83873

FINAL FOR SUBMISSION
8/4/94

ANL/CMB/PP--83873

Cloning, Characterization, and Regulation of the Human Type II IMP Dehydrogenase Gene

David A. Glesne[1,2] and Eliezer Huberman[1,3,*]

[1]Center for Mechanistic Biology and Biotechnology
Argonne National Laboratory
9700 South Cass Avenue
Argonne, Illinois 60439

[2]Department of Molecular Genetics and Cell Biology
[3]Committee on Genetics
University of Chicago
920 East 58th Street
Chicago, Illinois 60637

*To Whom Correspondence Should be Addressed
Phone: (708) 252-3819
FAX: (708) 252-3387

DISCLAIMER

ABSTRACT

Human type II inosine 5'-monophosphate dehydrogenase (IMPDH, EC 1.1.1.205) is the rate-limiting enzyme in *de novo* guanine nucleotide biosynthesis. Regulated IMPDH activity is associated with cellular proliferation, transformation, and differentiation. We cloned and sequenced the entire gene for type II IMPDH and here provide details regarding the organization of the gene and the characterization of its promoter. The gene spans approximately 5 kb and is disrupted by 12 introns. The transcriptional start sites were determined by S1 nuclease mapping to be somewhat heterogeneous but predominated at 102 and 85 nucleotides from the translational initiation codon. Through the use of heterologous gene constructs and transient transfection assays, a minimal promoter from −206 to −85 was defined. This promoter is "TATA-less" and contains several transcription factor motifs including four potential Sp1 binding sites. The minimal promoter is GC-rich (69%) and resembles a CpG island. Through the use of gel mobility shift assays, nuclear proteins were shown to specifically interact with this minimal promoter. Stable transfectants were used to demonstrate that the down-regulation of IMPDH gene expression in response to reduced cellular proliferation occurs by a transcriptional mechanism.

INTRODUCTION

Inosine 5'-monophosphate dehydrogenase (IMPDH, EC 1.1.1.205) is the rate-limiting enzyme in *de novo* guanine nucleotide biosynthesis, catalyzing the NAD-dependent formation of XMP from IMP. This is the penultimate step in purine biosynthesis and the first step in this pathway specific to guanine nucleotide formation. Increased IMPDH activity was first found in a screen of enzymatic activities in a series of rat hepatomas (1). This rise in activity correlated positively with the growth rate of the individual hepatomas (1). Subsequently, increased IMPDH expression and activity has been found in a variety of tumors and tumor cell lines (2). The importance of IMPDH activity and guanine nucleotide levels in cellular proliferation and maturation has been further substantiated by the findings that the inhibition of IMPDH activity with the inhibitors mycophenolic acid or tiazofurin resulted in the cessation of cell replication and the induction of cellular differentiation in leukemia (3-6), breast tumor (7), and melanoma (8) cell lines. These effects could be circumvented by the restoration of guanine nucleotide levels through salvage of exogenously added guanosine. Regulation of IMPDH activity has also been shown to be important during T-lymphocyte activation during which IMPDH activity is increased ten-fold (9). Inhibitors of IMPDH inhibit T-cell activation (10) and are clinically immunosuppressive (11). Additionally, IMPDH may be a target of action of the tumor suppressor p53. Decreased IMPDH activity and a suppressed phenotype was found in p53 temperature-inducible murine cells at the permissive temperature, with these effects being circumvented by the addition of exogenous purine bases (12).

In humans, two distinct, highly similar (84% amino acid identity) IMPDH cDNAs termed

type I and type II have been identified (13, 14). In a comparison of the expression of these two mRNAs between normal lymphocytes and leukemic cell lines, it was found that type I IMPDH expression was similar in these cells, while type II IMPDH mRNA levels were specifically elevated in the tumor cell lines (15). Additionally, type II IMPDH mRNA levels were elevated when cells were stimulated to proliferate (16) and were depressed when cells were induced to differentiate (5-8, 16), while type I IMPDH mRNA levels remained constant. Additionally, type II IMPDH mRNA levels are subject to end-product regulation (17). Increases in guanine ribonucleotide pools cause decreased type II IMPDH mRNA levels, while depleted guanine ribonucleotide pools result in elevated type II IMPDH mRNA levels (17).

Whereas IMPDH activity and gene expression have been shown to be regulated by diverse stimuli and biological events, no specific regulatory mechanisms have been identified except in the case of end-product regulation which occurs by a nuclear posttranscriptional event (17). To identify the regulatory mechanisms, elements, and components that govern type II IMPDH expression during cellular proliferation, transformation, differentiation, and in response to fluctuations in guanine ribonucleotide levels, we sought to isolate and characterize the gene that encodes this protein. Here we report the isolation, complete sequence and structure of the gene, and the characterization and regulation of the promoter.

MATERIALS AND METHODS

Gene isolation

We previously reported the isolation of two yeast artificial chromosome (YAC) clones containing the full gene for type II IMPDH (18). One YAC (149C12) was used as a substrate to generate a PCR probe consisting largely of IMPDH intron sequences. Primers IMP2ut2 (5'-TCGGAGACACGCGGCGGTGT-3') and IMP2oli5 (5'-GTACCCAGGGAGAATGAGAA-3') amplified a 600-bp fragment that was sequenced and found to contain a single 400-bp intron. This PCR fragment was used as a probe to screen 5×10^5 phage clones from a human peripheral blood genomic library (Promega, Madison, WI). Two clones, designated FFE-7 and FFE-9, were isolated and subjected to preliminary mapping experiments. FFE-9 was determined to be truncated for the 3' portion of the gene, while FFE-7 was deemed to contain the entire coding sequence for type II IMPDH.

5' end analysis

To determine the starting point of type II IMPDH mRNA transcripts, a 1.1-kb *Nco*I fragment was subcloned into pGEM-5 (Promega) and sequenced. The fragment terminated at the translation initiation site and contained all of the previously reported UTR sequence (13) of the cDNA. The plasmid was linearized with *Aat*II or *Eco*RI, the ends were blunted, and antisense riboprobes were prepared by using the Riboprobe System (Promega), T7 RNA polymerase, and ^{32}P-UTP (NEN, Wilmington, DE) under the conditions specified by the manufacturer. The DNA template was destroyed with RNase-free DNase (Promega) and the probe purified by phenol extraction and ethanol precipitation. One $\times 10^5$ cpm of riboprobe was hybridized to 2 µg of either

human poly(A)$^+$ RNA or yeast tRNA in 80% formamide, 40 mM PIPES (pH 6.7), 400 mM NaCl, and 1 mM EDTA, for 12 h at 45°C. Buffer was added to bring the reaction to 300 mM NaCl, 50 mM sodium acetate (pH 4.5), 5 mM $ZnSO_4$, and 20 μg/ml single stranded calf thymus DNA. Three hundred units of S1 nuclease were added, and digestion proceeded at 30°C for 1 h. The reaction was stopped by the addition of 0.25 volumes of 4 M ammonium acetate, 20 mM EDTA, and 40 μg/ml tRNA. The reactions were ethanol-precipitated and resuspended in formamide loading dye and electrophoresed through a sequencing gel alongside a sequencing reaction. The gel was dried and exposed to autoradiography.

Sequence analysis and mRNA size determination

To determine the size of the coding portion of the IMPDH gene, PCR was performed with primers complementary to sequences in the 5' UTR (IMP2ut2) and the 3' UTR (IMP2R2: 5'-CCGAGGAGGTGTGCTGGAT-3'). By this analysis, the coding portion of the gene was contained in 5.0 kbp of DNA and was therefore of suitably small size for complete sequence determination. Several strategies for sequencing were employed. Initially, 1.0 to 3.0-kbp restriction fragments were subcloned into pGEM (Promega) or pBluescript (Stratagene, La Jolla, CA) vectors. The inserts were subjected to nested deletion with exonuclease III (19) and the resultant clones sequenced by using universal vector primers (T7, T3, and SP6). Subsequently, restriction sites identified in the initial experiments were used to clone smaller fragments and consequently to obtain the complete sequence of the gene. All sequencing was done by using the thermal-cycling-based femtomole sequencing kit (Promega) and ^{32}P end-labeling of primers. Both strands of the gene were completely sequenced. To obtain an accurate estimate of the size of type II IMPDH mRNA species, we performed agarose gel electrophoresis of total human RNA from

various tumor cell lines (HT-1080 fibrosarcoma, SK-MEL-131 melanoma, HL-60 promyelocytic leukemia) in the presence of methylmercuric hydroxide with use of the buffer components as described (20). IMPDH mRNA was detected by probing blots of these gels with a cDNA probe (13), and an estimate of the size was obtained by comparison to RNA size standards (GIBCO-BRL, Bethesda, MD).

Promoter determination and analysis

The 1,088-bp *Nco*I fragment containing the 5' UTR and the putative promoter was rendered blunt-ended by treatment with the Klenow fragment of DNA polymerase I (Promega) and dNTPs. This fragment was subcloned into the *Hin*dIII site of plasmid pSV0-CAT, a promoterless plasmid containing the bacterial chloramphenicol acetyl transferase (CAT) gene, creating plasmid pNB-IMP-CAT. Constructs pNBΔS-IMP-CAT, pNBΔSD-IMP-CAT, and pNBΔSB-IMP-CAT were made by digesting pNB-IMP-CAT with *Stu*I, *Stu*I and *Dra*III, or *Stu*I and *Bss*HII, respectively, and then blunting the ends and recircularizing the plasmids by ligation. The sequences at the boundaries of these deletions were verified by DNA sequencing with use of a type II IMPDH oligonucleotide primer. Two picomoles of each plasmid along with 2 μg of pSV-β-Galactosidase plasmid (Promega), was electroporated into either HT-1080 or SK-MEL-131 cells. After 2 d, RNA was isolated and analyzed by agarose-formaldehyde electrophoresis and Northern blotting as previously described (17) using CAT and β-galactosidase probes. mRNA levels were determined by densitometric analysis with a model CS-910 chromatogram scanner (Shimadzu Co., Kyoto, Japan).

DNA protein interactions in the promoter

The 209-bp *Dra*III to *Nco*I fragment containing sequences from −206 to +3 with respect

to the translation initiation codon was labeled with ^{32}P-dCTP and Klenow fragment and was used as a target for gel-shift assays (21). Nuclear extracts were prepared from SK-MEL-131 cells as previously described (22) with published modifications (23). Reactions contained 12% glycerol, 12 mM HEPES (pH 7.9), 7.5 mM $MgCl_2$, 60 mM KCl, 1 mM DTT, 70 μM EGTA, 300 μg/ml BSA, 10 μg crude nuclear extract, 0.5 ng labeled probe, and various amounts of poly (dI/dC) (Sigma Chemical Co., St Louis, MO). Reactions were performed at 30°C for 20 min and then were electrophoresed through a 4% polyacrylamide gels. The gel was then dried and exposed to autoradiography.

Isolation and characterization of stable transfectants

Plasmids pNB-IMP-CAT (10 μg) and pRSV-NEO (3 μg) were linearized and introduced into either HT-1080 or SK-MEL-131 cells by electroporation. After 2 d of recovery, 500 μg/ml of GENETICIN (GIBCO-BRL) was added and colonies allowed to form for 10 d. Transfectants were picked and screened for the expression of a properly processed CAT transcript by Northern analysis. To confirm that the regulation of type II IMPDH mRNA levels in response to fluctuations in guanine ribonucleotide levels was not mediated by a transcriptional mechanism, stable transfectants were grown in the presence of 300 μM guanosine or 1.6 μM mycophenolic acid (Sigma). To analyze the regulation of the type II IMPDH promoter by cellular proliferation rates, several SK-MEL-131 transfectant clones were plated and then grown in RPMI-1640 medium (GIBCO-BRL) containing fetal bovine serum (INTERGEN, Purchase, NY) at either 15% or 0.15% for 2 d. Cell numbers were determined by hemocytometer chamber counting and IMPDH promoter activity was determined by Northern analysis.

RESULTS

Isolation and sequence of the type II IMPDH gene

Through the use of an intron-containing probe, two genomic clones for type II IMPDH, FFE-7 and FFE-9, were isolated from a genomic library. These clones were initially mapped by restriction digestion with enzymes that cleave the gene (*Sac*I, *Bam*HI, *Eco*RI, *Nco*I) and Southern blotting with use of 5' and 3' type II IMPDH cDNA probes. It was determined that FFE-9 was truncated in the 3' end, while FFE-7 contained the full gene. The observed hybridization patterns for clone FFE-7 were identical to those obtained from blots of restricted human genomic DNA and restricted DNA from the two previously described (18) type II IMPDH YAC clones, 149C12 and 239B6. To determine the 5' end of the IMPDH transcript, a 1.1-kb *Nco*I fragment containing the previously reported 41-bp untranslated region (13) and over 1 kb of upstream sequence was subcloned and sequenced. Two antisense riboprobes initiating from the translation start codon but differing in their length were generated and used in S1 nuclease protection experiments. The protected fragments (Fig. 1, Lanes 1, 2) demonstrated some heterogeneity but revealed predominantly one major and one minor cap site for the transcript. The riboprobes protected no fragments when hybridized to yeast tRNA (Fig. 1, Lanes 3, 4). By running two sequencing reactions in parallel and taking into account a 5% slower mobility of RNA bands under these gel conditions, the major cap site was localized to nucleotide (nt) -102 and the minor cap site to nt -85 with respect to numbering of the A of the AUG codon as +1. There is no TATA box upstream of these cap sites, but there are four Sp1 (24) binding sites starting at -170, -165, -152, and -140, as well as a binding site motif used by ATF (25), or in response to cAMP (26) or

glucocorticoids (27). The region from the minor start site at −85 to the *Dra*III site at −206 is GC-rich (69 %) and contains 20 CpG dinucleotides.

Earlier estimates of the size of the type II IMPDH gene established that the gene was smaller than 12.5 kb (18). To determine if the gene was small enough to be an effective substrate for PCR, two primers, one just 5' of the initiation codon and one just 3' of the translation termination codon, were used in an attempt to amplify the whole gene. These primers generated an approximately 5-kb PCR product with use of either clone FFE-7, or a previously described YAC clone of type II IMPDH (18), as a template. Because of this relatively small size, we decided to sequence the entire gene rather than just the intron-exon junctions. The entire gene sequence is available from Genbank under accession number L33842. The gene for type II IMPDH is disrupted by 12 relatively small introns (average size of 278 bp) (Fig. 2). The donor and acceptor intron splice sites (Table 1) all conform to proposed consensus sequences (28). The phases of the introns (29) are clustered with introns 2-5 of type 0, introns 6-9 of type 1, and introns 10-12 of type 2. A computer search through Genbank revealed the presence of two *Alu*-like sequences in intron 5. Sequences from bases 2880-3040 and from bases 3160-3320 are greater than 70% identical to previously reported *Alu* sequences in Genbank. The data obtained from S1 nuclease mapping and DNA sequence analysis predict an mRNA size for type II IMPDH of 1,709 nts plus a poly(A) tail. To determine if this predicted size was in agreement with observed mRNA size, we performed agarose electrophoresis under conditions in which the mobility of RNA is proportional to its molecular weight (20). With this system, it was determined that the size of the processed type II IMPDH mRNA transcript was between 1,800 and 1,900 nts (data not shown).

Definition and characterization of the type II IMPDH promoter

To demonstrate the activity of the region upsteam of the cap sites as a promoter, a fragment containing sequences from -1084 to +3 with reference to the AUG as +1 was inserted in front of a promoterless CAT gene to create pNB-IMP-CAT. This construct was introduced into HT-1080 fibrosarcoma and SK-MEL-131 melanoma cells and promoter activity determined by Northern blot analysis. This fragment drove expression of the heterologous gene and generated transcripts of the size expected from the fusion of the CAT gene and the type II IMPDH untranslated region as determined by our S1 nuclease protection anlysis (data not shown). The level of CAT expression was highly dependent on the proliferative state of the transfected cells, being barely detectable at 12 h, 20% of maximal at 24 h, and then increasing to the maximum determined level as cells recovered and proliferated. To define a minimal promoter for the gene, we made three deletions from this construct by using appropriately spaced restriction sites (Fig. 3). With reference to the AUG as +1, the parental construct (pNB-IMP-CAT) contains sequences to -1084, the first deleted construct (pNBΔS-IMP-CAT) contains sequences to -490, the second (pNBΔSD-IMP-CAT) contains sequences to -206, and the third (pNBΔSB-IMP-CAT) contains sequences to -104. Equivalent amounts of these plasmids were cotransfected with p-β-Galactosidase into either HT-1080 or SK-MEL-131 cells. These cells were analyzed 2 d later for CAT and β-galactosidase gene expression and relative transcription from the IMPDH promoter was determined. The deletions in the first two constructs had only minimal effect on transcriptional initiation from the IMPDH promoter in both cell types (Fig. 3). The deletion from bp -206 to -104 in pNBΔSB-IMP-CAT resulted in substantial (96% of the parent construct) abrogation of transcriptional initiation, and those transcripts that were generated were of a highly

heterogeneous size compared with the relatively homogeneous size of the mRNAs produced by the other constructs. Therefore, using the definition of a minimal promoter as one that produces significant levels of accurately initiated transcripts, we designate the 122-bp region from −206 to the minor cap site at −85 as the minimal promoter for type II IMPDH.

To demonstrate specific DNA-protein interactions within this minimal promoter, we subjected a fragment encompassing sequences from −206 to +3 to gel mobility shift analysis. As seen in Figure 4, this labeled fragment was nonspecifically bound by nuclear protein from SK-MEL-131 cells in the absence of competitor DNA (Lane 2). When 3-30 μg of competitor poly(dI/dC) was added to the reactions (Lanes 3-6), a series of three complexes (labeled I, II, and III) was observed. The sequence of the fragment used in this assay (Fig. 4, lower panel) contains four potential Sp1 binding sites (underlined), with the two most distal elements overlapping, as well as a sequence 17 nts upstream from the major cap site used by numerous transcription factors (bolded sequence).

Regulation of the type II IMPDH promoter

To confirm that the previously demonstrated regulation of type II IMPDH gene expression in response to alterations in guanine ribonucleotide pools was not due to a transcriptional mechanism (17), we generated HT-1080 and SK-MEL-131 cell line clones that stably express the bacterial CAT gene under control of type II IMPDH upstream sequences from −1084 to +3. We then manipulated guanine nucleotide pools by treatment with either exogenous guanosine or with the IMPDH inhibitor mycophenolic acid (MPA). Our results indicated that treatment of either HT-1080 (not shown) or SK-MEL-131 transfectants with 300 μM guanosine for 12 h resulted in a decrease of IMPDH steady state mRNA levels to 15% of the control level,

whereas treatment with 1.6 μM MPA for 4 h resulted in a four-fold increase in steady-state mRNA levels. To the contrary, promoter activity as measured by CAT mRNA levels varied by less than 10% (Fig. 5).

As a preliminary investigation into the mechanism by which type II IMPDH gene expression is regulated by cellular proliferation rates, SK-MEL-131 transfectants were analyzed for CAT expression when growing in full medium supplemented with 15% fetal bovine serum and after a shift down to medium containing low (0.15%) serum. After 2 d of culture, cellular proliferation of the cells shifted to low serum had dropped to a rate such that cell numbers were 25% of the numbers attained by growth in full medium. As a consequence of this decreased cell replication, type II IMPDH mRNA levels were reduced to 40% of control and transcriptional initiation at the type II IMPDH promoter dropped to less than 30% of initiation at the promoter in rapidly dividing cells.

DISCUSSION

We have described the isolation and characterization of the complete gene for type II IMPDH. We chose to use a probe consisting largely of intron sequences for genomic clone isolation because of the high sequence identity between types I and II IMPDH, as well as the previously proposed possibility of pseudogenes (18) or IMPDH-like loci (30). Through the use of this intron probe, we identified two overlapping phage clones, one of which contained the entire gene for type II IMPDH, which we sequenced. The following is our evidence that this is indeed the gene for type II IMPDH: (i) the exon sequences are identical to previously reported cDNA sequences; (ii) the predicted and observed restriction fragment patterns are identical to those generated from digests of human genomic DNA or YAC clone DNA; (iii) all sequences at intron exon boundaries conform to previously proposed consensus sequences; (iv) the gene has a promoter functional in two different human cell types which displays activity that is proliferation-dependent. Our mapping of the start site for transcription of the gene would produce an mRNA of 1,709 nts plus the poly(A) tail. This size is in conflict with earlier size estimates (13, 14) of 2,300 nts, or more recently of 2,000 nts (9). However, we have found that in more stringent denaturation conditions employing methyl mercuric hydroxide gel electrophoresis, IMPDH mRNA migrates at between 1,800-1,900 nts which would be in accord with this predicted mRNA size. Earlier overestimates of the mRNA size may be due to stable secondary structure causing the mRNA to migrate anomalously. The approximately 5-kb gene is relatively small, yet is disrupted by a relatively large number of introns. The clustering of like intron phases raises the possibility that functional domains may have evolved by "exon-shuffling" (31). Alternatively,

from an "introns late" perspective, IMPDH exons could have been used as modules for the creation of other proteins (32). At present, little is known about the functional organization of this protein aside from the identification of cysteine-331, encoded in exon 9, as part of the active site of type II IMPDH (33). As more information becomes available concerning structure-function relationships for this protein, an examination into protein domain organization and exon organization correlations will be possible. Evolutionary questions can then be addressed as more is known about gene sequences and gene structures for additional IMPDH-related proteins.

By deletion analysis, we have defined the minimal promoter necesary for accurate transcription of the gene as sequences from the *Dra*III site at nt −206 to the minor cap site at nt −85. Deletion to nt -104 results in substantial transcript production, but these transcripts are highly heterogeneous and this region can therefore not be defined as sufficient for producing accurate transcription. Sequences upstream of nt −206 may contain enhancers or elements that modulate transcription in response to specific stimuli. This 122-bp minimal promoter is GC-rich (69%) and contains 20 CpG dinucleotides, and therefore resembles a CpG island (34). Such sequences have been shown to be associated with the promoters of many vertebrate genes (34, 35). The minimal promoter for type II IMPDH contains no canonical TATA sequence but four elements for Sp1 binding, and is thus similar to many other promoters for genes encoding proteins involved in cellular metabolism (36). Multiple Sp1 binding sites are almost always found in TATA-less promoters (36). Nuclear proteins specifically interact with this region as evidenced by gel mobility shift assays, but whether these proteins are in fact Sp1 or other transcription factors will require further analysis.

It has been previously reported that IMPDH may be a target for action of the p53 tumor

suppressor (12). There is a p53 DNA binding motif starting at nt −371 of the IMPDH promoter, but, in *in vitro* studies, it has been determined that two of these motifs, separated by 0-13 bp, are required for p53 binding (37). However, transcription of the type II IMPDH gene may be regulated by this protein by other mechanisms, as p53 has also been shown to repress gene expression in a non-sequence dependent manner by protein-protein interaction (38),

Our finding that transcriptional activity of the promoter in transient transfection assays was dependent on the growth state of the cells implied that the regulation of IMPDH activity in response to changes in proliferative rates occurs by a transcriptional mechanism. This proliferation-associated transciptional regulation was confirmed by using stable transfectants. When cell proliferation was inhibited by the removal of serum growth factors, type II IMPDH transription was substantially reduced. In addition, we have previously demonstrated through the use of nuclear run-off assays that transcriptional mechanisms do not mediate the regulation of IMPDH mRNA steady state levels in response to alterations in guanine ribonucleotide pools (17). That finding has been confirmed here through the use of stable transfectants. Thus, type II IMPDH gene expression appears to be regulated at at least two levels: transcriptionally during changes in cellular proliferation rates, and posttranscriptionally in response to alterations in cellular guanine ribonucelotide pools. At present it is not known whether the changes in IMPDH gene expression and/or protein activity associated with diverse processes such as proliferation, differentiation, and malignant transformation are all mediated by the same regulatory mechanisms and components or are functionally discrete regulations. The isolation and characterization of this gene will allow one to answer this question and identify regulatory mechanisms, sequences, and components that modulate type II IMPDH gene expression during cellular replication,

transformation, and differentiation, and in response to fluctuations in guanine ribonucleotide pools.

ACKNOWLEDGEMENTS

This work was supported by the U. S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38, and by an Argonne National Laboratory Graduate Fellowship to D. Glesne.

REFERENCES

1. Jackson,R.C. and Weber,G. (1975) *Nature*, **256**, 331-333.

2. Collart,F.R., Chubb,C.B., Mirkin,B.L. and Huberman, E. (1992) *Cancer Res.*, **52**, 5826-5828.

3. Knight,R.D., Mangum,J., Lucas,D.L., Cooney,D.A., Khan,E.C. and Wright, D.G. (1987) *Blood*, **69**, 634-639.

4. Olah,E., Natsumeda,Y., Ikegami,T., Kote,Z., Horanyi,M., Szelenyi,J., Paulik,E., Kremmer,T., Hollan,S.R., Sugar,J. and Weber,G. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 6533-6537.

5. Collart,F.R. and Huberman,E. (1990) *Blood*, **75**, 570-576.

6. Kiguchi,K., Collart,F.R., Henning-Chubb,C. and Huberman,E. (1990) *Exp. Cell Res.*, **187**, 47-53.

7. Bacus,S., Kiguchi,K., Chin,D., King,C.R. and Huberman,E. (1990) *Mol. Carcinog.*, **3**, 350-362.

8. Kiguchi,K., Collart,F.R., Henning-Chubb,C. and Huberman,E. (1990) *Cell Growth Differen.*, **1**, 259-270.

9. Dayton,J.S., Lindsten,T., Thompson,C.B. and Mitchell,B.S. (1994) *J. Immunol.*, **152**, 984-991.

10. Turka,L.A., Dayton,J., Sinclair,G., Thompson,C.B. and Mitchell,B.S. (1991) *J. Clin. Invest.*, **87**, 940-948.

11. Tajima,A., Hata,M., Ohta,N., Ohtawara,Y., Suzuki,K. and Aso,Y. (1984)

Transplantation, **38**, 116-118.

12. Sherley,J.L. (1991) *J. Biol. Chem.*, **266**, 24815-24828.

13. Collart,F.R. and Huberman,E. (1988) *J. Biol. Chem.*, **263**, 15769-15772.

14. Natsumeda,Y., Ohno,S., Kawasaki,H., Konno,Y., Weber,G. and Suzuki,K. (1990) *J. Biol. Chem.*, **265**, 5292-5295.

15. Konno,Y., Natsumeda,Y., Nagai,M., Yamaji,Y., Ohno,S., Suzuki,K. and Weber,G. (1991) *J. Biol. Chem.*, **266**, 506-509.

16. Nagai,M., Natsumeda,Y. and Weber,G. (1992) *Cancer Res.*, **52**, 258-261.

17. Glesne,D.A., Collart, F.R. and Huberman, E. (1991) *Mol. Cell. Biol.*, **11**, 5417-5425.

18. Glesne,D., Collart,F., Varkony,T., Drabkin,H. and Huberman, E. (1993) *Genomics*, **16**, 274-277.

19. Henikoff,S. (1984) *Gene*, **28**, 351-359.

20. Bailey,J.M. and Davidson,N. (1976) *Anal. Biochem.*, **70**, 75-85.

21. Garner,M.M. and Revzin,A. (1981) *Nucleic Acids Res.*, **9**, 3047-3060.

22. Dignam,J.D., Lebovitz,R.L. and Roeder,R. (1983) *Nucleic Acids Res.*, **11**, 1475-1489.

23. Swick,A.G. Blake,M.C., Kahn,J.W. and Azizkhan,J.C. (1989) *Nucleic Acids Res.*, **17**, 9291-9304.

24. Kadonaga,J.T., Courey,A.J., Ladika,J. and Tjian,R. (1988) In Franza,B.R. *et al.*(ed.), The control of human retrovirus gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 239-250.

25. Hai,T., Liu,F., Allegretto,E.A., Karin,M. and Green,M.R. (1988) *Genes Dev.*, **2**, 1216-1226.

26. Roesler,W.J., Vanderbark,G.R. and Hanson,R.W. (1988) *J. Biol. Chem.*, **263**, 9063-9066.

27. Yamamoto,H.R. (1985) *Ann. Rev. Genet.*, **19**, 209-252.

28. Mount,S.M. (1982) *Nucleic Acids Res.*, **10**, 459-472.

29. Patthy,L. (1987) *FEBS Lett.*, **214**, 1-7.

30. Doggett,N.A., Callen,D.F., Chen,Z.L., Moore,S., Tesmer,J.G., Duesing,L.A. and Stallings, R.L. (1993) *Genomics*, **18**, 687-689.

31. Gilbert,W. (1978) *Nature*, **271**, 501.

32. Patthy,L. (1991) *Bioessays.* **13**, 187-192.

33. Antonino,L.C., Straub,K. and Wu,J.C. (1994) *Biochem.*, **33**, 1760-1765.

34. Bird,A.P. (1986) *Nature*, **321**, 209-213.

35. Gardiner-Garden,M. and Frommer,M. (1987) *J. Mol. Biol.*, **196**, 261-282.

36. Azizkhan,J.C., Jensen,D.E., Pierce, A.J. and Wade.M. (1993) *Crit. Rev. Euk. Gene Expr.*, **3**, 229-254.

37. El-Deiry,W.S., Kern, S.E., Pietenpol,J.A., Kinzler,K.W. and Vogelstein,B. (1992) *Nature Genetics*, **1**, 45-49.

38. Seto,E., Usheva,A., Zambetti,G.P., Momand,J., Horikoshi,N., Weinmann,R., Levine,A.J. and Shenk,T. (1992) *Proc. Natl. Acad. Sci., USA*, **89**, 12028-12032.

Table 1. Type II IMPDH exon-intron junction sequences

Intron	Donor[a]	Type[b]/Codon(s)	Acceptor[a]
1	CAAgtgcgg	2/Asn33	tcccttccctcgcagTGA
2	GTGgtgagt	0/Val49,Asp50	cgtttgtctcctcagGAC
3	GCGgtgagc	0/Ala83,Leu84	ctttttatcctgtagCTT
4	AAGgtcaga	0/Lys108,Lys109	accatccctttccagAAG
5	GAGgtgggt	0/Glu177,Ile178	tcacctcccacgtagATA
6	AGGgtaagt	1/Gly206	ctgccctgaccacagGAA
7	TGGgtgagc	1/Asp274	tacttcttgtcctagACT
8	ATGgtaagg	1/Val304	tttccttcaccatagTGG
9	AAGgtaaga	1/Val336	cctccatctcaacagTGA
10	CAGgtggga	2/Ser432	tgcctctccctgcagTGA
11	CCGgtgagc	2/Arg480	tctgcccttcttcagAGC
12	TTCgtaagt	2/Ser508	tccttctgcctgcagGTA
		IMPDH consensus[c]	
	VDGgtrrgh		yyyyyyyyyyydyagD
		PROPOSED consensus[d]	
	MAGgtragt		yyyyyyyyyynyagG

[a]Boundary sequences are presented with capital letters denoting coding sequences and lowercase letters denoting intron sequences

[b]See reference 28

[c]Consensus sequences are presented with letter codes: v= A, C, or G; d= A, G, or T; r= A or G; h= A, C, or T; y= C or T; m= A or C; n= A, C, G, or T

[d]See reference 27

FIGURE LEGENDS

Figure 1. Determination of type II IMPDH mRNA start sites.

A 1.1-kb *Nco*I fragment containing the previously reported 5' UTR plus additional upstream genomic sequences was subcloned into pGEM 5 (Promega). The plasmid was linearized with either *Aat*II or *Eco*RI, the ends blunted, and riboprobes were synthesized as described in the Materials and Methods section. These probes were hybridized to either human poly(A)$^+$ RNA or yeast tRNA and then subjected to digestion with S1 nuclease. The resultant products were then electrophoresed through denaturing polyacrylamide sequencing gels alongside two standard sequencing reactions. The left eight lanes shown are the two sequencing reactions. Labeled lanes are the protected fragments from hybridization of (1) *Aat*II probe to human mRNA, (2) *Eco*RI probe to human mRNA, (3) *Aat*II probe to yeast tRNA, (4) *Eco*RI probe to yeast tRNA. The sizes of the protected probe are given in nucleotides and reflect a 5% slower mobility of RNA through the gel under these conditions.

Figure 2. Structure and sizes of introns and exons of the type II IMPDH gene.

The upper cartoon represents the structure of the gene for type II IMPDH. Exonic sequences are represented by filled boxes, and intronic sequences are represented by open boxes. The narrower boxes at both ends represent the 5' and 3' UTRs. The scale is given by the bar in the upper righthand corner of the figure. The lower table lists the exact size of the exons and introns of the gene. The size of the first exon is given by using the major transcritional start point as its 5' boundary. The size of the thirteenth exon is given by using the poly(A) addition site as its 3' boundary.

Figure 3. Deletion analysis of the type II IMPDH promoter.

Plasmid pNB-IMP-CAT was constructed by cloning the 1088 bp *Nco*I fragment containing nts −1084 to +3 of the gene in front of the promotorless CAT gene of pSV0CAT. Plasmids pNBΔS-IMP-CAT, pNBΔSD-IMP-CAT, and pNBΔSB-IMP-CAT, deleted to nt −490, −206, and −104, respectively, were constructed as described in the Materials and Methods. The upper bar represents the full *Nco*I fragment with restriction sites labeled (N= *Nco*I, S= *Stu*I, D= *Dra*III, and B= *Bss*HII) and the two predominant transcriptional initiation sites denoted by the arrow. Two picomoles of each plasmid, along with 2 μg of pSV-β-Galactosidase plasmid (Promega), was transfected into HT-1080 or SK-MEL-131 cells. Two days later RNA was isolated, and steady-state levels of CAT and β-galactosidase mRNA were determined by Northern analysis and densitometric scanning of autoradiographs. The activity of each construct was standardized and the relative transcription (R.T.) was determined by using the ratio of pNB-IMP-CAT to pSV-β-Galactosidase as 100% activity. The results shown are from four independent transfections of HT-1080 cells with the standard deviation (S.D.) given. Results from SK-MEL-131 transfections varied by less than 10% from the results obtained in HT-1080 transfections.

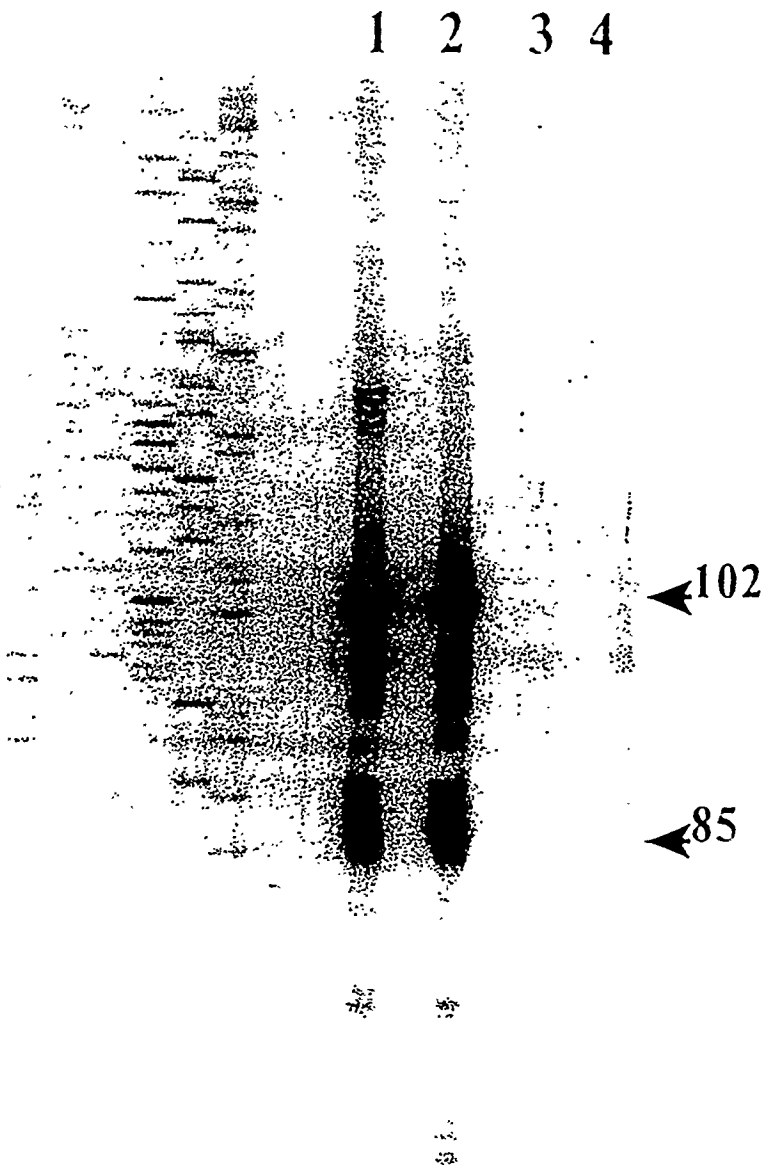
Figure 4. Interaction of nuclear proteins with the minimal type II IMPDH promoter.

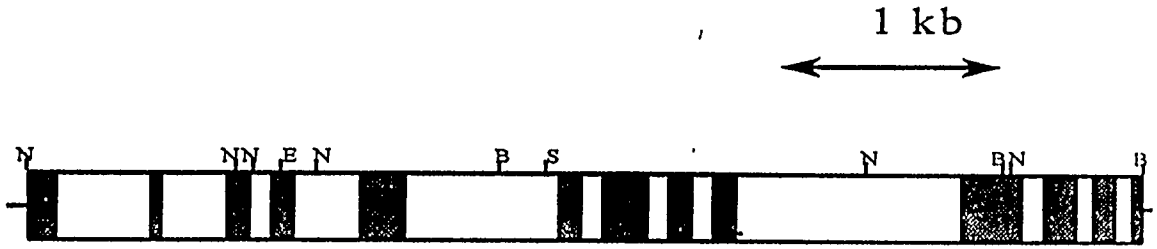
Nuclear extracts were prepared from SK-MEL-131 cells as referenced in the Materials and Methods section. A ^{32}P-labeled *Nco*I/*Dra*III fragment containing the minimal promoter was used as a probe to detect specific DNA protein interactions by using a gel mobility shift assay. Lanes are (1) probe alone; (2) probe and 10 μg of nuclear extract; 3-6) probe, 10 μg nuclear extract, and 3, 9, 15, and 30 μg respectively of poly(dI/dC) competitor DNA. The F denotes free probe, while I, II, and III indicate specific DNA protein complexes. In the lower portion of the panel, the

sequence of the fragment used in this assay is given. The lowercase letters represent the minimal promoter up to the major cap site. Uppercase letters are expressed sequences. The four potential Sp1 binding sites are underlined (the first two sites overlap), while a binding site for multiple transcription factors is in bold face. The major and minor cap sites are marked with an asterisk, and the translation initiation codon is italicized.

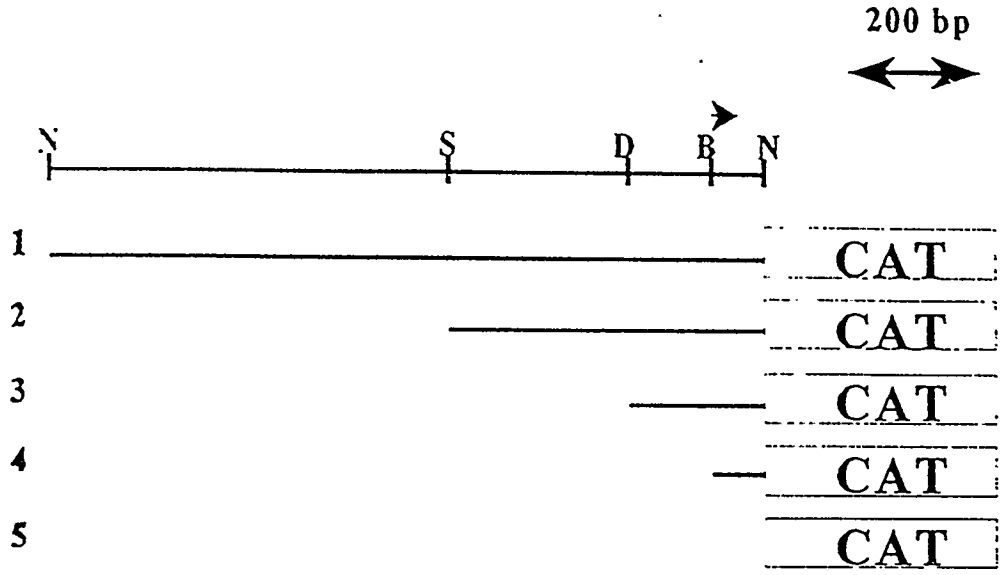
Figure 5. Regulation of the type II IMPDH promoter.

SK-MEL-131 clones that stably express the bacterial CAT gene driven by the type II IMPDH upstream sequences to −1084 were derived as described in the Materials and Methods section. Clone SK-3 was grown in the full media (C) or in the presence of 300 μM guanosine for 12 h (G), or 1.6 μM MPA for 4 h. Clone SK-1 was grown for 2 d in the presence of either 15% fetal bovine serum (+) or 0.15% fetal bovine serum (−). RNA was purified from the cells and steady state levels of CAT, type II IMPDH, and 18 s rRNA were determined by Northern analysis. Quantitation was determined by densitometric scanning of autoradiographs from two independent repetitions from three SK-MEL-131 stable transfectants (SK-1, SK-3, and SK-7).

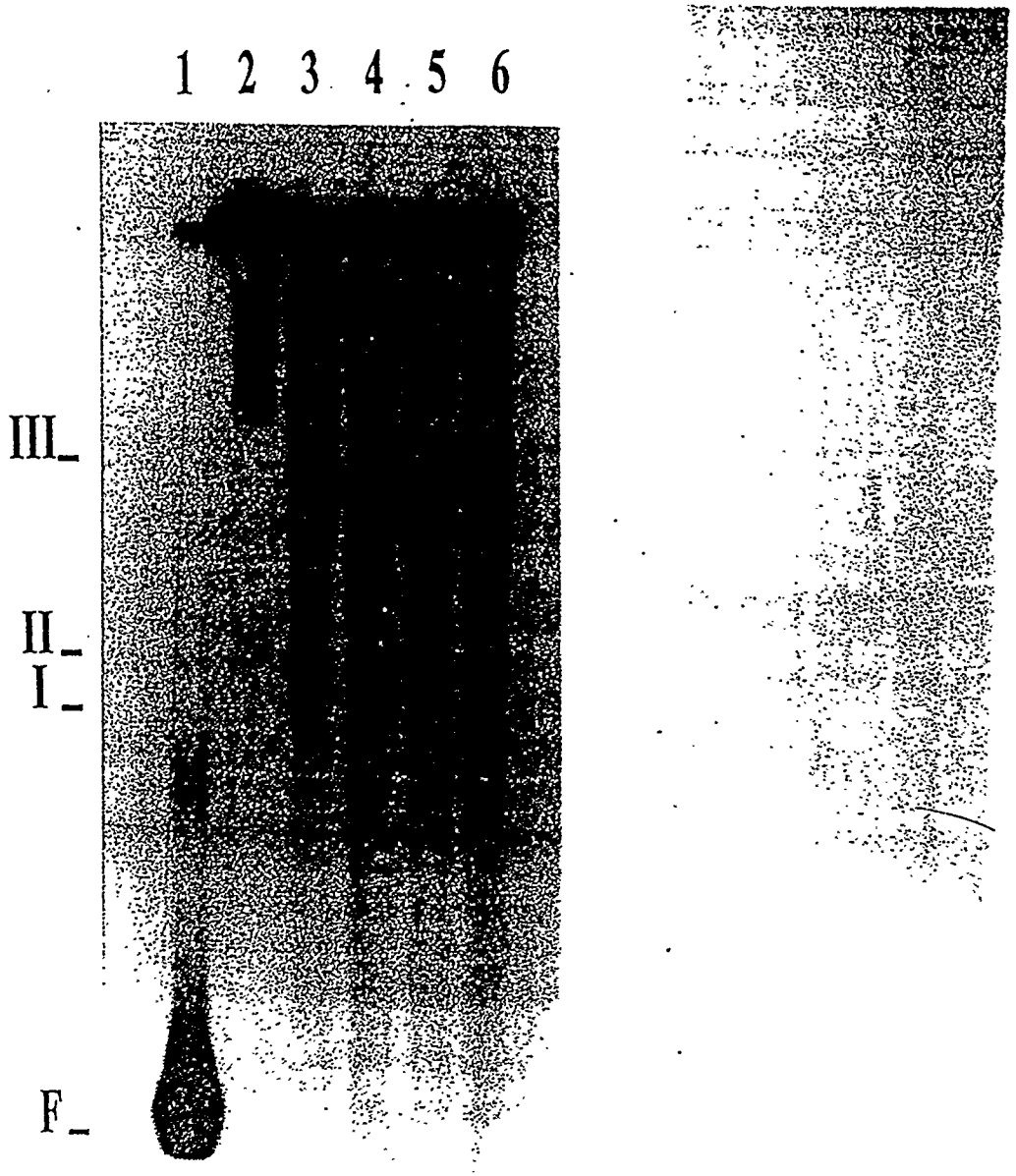

1
2
3
4
102
85



EXON	BP	INTRON	BP
1	200	1	415
2	49	2	223
3	102	3	108
4	75	4	331
5	207	5	662
6	88	6	73
7	201	7	78
8	90	8	99
9	97	9	1053
10	289	10	94
11	144	11	80
12	84	12	90
13	63		



	CONSTRUCT	R. T.
1	pNB-IMP-CAT	100
2	pNBΔS-IMP-CAT	74 ± 26
3	pNBΔSD-IMP-CAT	75 ± 12
4	pNBΔSB-IMP-CAT	4 ± 4
5	pSV0-CAT	N. D.

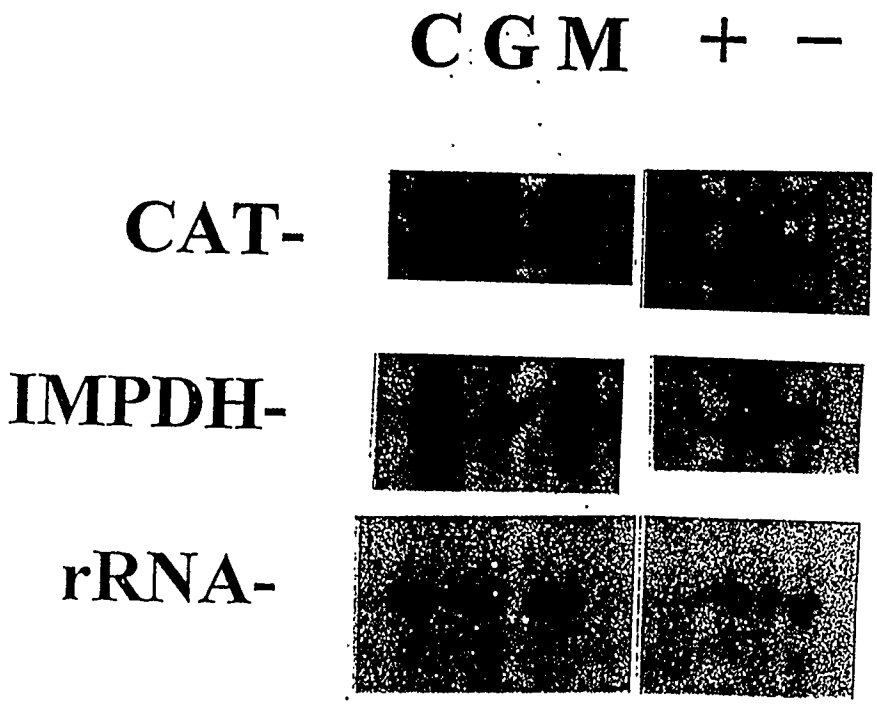


gtgctatacgcatgcgctgtttcttcagcgccagctccgcccccgccgcagcgaggcgggtaggttccgcccgcgcgc

actacgccctgacgtcagcgtcgcgcG*CAGCAGTGACGAAATCG*GCTGGTTTATATT

GGCGCGGCCCAGACGGCAGAGGTCTCTGCGGCGCGGTCCTCGGAGACA

CGCGGCGGTGTCCTGTGTTGGCCA


CGM + −
CAT-
IMPDH-
rRNA-