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CLONING AND SEQUENCE OF THE HUMAN TYPE II IMP DEHYDROGENASE GENE+

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Human type II inosine 5'-monophosphate dehydrogenase (EC 1.1.1.205) is the rate-limiting enzyme in *de novo* guanine nucleotide biosynthesis. Regulated inosine 5'-monophosphate dehydrogenase activity is associated with cellular proliferation, transformation, and differentiation. We cloned and sequenced the entire gene for type II inosine 5'-monophosphate dehydrogenase and here provide details regarding the organization of the gene and its transcriptional start sites. 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.

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

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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 leukemic (3-5), breast tumor (6), and melanoma (7) 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 (8). Inhibitors of IMPDH inhibit T-cell activation (9) and are clinically immunosuppressive (10). 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 (11).

In humans, two distinct, highly similar (84% amino acid identity) IMPDH cDNAs termed type I and type II have been identified (12, 13). 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 (14). Additionally, type II IMPDH mRNA levels were elevated when cells were stimulated to proliferate (15) and were depressed when cells were induced to differentiate (4-7, 15), while type I IMPDH mRNA levels remained constant. Additionally, type II IMPDH mRNA levels are subject to end-product regulation (16). 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 (16).

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 (16). 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 this gene.

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 (17). 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 (12) 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 × 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

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 (18) 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.

RESULTS AND DISCUSSION

Isolation and sequence of the type II IMPDH gene

Because of the possibility of IMPDH pseudogenes (17) or IMPDH-like loci (19), an intron-containing probe was used to isolate two genomic clones for type II IMPDH, FFE-7 and FFE-9, 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 (17) 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

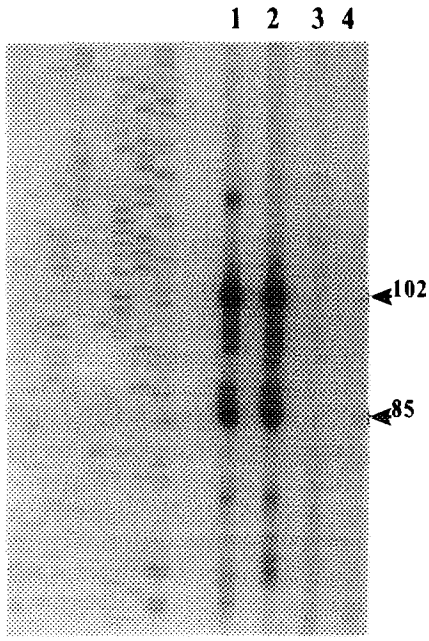
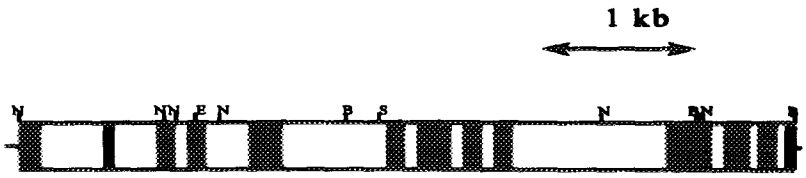


Figure 1. Determination of type II IMPDH mRNA start sites.
A 1.1-kb *Nco*I fragment containing the previously reported 5' UTR plus additional upstream genomic sequences was subcloned into pGEM 5 (Promega). The plasmid was linearized with either *Aat*II or *Eco*RI, the ends were blunted, and riboprobes 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.

reported 41-bp untranslated region (12) 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 (20) binding sites starting at -170, -165, -152, and -140, as well as a binding site motif used by ATF (21), or in response to cAMP (22) or glucocorticoids (23). The



EXON	BP	INTRON	BP
1	200	1	445
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		

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.

region from the minor start site at -85 to the *Dra*III site at -206 is GC-rich (69 %) and contains 20 CpG dinucleotides and therefore resembles a CpG island (24).

Earlier estimates of the size of the type II IMPDH gene established that the gene was smaller than 12.5 kb (17). 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 (17), as a template (data not shown). 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

Table 1. Type II IMPDH exon-intron junction sequences

Intron	Donor[a]	Type[b]/Codon(s)	Acceptor[a]
1	CAAgtgcgg	2/Asn33	tcccttccctegcagTGA
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 26.
[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 25.

(25). The phases of the introns (26) 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. This size is within range of previously reported size estimates of the mature transcript (8, 12, 13). 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.

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