Isolation of a full-length cDNA clone encoding a N-terminally variant form of the human retinoid X receptor β

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Retinoid X receptors (RXRs) and retinoic acid receptors (RARs) belong to a subgroup of the nuclear hormone receptor superfamily that is involved in retinoic acid mediated activation of target genes. The RXRs are highly conserved in the animal kingdom (1–5). A human RXRβ gene has recently been cloned from HELA cells (3). Here we report the isolation of a full-length cDNA clone encoding an N-terminally variant form of the human RXRβ. The gene was identified by nucleic acid screening of a cDNA library constructed from the human BURKITT lymphoma line DAUDI. A 561 base pair fragment of the murine RXRβ gene (H-2RIIBP, [1]) encompassing the zinc-finger coding region was used as a probe. Several positive clones, each 2.9 Kb in size, were identified. One of these clones, designated DAUDI.6, was shown to contain a large open reading frame that was preceded by two in-frame stop codons. Sequence analysis of this open reading frame predicted a 533 amino acid protein. The C-terminal region of this molecule from amino acid position 74 to the end was found to be perfectly homologous to the previously published human RXRβ (3). In contrast, the amino acid composition of the 74 N-terminal residues is quite distinct between the two human RXRβs (referred to as hRXRβ1 [DAUDI.6, this report] and hRXRβ2 (3), Figure 1) with a sequence homology of only 26.5%. Interestingly, the nucleotide sequence of this region is almost perfectly conserved between the two genes. The amino acid sequence diversity arises from various frame-shifts which are due to several insertions of one or 16 base pairs in the open reading frame of hRXRβ1 (Figure 1). Note that hRXRβ1 contains two additional in-frame ATG initiator codons which may serve as alternative translational start sites since they are located within a favorable Kozak motif (6). There is also one single base insertion in the 5′ untranslated region of the hRXRβ1. The authenticity of these results was confirmed by oligonucleotide typing of cDNA derived from DAUDI cells, HELA cells and a variety of other human cell lines. A fragment of the hRXRβ1 gene (residues 40 to 458) was PCR-amplified from the cDNA template and hybridized to oligonucleotide probes corresponding to the regions of sequence disparity (including the 16 base pair insertion). Under stringent washing conditions, this method can be used to detect single base mismatches. hRXRβ1 and hRXRβ2 may represent isoformes of the human RXRβ which differ only in their N-terminal sequences and have arisen from alternative splicing and/or differential promoter usage. The existence of such isoformes has previously been shown for RARα (7), β (8) and γ (9). The complete cDNA sequence of DAUDI.6 has been deposited at the EMBL database (Accession Numbers X63522 and X63523).

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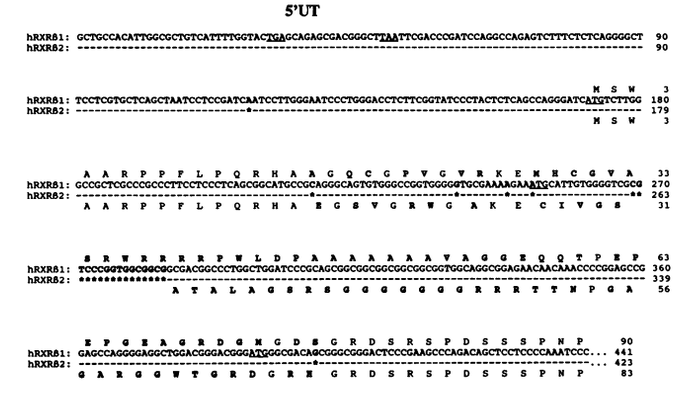

Figure 1. Alignment of the N-terminal nucleotide and amino acid sequence of the two hRXRβ isoformes. Only the 5′ untranslated region (5′UT) and the first 441 (hRXRβ1) or 423 (hRXRβ2) codons are shown. Two in-frame stop codons and three in-frame ATG start triplets are underlined. Position numbering is indicated on the right side of the sequence. Dashes indicate sequence identity, stars indicate gaps introduced in the hRXRβ2 sequence for optimal alignment. Residues differing between hRXRβ1 and hRXRβ2 are printed in bold. hRXRβ1 is the gene identified in this study; hRXRβ2 is the sequence identified previously (3).