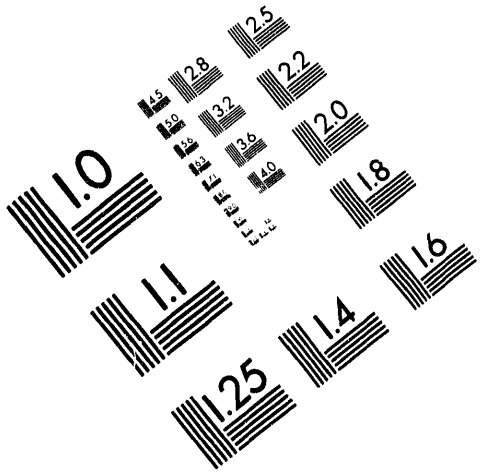

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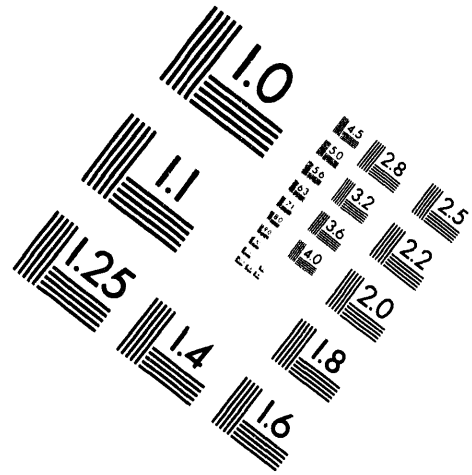

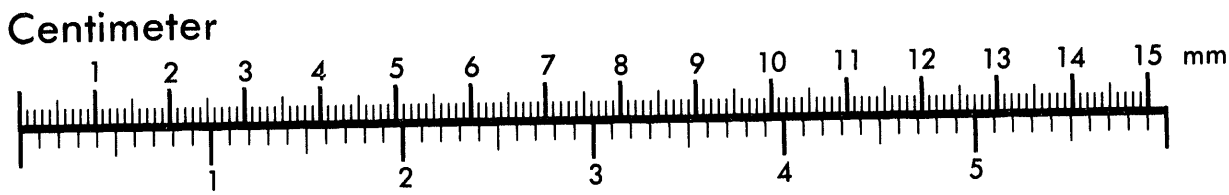

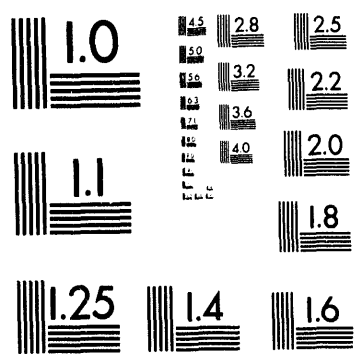

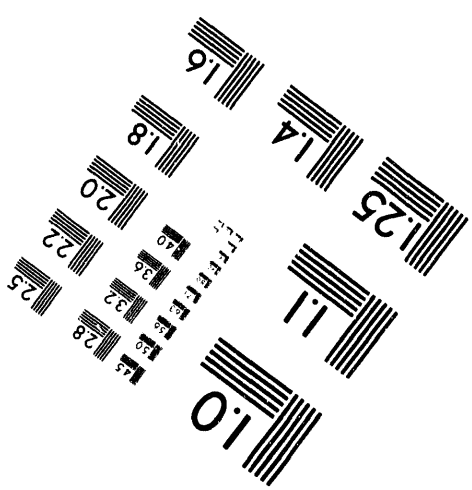

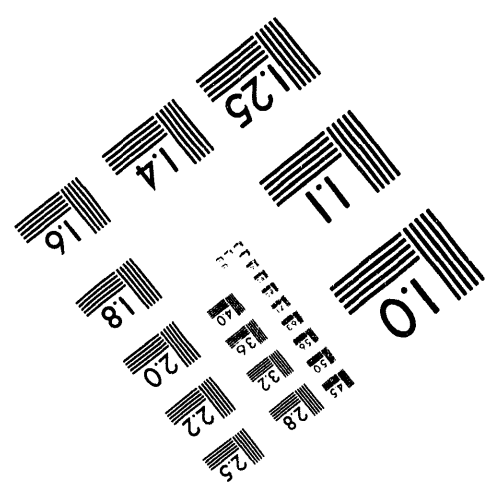

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Chromosomal Localization and Structure of the Human Type II IMP Dehydrogenase Gene

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Running title: IMPDH gene localization and structure.

We determined the chromosomal localization and structure of the gene encoding human type II inosine 5'-monophosphate dehydrogenase (IMPDH, EC 1.1.1.205), an enzyme associated with cellular proliferation, malignant transformation, and differentiation. Using polymerase chain reaction (PCR) primers specific for type II IMPDH, we screened a panel of human-Chinese hamster cell somatic hybrids and a separate deletion panel of chromosome 3 hybrids and localized the gene to 3p21.2→p24.2. Two overlapping yeast artificial chromosome clones containing the full gene for type II IMPDH were isolated and a physical map of 117 kb of human genomic DNA in this region of chromosome 3 was constructed. The gene for type II IMPDH was localized and oriented on this map and found to span no more than 12.5 kb.

IMPDH is the rate-limiting enzyme in *de novo* guanine nucleotide biosynthesis. It catalyzes the NAD-dependent conversion of IMP into XMP, which is rapidly converted to GMP. IMPDH activity correlates positively with increased proliferation of both normal and malignant tissues and cells (Jackson and Weber, 1975) as well as malignant transformation (Itoh *et al.*, 1989). Specific inhibition of IMPDH activity by treatment with mycophenolic acid or tiazofurin inhibits cell multiplication and induces differentiation in a number of cell systems (Wright, 1987; Kiguchi *et al.*, 1990). In humans, there are two separate IMPDH proteins encoded by two distinct genes, termed type I and type II (Collart and Huberman, 1988; Natsumeda *et al.*, 1990). Regulation of IMPDH activities during growth, transformation, and differentiation is due

to specific changes in the expression of type II mRNA while type I remains constitutively expressed (Nagai *et al.*, 1992).

To localize type II IMPDH to a particular chromosome, PCR primers (2L3: 5'-ATTGCTGCCATCCAACACTCA-3'; 2R2: 5'-CCGAGGAGGTGTGCTGGAT-3') were designed so as to amplify a 190-bp DNA fragment from an IMPDH type II cDNA clone (Genbank accession #J04208). However, amplification of human genomic DNA resulted in a single band of 360 bp, 170 bp larger than expected. This band was sequenced (Genbank accession #L08114) and the larger size determined to be due to amplification across two introns. Both introns are of the type 2 variety (Patthy, 1987) with the first 80-bp intron dividing the codon for arginine 480 and the second 90-bp intron dividing the codon for serine 508. Both sets of splice acceptor and donor sites conform to the consensus sequences previously proposed (Mount, 1982). We then used primers 2L3 and 2R2 and a PCR based method to screen a panel of DNAs from 25 different human-Chinese hamster somatic cell hybrids. The presence or absence of the 360-bp type II IMPDH PCR product was tabulated against the presence or absence of individual chromosomes from the tested hybrids (Table 1). By this method, IMPDH type II cosegregated with human chromosome 3 at a discordancy of 0%. Amplification of the panel with two type I IMPDH primer pairs resulted in amplification of DNA from a variety of cell hybrids (data not shown). However, discordancy analysis with each primer set did not lead to the unambiguous assignment of type I to a specific chromosome. Localization on chromsome 3 was excluded by these analyses, so the genes for type I and II IMPDH are not syntenous. In addition,

hybrid amplification results were not the same using each of the two type I IMPDH primer sets. These results can be interpreted in several ways. First, there may be multiple, chromosomally-dispersed genes encoding type I IMPDH. Alternatively, there may be one copy of the type I gene but multiple, dispersed pseudogenes. In either case, chromosomal assignment of type I IMPDH was not possible using this particular approach.

To more precisely sublocalize IMPDH type II to a region of chromosome 3, we used PCR and our specific type II primers to screen a panel of somatic cell hybrids that retain various portions of chromosome 3 (Drabkin *et al.*, 1990). This panel allows for the sublocalization of a gene to six regions of the short arm (p) and five regions of the long arm (q) of chromosome 3. PCR amplification results and chromosome content of the cell hybrids are shown in Figure 1. Particularly informative is the lack of amplification in hybrid 3;7/UC2E-1 (lane D) and the positive amplification in hybrid 3;21/Q131-91A-1 (lane E). These results define the end points for the sublocalization of IMPDH type II to 3p21.2→p24.2.

This region is of interest as deletions and loss of heterozygosity of the short arm are frequently detected in small cell lung carcinoma (Whang-Peng *et al.*, 1982) and renal cell carcinoma (Zbar *et al.*, 1987) leading to the postulate that one or more tumor suppressor genes may reside within 3p13→p23. While IMPDH type II is not itself a tumor suppressor, this gene may serve as an access point for chromosome walking or jumping in an attempt to identify suppressor genes involved in these diseases.

For the purpose of isolating the gene for type II and the surrounding genomic area, primers 2L3 and 2R2 were used to screen a portion of a human Yeast artificial chromosome (YAC) library (Albertsen *et al.*, 1990; generously provided by Drs. D. Cohen, D. Le Paslier and colleagues). Two YAC clones, 149C12 (117-kb insert) and 239B6 (640-kb insert), were isolated and shown to contain the full gene for type II IMPDH by hybridization (data not shown). Neither YAC contained the gene for type I IMPDH as determined by PCR. YAC clone 149C12 was subjected to partial digestion using restriction enzymes whose recognition site contains the 5'-CpG-3' dinucleotide and the resultant data was assembled into a physical map (Figure 2). In order to localize and orient the IMPDH gene with respect to the physical map of the region, we performed double digests with the enzymes shown on the map and performed Southern hybridization analysis using a 5' *Eco*RI cDNA fragment and a 3' *Bam*HI cDNA fragment as probes. The gene for IMPDH type II is at most 12.5 kb in length and is contained entirely between the *Cla* I site at +12.5 kb and the *Not* I site at +25.5 kb of our physical map. With respect to the orientation of the YAC arms, the direction of transcription is from right to left. The isolation of this gene will allow for the identification of regulatory elements involved in the guanine nucleotide-mediated nuclear posttranscriptional regulation of type II IMPDH gene expression (Glesne *et al.*, 1991) as well as investigation into the regulation of this gene during cellular proliferation, malignant transformation, and differentiation.

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FIGURE LEGENDS

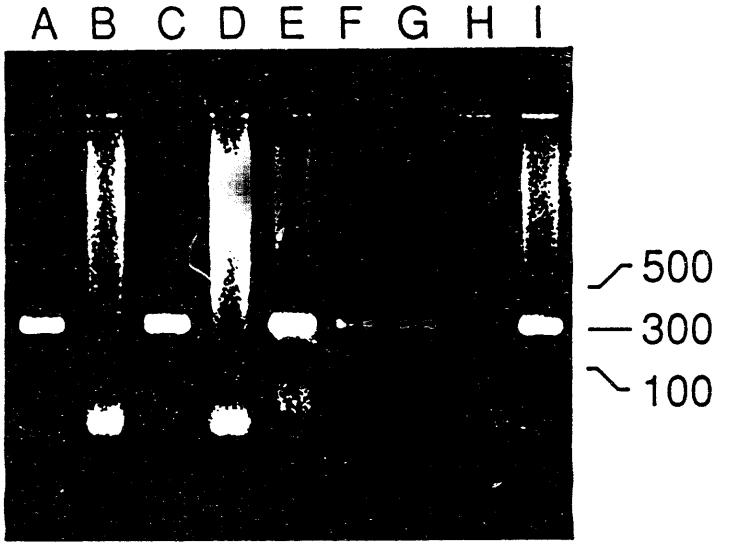
Figure 1. Sublocalization of type II IMPDH on chromosome 3. PCR was performed using human, Chinese hamster, and human-Chinese hamster hybrid cell DNA from a deletion panel for chromosome 3 and primers 2L3-2R2. The products were electrophoresed and stained with ethidium bromide (left). The substrate DNA was (A) UCTP 2A3, (B) UCH12, (C) R1-1, (D) 3;7/UC2E-1, (E) 3;21/Q131-91A-1, (F) R227-3A, (G) R148-3, and (H) genomic Chinese hamster, (I) genomic human. The chromosomal content of each hybrid is represented graphically by the vertical lines (center) with respect to the banding pattern of human chromosome 3 (right). A "+" or "-" below the vertical bar indicates whether or not type II IMPDH was amplified from the hybrid.

Figure 2. Physical map of YAC clone 149C12. Partial digests of YAC clone 149C12 were separated by Transverse Alternating Field Electrophoresis (conditions: 22 h, 350 mA, 5 s switch times) and Southern analysis was performed individually using both the 2.6-kb or 1.6-kb *Pvu*II/*Bam*HI fragment from pBR322. These fragments hybridize respectively to the left and right arms of the YAC and produce a banding pattern so as to identify the distance of each site from the arm. The scale is given (upper right). Type II IMPDH maps to the region indicated by the dark bar below the physical map

of the YAC. The direction of transcription is indicated by the arrow.

Table 1. Segregation analysis of type II IMPDH. PCR was performed with type II IMPDH primers and a panel of human-Chinese hamster somatic cell hybrid DNAs (BIOS, New Haven, CT) as substrate. Rows correspond to human-Chinese hamster hybrids. Columns correspond to human chromosome numbers. A "+" indicates the presence of the human chromosome in the hybrid. The final column shows the results of PCR amplification with type II IMPDH primers. For discordancy analysis, a correlation of the presence or absence of IMPDH amplification and the presence or absence of a chromosome was tabulated and is shown in the penultimate set of rows (IMPDH/chromosome). Discordancy was calculated as the sum of (+/-) and (-/+) divided by the sample number (n=25).

	Human Chromosome																								IMP gene
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
Hybrid 867	+	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	+	+	−	−	−	−	−	−
854	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
423	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
860	−	−	+	−	+	+	−	−	−	+	−	−	−	−	−	−	−	−	+	−	+	−	−	−	+
803	−	−	−	+	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	+	+	−	−
909	−	−	−	−	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−
1006	−	−	−	+	+	−	+	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	−	−
811	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
967	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
734	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
968	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−
683	−	−	−	−	+	−	−	−	−	−	+	+	−	+	−	−	−	−	+	−	+	+	−	−	−
507	−	−	+	−	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	+	−	+	−	+	+
750	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−	−
1099	+	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	+	−	−	−
324	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
940	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
983	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
937	+	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	+	−	−	−	+	−	−	−	−
1079	−	−	+	−	+	−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+
756	−	−	−	−	+	+	+	−	−	−	−	+	+	+	−	−	−	−	+	+	+	−	−	+	−
904	−	−	−	−	+	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	+	−
862	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1049	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
212	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Correlation (IMPDH/chromosome)																									
+/+	0	0	4	0	3	1	0	0	0	2	0	1	0	1	1	1	0	0	1	1	1	1	0	1	
+/−	4	4	0	4	1	3	4	4	4	2	4	3	4	3	3	3	4	4	3	3	3	3	4	3	
−/+	3	1	0	2	19	3	2	5	3	1	3	3	6	6	3	2	2	4	6	2	6	3	3	3	
−/−	18	20	21	19	2	18	19	16	18	20	18	18	15	15	18	19	19	17	15	19	15	18	18	18	
Discordancy (%)	28	20	.0	24	80	24	24	36	28	12	28	24	40	36	24	20	24	32	36	20	36	24	28	24	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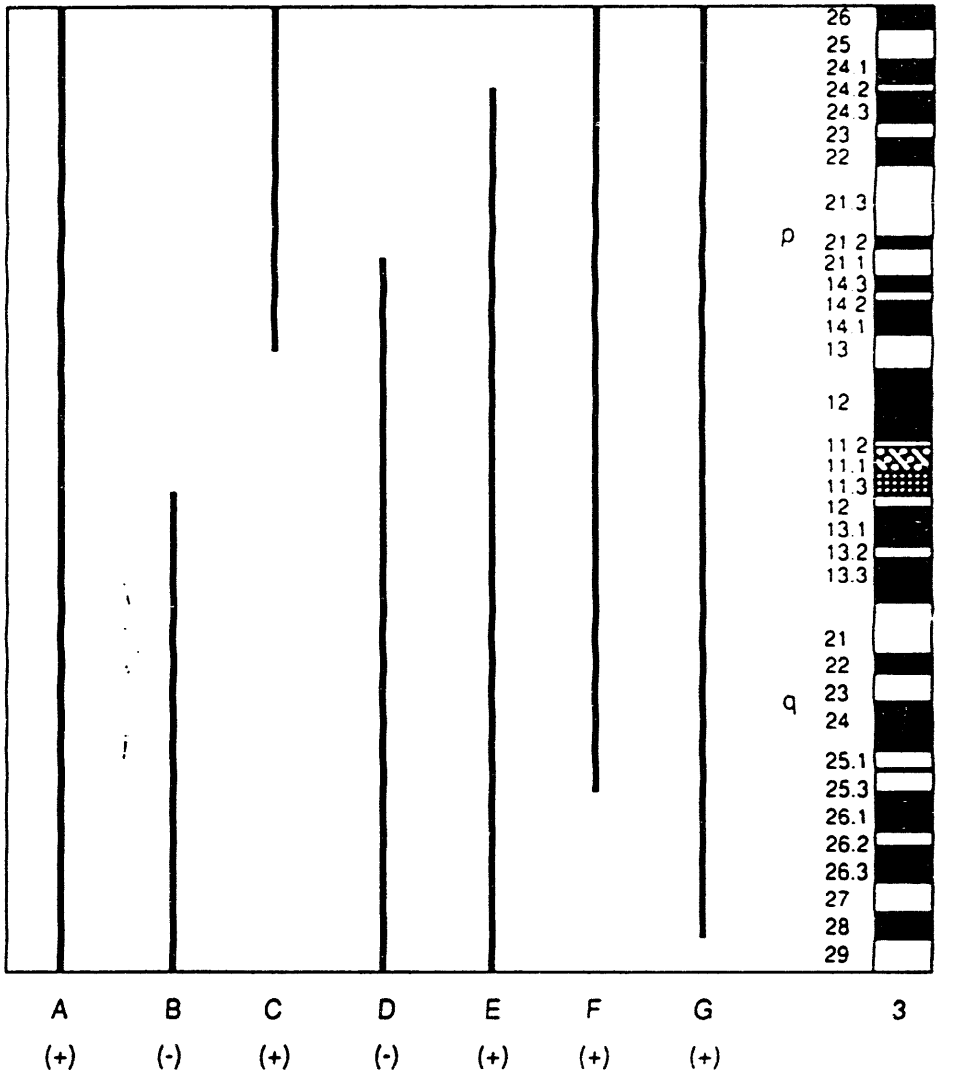
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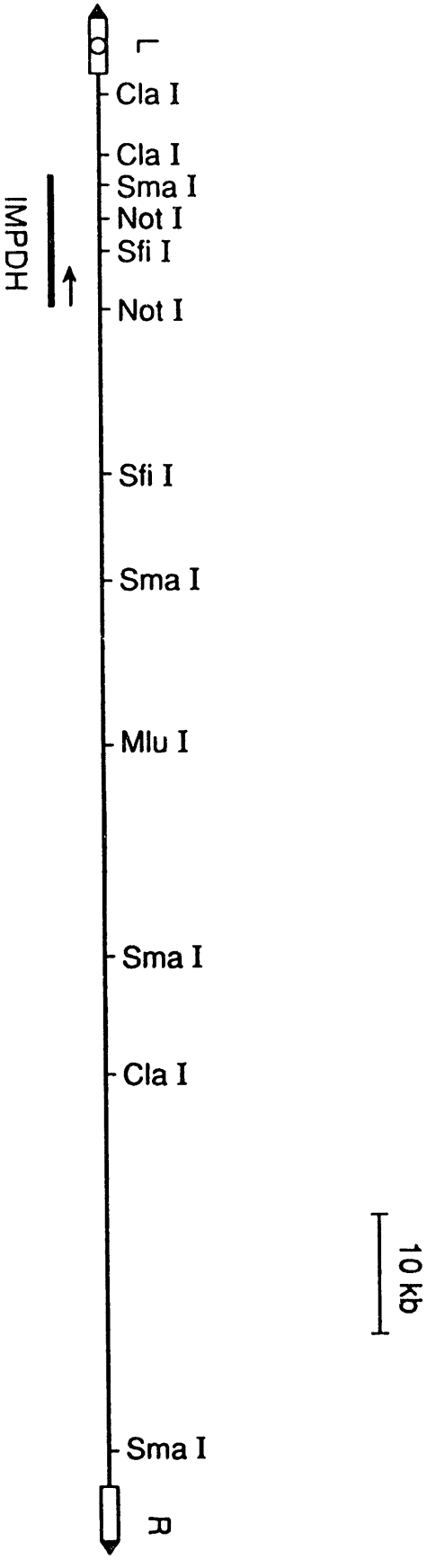
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