Molecular Basis for the Heterogeneity of Human Tyrosinase

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Molecular Basis for the Heterogeneity of Human Tyrosinase. Tohoku J. exp. Med., 1988, 156 (4), 403-414 — A cDNA clone, pH\(\gamma\)1, representing human tyrosinase mRNA was isolated by screening a melanoma cDNA library with a synthetic oligonucleotide complementary to a segment of the human tyrosinase cDNA, Pmel 34 [Kwon et al. (1987) Proc. nat. Acad. Sci. USA 84, 7473-7477]. However, there are a number of differences in the nucleotide sequence between two cDNAs, pH\(\gamma\)1 and Pmel 34, particularly in the region coding for the carboxyl terminus of the enzyme (putative exon 5). We therefore cloned the genomic DNA segment carrying the exon 5 of the human tyrosinase gene by screening a human placental genomic DNA library with a cloned cDNA probe. The nucleotide sequences of human tyrosinase cDNA as well as a part of its gene were determined. Mature human tyrosinase is composed of 511 amino acids with a molecular weight of 58,000. We provide evidence for the presence of at least two species of human tyrosinase mRNA generated by alternative splicing in human pigmented melanoma cells. ——— tyrosinase; melanin pigment; cDNA; gene; alternative splicing

Tyrosinase (EC 1.14.18.1) is a multi-functional copper-containing enzyme that is essential for melanin biosynthesis in pigment cells (Lerner et al. 1950). It catalyzes the conversion of tyrosine to dihydroxyphenylalanine (dopa), and dopa to dopaquinone, which are referred to as tyrosine hydroxylase activity and dopa oxidase activity, respectively (Pomerantz 1966). Previous studies showed by polyacrylamide gel electrophoresis the presence of three or four different forms of tyrosinase (Shimao 1962; Burnet et al. 1967; Pomerantz and Li 1973; Hearing et al. 1981). However, no information is available whether such a heterogeneity of tyrosinase resides in the gene level.

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We have isolated a pigment cell-specific mouse cDNA, pMT4, and provided evidence that it encodes tyrosinase (Shibahara et al. 1986). Recently, other groups have isolated and sequenced another cDNAs encoding mouse tyrosinase (Yamamoto et al. 1987; Kwon et al. 1988; Müller et al. 1988) and a cDNA for human tyrosinase, Pmel 34 (Kwon et al. 1987). Furthermore, it was shown that the mouse tyrosinase cDNA, pmcTyr1 (Müller et al. 1988), encodes a protein possessing tyrosine hydroxylase activity, namely functional tyrosinase. The amino acid sequence deduced from the nucleotide sequence of pmcTyr1 shows about 40% homology with the entire region of mouse tyrosinase deduced from pMT4 (Shibahara et al. 1986). It is therefore conceivable that there may be at least two tyrosinase isozymes encoded by two distinct genes, and that the enzyme protein encoded by pMT4 may possess dopa oxidase activity instead of tyrosine hydroxylase activity. In this report, however, we have termed the protein encoded by Pmel 34 (Kwon et al. 1987) or pmcTyr1 (Müller et al. 1988) as tyrosinase.

Comparison of mouse and human tyrosinase reveals that the two enzymes are about 80% identical except for the carboxyl termini (Kwon et al. 1987; Müller et al. 1988). In spite of the differences in the deduced amino acid sequences of carboxyl termini between human and mouse tyrosinase, the nucleotide sequences encoding the carboxyl termini have about 75% homology. Furthermore, the molecular weight (Mr) of mature human tyrosinase deduced from Pmel 34 is about 63,000 (Kwon et al. 1987), which appears too big for human tyrosinase of Mr 58,000 (Nishioka 1978). We therefore wanted to reexamine the nucleotide sequence of human tyrosinase cDNA. Since our aim is to clarify the molecular basis of human albinism, it is of vital significance to know the correct amino acid sequence of tyrosinase. Consequently, we cloned and sequenced the cDNA encoding human tyrosinase, and deduced its amino acid sequence. Furthermore, we present evidence that there are at least two mRNA species for human tyrosinase, which may be generated by alternative splicing. Our observations could provide the molecular basis for the heterogeneity of tyrosinase.

**Materials and Methods**

**Preparation of RNA**

Total RNA was prepared from MeWo human melanoma cells (Brüggen et al. 1981). Poly (A)+ RNA was isolated by oligo(dT)-cellulose chromatography (Aviv and Leder 1972).

**Isolation of human tyrosinase cDNA clone and genomic clone**

A cDNA library was constructed by the method of Okayama and Berg (1982) using 2 µg of MeWo melanoma poly(A)+ RNA, and screened with the 32P-end-labeled synthetic oligonucleotide that is complementary to the nucleotide residues 244/276 of Pmel 34 (Kwon et al. 1987). This region was chosen because of a good similarity between the human and mouse cDNA sequences (Kwon et al. 1987; Yamamoto et al. 1987). The human genomic DNA library used was a collection of recombinant phage that contain human placental DNA fragments generated by partial digestion with Sau3AI and joined to EMBL3 arms. The
hybridization probe was the \(^{32}\text{P}\)-labeled \text{PvuII}/\text{NdeI} fragment (931/1794) derived from \text{pHT}\(_1\) (see Fig. 1). Nucleotide sequences were determined by the method of Maxam and Gilbert (1980).

**RNA blot hybridization**

MeWo RNA and HeLa cell RNA were subjected to electrophoresis as described previously (Shibahara et al. 1986), transferred to a nitrocellulose filter, and hybridized with \(^{32}\text{P}\)-labeled fragment of human tyrosinase cDNA. The DNA probe used in this study was labeled with \([\alpha-^{32}\text{P}]\text{dCTP}\) by the method of Feinberg and Vogelstein (1983), and is indicated in the figure legend. The markers were human rRNA.

**S1 nuclease-mapping analysis**

The S1 probe was the PstI/BglII fragment (vector/1314) derived from the \text{pHT}\(_1\) (see Fig. 1). The PstI site is located in the Okayama-Berg vector (1982). The probe was hybridized to total RNA (20 \(\mu\text{g}\)) prepared from MeWo melanoma cells or HeLa cells, and digested with S1 nuclease as described (Müller et al. 1987).

**Reagents**

Restriction endodeoxyribonucleases were obtained from Bethesda Research Laboratories (Rockville, MD, USA), Pharmacia (Uppsala, Sweden) and Boehringer (Mannheim, FRG), oligo (dT)-cellulose type 7 and Okayama-Berg vector from Pharmacia. EMBL3 arms, \([\alpha-^{32}\text{P}]\text{dCTP}\) (specific activity, 3,000 Ci/m mole) and \([\gamma-^{32}\text{P}]\text{ATP}\) (specific activity, 5,000 Ci/m mole) were purchased from Amersham International (Buckinghamshire, UK).

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**Fig. 1.** Restriction map and sequencing strategy of cloned cDNA, \text{pHT}\(_1\), encoding human tyrosinase. The restriction map shows only relevant sites. The protein-coding region is indicated by an open box, in which the putative transmembrane segment is stippled. The closed box indicates the putative signal peptide. The arrows indicate the direction and extent of sequence determinations. The short vertical lines and the open circle at the end of arrows indicate the sites of 5'-end labeling located in the cDNA and the vector DNA, respectively. The location of an intron (putative intron 4) is indicated by a triangle. The S1 probe used in Fig. 5 is also shown.
RESULTS AND DISCUSSION

cDNA cloning of human tyrosinase

From $2 \times 10^4$ transformants of MeWo human melanoma cDNA library, we have isolated three hybridization-positive clones and characterized the longest cDNA clone, pH\(\gamma\)1 (Fig. 1). The pH\(\gamma\)1 is not a full-length cDNA, but contains the entire protein-coding region for mature tyrosinase. The size of human tyrosinase mRNA was estimated by blot hybridization analysis of total RNA prepared from MeWo human melanoma cells (Fig. 2, lane 1). The tyrosinase mRNA is about 2,000 nucleotides long, and is not detectable in HeLa cells (lane 2), suggesting that this mRNA is pigment cell-specific.

Nucleotide sequence of human tyrosinase cDNA

The nucleotide sequence of the message strand of the human tyrosinase cDNA, pH\(\gamma\)1 is shown in Fig. 3. The pH\(\gamma\)1 lacks the region near 5'-terminus of its mRNA including the initiating methionine. However, based on the nu-

![Fig. 2. Autoradiogram of blot-hybridization analysis of human tyrosinase mRNA. Each lane contained 8 \(\mu\)g total RNA prepared from MeWo melanoma cells (lane 1) and HeLa cells (lane 2). The hybridization probe was the \(^{32}\)P-labeled HpaII/HpaII fragment (213/1248) derived from pH\(\gamma\)1. The size markers were human rRNA.](image-url)
cleotide sequences of human (Kwon et al. 1987) and mouse (Yamamoto et al. 1987; Müller et al. 1988) tyrosinase cDNAs as well as of mouse tyrosinase gene (Ruppert et al. 1988), we tentatively assign the CAT codon (nucleotide residues 39/41) as the first amino acid residue of the mature human tyrosinase. The synthetic oligonucleotide used as a hybridization probe is complementary to the nucleotide residues 269/299 of the pHTγ1, Fig. 3). The translation termination codon TAA is present in the assigned reading frame at position (1572/1574) and is followed by about 300 nucleotides of the 3′-untranslated region.

There are a number of differences in the nucleotide sequences of the cDNAs encoding human tyrosinase, pHTγ1 and Pmel 34 (Kwon et al. 1987) (marked in Fig. 3). For example, two deletions are present in the protein-coding region near amino-terminus of Pmel 34. The G residue at the position 108 and the CC dinucleotide at the positions 118/119 of pHTγ1 are deleted in Pmel 34. These deletions present in Pmel 34 resulted in frame shifts but only in small regions. The sequence of mouse tyrosinase cDNA pmcTyr1 (Müller et al. 1988) favors the sequence of pHTγ1. Furthermore, there are two base changes (C residue at 559 and G residue at 907 of pHTγ1), which also result in amino acid substitutions. In the putative exon 3 region indicated by a dotted underline in Fig. 3, the Pmel 34 contains four deletions of single nucleotide (nucleotide residues 1101, 1104, 1120 and 1148 of pHTγ1) and one insertion of the G residue (1152 of Pmel 34), which therefore caused frame shifts only in short regions. Furthermore, there are two base changes (1135 and 1189 of pHTγ1), which also resulted in amino acid substitutions. These nucleotide differences are of particular significance, because these regions code for the putative copper-binding site (see Fig. 4). The nucleotide sequence of mouse tyrosinase cDNA, pmcTyr1 (Müller et al. 1988) reveals the deletion of 148 nucleotides in the aberrant mouse tyrosinase cDNA, Tyrs-33 (Yamamoto et al. 1987) (Fig. 3), and always favors the sequence of pHTγ1. Recently, Kwon et al. (1988) corrected a part of the sequence errors in human tyrosinase cDNA, Pmel 34 (Kwon et al. 1987) and their corrected sequence is identical to the sequence of pHTγ1 (nucleotide residue 1135/1180).

In the 3′-region, there are two deletions of single nucleotide (1644 and 1667 of pHTγ1), two insertions of single nucleotide (1515 and 1583 of Pmel 34) and one base change (1468 of pHTγ1). The most critical difference is the insertion of A residue (nucleotide residue 1515 of Pmel 34), which caused a frame shift and changed the deduced amino acid sequence of the entire carboxyl terminus. This frame of Pmel 34 thus codes for a longer peptide. In order to confirm the nucleotide sequence of the pHTγ1, we have isolated a genomic clone harboring the 3′-end of the pHTγ1 (nucleotide residues 1351/1866, putative exon 5) and determined its nucleotide sequence. Sequence analysis of the cloned gene reveals that the location of the intron (putative intron 4) is between the nucleotide residues 1350 and 1351 of the pHTγ1 (indicated by a vertical line in Fig. 3). The nucleotide sequence of the putative exon 5 is same as that of pHTγ1. Moreover,
TACTGCCCTGCTGGAGTTTCCACACCTCCCGCTGGCCATTTCCCTAGACCGCTGTCTCC
TyrCysLeuLeuLeuTrpSerPheGlnThrSerAlaGlyHisPheProArgAlaCysValSer
-11
63 TCTAGAAGCTGAGGAGAAGGAGTCTGCTACCAGGTCGGAGGGGACAAGGAGCTCCGG
SerLysAsnLeuMetGluLysGluCysProProTrpSerGlyAspArgSerProCys
10
123 GCCGACGCTCTAGAGGATCTGCAATATCTTCTGCTTACTGGAACACTCTTGG
GlyGlnLeuSerGlyArgGlySerCysGlnAsnIleLeuLeuSerAsnAlaProLeuGly
30
183 CCTCAAATTTCTCTCCACAGGGGTGAGTCGGTTCCTCGCCCTTTGATATA
ProGlnProProPheThrGluValAspAspArgGluSerThrProValPheThrAsn
50
243 AGGCATCCTGAGCTGCTGGAACACTCTATGGAGATCTGGAAGATCTGCAATCTTG
ArgThrCysGlnCysSerGlyAsnProMetGlyPheAsnCysGlyAsnCysLysPheGly
70
303 TTGCGAGGACCAAATCTGCAAGGACTCCTTGGTGAAGAAGACACTTCTGG
PheThrGlyProSerCysThrGluArgLeuValArgAsnIleAsnIleAspProLeu
90
363 AGTTCCCGAGGACAGAATTTTTTTTGGCTACACCTTACTATTACAAAGCATACCATACCA
SerAlaProGluLysAspLysPheAspPheAsnLeuThrLeuThrLeuAlaYseHisThrIleSer
110
423 TCAGACTATGTCCACCCATAGGGAGATCCATGCGGACACAAATGAAATGAGCAACACCCTG
SerArgThrValIleProIleGlyThrThrGlyGlnMetLysGluSerGlySerProAspMet
130
483 TTTAACGACATATATTATTGACCTCTCTGTCCTGAGGTCAATTATATTGTTGCAATGAT
PheAspIleAsnIleAspProLeuPheValThrProMetHisThrThrValSerMetAsp
150
543 GCACTTGCGGCTGGACAGGACAGATGTTGTTGCCATAGGACAGACACTCAGCATCAG
AlaLeuLeuGlyGluThrAspArgIleSerAsnProPheAspAlaHisGluAlaProAla
170
603 TTTCGCGCTTGGCATGACTCCTTTCTCTCTGTTGCGGAGGACAAAACCAAACTGACCCTG
PheLeuProTrpHisArgLeuPheLeuLeuArgTrpGluGlnIleIleGlnLeuThr
190
663 GGAGATGAAACCTCAGATTCCATATTGGGACCTGCGGCGGACAGAAAGATGTGACATT
GlyAspGluAsnProProPheProAspArgPheAsnAspIleGluSerProAspIleGluAsnSerProGlu
210
723 TGCCAGCATGAGTGCACTGGAGCTGACCAAGGCAAATCTACCTACTGACGCCAGCA
CysThrAspGluMetGluGlnHisProProAsnProAsnLeuLeuLeuLeuSerProAla
230
783 TCATTCTCCTCTCCTGCGGAGATGCTGSGCTGACGAGGATCAACAGCCACACTG
SerPhePheSerSerTrpGlnIleValCysSerArgLeuGluThrAsnSerHisGln
250
843 TCTTTATGCGACATGGGACCCCGGGAGGACTTTTTACCGGCTAAATCCCTGGAACAGAAA
SerLeuCysAsnGlyThrProGluProArgAspArgProGluAspGlyAsnHisAspLys
270
903 TCCAGACCAAGCCGCTCCCCCTCTGATGTTGAAATTTTGGCTGAGTGTGCACAAA
SerArgThrProArgLeuProSerSerAlaAspValPheCysLeuLeuSerLeuSer
290
963 TGAATCCTGCGCTTCTGCGATGCTGAGTATACTGCGGAGCTGCGCGAGCATGCAGCTGCTG
TyrGluSerGlySerAspLysAlaAsnAspPheSerArgThrLeuGluGly
310
1023 TTGCTGATGCTCTCTACTGAGTGGCTGAGTGGACCTCTTACGACGACGACATGCTGCTG
PheAlaSerProLeuThrGlyIleIleSerAspAspSerGluSerGlySerLeuAsnAspProIlePhe
330
1083 CACATCTATGAAATGGGCAACTGCCCCAGGATCAGGACTTGGCAGCTACAGACATGCTG
HisIleThrMetAsnGlyThrMetSerGlnValGlnGlySerAlaAsnAspProIlePhe
350
1143 CTCTCTCAGCTCATTTGTGACATATTTTTGAGACGTCGGTTCAGGACAGGACGGCATCAG
LeuLeuHisHisAlaAspPheValIlePheGluGlnTrpLeuArgArgHisArgPro
370
380
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The deduced sequence of the first 18 amino acid residues of the mouse tyrosinase (Müller et al. 1988) has a feature characteristic of the signal peptide of the nucleotide sequences of mouse tyrosinase cDNAs, MTY811 and pmcTyr1 favor the sequence of pHTγ1. Blot hybridization analysis of human placental DNA suggested that there is a single gene for human tyrosinase (data not shown). It is therefore unlikely that the differences in the sequence of pHTγ1 are caused by cloning artifacts, such as errors of reverse transcription.

Amino acid sequence of human tyrosinase

The deduced sequence of the first 18 amino acid residues of the mouse tyrosinase (Müller et al. 1988) has a feature characteristic of the signal peptide of
secretory and membrane-associated protein. Although both human tyrosinase cDNAs, Pmel 34 (Kwon et al. 1987) and pHTγ1, lack the 5’-end of mRNA, comparison of the nucleotide sequence of Pmel 34 with mouse tyrosinase cDNAs (Müller et al. 1988) suggests that Pmel 34 may lack only one amino acid residue, namely initiating methionine. We therefore assume that a possible cleavage site for a signal peptide is after the glycine residue at position −1 (Fig. 3). Mature

| 1 | HFPRACVSSKNLMEKECCPPWSGDRSCGCQLSGRGSCQNLILLSNAFLQPFVFPGYVGDRE |
| 2 | HFPRACASSKNLLKECCPPQMDGSGCQQLSGRGSCQDIILLSSAPDPQFFPGYVGDRE |
| 61 | SWPSVFYNTCQCSNFMNCGCNGKCFGWPGQCETERRLLYVRNIFDLSAPEKDFAYL |
| 121 | TLAKHTISSDYVIPGTYQKMGSTPMDNINTYLDLFWHVHYVSMADALLGGSEIWRDI |
| 181 | DFAHEAPAPLPWRLFFLYWEQEIQLIIKLTGDENFTIPYWDWRDAEKDICTDEYMGQHP |
| 241 | NPNLLSPASFFSWQYICRLEYNHQSLNGTPEGPLRNPYNKDSRTTPRPLLPSADV |
| 301 | EFCLSLTQYESGMDKAANFSRNTLEQASPLGTQADASQSMXNAHTYMNQTMSQV |
| 361 | GSANPPIFLLLLHAFVDSIEQWLRRHRPQEVEANAPIGHNRRESYMVFIPRYNGDF |
| 421 | FISSKDLGDSYLDSPDSQFDYIKSYLEQASRIWSWGLGAAMVAGATALLAGVSL |
| 481 | LCRXKR..QLPEEQPQPLMEKECXYL.YQSHL |

Fig. 4. Homology in the amino acid sequences of human and mouse tyrosinase.

The sequences are shown by the single-letter code. The top line indicates the mature human tyrosinase deduced from the nucleotide sequence of pHTγ1, and the bottom line indicates the mouse tyrosiase deduced from pmcTyr1 (Müller et al. 1988). The asterisk indicates the identical amino acids found in both sequences; (.) the chemically similar amino acids. Putative copper-binding regions are indicated by underlines.
human tyrosinase is thus composed of 511 amino acids of Mr 58,000; this value is similar to that reported for the purified human tyrosinase (Nishioka 1978) as well as those for mature mouse tyrosinase deduced from MTY 881 (Kwon et al. 1988) and pmcTyr1 (Müller et al. 1988). Human tyrosinase also contains a putative transmembrane segment near the carboxyl terminus (residues 456-481) as indicated by an underline in Fig. 3.

The alignment of the amino acid sequences of human and mouse tyrosinase is shown in Fig. 4. Human tyrosinase is shorter than mouse enzyme by four amino acids at the carboxyl terminus. The degree of sequence homology is about 86%. Human tyrosinase contains 17 histidine residues, and all of them are also conserved in mouse enzyme (Müller et al. 1988). Recently, Lerch (1988) compared the amino acid sequences of bacterial, fungal and mouse tyrosinase and indicated two regions as potential copper-binding sites, which is also supported by a structural similarity between tyrosinase and hemocyanins, the copper-containing dioxygen carriers (R. Lontie, personal communication). Based on these comparisons, we tentatively assign two regions, amino acid residues 154-220 and 343-385, as the copper-binding sites of human tyrosinase (Fig. 4), which could provide the ligands of the first copper atom, designated as Cu(A), and the second copper atom, Cu(B), respectively (Lerch 1988). In each copper-binding site, three or four histidine residues are present in both human and mouse tyrosinase. It is therefore conceivable that some of these histidine residues may serve as the ligands of two copper of tyrosinase (Nishioka 1978).

Mature human and mouse tyrosinase contain 16 cysteine residues at equivalent positions (Fig. 4). Ten cysteine residues are present within the 100 residues at the amino-terminus. Five cysteine residues are clustered between two copper-binding regions. There are six potential N-glycosylation sites (Marshall 1974; Bause 1983) conserved in both human and mouse tyrosinase (asparagine residues 68, 93, 143, 212, 319 and 353). Since human tyrosinase contains four asparagine-linked sugar chains per molecule (Ohkura et al. 1984), some of these residues are the functional N-glycosylation sites.

Presence of aberrant species of human tyrosinase mRNA

The aberrant mouse tyrosinase cDNA, Tyrs-33 (Yamamoto et al. 1987) lacks 148 nucleotides, which are the entire sequence of the exon 3 of the mouse tyrosinase gene (Ruppert et al. 1988). The deleted sequence of the Tyrs-33 corresponds to the nucleotide residues 1021/1168 of the pHTγ1 (Fig. 3, indicated by a dotted underline). It is therefore conceivable that mouse tyrosinase cDNA, Tyrs-33, is derived from an aberrant mRNA generated by alternative splicing of the primary transcripts as reported (Müller et al. 1988; Ruppert et al. 1988). The mouse tyrosinase deduced from Tyrs-33 (Yamamoto et al. 1987) thus lacks a putative Cu(B)-binding region encoded by the exon 3 (Ruppert et al. 1988). We therefore assumed that the locations of introns in the human tyrosinase gene are
Fig. 5. Presence of aberrant species of human tyrosinase mRNA. The presence of aberrant mRNA was analyzed by S1 mapping. The S1 probe is shown in Fig. 1. The size markers are end-labeled φX174 DNA fragments (M1) and pUC8 DNA fragments (M2) (see Materials & Methods) and given in base pairs. The arrow indicates the protected fragments. Lane A, control with HeLa RNA and lane B, with MeWo RNA.

between the nucleotide residues 1020 and 1021, and 1168 and 1169 of pHTγ1 (indicated by dotted vertical lines in Fig. 3) and analyzed RNA prepared from MeWo human melanoma cells in order to confirm the presence of such an aberrant tyrosinase mRNA. The S1 probe was the PstI/BglII fragment (vector/1314) shown in Fig. 1 and the BglII site is located about 150 nucleotides downstream from the putative intron/exon junction (Fig. 3). The results showed protected fragments of about 150 nucleotides (Fig. 5), indicating that the sequence of some tyrosinase mRNA is different around the nucleotide residue 1168 of the pHTγ1. Since we used total RNA, it is possible that our RNA preparation contains heterogenous nuclear RNA prior to splicing. The protected fragments therefore may represent such unspliced RNAs. Alternatively, the results may indicate the presence of aberrant RNAs like the one for mouse tyrosinase cDNA, Tyrs-33 (Yamamoto et al. 1987). Considering all these observations, we propose that there are at least two distinct species of human tyrosinase mRNAs, which are
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generated by alternative splicing. Since such aberrant tyrosinase encoded by Tyrs-33 lacks the Cu(B)-binding site, it may not be functional. Further studies are required to identify the physiological role of such aberrant tyrosinase.

Recently, we have confirmed that the tyrosinase gene is present and its overall structural organization seems to be unchanged in a patient with oculocutaneous albinism (Tomita, Tagami and Shibahara, unpublished observations). We are currently attempting to isolate and characterize the tyrosinase gene of this patient. The sequence data presented in this paper will enable us to look for the mutations in the tyrosinase gene of albino patients.

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References


