Nucleotide Sequence of the Putative Human Tyrosinase Pseudogene

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TAKEDA, A., MATSUNAGA, J., TOMITA, Y., TAGAMI, H. and SHIBAHARA, S. Nucleotide Sequence of the Putative Human Tyrosinase Pseudogene. Tohoku J. Exp. Med., 1991, 163 (4), 295-297 —— We have cloned and sequenced the putative human tyrosinase pseudogene, which shares more than 98% nucleotide homology with exon 4 and exon 5 of the human tyrosinase gene including their flanking introns. Because of such a high homology, both the tyrosinase gene and its pseudogene could be amplified from genomic DNA by polymerase chain reaction. The nucleotide sequences presented thus enable us to discriminate the tyrosinase gene from its related sequences and are invaluable for a gene diagnosis of oculocutaneous albinism. —— melanin pigments; oculocutaneous albinism; inherited disease; gene diagnosis; chromosome 11

Tyrosinase (EC 1.14.18.1) is a bifunctional copper-containing enzyme responsible for the conversion of tyrosine to dihydroxyphenylalanine (DOPA) and DOPA to dopaquinone, and plays a key role in melanin biosynthesis. The human tyrosinase gene of greater than 70 kb is organized in five exons (Tomita et al. 1989; Takeda et al. 1990) and mapped to chromosome 11, region q14–q21 (Barton et al. 1988). The human genome contains the additional sequences cross-hybridizing to exon 4 and exon 5 of the tyrosinase gene (Takeda et al. 1989a), located on the short arm of chromosome 11 near the centromere (p11.2–cen) (Barton et al. 1988). Southern blot analysis of genomic DNA, prepared from three unrelated patients affected with tyrosinase-negative oculocutaneous albinism (OCA) and one normal individual, revealed that the exon 4 probe hybridized to two fragments of about 13- and 2.7-kb in the HindIII-digested DNA and the exon 5 probe hybridized to two fragments of about 6.8- and 1.8-kb in the BglII-digested DNA (Takeda et al. 1989a). We then confirmed that the 13 kb-HindIII and the 1.8 kb-BglII fragments contain the authentic exon 4 and exon 5 of the tyrosinase gene, respectively (Tomita et al. 1989; Takeda et al. 1990), suggesting that the 2.7 kb-HindIII and the 6.8 kb-BglII fragments represent the

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Abbreviations: OCA, oculocutaneous albinism; DOPA, dihydroxyphenylalanine; kb, kilobase pair(s); PCR, polymerase chain reaction.

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a cross-hybridizing sequences located on the short arm of chromosome 11 (Barton et al. 1988). Here we have cloned and sequenced the cross-hybridizing DNA segments, sharing more than 98% nucleotide homology with either exon 4 or exon 5 including their flanking introns of the human tyrosinase gene (Fig. 1A and B) (Shibahara et al. 1988; Takeda et al. 1989b, 1990). The amino acid sequence predicted from the putative exons of these DNA segments is identical to that of human tyrosinase (amino acid residues 377-511) except for a single amino acid at position 388 (codon 406) (Fig. 1A). Such a homology in the nucleotide sequences may cause a problem in analyzing the tyrosinase gene of patients affected with OCA by polymerase chain reaction (PCR), because point mutations in the tyrosinase gene could lead to a phenotype of OCA (Tomita et al. 1989; Takeda et al. 1990). Indeed, we were able to amplify both the tyrosinase gene and its related sequences from genomic DNA using oligonucleotide primers flanking the exon 4 or 5 of the tyrosinase gene. The nucleotide sequences presented thus enable us to discriminate the amplified DNA segments of the tyrosinase gene from those of its related sequences. Furthermore, since we were unable to detect the transcripts of these related sequences in the PCR products amplified from cDNA of human pigmented melanoma cells (data not shown), they may represent a pseudogene rather than a part of other functional genes.

Fig. 1. Nucleotide sequence of the putative human tyrosinase pseudogene. A, exon 4-related sequence; B, exon 5-related sequence. Putative exon regions are displayed in capital letters. Nucleotide sequences, different from the functional tyrosinase gene, are underlined. An asterisk indicates the base change causing the Pro (CCT)→Leu (CTT) substitution. An insertion of three nucleotides is double-underlined. The overline represents the NcoI site, which is absent in the tyrosinase gene.

References
1) Barton, D.E., Kwon, B.S. & Francke, U. (1988) Human tyrosinase gene, mapped to chromosome 11 (q11→q21), defines second region of homology with mouse...


