control of expression of the gene was examined by introducing the construct into mouse NIH 3T3 cells. Deletion analysis indicated that a sequence from −321 to +9 carries the basic promoter activity while an adjacent region, spanning from +352 to +525 stimulates the frequency of transcription.

Reactive oxygen species produced during normal cellular metabolism damage DNA and its precursors (1). An oxidized form of guanine base, 8-oxo-7,8-dihydroguanine (8-oxoguanine),1 is regarded as most critical in terms of mutagenesis as well as carcinogenesis (2–4). During DNA replication, the 8-oxoguanine nucleotide can pair with cytosine and adenine nucleotides, with an almost equal efficiency, and transversion mutation ensues (5–7).

Organisms are equipped with elaborate mechanisms to counteract such mutagenic effects of 8-oxoguanine, and enzymes responsible have been identified in the bacterium Escherichia coli. Two glycosylases, encoded by the mutM and the mutY genes, function to prevent mutation caused by 8-oxoguanine in DNA (8–12). The MutM protein removes 8-oxoguanine paired with cytosine, and the MutY protein removes adenine paired with 8-oxoguanine. Oxidation of guanine proceeds also in the form of free nucleotides, and an oxidized form of dGTP, 8-oxo-dGTP, is a potent mutagenic substrate for DNA synthesis (13). The MutT protein of E. coli hydrolyzes 8-oxo-dGTP to the monophosphate, and lack of the mutT gene increases the occurrence of A:T to C:G transversion several thousand-fold over the wild type level (13–15).

Mammalian cells contain enzymes similar to those of the MutM, MutY, and MutT proteins (16–20). Among them, the mammalian counterpart of MutT protein has been studied most extensively. The human enzyme specifically hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP, with a relatively low Km, value, as compared with other deoxyribonucleoside triphosphates (20, 21). As the enzyme inhibits the misincorporation of 8-oxoguanine opposite the adenine residue of template DNA in an in vitro reconstituted DNA synthesis system, the mammalian 8-oxo-dGTPase probably has the same antimutagenic capacity as the E. coli MutT protein. The finding that expression of cDNA for mammalian 8-oxo-dGTPase in E. coli mutT– mutated cells can revert the elevated level of spontaneous mutation frequency to normal (22, 23) would support this view.

To elucidate the roles of 8-oxo-dGTPase in carcinogenesis, it is necessary to construct an animal model with altered levels of the enzyme activity. It is of interest to determine whether the frequency of occurrence of tumors would increase in mice defective in the 8-oxo-dGTPase gene. We isolated the genomic sequence for mouse 8-oxo-dGTPase protein, identified the exon/intron region of the gene, and characterized the promoter in relation to the regulation of expression of the gene.

EXPERIMENTAL PROCEDURES

Cells and Culture—The mouse embryonic stem cell line CCE was obtained from M. Katsuki, and mouse fibroblast cell lines NIH 3T3 and Balb/c 3T3 were a gift from Y. Nakabeppu. CCE cells were cultured on a feeder layer in Dulbecco's modified Eagle's medium supplemented with 20% fetal bovine serum and 105 units/ml of leukemia inhibitory factor (24), at 37 °C in a humidified atmosphere of 5% CO2. Balb/c 3T3 and NIH 3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and with 10% calf serum, respectively, at 37 °C in a humidified atmosphere of 5% CO2.

Chemicals—[α-32P]dCTP and [γ-32P]ATP were purchased from Amersham Corp., and [14C]chloramphenicol was purchased from DuPont NEN. DNA labeling kits were purchased from Nippon Gene (Toyama, Japan). Restriction enzymes, T4 DNA ligase, T4 polynucleotide kinase, T4 DNA polymerase, and Klenow fragment were obtained from Toyobo Co. (Osaka, Japan). Calf intestine alkaline phosphatase and acetyl coenzyme A were purchased from Boehringer Mannheim and Pharmacia Biotech Inc., respectively. The cloning vectors, pBluescript KS− and pBluescript II KS+, were purchased from Stratagene. S1 nuclease and Moloney murine leukemia virus reverse transcriptase were obtained from Promega Corp.
from Life Technologies, Inc., and recombinant RNasin was from Promega. Sources of other materials are given throughout.

Isolation of Mouse MTH1 Genomic Clones—Two genomic DNA libraries prepared from the 129sv mouse and its embryonic stem cell line, CCE, were screened by plaque hybridization using as a probe the mouse MTH1 cDNA sequence (22). Positive clones were plaque-purified and subjected to further analyses. The region of the phage clones corresponding to the cDNA sequence were identified by Southern blot analysis. To obtain the 5'-region of the MTH1 gene, the library was rescreened using as a probe an ~500-bp KpnI-EcoRI genomic fragment of LmMTH2. Various DNA fragments derived from the phage clones were subcloned into pBluescript II KS+ for further analyses. Nucleotide sequence was determined by the dye terminator method with a model 373A automated DNA sequencer (Applied Biosystems, Inc.). Gene Works Release 2.5 (nucleic acids and protein sequence analysis software) (IntelliGenetics) was used to handle the sequences.

Southern Blot Analysis—Genomic DNA was isolated from CCE cells, as described (25). The DNA (8 µg) was digested with EcoRI and BamHI, and applied to electrophoresis on 0.8% agarose gels and transferred onto a Hybond N+ nylon membrane (Amersham) by the alkali transfer method (25). The filter was hybridized with the 5'- and/or the 3'-region of the mouse MTH1 cDNA. These probes were labeled with [α-32P]dCTP, using a DNA labeling kit. The filter was washed in 0.2 × SSC and 0.1% SDS at 65 °C for 30 min. Data were processed using a Fujix BAS 2000 Bio-image analyzer.

Preparation of RNA—Total RNAs was isolated from mouse cell lines, Balb/c 3T3 and CCE, using the guanidium thiocyanate-CsCl method (26). Poly(A)+ RNA was prepared with the use of an mRNA purification kit (Pharmacia). For Northern blot analysis, total RNAs were isolated from CCE and from various tissues of 10-week-old C57BL/6J mice (CLEA, Inc., Tokyo, Japan), using ISOGEN (Nippon Gene).

Northern Blot Analysis—20 µg of total RNAs were applied to electrophoresis on a 1.2% agarose-formaldehyde gel, and the RNAs separated were transferred onto a nitrocellulose membrane (BA-85, Schleicher & Schuell) in 20 × SSC by capillary blotting (25). The filter was hybridized using a 503-bp Neol-BamHI fragment of mouse cDNA as a probe (22). The labeled 18 S ribosomal RNA gene probe (27), obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan), was used to quantify amounts of RNA species on the blot. The filter was washed in 0.1 × SSC and 0.2% SDS at 37 °C for 30 min. Data were processed using a Fujix BAS 2000 Bio-image analyzer.

S1 Mapping—To generate probes for S1 mapping, a 330-bp BamHI-SmaI fragment containing part of exon 1 was excised from the genomic subclone ES5, and a 703-bp SmaI-EcoRI fragment was excised from the cDNA clone M-2 (22). The two fragments were ligated and inserted into the BamHI/EcoRI site of pBluescript KS+ to produce construct pH201, which contains the 5'-upstream region for the gene, placed adjacent to the cDNA portion. A 1-kb BamHI-EcoRI fragment, excised from pH201, was inserted into the BamHI/EcoRI site of M13mp19. Single-stranded DNA was isolated from the plaque and annealed with the 5'-end-labeled primer HI-2, a 25-mer synthetic antisense oligomer priming at position 945 (5'-GCTGAGGTGGCTGAGGCGACCTTGATC-3'), complementary to a region corresponding to positions 2 to 23 from the ATG codon. The annealed primer was used to elongate the sequence, and the product was digested with SaeI. The digest was electrophoresed on a 1% denaturing polyacrylamide gel, and a labeled 267-base single-stranded DNA fragment, which can be used as a probe, was recovered from the gel.

The S1 mapping was carried out as described (28). Poly(A)+ RNA derived from CCE cells was annealed with a labeled probe (5 × 10^6 cpm) by heating at 80 °C for 10 min, followed by additional incubation overnight at 55 °C. The resulting DNA/RNA hybrids were treated with T1 nuclease (100, 300, 500 units/reaction) at 16 °C for 30 min, and the products were applied to a 6% denaturing polyacrylamide gel. Sequences produced on pH201 with the same primer (Sanger's dideoxytermination method) were applied as standards (29). Bands were monitored using a Fujix BAS 2000 Bio-image analyzer.

Primer Extension—Poly(A)+ RNAs derived from CCE cells and Balb/c 3T3 cells were annealed with the primer HI-2, the 5'-end labeled by γ-[32P]ATP. Hybridization was performed in 10 mli Tris-HCl, pH 8.3, 0.25 M NaCl, 1 mM EDTA at 65 °C for 1 h. The DNA strand was extended using 50 units of M-Mukue marine virus reverse transcriptase in an appropriate buffer in the presence of 2.1 µg of actinomycin D and 60 units of recombinant RNasin for each reaction at 42 °C for 90 min. The reaction products were ethanol-precipitated and analyzed on a 6% denaturing polyacrylamide gel. The data were processed using a Fujix BAS 2000 Bio-image analyzer.

Plasmid Construction for Chloramphenicol Acetyltransferase (CAT) Assay—pBLCAT30 and pBLCAT20 are derivatives of pBLCAT3 and pBLCAT2 (30), respectively, in which an additional polyadenylation signal derived from SV40 VP1 gene was placed upstream of the CAT gene to minimize the read-through transcription from cryptic transcription initiation sites on the vector sequence. pBLCAT2, a derivative of pBLCAT30, has minimal CAT activity and thus served as a negative control in the CAT assay, while pBLCAT20 containing the herpes simplex virus thymidine kinase promoter served as a positive control. A 6.0-kb XhoI-SmaI fragment and a 6.5-kb XhoI-SpeI fragment, derived from genomic clone LmMTH1, were subcloned in the sense orientation at the polylinker sites of pBLCAT32 plasmid, and the resulting constructs were designated as pH101 and pH103, respectively. The constructs pH102, pH105, and pH107 are the deletion mutants derived from pH101, while pH104, pH106, and pH108 are those from pH103. pH109 and pH110 are deletion mutants derived from pH104 and pH103, respectively. A 477-bp SmaI-SpeI fragment was placed in the same orientation upstream of the 1.46-kb site of pH107, and the resulting plasmid was designated pH111. When the same fragment was placed in reverse orientation, the resulting plasmid was named pH113. pH112 is a deletion mutant derived from pH111.

DNA Transfection and CAT Assay—NIH 3T3 cells (5 × 10^5 cells in a 10-cm dish) were plated 24 h before transfection and transfected by the method of Chen and Okayama (31) with minor modifications. A mixture of DNA (30 µg) containing a CAT construct (2.8 pmol/dish), plasmid pYNS3214lacZ (2 µg) (32), and pBluescript KS+ was applied, together with 0.5 ml of 0.25 M CaCl₂ and 0.5 ml of 2 × N,N-bis[2-hydroxyethyl]-2-aminothanesulfonic acid-buffered saline (pH 7.06), onto cells. After incubation at 35 °C for 24 h under 3% CO₂, the cells were washed with phosphate-buffered saline and incubated with a fresh culture medium at 37 °C for 24 h under 5% CO₂. Cells were harvested, and lysates were prepared for assays of β-galactosidase and CAT activities. CAT assay was performed using 1.5 µl of [14C]chloramphenicol (50 µCi/mmol, 100 µM/cm²) and 20 µl of 4 µm acetyl coenzyme A. Nonacylated and acetylated chloramphenicol spots on the TLC plates were quantified and processed using a Fujix BAS 2000 Bio-image analyzer. The β-galactosidase assay was carried out as described (33).

RESULTS

Organization of the Mouse MTH1 Gene—To observe the gross structure of the mouse MTH1 gene, Southern blot analysis was performed using as probes the whole and the 5'- and 3'-regions of cDNA (Fig. 1). Genomic DNA was prepared from cultured cells of the mouse CCE line and digested with restriction enzyme EcoRI or BamHI. When examined with whole
respectively. The fragment used for isolation of clone LmMMTH3 and LmMMTH4 is indicated by a hatched box. Restriction enzyme sites are shown: EcoRI, BamHI, SpeI, and ApaLI. The SpeI and ApaLI sites shown are only those used for sequencing. The initiation codons ATG and the termination codon TAA are within exons 3 and 5, respectively. The fragment used for isolation of clone LmMMTH3 and LmMMTH4 is indicated by a hatched box.

cDNA as a probe, the BamHI digestion yielded a single 5.4-kb band, whereas the EcoRI digest gave three fragments corresponding to sizes of 7.7, 2.0, and 1.3 kb. Judging from the intensity of the 5.4-kb BamHI band, we concluded that the band represented two independent fragments. Indeed, hybridization with either the 3'-end or the 5'-end probe gave the 5.4-kb band. In the case of EcoRI digestion, only the 7.7-kb band appeared upon hybridization with the 5'-end probe, while the 2.0- and 1.3-kb bands appeared with the 3'-end probe. Taken together, it appears that there is a single gene for MTH1 with an approximate size of 11 kb.

Two mouse genomic DNA libraries were screened by plaque hybridization, using as a probe 32P-labeled mouse cDNA, which covers almost the entire transcribed region. Positive clones were plaque-purified and grown for preparation of DNAs. Among them, clone LmMMTH2 carried a region covering the 7.7-, 2.0-, and 1.3-kb EcoRI fragments. Since LmMMTH2 lacks the 3'-most region of the gene, the library was rescreened, and two additional clones, LmMMTH3 and LmMMTH4, were isolated. The sequences were aligned according to patterns of restriction enzyme digestion, and the exon regions were identified by Southern blot hybridization (data not shown). As shown in Fig. 2, the gene spans about 9 kb and consists of five exons.

The nucleotide sequences of the exons and their flanking regions were then determined (Fig. 3). The coding sequence resides on exons 3, 4, and 5. Consensus sequences for splicing, i.e. 5'-GT...AG-3', are present at each exon/intron junction. Comparison of the genomic sequence and the cDNA sequence (22) revealed a complete match for both sequences.

A Pseudogene for Mouse MTH1—Southern blot analysis of BamHI and EcoRI digests of the mouse genomic DNA gave faint bands with sizes of 11.5 and 6.0 kb, respectively (see Fig. 1). These fragments were not found in the region of the mouse MTH1 gene (Fig. 2).

In the course of isolation of the mouse genomic clones, we obtained one clone, named LmMMTH5, the restriction patterns of which were completely different from those of the MTH1 gene region (Fig. 4A). The sequence found in clone LmMMTH5 shows an 66.7% homology with the mouse cDNA sequence but carries many base changes, deletions and insertions (Fig. 4B). Several translation initiation codons and stop codons are found when this region is translated in any of three frames; thus, this sequence is regarded as a pseudogene for the mouse MTH1 gene. There are direct repeats of six bases, ACCACT, in both upstream and downstream flanking regions; these may be generated at integration sites when the processed gene for 8-oxo-dGTPase is inserted into the genome during the course of evolution.

Expression of the MTH1 Gene—Northern blot analysis was made to determine levels of expression of the MTH1 gene in mouse tissues. Total RNAs extracted from various tissues of C57BL/6J mice and also from mouse CCE embryonic stem cells were subjected to hybridization with MTH1 cDNA as well as an 18 S ribosomal RNA gene, as probes. As shown in Fig. 5, a band corresponding to a 1.2-kb MTH1 mRNA was detected in all of the samples examined, although intensities of the bands differed considerably.

Intensities of the bands were quantified using an image analyzer, and the amounts of MTH1 mRNA, as standardized for 18 S ribosomal RNA, are shown in Table I. Relatively large amounts of mRNA were present in thymus, testis, heart, kidney, and lung. Lesser but significant amounts of MTH1 mRNA were present in other tissues, with the brain having the lowest value.

The content of MTH1 mRNA in CCE cells was exceedingly high, as compared with adult mouse tissues, a finding in accord with observations that CCE cells, with an intense proliferating capacity, have a high level of MTH1 protein (22). High oxygen consumption may correlate with high levels of oxidative damage (34), and the level of expression of the MTH1 gene might be regulated in this context.

Transcription Initiation Sites and the Promoter—To determine the transcription initiation site for the MTH1 gene, S1 mapping and primer extension were done (Fig. 6). Poly(A)+ RNA was prepared from CCE cells, which exhibit a sufficiently high level of expression, and used for analyses. In both S1 mapping and primer extension, multiple transcription initiation sites were detected over the 50-bp region, among which the most 5' site with a strong signal corresponds to the 5'-end of the previously cloned cDNA (22). We obtained a similar result with poly(A)+ RNA prepared from Balb/c 3T3 cells. Based on these analyses, the major transcription initiation site was deduced and defined as +1.

To characterize the promoter, sequences of the EcoRI-SpeI and SpeI-ApaLI fragments, which were derived from clone
LmMMTH2 and contain exons 1, 2, and 3 as well as introns 1 and 2, were determined (Fig. 7). Consensus sequences for some cis-elements are depicted in the figure, together with other relevant sequence data. The nucleotides of which are numbered on the left. Identical nucleotides between the sequences are indicated by dots, gaps by dashes, and deviations by the respective bases below the cDNA sequence. Gaps inserted in the alignment are to maximize homology. The direct repeat of six bases, ACCACT, is boxed.

Deletion Analyses for the Promoter—Various lengths of the upstream region for the MTH1 gene were placed upstream of the reporter CAT gene, and the constructs were introduced into NIH 3T3 cells together with reference plasmid pYN3214:lacZ, which expresses β-galactosidase activity. In each assay, CAT and β-galactosidase activities were determined, the former value being divided by the latter to express levels of CAT gene expression.

The results are summarized in Fig. 8. Inspection of data with a group of plasmids carrying various regions of promoters beginning from the +9 SmaI site, located 8 bases downstream of the major transcription initiation site, revealed that a 330-bp sequence from +232 to +9 carries the basic promoter activity (pHI105), as shown in Fig. 8A. Extension of the region up to 6 kb (for pHI101) caused only a 2-fold increase in transcription-promoting activity. On the other hand, shortening of the basic region led to a complete loss of the promoter activity (pHI107 carrying the +2146 to +9 region).

When analyses were extended further downstream to the +525 site, a new picture emerged (Fig. 8B). A construct carrying a region from +146 to +525 (for plasmid pHI108) had a

FIG. 4. Comparison of sequences for the MTH1 gene and the pseudogene.

A, restriction map of the phage LmMMTH5 carrying the pseudogene. Restriction enzyme sites are shown: EcoRI (E), BamHI (B), PstI (P), and ClaI (C). The PstI and ClaI sites shown are only those used for sequencing. The location of the pseudogene is indicated by a filled box. B, aligned above the pseudogene sequences is the sequence of mouse cDNA, the nucleotides of which are numbered on the left. Identical nucleotides between the sequences are indicated by dots, gaps by dashes, and deviations by the respective bases below the cDNA sequence. Gaps inserted in the alignment are to maximize homology. The direct repeat of six bases, ACCACT, is boxed.
FIG. 5. Northern blot analysis of RNAs from various mouse tissues. Total RNAs were extracted from tissues of C57BL/6J mice and from the mouse cell line CCE. Each RNA sample (20 μg) was subjected to blotting analysis, using a 503-bp fragment of pTF1 (22), carrying mouse MTH1 cDNA, as a probe. Lane 1, brain; lane 2, thymus; lane 3, heart; lane 4, lung; lane 5, liver; lane 6, kidney; lane 7, spleen; lane 8, stomach; lane 9, small intestine; lane 10, large intestine; lane 11, testis; lane 12, CCE cell. To quantify the amounts of RNAs, the blot was reprobed with the 18 S ribosomal RNA gene (lower panel).

Table I

MTH1 mRNA in mouse tissues and cells

Intensities of bands of MTH1 mRNA, measured by image analyzer and intensities of the bands of 18 S ribosomal RNA are shown. Ratios of MTH1 mRNA to 18 S ribosomal RNA intensity are listed in the last column.

| Tissues/cell | MTH1 mRNA | 18 S ribosomal RNA | Ratio |
|-------------|------------|---------------------|-------|
| Brain       | 1.50       | 9.70                | 0.16  |
| Thymus      | 7.06       | 12.30               | 0.57  |
| Heart       | 3.30       | 9.88                | 0.34  |
| Lung        | 2.97       | 10.80               | 0.28  |
| Liver       | 4.15       | 18.96               | 0.22  |
| Kidney      | 5.38       | 17.34               | 0.31  |
| Spleen      | 3.79       | 19.21               | 0.20  |
| Stomach     | 3.07       | 14.00               | 0.22  |
| Small intestine | 3.35     | 18.11               | 0.19  |
| Large intestine | 4.05     | 18.48               | 0.22  |
| Testis      | 4.65       | 13.53               | 0.34  |
| CCE (embryonic stem cell) | 11.33 | 9.47 | 1.20 |

FIG. 6. Determination of the transcription initiation sites for the mouse MTH1 gene by S1 nuclease mapping and primer extension. A, S1 mapping. The probe was a 273-base single-stranded DNA corresponding to the 5′-flanking region of genomic DNA. The labeled probe was annealed with poly(A)+ RNA from CCE cells, and then these DNA/RNA hybrids were digested with various amounts of S1 nuclease at 16 °C for 20 min. Lane 1, poly(A)+ RNA (10 μg) digested with 500 units of S1 nuclease; lane 2, poly(A)+ RNA (5 μg) digested with 500 units of S1 nuclease; lane 3, poly(A)+ RNA (5 μg) digested with 300 units of S1 nuclease; lane 4, poly(A)+ RNA (5 μg) digested with 100 units of S1 nuclease. B, primer extension. The primer HI-2 was labeled with 32P and annealed with 5 μg of poly(A)+ RNA from CCE cells or Balb/c 3T3 cells. The reaction was performed at 42 °C for 90 min. Lane 5, Balb/c; lane 6, 3T3/CCE. The nuclease sequence of pH1201 plasmid DNA from primer HI-2 was read on the same gel. Closed circles and stars show possible transcription initiation sites identified with S1 mapping and primer extension, respectively. The arrows indicate the major transcription initiation site, which was denoted as +1.

Discussion

S-Oxo-dGTP can be generated not only by direct oxidation of dGTP but also by phosphorylation of 8-oxo-dGDP (39). Mammalian cells contain powerful nucleoside diphosphate kinase activity that converts ribo- and deoxyribonucleoside diphosphates, including 8-oxo-dGDP, to the corresponding nucleoside triphosphates. Once 8-oxo-dGTP is formed, it can be incorporated into cellular DNA to yield transversion mutations. The enzyme 8-oxo-dGTPase is present in bacteria and mammalian cells (13, 20–23, 40–42) and appears to function in order to prevent this misincorporation. The enzyme specifically hydrolyzes 8-oxo-dGTP to the monophosphate, and the 8-oxo-dGMP thus formed cannot be rephosphorylated. Guanylate kinase that acts on both GMP and dGMP for phosphorylation is totally inactive for 8-oxo-dGMP (39). By the action of nucleotidase, 8-oxo-dGMP is further degraded to 8-oxodeoxyguanosine, a form readily excretable through the cellular membrane.

The biological significance of the 8-oxo-dGTPase has been demonstrated by studies of E. coli mutant strains. Lack of the

significant high level of CAT expression. It should be noted that each of the two component sequences alone has no transcription-promoting activity, as evidenced by results with plasmid pH1107 (−146 to +9) and pH1109 (+49 to +525). It appears that the potential transcription promoting-activity carried by pH1107 is activated by an enhancer present in pH1109.

A distinct enhancer activity was detected when placing the SmaI-SpeI fragment, carrying the +49 to +525 region, or a shorter one upstream of the −146 to +9 region of pH1107 (Fig. 8C). The same level of enhancer activity was maintained even if the sequence was placed in the opposite direction. Since a shorter sequence, corresponding to the PstI-SpeI fragment, is effective for transcription promotion, a certain sequence within this region may be responsible for this activity.

Since there are putative AP-1 binding sites (TGACCTCA and TGACACA) in this region, the sequences were changed to GGGCCC (ApaI restriction sequence), and their transcription-promoting activities were examined; the mutant constructs retained full activity (data not shown). Some other sequence yet to be defined may be responsible for this transcription activation.

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mutT gene encoding the enzyme causes an increased level of A:T to C:G transversion several thousand-fold over the wild type cells (13–15), and a significant increase in the 8-oxoguanine content in DNA of mutT cells occurs (12). Furthermore, there is evidence that the elevated level of spontaneous mutation frequency of mutT cells reverts to normal when DNA for mouse 8-oxo-dGTPase is expressed in such cells (22). It seems likely that mouse 8-oxo-dGTPase has the same antimutagenic capacity as E. coli MutT protein.

To better understand the roles of mammalian 8-oxo-dGTPase in the control of spontaneous mutagenesis as well as carcinogenesis, mice defective in their own gene for 8-oxo-dGTPase (the MTH1 gene) would be useful. Isolation and characterization of the genomic sequence is the first step toward this goal, and this was achieved in the present work. We screened two genomic DNA libraries, derived from mouse 129Sv and its embryonic stem cell line, CCE, by using cDNA as a probe, and we isolated the gene encoding the 8-oxo-dGTPase protein. This gene has 5 exons, among which the coding sequence resides on the third through fifth exons, and spans approximately 9 kb.

Part of the human and rat gene for 8-oxo-dGTPase has been isolated (23, 42). The human gene is composed of four exons, while the rat gene has three exons. A comparison of the gross structures of the three types of genes is shown in Fig. 9. The overall structure of the mouse gene is similar to those of the human and rat counterparts. A comparison of DNA sequences revealed that sizes of the exons for the three species of the genes are practically identical, although sizes of the introns do differ.

To determine transcription characteristics of the MTH1 gene, we first determined expression levels of the gene in various mouse tissues. All organs examined contained substan...
tial amounts of mRNA with a high value seen in the thymus, testis, heart, kidney, and lung, when comparisons were made on the basis of 18S ribosomal RNA content. Even a higher level of expression was observed in the embryonic stem cell line CCE, with an intense proliferating capacity. On the other hand, the brain showed a low level of gene expression. These results of the mRNA level are almost in accord with the values of 8-oxo-dGTPase, established by Western blot analysis (22). The gene expression may be regulated to cope with oxidative stress.

We isolated the putative promoter region that resides upstream of the first exon and sequenced most of the region. In S1 mapping and primer extension analyses, multiple transcription initiation sites were detected. The promoter region is GC-rich and contains numerous AP-1 and AP-2 binding sites, while it exhibits strong, positive effects, and attachment of the fragment to a small region of the promoter led to a significant stimulation of the transcription. Modulation of the gene expression in vivo by manipulating these sequences is the subject of ongoing studies.

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