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TYPE I INOSINE MONOPHOSPHATE DEHYDROGENASE: EVIDENCE FOR A SINGLE FUNCTIONAL GENE IN MAMMALIAN SPECIES

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Human inosine monophosphate dehydrogenase activity is the result of the expression of two independent but closely related genes, termed IMPDH type I and type II. We have documented the presence of multiple, processed pseudogenes of type I IMPDH in human and Rhesus monkey genomic DNA, as well as a single functional gene encoding low levels of type I mRNA in human brain, heart, kidney and placenta. Single copy genes for each IMPDH isoenzyme were also found in rat, mouse, dog, cow, and chicken DNA and distinct mRNA species for type I and type II were identified by Northern blots in mouse and hamster RNA. Northern blot analysis of chicken RNA revealed a single mRNA species that hybridized to human IMPDH type I and II probes. These data document the high degree of evolutionary conservation of these two genes among mammals. © 1993 Academic Press, Inc.

The enzyme inosine monophosphate dehydrogenase (IMPDH; EC1.1.1.205) catalyzes the rate-limiting step in the *de novo* synthesis of guanine nucleotides from IMP and its activity is essential for cellular proliferation (1). It has been demonstrated that human IMPDH enzymatic activity is the result of the expression of two distinct genes with corresponding mRNA species of approximately 3 kb (IMPDH I) and 2 kb (IMPDH II), respectively (2, 3). The resulting proteins both have 514 amino acids and are 84% identical in sequence. Several reports have indicated that type II IMPDH is expressed at markedly higher levels in proliferating leukemic cell lines than in resting peripheral blood cells, whereas type I is expressed at similar levels in both cell types (3-7). Whether distinct functions can be attributed to these two isoenzymes that appear to be regulated differently (8) remains unclear. In order to gain a better understanding of the relative importance of these two isoenzymes, we have asked whether one or both genes are present and functional in other species. In addition, we have asked whether there is differential expression of type I IMPDH among other human tissues and cell types.

Abbreviation: IMPDH, inosine monophosphate dehydrogenase.

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MATERIALS AND METHODS

Cell culture and reagents. RPMI-1640 tissue culture medium, penicillin, streptomycin, and fetal calf serum were purchased from Gibco Laboratories (Grand Island, NY). Ionomycin was obtained from Calbiochem Behring Corp. (San Diego, CA) and was suspended in dimethylsulfoxide at 1 mg/ml and stored at -20°C. PMA was purchased from Sigma Chemical Co. (St. Louis, MO) and was reconstituted as a 1 mg/ml solution in dimethylsulfoxide. Fresh dilutions of PMA (at 5 μg/ml in PBS) were prepared as needed for each experiment. $CD28^+$ T lymphocytes were purified by negative selection as described (9). Peripheral blood T lymphocytes were cultured at a density of 1 x 10^6 cells/ml in RPMI-1640 containing 10^5 U/liter penicillin, 100 μg/liter streptomycin, and 10% fetal calf serum. PMA was used at a final concentration of 3 ng/ml, and ionomycin at 125 ng/ml. The Jurkat T cell line was obtained from Jeffrey Leiden (University of Chicago, Chicago, IL).

Northern blots. Total cellular RNA was isolated using TRI reagent (Total RNA Isolation reagent from Molecular Research Center, Inc., Cincinnati, OH). RNA samples from human brain, heart, and kidney were purchased from Clontech (Palo Alto, CA). Northern blots and hybridizations were performed as previously described (10).

Southern blots. Genomic DNA was isolated from Jurkat cells using sarcosyl or from whole blood as described (11). Southern blots were performed (10) using 15 μg per lane digested with the indicated restriction enzyme. The Interspecies Zoo-blot was purchased from Clontech (Palo Alto, CA). Hybridization was carried out with ^{32}P-labelled IMPDH I or II cDNAs as previously described (8) at 42°C in 50% formamide, 5X Denhardt's, 6X SSC, 0.5% SDS, and 100μg/ml ssDNA. Washes were performed using 3X SSC, 0.5% SDS at 25°C, 33°C, 42°C, and 55°C for 20 minutes sequentially with exposure to X-ray film after each wash.

RNase mapping. RNase mapping was performed according to Melton *et. al.* (12). Three constructs were generated to contain overlapping regions of the IMPDH type I cDNA coding sequence: Nde 1 to Pst 1, base pairs 601-1205; Bst X1 internal fragment, base pairs 843-1498; and Eco R1 to the TGA termination codon, base pairs 1434-2145 (3). Each vector was linearized at the 5' end of the insert and transcribed *in vitro* from the Sp6 or T7 promoter to generate [^{32}P]CTP labelled antisense RNA probes. Probes were hybridized overnight at 45°C and the hybrids were digested at 30°C for 45 minutes with 40μg/ml RNase A and 2μg/ml RNase T1. The samples were heated to 85°C and run on a 8M urea, 6% polyacrylamide gel.

RESULTS AND DISCUSSION

Type I IMPDH has multiple processed pseudogenes.

Figure 1 shows a Southern blot of genomic DNA from cultured Jurkat T cells probed with the type II (Panel A) or type I (Panel B) IMPDH cDNA coding regions. These results confirm the presence of two distinct genes, as has been reported previously (4), and support the presence of a single copy gene for type II. In contrast, the multiple bands hybridizing with the type I probe strongly suggest the presence of pseudogenes. Therefore, the blots were reprobed with non-overlapping 5' (Figure 2A) or 3' (Figure 2B) type I probes. The similarity of these two blots provides evidence for the presence of multiple processed pseudogenes for type I in human DNA. An identical result was obtained with DNA from human peripheral blood (data not shown), demonstrating that this result was not idiosyncratic to this cultured cell line.

A number of processed pseudogenes, defined as nonfunctional genes with a high degree of sequence identity but without intervening sequences, have been described in the human genome, including those for ß-tubulin, ß-actin, dihydrofolate reductase, and glyceraldehyde-3-phosphate dehydrogenase (13-19). The characteristics of processed pseudogenes suggest that they are derived from the corresponding mature mRNA and inserted at random into the genome, but they are by definition non-functional (20). In order to determine whether more than one functional gene for IMPDH I exists in the human genome, Northern blot analysis was performed on RNA from human brain, heart, and kidney, as well as from stimulated peripheral blood T

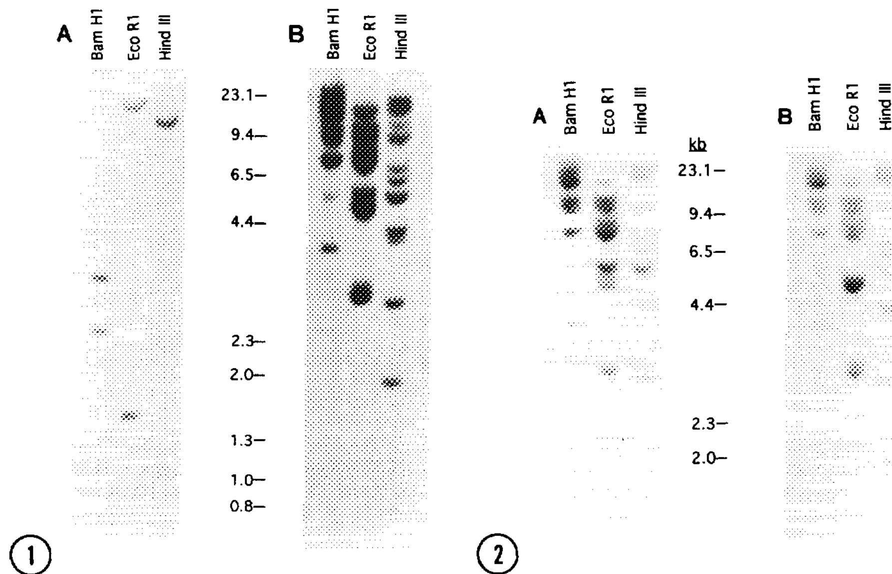

Figure 1. Southern blot of human genomic DNA probed with human IMPDH type II (panel A) or human IMPDH type I (panel B) cDNA coding regions. Each lane contains 15 μg DNA digested with BamH1, EcoR1, or Hind III. The positions of λ Hind III size markers (kb) are shown.

Figure 2. Southern blot of human genomic DNA probed with human IMPDH type I 5' (bp 601-1204) or 3' (bp 1434-2145) probes. DNA was digested with BamH1, EcoR1, or Hind III, as indicated.

lymphocytes and Jurkat T cells (Figure 3A). A single 3 kb mRNA species was observed in all samples, with the exception of peripheral blood T lymphocytes where a second band of 4 kb was observed (8). RNase protection assays performed on RNA from human heart (Figure 3B), as well as from stimulated peripheral blood T lymphocytes (8), demonstrates only a single protected RNA species over the entire coding region, indicating that only a single gene is being expressed in these tissues. It is likely that the 4 kb band in stimulated T lymphocytes arises from alternative splicing in the 5' or 3' untranslated region (8).

Both type I and type II IMPDH genes are present in mammals and avian species.

A zoo blot probed with the human IMPDH type I cDNA probe (Figure 4A) demonstrates that Rhesus monkey DNA also contains multiple IMPDH I pseudogenes, whereas rat, mouse, dog, and cow DNA appear to contain a single copy gene. The human type II IMPDH probe hybridizes to a completely distinct set of bands than does the type I probe in these species, supporting the presence of distinct type I and II genes. In contrast, avian DNA hybridizes less intensely with the type I than with the type II probe, but the band is of the same size, suggesting that only one IMPDH gene is present. However an additional Southern blot of chicken and turkey genomic DNA digested with Bam H1 and Sac 1 showed distinct band patterns with the type I and type II probes, demonstrating the existence of two IMPDH genes in these avian species (data not shown). Northern blot analysis of murine S49.1 cell line, Chinese Hamster Ovary cell, and chicken thymus RNA is shown in Figure 5. Although distinct type I and II mRNAs are present in the rodent cells, only a single band of the size of the type II mRNA is present in the chicken

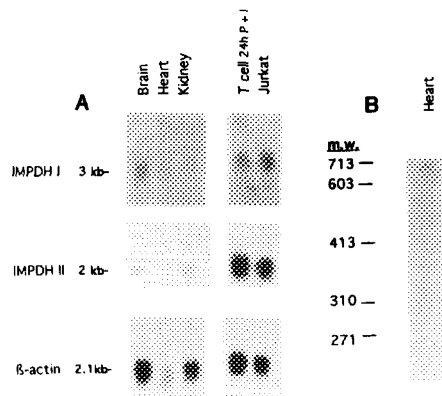

Figure 3. Expression of IMPDH type I and type II mRNAs in human tissues and T lymphocytes. A. Northern blots were sequentially probed with cDNA probes for IMPDH type I, IMPDH type II, and ß-actin. Purified T lymphocytes were stimulated for 24 h with 3 ng/ml PMA (P) and 125 ng/ml ionomycin (I). Each lane contains 12 μg total RNA. B. RNase protection assay of IMPDH type I. RNA from human heart.

RNA, suggesting that IMPDH is either not expressed in chicken thymus or hybridizes only weakly to the human cDNA probe. These results suggest that the existence of two genes is a general phenomenon in mammals.

IMPDH cDNAs have been sequenced from mouse (21), hamster (2), *Drosophila melanogaster* (GenBank L14847), *trypanosoma brucei* (GenBank M97794), *Leishmania donovoni* (22), *Escherichia coli* (23), and *Bacillus subtilis* (24). The human type II cDNA is 89% identical at the DNA level to the published hamster sequence and the hamster and mouse

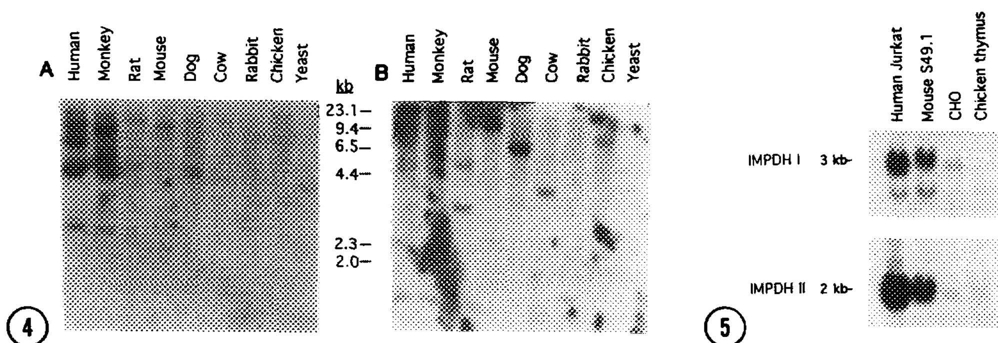

Figure 4. Hybridization of human IMPDH cDNAs to mammalian, avian and yeast DNA. The blot was probed with the cDNA for human IMPDH type I (panel A) and human IMPDH type II (panel B) and washed under low stringency conditions (materials and methods).

Figure 5. Expression of IMPDH type I and type II mRNAs in human, mouse, hamster, and chicken cells. Lane 1: human Jurkat T cell line; lane 2: S49 mouse lymphoma cell line; lane 3: Chinese hamster ovary cells; and lane 4: chicken thymus. 20 μg total cellular RNA was run in lanes 1, 2, and 4 and 2 μg polyadenylated RNA was run in lane 3. The blot was sequentially probed with cDNAs for human IMPDH type I and IMPDH type II. The lower band seen with the type I probe represents cross-hybridization to the abundant type II mRNA species. Blots were washed at low stringency (3x SSC, 0.5% SDS at 43°C).

published sequences are 95% identical to each other (21). We have recently obtained a distinct mouse "type I" IMPDH cDNA by PCR that is approximately 90% identical to the human type I sequence but only 75% identical to the published murine sequence (Dayton, unpublished). In contrast, the sequences of the published non-mammalian cDNAs are equivalently divergent from the human type I and type II genes, as determined by a molecular phylogenetic approach (K. Wilson and B. Ullman, personal communication). These observations are consistent both with the presence of a single primordial gene in these non-vertebrate species and with the existence of distinct type I and type II genes in mammals.

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