

Nucleotide sequence of murine purine nucleoside phosphorylase cDNA

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Murine purine nucleoside phosphorylase (PNP) has been cloned and sequenced. An ICR macrophage murine cDNA library (Clontech Laboratories) was screened with a 246 base pair (bp) RNA probe to the 3′ coding region of human PNP (1) (obtained from S.R.Williams). Seven clones were obtained, their sequence spanning 1161 bp. The deduced amino acid (AA) sequence of an open reading frame from bp 3 to 770 was found to be homologous to the human AA sequence, but the first 34 AA (100 bp) was missing. The 5′ end of the gene was obtained by the RACE procedure (2) using cDNA generated from polyA+ RNA isolated from C57BL/6J mouse liver (3) and sequenced. The fragment obtained included the 100 bp 5′ coding region and 77 bp of 5′ non coding sequence. The entire cDNA sequenced is 1337 bp with the PNP coding region spanning bp 77 to 946 of which the first 100 bp is C57BL/6J and the remaining ICR. The entire coding region of PNP was obtained for C57BL/6J by polymerase chain amplification (PCR) (4) using primers to the 5′ (bp 51−70) and 3′ (bp 1004−1025) non-coding sequences. Two clones were isolated from two separate PCR's and sequenced via the chain termination method (5).

Four alleles have been described at the Np-1 locus in the mouse (6, 7, 8). Mice of the C57 and C58 background have been designated Np-1^d, whereas most inbred strains are Np-1^a (7, 8). We observed six single base changes between the ICR and C57BL/6J sequence and only one of these, C to G, at bp 603 corresponded to an AA change, threonine to serine at residue 176. The characterization of the murine PNP cDNA enables the molecular analysis of mutations which have recently been recovered at the Np-1 locus in the mouse (9).

The murine sequence has a putative poly-adenylation signal at bp 1315 (AATAAA) which is 15 bp upstream of the poly A tail. This feature was not revealed in the human cDNA, which did not contain the entire 3′ non-coding region. Comparison of human and murine non-coding regions shows little or no homology. The deduced C57BL/6J mouse AA sequence is identical in length and 84.4% homologous to human PNP with a similarity of 89.6%. The murine and human coding sequences are 84.1% homologous.

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