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Regulation of the Human Inosine Monophosphate Dehydrogenase Type I Gene

UTILIZATION OF ALTERNATIVE PROMOTERS*

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Catalysis of guanine nucleotide formation from IMP in the *de novo* purine synthetic pathway is carried out by two isoforms of the enzyme inosine monophosphate dehydrogenase (IMPDH) that are catalytically indistinguishable but are encoded by separate genes. In order to assess the potential for cell type-specific expression of IMPDH activity, we have characterized the IMPDH type I gene and identified three major RNA transcripts that are differentially expressed from three different promoters. A 4.0-kilobase pair (kb) mRNA containing 1.3 kb of 5′-untranslated region is expressed in activated peripheral blood lymphocytes and to a far lesser extent in cultured tumor cell lines. The P1 promoter that regulates the transcription of this mRNA has a high degree of sequence identity to an Alu repetitive sequence. A transcript of 2.7 kb is found in a subset of the tumor cell lines examined, whereas a 2.5-kb mRNA species is universally expressed and is the prevalent mRNA in most cell lines and tissues. The relative strengths of the three promoter regions and the effects of variable extents of 5′-flanking sequence on the P3 promoter differ in Jurkat T, as compared with Raji B lymphoid cell lines, demonstrating a complex cell type-specific transcriptional regulation of IMPDH type I gene expression.

Inosine 5′-monophosphate dehydrogenase (IMPDH;[1] EC 1.1.1.205) is an essential, rate-limiting enzyme in the *de novo* guanine nucleotide synthetic pathway. It catalyzes the conversion of IMP to XMP at the purine metabolic branch point and provides sufficient guanine nucleotides for important cellular processes. The observations that the activity of IMPDH is tightly linked with both cellular proliferation and transformation (1–3) have led to an interest in developing IMPDH inhibitors for clinical use. Inhibition of IMPDH activity is associated with depletion of intracellular guanine nucleotide pools and has been shown both to inhibit cellular proliferation (4, 5) and to induce cell differentiation (6–9). The specific reversibility by exogenous guanine of the biologic effects of IMPDH inhibitors on cell division and differentiation is a unique feature of such compounds that differentiates them from other inducers of cellular differentiation. IMPDH inhibitors have also been demonstrated to have considerable efficacy as immunosuppressive agents (10, 11) and prevent both B and T lymphocyte activation (12, 13).

Human IMPDH activity is composed of the activities of two separate but very closely related IMPDH isoenzymes, termed type I and type II, that are encoded by separate genes located on chromosomes 7 and 3, respectively (14, 15). The corresponding cDNAs have been isolated (16, 17) and encode two distinct protein subunits of 56 kDa with 84% sequence identity. The two IMPDH proteins are tetrameric and are indistinguishable in their catalytic activities, substrate affinities, and K_i values for known inhibitors (18–20).

The increased IMPDH activity observed in replicating or neoplastic cells is largely due to increased expression of the type II IMPDH mRNA, whereas expression of the type I mRNA has been thought to be relatively unchanged by cell proliferation or transformation (21, 22). Conversely, type II mRNA levels are sharply decreased in response to the induction of cell differentiation, whereas type I mRNA remains constitutively expressed (22). A survey of relative IMPDH mRNA levels in human tissues demonstrated significant differences in the pattern of distribution of the type I transcript, with relatively high levels in kidney, pancreas, colon, and peripheral blood leukocytes as well as in fetal heart, brain, and kidney (23). Expression levels of the type II transcript, while generally higher than type I, were far less variable in different tissue types. These data support the concept that the two genes are regulated differently in resting, proliferating, and neoplastic cells and that these differences may have important consequences for cellular function.

In view of the important role of IMPDH activity in the immune response, we have previously studied IMPDH type I and II expression during T lymphocyte activation and documented that IMPDH type I mRNA levels increase approximately 10-fold in response to a variety of stimuli. In addition, T cell activation results in the appearance of two distinct type I mRNA transcripts, the larger of which is not readily appreciated in Northern blots of RNA from other cell types (24). This heterogeneity in IMPDH type I expression has led us to characterize the IMPDH type I gene and compare it with the recently analyzed IMPDH type II gene (25) with the goal of elucidating possible mechanisms involved in the tissue-specific expression of the IMPDH type I gene.

MATERIALS AND METHODS

Isolation and Mapping of the IMPDH Type I Gene—The cloning of the IMPDH type I gene was complicated by the presence in the human

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[1] The abbreviations used are: IMPDH, inosine 5′-monophosphate dehydrogenase; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pair(s); PBL, peripheral blood lymphocyte; CAT, chloramphenicol acetyltransferase; UTR, untranslated region; XMP, xanthosine 5′-monophosphate.

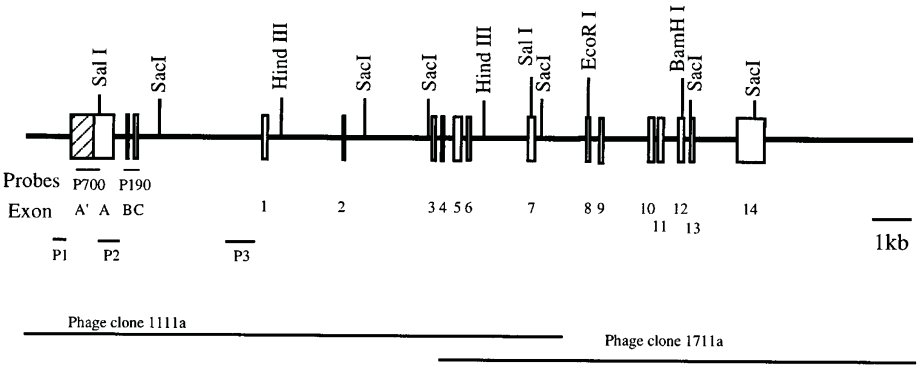

FIG. 1. **Schema and partial restriction map of the human IMPDH type I gene.** Exons are represented by *boxes* and *numbered* at the *bottom*. The *shaded box* (A′) indicates sequences between the 5′-end of the published cDNA (exon A) and the upstream transcription start site determined in the present study. P1, P2, and P3 refer to the locations of the three promoter regions. The locations of probes p700 and p190 used in Northern hybridizations are also shown. Exons are numbered according to their position in the coding sequence, with exon 14 containing 713 bp of 3′-untranslated sequence.

genome of multiple processed pseudogenes (26). To obtain IMPDH I genomic clones, a human leukocyte genomic library in the λ phage FIX II vector (provided by Dr. J. Lowe, University of Michigan, Ann Arbor) was sequentially screened using oligonucleotides complimentary to the 5′- and 3′-untranslated regions (UTRs) of the type I cDNA (17). Two positive clones were isolated from the approximately 1.8×10^6 plaques screened. Clone 1111a was obtained by sequential hybridization to three oligonucleotides corresponding to the 5′-UTR of the cDNA (bp −85 to −108, −523 to −546 and −448 to −471, relative to the ATG). Clone 1711a was obtained using two oligonucleotides corresponding to the 3′-UTR (bp 1614–1637 and 2119–2142). The identity of each clone was confirmed by hybridization with the cDNA of the entire coding region. The probes were either end-labeled with [γ-^{32}P]ATP (6000 Ci/mmol; Amersham Corp.) using T4 polynucleotide kinase (Promega, Madison, WI) or labeled using a random primer labeling kit (Promega) with [α-^{32}P]dCTP (3000 Ci/mmol; Amersham) to a specific activity of $5–10 \times 10^8$ cpm/μg. Hybridizations were performed in a 10% dextran sulfate, 1% SDS, and 5.8% NaCl solution at 60 °C for 16–24 h, and the blots were washed twice with 1 × SSC, 0.2% SDS at room temperature for 10 min, and once at 50–55 °C for 30 min for oligonucleotide probes. Hybridization using the cDNA probe was performed at 65 °C and with a final wash in 0.1 × SSC, 0.1% SDS at 65 °C for 30 min. Both clones were digested with the restriction endonuclease *Sac*I and the resulting fragments subcloned into pGEM7Zf(+) vector. Mapping of the gene was performed using Southern blot and PCR analysis. The entire coding region and all exon-intron boundaries were sequenced using the dideoxynucleotide chain termination method (27) and Sequenase 2.0 (U.S. Biochemical Corp.). For the promoter regions, both automated double-stranded sequencing and sequencing of single-stranded DNA were performed in regions with high GC content.

Northern Blot Hybridization—Total cellular RNA was isolated using TRI-reagent (MRC, Cincinnati, OH). Total cellular RNA (15 or 20 μg) was electrophoresed on 1% agarose, 2.2 M formaldehyde gels and transferred to Zeta-Probe GT membranes (Bio-Rad). Prehybridizations were performed in 1% SDS, 0.1 M NaCl for 1 h at room temperature. Hybridizations were carried out in high efficiency hybridization solution containing 50% formamide (MRC) with ^{32}P-labeled probe at $2–3 \times 10^6$ cpm/ml at 42 °C for 24–48 h. Oligonucleotide probes were washed in 1 × SSC, 0.2% SDS at 50 °C for 30 min, the p190 probe was washed in 0.1 × SSC, 0.1% SDS at 50–60 °C for 30 min, and the p700 and 1.5-kb cDNA probes were washed in 0.1 × SSC, 0.1% SDS at 65 °C for 30 min.

Human peripheral blood lymphocytes (PBLs) were isolated from buffy coats obtained from the American Red Cross (Charlotte, NC) using Histopaque-1077 (Sigma) gradient centrifugation. Monocytes and macrophages were removed using the adherence method (28). Purified PBLs were incubated at a concentration of 1.5×10^6/ml in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, Inc., Grand Island, NY), 100 units/ml penicillin, and 100 μg/ml streptomycin for 24 h in the presence or absence of 10 ng/ml of phorbol 12-myristate 13-acetate and 250 ng/ml of ionomycin (Calbiochem). The extent of lymphocyte activation was monitored by measuring [^{3}H]thymidine incorporation into DNA at 24 and 48 h of activation.

Primer Extension—An oligonucleotide primer complimentary to bases 73–99 in exon 1 was end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Probe (1×10^5 cpm) was mixed with 20 μg of total RNA from Jurkat and Raji cells or tRNA as a control and precipitated. Annealing was performed in 20 μl of 1 × avian myeloblastosis virus reverse transcription buffer (Promega) and incubated sequentially at 75 °C for 15 min and 60 °C for 30 min and slowly cooled to 40 °C. Extension was performed in the presence of 20 units of avian myeloblastosis virus reverse transcriptase (Promega), 1 mM dNTPs, and 10 units of RNasin (Promega) at 42 °C for 90 min. The mixture was then digested with 20 μg/ml DNase-free RNase (Boehringer Mannheim) at 37 °C for 20 min followed by phenol/chloroform extraction and ethanol precipitation. The resulting products were resuspended in 5 μl of gel loading buffer, and 3 μl was analyzed on a denaturing 8 M urea, 6% polyacrylamide gel. The gel was dried and autoradiographed at −70 °C for 2 days.

Ribonuclease Protection Assay—A pGEM-320 construct, containing genomic DNA extending 280 bp upstream and 45 bp downstream of the ATG translation initiation site was used for RNase protection assays using the Ribonuclease Protection Assay kit (Ambion, Austin, TX). The construct was digested with the restriction endonuclease *Bgl*I, generating a 1.6-kb fragment containing the SP6 promoter and 115 bp 5′ to the ATG initiation codon. This fragment was gel-purified and transcribed *in vitro* using SP6 RNA polymerase (Promega) to generate a [^{32}P]CTP-labeled 235-bp antisense RNA probe. The labeled probe (5×10^5 cpm) was added to 30 μg of total RNA from Jurkat and Raji cells and 30 μg of tRNA, and ethanol-precipitated. Hybridizations were performed in 20 μl of hybridization buffer at 45 °C for 20 h, and samples were digested with a RNase A and T mixture (1:100 dilution) at 37 °C for 30 min and precipitated in the presence of 2 μg of tRNA. The pellets were resuspended in 5 μl of gel loading buffer, and 3 μl was analyzed on 6% denaturing polyacrylamide gels.

Plasmid Constructs—The P1 and the P2 promoter regions were obtained by PCR using the following primers: 32F (5′-TGTAATCCCAGCATTTTGGGA-3′) and 33R (5′-GCTCTGTCGCCCAGGCTGGAGT-3′) for the P1 region; 7F (5′-TTCTTTCCAGTCCCACCCGTGTAG-3′) and 18R (5′-TGGCGTTTCGGGAAGTTA-3′) for the P2 region (see Fig. 3). The amplified regions were cloned into the PCR™ II vector (Invitrogen, San Diego, CA) and subcloned into the *Hin*dIII site of the pCAT-Basic vector (pCATB) in 5′ → 3′ orientation upstream of the chloramphenicol acetyltransferase (CAT) gene. Fragments of 0.4, 0.7, 1.4, and 2.7 kb 5′ to exon 1 were isolated by restriction enzyme digestions, as indicated in Fig. 6, gel-purified, and subcloned into the *Sal*I site of pCATB. The pCATB/0.7 construct is designated as P3. The orientation of each construct was verified by direct sequencing.

Transient Transfection and CAT Assays—Plasmid DNA used for transfection experiments was prepared using QIAGEN columns (Chatsworth, CA), and the DNA was quantitated by both spectrophotometry and ethidium bromide staining. To determine the transcriptional activity of the putative promoters, 20 μg of each construct was added to 10^7 exponentially growing Jurkat or Raji lymphoblasts in a total volume of 500 μl of serum-free RPMI 1640 medium. Electroporations were performed using a Bio-Rad Gene Pulser set at 250 V and 960 microfarads. Cells were plated in 20 ml of RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin and cultured for 44–48 h at 37 °C in a humidified atmosphere in the presence of 5% CO_2. A β-galactosidase plasmid under control of the β-actin promoter (pβAc-lacZ) (kindly provided by Lorraine Gudas, Cornell University) was used in each experiment to control for transfection efficiency. After harvesting, cells were washed twice in cold phosphate-buffered saline and extracted in 150 μl of 0.25 M Tris-HCl (pH 8.0) with three cycles of freeze-thawing. After centrifugation at $14,000 \times g$ for 5 min, supernatants were used for protein quantitation using the Micro BCA protein assay reagent kit (Pierce) and for assay of β-galactosidase activity by a modification of a published technique (29) using chlorophenolred β-D-galactopyranoside (Boehringer Mannheim) as a substrate. For CAT assays, aliquots of supernatant were heated at 60 °C for 10 min followed by centrifugation at $14,000 \times g$ for 5 min, and 50 μl of supernatant were then incubated with 0.125 μCi of [^{14}C]chlor-

TABLE I
Comparison of exon and intron sizes for the IMPDH type I and II genes

	Exons																	
	A + A′	B	C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
IMPDH type I	~1200	44	64	99	49	102	75	207	88	200	91	96	144	145	144	84	735	
IMPDH type II				148	49	102	75	207	88	200	91	96	144	145	144	84	75	
	Introns																	
	A	B	C	1	2	3	4	5	6	7	8	9	10	11	12	13		
IMPDH type I	265	88	~3200	~2000	~2300	137	278	149	~1500	~1400	236	~1300	94	288	93	~1200		
IMPDH type II				445	225	107	327	657	73	77	99	1065	83	94	80	89		

amphenicol (Amersham) and 25 μg of *n*-butyryl coenzyme A (Sigma) for 2–6 h, depending on the transfection efficiency of each experiment. Reactions were extracted with xylenes and counted in a Beckman scintillation counter (model LS 7800; Beckman, Irvine, CA). CAT activity was normalized for extract protein concentration and calculated as cpm/mg/min of assay.

RESULTS

Cloning and Mapping of the IMPDH Type I Gene—Two phage clones (1111a (14 kb) and 1711a (13 kb)) were isolated that overlap by 3.6 kb in the intragenic region and extend approximately 6.3 kb 5′ and 4.7 kb 3′ from the ends of the coding region. A physical map of the gene was established using a combination of restriction mapping, PCR, and sequence analysis of the two genomic clones (Fig. 1). The entire IMPDH type I gene is approximately 18 kb in length. According to the published cDNA sequence (17), the 5′-UTR is composed of 600 bp, which we have designated as exons A-C in Fig. 1. Exon A′ is the region between exon A and an upstream transcription initiation site described below. The coding region of the gene contains 14 exons, the last of which includes 713 bp of 3′-UTR. All exon-intron boundaries contain the canonical splice donor (AG) and acceptor (GT) consensus sequences, with the exception of a GC splice acceptor site at exon 4. Within the coding region, 16 bases differ from the published cDNA sequence (17). Six of these base changes result in amino acid differences: bp 86 (A → G; aspartic acid → glycine); 327 (C → G; asparagine → lysine); 819 (C → G; phenylalanine → leucine); 820 (C → G; histidine → aspartic acid); 1255 (C → G; proline → alanine); and 1489 (C → G; proline → alanine).

Comparison of the Genomic Structures of the IMPDH Type I and II Genes—The type I gene is significantly larger than the 5.8-kb type II gene (25). However, as shown in Table I, the exons of the coding region are identical in size and contain the same amino acids at each exon boundary with the exception of an isoleucine → valine substitution at the exon 5/6 boundary. The type I gene contains substantially larger introns, seven of which are over 1 kb in length, and has approximately 1.3 kb of 5′-UTR and 713 bp of 3′-UTR, as compared with 50 and 53 bp in the type II gene, respectively (16, 25). In addition, nine ATG codons are present in the 5′-UTR of the type I gene.

Analysis of IMPDH Type I Transcripts—Previous studies have demonstrated two IMPDH type I mRNA species of approximately 3 and 4 kb on Northern blot analysis of RNA from peripheral blood T lymphocytes (24). RNase protection assays suggested that the larger mRNA species of 4 kb contained additional 5′ or 3′ sequences, since the coding regions of both mRNA species were identical. In order to determine the composition of these mRNAs, Northern blot hybridization was performed on total cellular RNA isolated from resting or activated human PBLs using probes corresponding to different exons. Using a cDNA probe corresponding to the coding region (exons 1–14), the expression of both a 4- and a 2.5-kb RNA species, as accurately assessed using RNA markers, was markedly induced as a result of phorbol 12-myristate 13-acetate plus ionomycin treatment of PBLs (Fig. 2*A*, *lanes 1* and *2*). When a 700-bp probe encompassing 500 bp in exon A′ and 200 bp in exon A (p700; Fig. 2*A*, *lanes 3* and *4*) and a 190-bp probe spanning the region from exon B to exon C (p190; Fig. 2*A*, *lanes 5* and *6*) were used as probes, only the 4-kb mRNA was observed, indicating that this mRNA species originates upstream of the 2.5-kb mRNA and contains a significantly longer 5′-UTR. In contrast, both mRNA species were readily detected by two oligonucleotides derived from exon 1 sequence (Fig. 2*A*, *lanes 7*

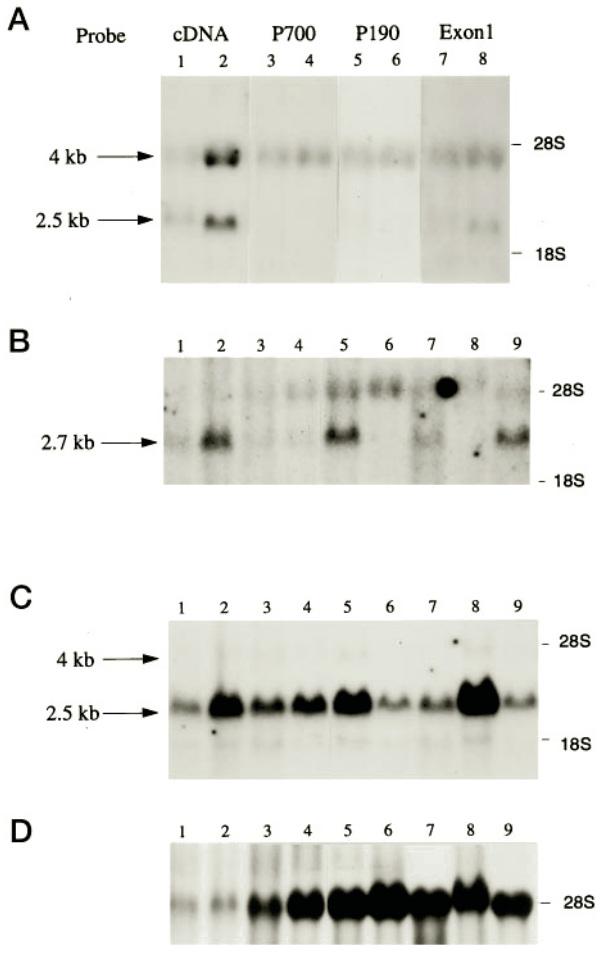

FIG. 2. **Differential expression of three IMPDH type I mRNA species.** *A*, Northern blot analysis of IMPDH type I mRNA was performed on 15 μg of total cellular RNA from resting (*lanes 1*, *3*, *5*, and *7*) or activated (*lanes 2*, *4*, *6*, and *8*) PBLs using probes corresponding to different exons as described under "Results." *B*, 20 μg of total RNA from K562 (*lane 1*), U937 (*lane 2*), KT-1 (*lane 3*), MOLT-4 (*lane 4*), Jurkat (*lane 5*), MGL-8 (*lane 6*), Nalm-6 (*lane 7*), Raji (*lane 8*), and H1437 (*lane 9*) cell lines were hybridized with the p190 probe. (*C*). The blot used in *B* was rehybridized with an IMPDH type I cDNA coding region probe. *D*, the blot used in *B* was rehybridized with a 28 S rRNA probe. The three IMPDH type I mRNA species (2.5, 2.7, and 4 kb) are indicated by *arrows*.

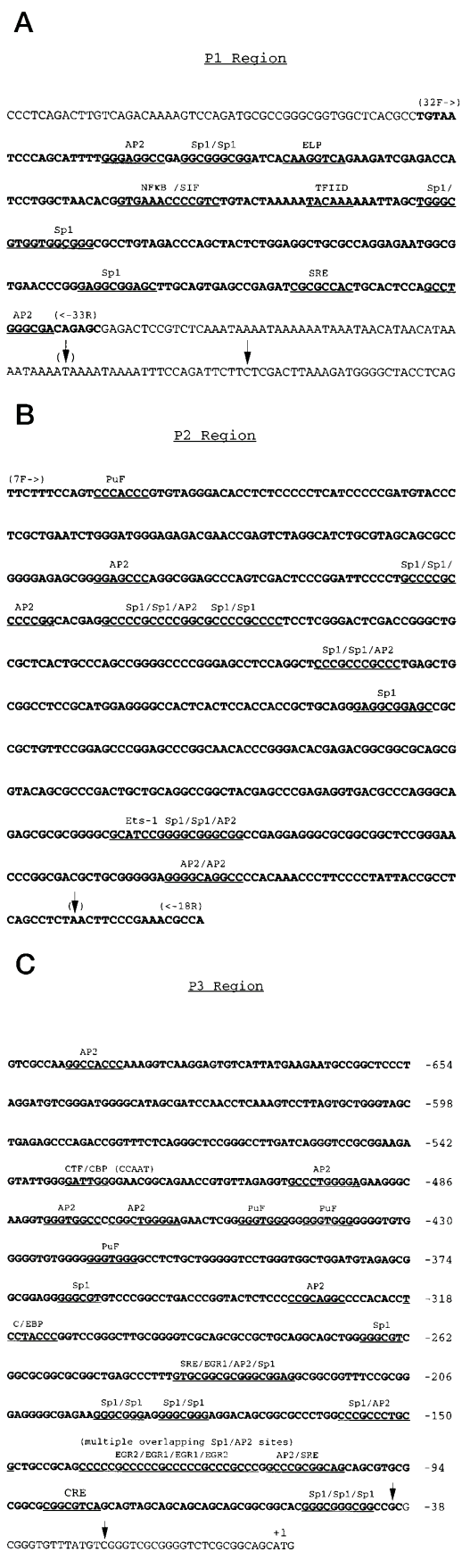

FIG. 3. **Sequences of the IMPDH type I P1 (*A*), P2 (*B*), and P3 (*C*) promoters.** The three putative promoter regions used to assay CAT activity are shown in *boldface type*. Putative transcription factor binding sites based on consensus sequences are indicated by *underlining*. The primers (32F, 33R, 7F, and 18R) used for PCR are indicated. Transcription initiation sites based on primer extension analysis and confirmed by RNase protection assays are shown by *arrows*. The transcription start sites determined by primer extension only are shown by *arrows* with *parenthesis*. The P3 sequence is numbered relative to the A of the ATG start codon (+1) in exon 1.

and *8*). Northern blot analysis was also performed on total cellular RNA isolated from nine tumor or transformed cell lines: an erythroleukemia line, K562; a monocytic leukemia line, U937; three T cell leukemia cell lines, KT-1, MOLT-4, and Jurkat; three B lymphoblast cell lines, MGL-8, Nalm-6, and Raji; and a lung cancer cell line, H1437 (Fig. 2*B*, *lanes 1–9*). Upon hybridization with the p190 probe, as shown in Fig. 2*B*, a third RNA species of approximately 2.7 kb was detected with relatively high abundance in U937, Jurkat, and H1437 cells but was absent in MGL-8 and Raji cells, as well as in PBLs. In order to confirm that this 2.7-kb band is not identical to the major 2.5-kb mRNA transcript, the blot was rehybridized with the 1.5-kb cDNA coding region probe. As shown in Fig. 2*C*, all of the cell lines demonstrated an abundant 2.5-kb transcript, with U937 and Raji cells having the highest level of expression after normalized for 28 S ribosomal RNA (Fig. 2*D*). These results clearly indicate the existence of three IMPDH type I RNA transcripts that differ at their 5′ termini.

Identification and Functional Analysis of the IMPDH Type I Promoter Regions—To study the elements involved in the regulation of IMPDH type I transcription, approximately 2.7 kb 5′ of exon C and 700 bp 5′ of exon 1 were sequenced. Three regions were identified as putative promoter regions based on sequence analysis; each contains a number of putative transcription factor binding sites, and we have designated them as P1, P2, and P3, respectively (Fig. 1). As shown in Fig. 3*A*, the P1 region encompasses 245 bp, as defined by PCR primers 32F and 33R, is located immediately 5′ to exon A′, and includes five Sp1 and two AP2 sites, one serum response element site, one NFκB site that overlaps with a SIF consensus binding sequences, and one ELP site. There is no TATA box in this region, although a consensus sequence for transcription factor IID is present. Of interest is the fact that this sequence is 95% identical to the catarrhine-specific (CS) Alu repetitive sequence found widely throughout the genome (30), although the consensus Alu sequence does not function as a promoter in similar transfection experiments (data not shown). A highly AT-rich region of 52 bp, containing four AAAAT repeats plus stretches of A nucleotides separated by a single T or C is located 3′ to the P1 element. Primer extension analysis using RNA from both resting and activated PBLs demonstrated two possible transcription initiation sites downstream of the P1 promoter, as indicated in Fig. 3*A*. Ribonuclease protection analysis detected a single band corresponding to the more 3′ initiation site, although the absence of a longer protected fragment could have resulted from nonspecific cleavage by RNase A of the A-rich sequence (data not shown).

The P2 region (Fig. 3*B*), 595 bp in length, is located 118 bp 5′ to exon B, and contains 73% G + C residues, a number of Sp1 and AP2 binding sites, a PuF consensus binding site, and an Ets-1 consensus binding site. A putative transcription initiation site located 134 bp 5′ to exon B was determined by primer extension analysis using a primer located in exon C. The P3 region (Fig. 3*C*), 671 bp in length, is located immediately 5′ to exon 1 and also has a high G + C content of 73%. This region contains, in addition to a number of potential Sp1 and AP2 binding sites, three Egr-1 sites (two of them overlapping with Egr-2 sites), three PuF, two serum response elements, and a site for CCAAT/enhancer-binding protein (C/EBP), a cAMP-response element (CRE), and a CCAAT binding site. Primer extension was performed using total RNA isolated from Jurkat

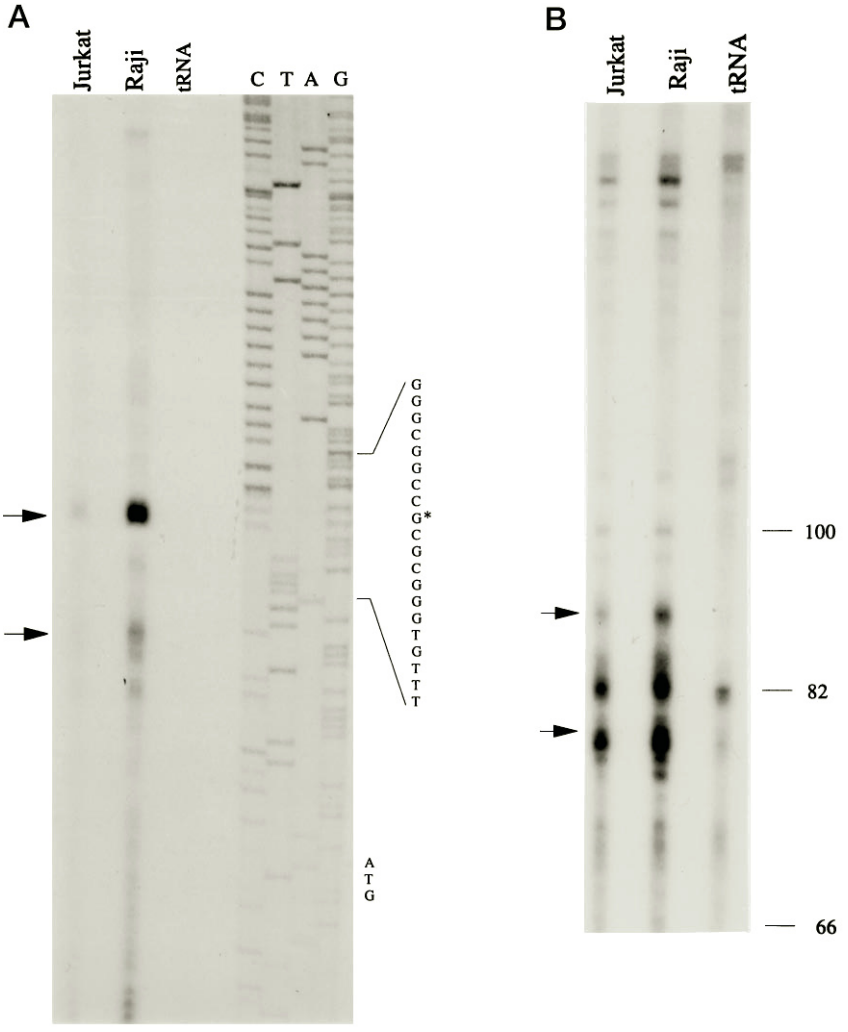

FIG. 4. **Determination of the transcription initiation site for the major 2.5-kb transcript.** *A*, primer extension analysis was performed using an oligonucleotide primer located in exon 1 and total cellular RNA from Jurkat or Raji cells or tRNA. The major and minor bands are indicated by *arrows*. The sequence obtained using the same oligonucleotide primer is shown on the *right*. The *asterisk* indicates the major transcription initiation site. *B*, ribonuclease protection assay performed with RNA from Jurkat, Raji cells and tRNA, and a 235-bp RNA probe as described under "Materials and Methods." The two major protected bands not seen in the tRNA lane are indicated by *arrows*. DNA markers in bp are shown.

and Raji cell lines and an oligonucleotide corresponding to the exon 1 region. Two transcription initiation sites were identified 40 bp (major) and 24 bp (minor) 5′ to the ATG initiation codon (Fig. 4*A*), and these results were confirmed by a ribonuclease protection assay in which two corresponding protected fragments were detected using RNA from Jurkat and Raji cells but not with the tRNA control (Fig. 4*B*). The greater intensity of the primer-extended bands and RNase-protected bands in Raji cells is consistent with the higher levels of the 2.5-kb mRNA in this cell line (Fig. 2*C*).

Functional studies of the P1, P2, and P3 regions were performed using transient transfection assays with CAT reporter gene constructs (Fig. 5). In both Jurkat and Raji cell lines, the activity of the P3 region is greater than that of the P1 and P2 regions. In the Jurkat T cells, constructs containing P1 or P2 result in an approximate 20-fold increase in CAT activity over the values obtained with the pCATB vector alone. In the Raji B cell line, in contrast, the P1 and P2 constructs resulted in a 1.2–6-fold increase in CAT activity, although P3 retained a level of promoter activity equivalent to that found in Jurkat cells. In order to define the P3 promoter region further, additional constructs containing variable regions 5′ to exon 1 were compared for their ability to promote transcription in Jurkat and Raji cells (Fig. 6). In Jurkat cells (Fig. 6*A*), the construct pCATB/0.7, containing a 671-bp *Apa*I-*Not*I fragment of P3, had the strongest promoter activity, while the activity of construct pCATB/2.7, containing additional 5′ sequences, is only about 15–20% that of the pCATB/0.7 construct. This profile differs markedly from that in Raji cells (Fig. 6*B*), where pCATB/2.7 has approximately 6-fold higher promoter activity than the pCATB/0.7 construct. These results strongly suggest that there are elements 5′ to the P3 region that may regulate transcription from this promoter in a cell type-specific fashion.

Transcriptional activity from the P3 element (pCATB/0.7) was compared with that from the 461-bp promoter for the IMPDH type II gene (25). CAT activity obtained with the type II promoter was 70-fold greater than that with the P3 promoter in both Jurkat and Raji cells (data not shown). This result is consistent with the observation that the expression of the type II IMPDH gene at the mRNA level is significantly higher than that of the type I gene in most tissues and cell lines tested (23) and suggests that transcriptional regulation may play a major role in determining the relative levels of expression of these two genes.

DISCUSSION

The co-existence in mammalian cells of two IMPDH genes with striking conservation of amino acid identity and catalytic function argues that each subserves an important and nonoverlapping function in cellular physiology or development. Since the expression of the IMPDH type II gene at the mRNA level has correlated more directly with cellular proliferation and transformation, it has been assumed that the IMPDH type I gene might provide a constitutive and noninducible level of guanine nucleotide biosynthesis (21, 22). However, the striking increase in IMPDH type I mRNA expression with T lymphocyte activation, the presence of a second mRNA transcript in these cells, and the variability in IMPDH type I gene expression in

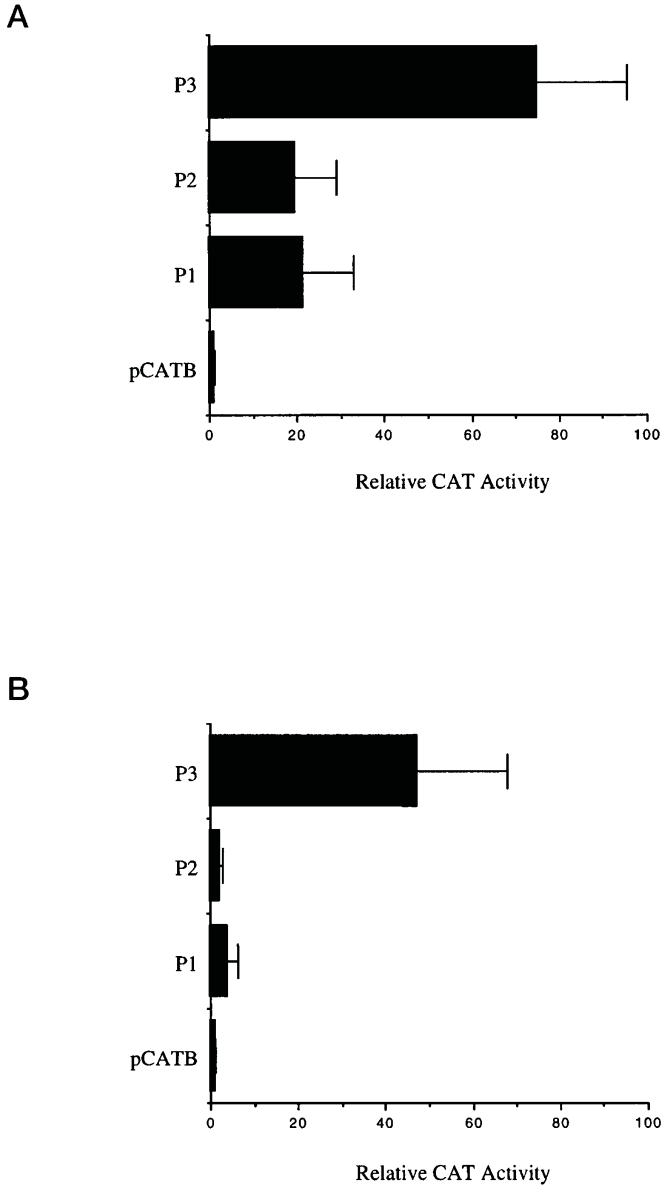

FIG. 5. **Transcriptional activity of the P1, P2, and P3 promoters in Jurkat (*A*) and Raji (*B*) lymphoid cells.** Cells were transiently transfected with plasmids containing the P1, P2, or P3 regions, as described under "Materials and Methods," and assayed for CAT activity. Relative CAT activity refers to -fold increase over values obtained by pCATB vector control, and *bars* represent the mean ± S.D. of four (Jurkat) and six (Raji) transfections performed in duplicate.

different tissues all support the concept that the regulation of expression of this gene may play a more significant role in cellular responses than had previously been appreciated. It was for this reason that a study of the structural characteristics of the IMPDH type I gene was undertaken.

The finding that the IMPDH type I gene retains the same exon structure as the smaller IMPDH type II gene argues for an early gene duplication event, while the preservation of the two functional genes throughout vertebrate evolution (26) supports an essential role for each. There are other striking examples of multigene families that account for tissue-specific differences in the expression of human enzymes important for nucleotide metabolism. Phosphoribosyl pyrophosphate synthetase (EC 2.7.6.1) catalyzes the synthesis of phosphoribosyl pyrophosphate, the primary precursor of the ribose monophosphate moiety of all nucleotides and a critical regulator of *de novo* purine and pyrimidine biosynthesis (31). Two isoforms, phosphoribosylpyrophosphate synthetase subunits I and II, are encoded by distinct genes (*PRPS1* and *PRPS2*) located on different regions of the X chromosome (32), and are expressed in a tissue-specific manner (33). A third phosphoribosyl pyrophosphate synthetase gene encoding a testes-specific isoform is autosomal in location (34). The tissue specificity of the expression of these genes has been attributed to the differences in 5′-flanking sequences and corresponding differences in transcriptional activity (35). Similarly, there are four isoforms of AMP deaminase (EC 3.5.4.6) encoded by three AMP deaminase genes (36). Recent studies have demonstrated alternative transcripts from the *AMPD2* gene that are expressed in a mutually exclusive pattern and that confer variable N-terminal extensions to their encoded peptides (37), while multiple RNA transcripts from the *AMPD3* gene are regulated by three distinct promoters in the 5′-flanking region (38). It appears from these and other examples that the tissue-, development-, and growth-regulated expressions of genes encoding enzymes essential for nucleotide metabolism may have important consequences for the development and survival of the organism as a whole.

The regulation of IMPDH type II gene expression has been studied in detail in resting and activated T lymphocytes and has been shown to be transcriptional in nature (25).[2] Stimulation of T cells with phytohemagglutinin plus interleukin-2 induces increased transcription from a single core promoter region that contains tandem cAMP-response element binding sites, an Sp1 site, and a novel palindromic octamer sequence.[2] In contrast, the regulation of IMPDH type I expression is more complex and is governed by the presence of three alternative promoters in the 5′-flanking region (Fig. 7). The P1 promoter gives rise to a 4.0-kb transcript that is primarily found in PBLs, and expression of this transcript increases markedly with T cell activation, as does expression of the 2.5-kb mRNA from the more generally utilized P3 promoter. The presence of an NFκB consensus binding site within the P1 region may be of direct relevance to this observation, since NFκB is involved in mediating the transcription of a number of genes following B and T lymphocyte activation, as well as in response to inflammatory cytokines (40). The 10-fold increase in IMPDH enzymatic activity and consequent increase in guanine nucleotide biosynthesis that occurs during T lymphocyte activation (5, 24) are prerequisites that enable these cells to enter S phase, and these events appear to be extremely important in T cell biological responses (13, 41). Although the relative contributions of IMPDH type I and II proteins to this increase in activity have been difficult to determine, given their high degree of amino acid identity, it is possible that the appropriate regulation of IMPDH type I gene expression is essential for the T cell mitogenic response.

The long 5′-UTR found initially in the published cDNA sequence (17) and demonstrated more fully as a result of our genomic characterization may play an additional role in regulating IMPDH type I activity. In the 4-kb IMPDH type I RNA transcript, there are 1.3 kb of sequence 5′ to the known translation start site that include nine AUG codons followed by open reading frames encoding putative peptides ranging from 3 to 85 amino acids. Two of these upstream AUG codons are in frame with the initiation codon for methionine in exon 1, raising the possibility that IMPDH type I isoforms with an additional 85 or 8 amino acids could be generated. Although we have been unable to identify size variants of IMPDH type I protein in Western blots of activated PBL extracts (data not shown), it is certainly possible that protein with N-terminal extensions

[2] A. G. Zimmermann, K. L. Wright, K. L., J. P.-Y. Ting, and B. S. Mitchell, submitted for publication.

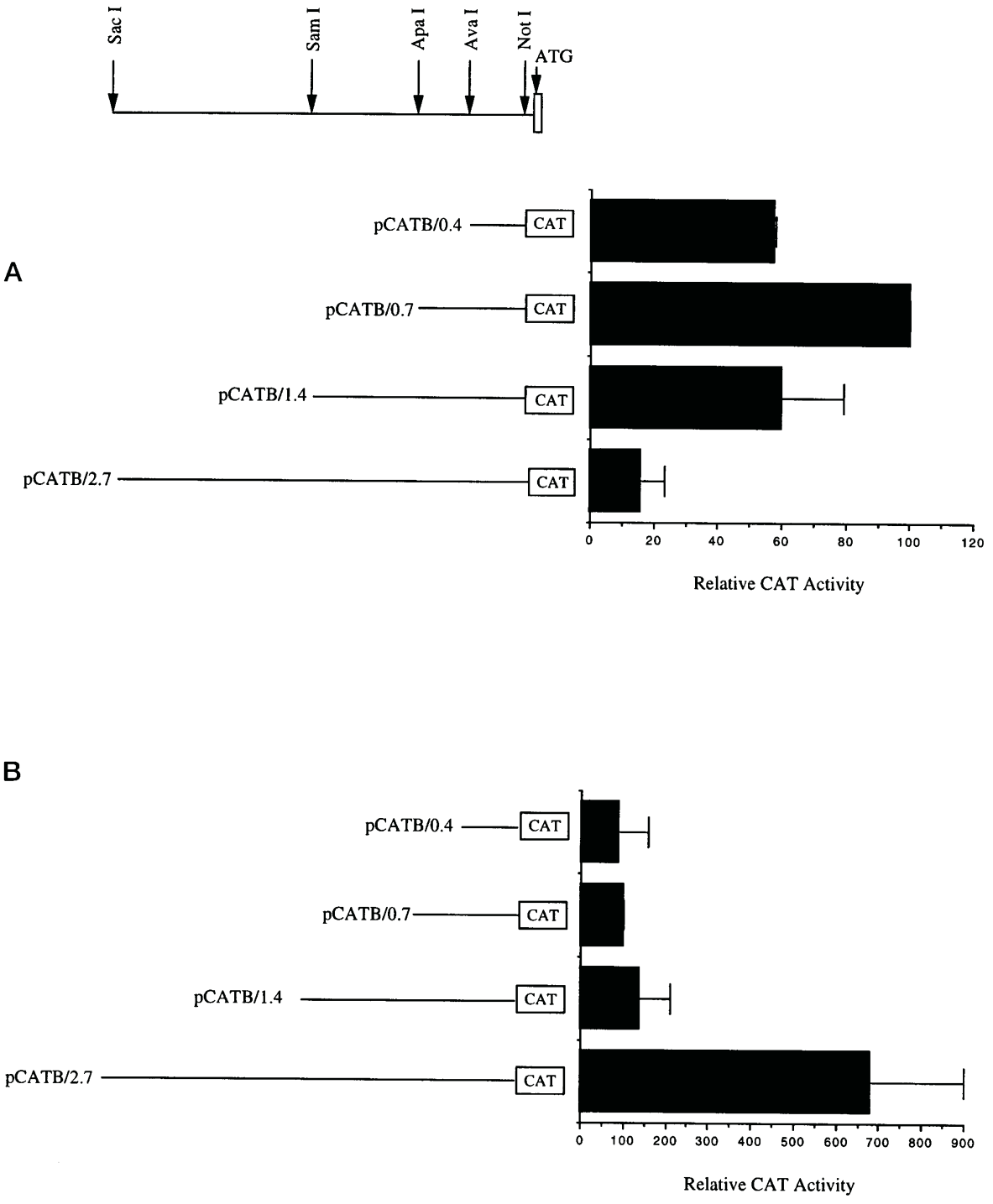

FIG. 6. **Delineation of P3 promoter activity in deletion constructs.** Jurkat (*A*) and Raji (*B*) cells were transfected with CAT constructs containing regions 0.4, 0.7, 1.4, and 2.7 kb 5′ to the ATG, as outlined under "Materials and Methods." CAT activity is expressed relative to the value obtained with the pCATB/0.7 construct, which has been set as 100. Data represent the mean ± S.D. of three experiments performed in duplicate.

could be synthesized. In addition, there is increasing evidence that 5′-UTR sequences longer than several hundred nucleotides may, as a consequence of their propensity to form secondary structure, regulate gene expression at the translational level (42). Although the presence of multiple AUG translation start codons have been shown to attenuate translation (43), there are also precedents for positive regulation, as in the induction of yeast GCN4 translation in response to amino acid availability (44) and in the Rous sarcoma virus RNA translation (45). Furthermore, tissue specificity of expression may be dictated by translational control mediated through this region (46, 47). Thus, although the 4-kb transcript is present in relatively low abundance in the tissues other than PBLs that have been surveyed to date, we cannot preclude a significant biologic role for this RNA species in regulating IMPDH type I enzyme synthesis.

The P2 promoter is GC-rich and appears to regulate the expression of a 2.7-kb transcript in a manner that is disproportionate to the expression of the 2.5-kb transcript in several cultured cell lines. Since this band can only be clearly dissociated from the 2.5-kb transcript by differential hybridization to the p190 probe, it is difficult to determine the extent to which

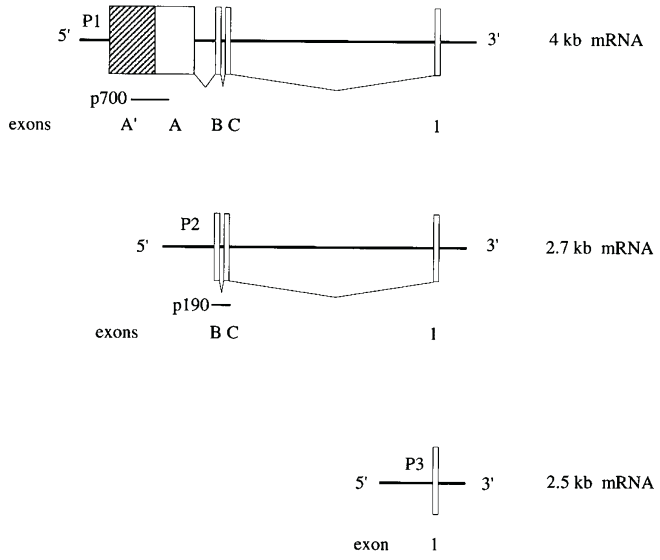

FIG. 7. **Schema of the use of alternative promoters to give rise to the three IMPDH type I mRNAs.**

it is present in the tissues that have been studied to date (23). Although the structure of the P2 region does not appear to contain transcription factor binding sites that are highly tissue-specific in nature, the observation that the relative expression of CAT from a reporter construct containing 595 bp of sequence from this region is significantly higher in Jurkat that in Raji cells, together with the presence of the 2.7 transcript in the former but not the latter cell line, would support the concept of tissue-specific expression.

The P3 promoter accounts for the major 2.5-kb transcript and contains a large number of Sp1 and AP2 binding sites that may be responsible for the basal expression of this gene. The presence of several NGFI-A/Egr-1 sites in close proximity to the transcription initiation sites offers one potential explanation for the up-regulation of the 2.5-kb mRNA with T cell activation, since Egr-1 is a zinc finger protein induced following interleukin-2 stimulation of T lymphocytes that appears to be important for subsequent cellular proliferation (48). In addition, the presence of three consensus binding sites for PuF further upstream raises the possibility that NM23.H2, a product of one of two *NM23* genes, could be involved in IMPDH I regulation. NM23.H2 is one of two subunits of nucleoside diphosphate kinase (EC 2.7.4.6), an enzyme that is important both in maintaining nucleoside triphosphate pools (49, 50) and in activating GTP-dependent proteins (51, 52). NM23.H2 has been shown to function as a transcription factor for the c-*myc* gene (53) and, together with NM23.H1, to be up-regulated in human PBLs following phytohemagglutinin stimulation with a time course that is consistent with the increase in IMPDH type I mRNA expression (24, 54). Whether or not these specific binding sites are important for the up-regulation of the 2.5-kb transcript in PBLs remains to be determined.

Of additional interest with regard to the P3 promoter region is the differential expression of CAT constructs containing variable amounts of 5′ sequence extending as far as the *Sac*I site 3′ to exon C (Fig. 1). Tissue specificity is evident in that maximal activity in Jurkat T cells is obtained with the 700-bp region 5′ to the ATG that includes the PuF and Egr-1 sites, whereas 6-fold greater relative activity is found in Raji cells when an additional 2 kb of upstream sequence is included in the construct. This observation again underscores the potential complexity of IMPDH type I gene transcriptional regulation. In contrast to the concept that the expression of IMPDH type I gene does not vary as a function of cell proliferation or transformation, our results support the view that the IMPDH type I gene is subject to differential regulation at the transcriptional level from three promoter regions in a highly tissue- or cell-specific manner. The list of enzymes whose expression is regulated by multiple promoters and alternative processing at the 5′-end, as well as by the presence of multiple genes with varying regulatory sequences encoding nearly identical proteins, is growing steadily (39). The biological roles of IMPDH type I that require this diversity of regulation remain to be determined.

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