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Characterization of the human rod transducin alpha-subunit gene

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ABSTRACT

The human rod transducin α subunit ($T_{r\alpha}$) gene has been cloned. A cDNA clone, HG14, contained a 1.1 kb insertion when compared with the human $T_{r\alpha}$ cDNA published by Van Dop *et al.* (1). Based on two overlapping clones isolated from a human genomic library, the human $T_{r\alpha}$ gene is 4.9 kb in length and consists of nine exons interrupted by eight introns. Northern blots of human retina total RNA showed that the gene is transcribed by rod photoreceptors into two species of mRNA, 1.3 kb and 2.4 kb in size. Apparently, this is the result of alternative splicing. Two putative transcription initiation sites were determined by primer extension and S1 nuclease protection assays. The putative promoter regions of the human and mouse $T_{r\alpha}$ genes have an identity of 78.1%. As found in the mouse gene (2), no TATA consensus sequence is present in the human gene.

INTRODUCTION

Transducins belong to a family of guanine nucleotide-binding proteins (G proteins) that play major roles in signal transduction (3, 4). The G proteins involved in the transmembrane signaling processes have three subunits (α, β and γ) and share common features in their mechanisms of action (3, 4, 5, 6, 7). Many G proteins have been identified and characterized in mammals including human, the major differences reside mainly in the α subunit. The amino acid sequences engaged in guanine nucleotide binding and GTP hydrolyzing activities, are found to be highly conserved (4, 7, 8). The β and γ subunits are either very similar or identical. It is interesting to note that the intron-exon organization of the mouse $T_{r\alpha}$ gene (2) is identical with the α-subunit genes of human G_o, G_i2, and G_i3 (2, 4).

It is not clear how many different G proteins are required to carry out the various signaling systems in mammalian cells. Rod and cone photoreceptors of bovine and human retinas each express a different transducin α-subunit (9, 10, 11, 12, 13), and share a common mechanism of visual transduction (14, 15, 16, 17).

In this report, the human $T_{r\alpha}$ gene and its 5′ flanking region have been isolated, and sequenced. The two sizes of transcripts for the human $T_{r\alpha}$ gene detected by Northern blot (13) appear to be derived by alternative splicing. The transcriptional initiation sites have also been determined. Significant identity between the human and mouse transducin α-subunit genes in the 5′ flanking region has been demonstrated. These results provide the basis for future studies on the regulatory elements that are involved in the expression of transducin α-subunit genes in normal and diseased human eyes.

MATERIALS AND METHODS

Isolation of cDNA And Genomic Clones for Human $T_{r\alpha}$

A 29-base oligomer complementary to residues 188–216 of the bovine $T_{r\alpha}$ cDNA sequence (9) was used to screen a human retina λgt10 cDNA library (kindly provided by Dr. J.Nathans). About 130 phage plaques out of 50,000 showed positive hybridization during the primary screening. Two different but overlapping clones (HG3 and HG14) were purified and characterized by sequencing. Both clones encoded human $T_{r\alpha}$. The cDNA HG3 was then used as a probe to screen a human leucocyte genomic library in EMBL3 (Clontech, Palo Alto, C-A). Thirteen phage plaques out of one million were positive. Two of these clones, HGLG4 and HGLG11, were characterized and are reported in this paper.

Subcloning and Sequencing

The cDNA clones were digested with *Eco* RI and subcloned into M13mp19. The genomic clones HGLG4 and HGLG11 were excised from the EMBL3 vector with *Sal* I. Restriction fragments generated by *Bam* HI, *Stu* I and *Bgl* I digestion were then subcloned into pUC and M13 vectors (18). Sequencing was performed by the dideoxy chain termination method (19) using Sequenase (United States Biochemical Corp,. Cleveland, OH) and the procedure of Dale (20). Some gaps were sequenced by using synthetic oligonucleotide primers.

RNA preparation

Human retina total RNA was prepared according to Chirgwin *et al.* (21). Poly (A^+) RNA was prepared from 100 mg of retinal tissue from individual donors by using the Micro-Fast Track mRNA isolation kit (Invitrogen Co., San Diego, CA).

Northern Blot Analysis

Approximately one half of the poly (A^+) RNA prepared from 100 mg of retinal tissue was loaded on a 1.2% agarose gel in

MOPS (3-[N-morpholino]-propanesulfonic acid) buffer containing formaldehyde and transferred to Nitroplus 2000 membrane (Micron Separations Inc., Westboro, MA). The blot transfer was hybridized at 42°C in the presence of 50% formamide with HG3 cDNA labeled with ^{32}P by random priming (Boehringer Mannheim, Indianapolis, IN).

Primer Extension Analysis

A synthetic 17-base oligonucleotide complementary to residues 119 to 135 of the cDNA sequence published by Van Dop *et al.* (1) was used as a primer. After end-labeling with T4 polynucleotide kinase (Bethesda Research Lab., Gaithersburg, MD), this oligonucleotide was annealed for 1 hour at 55°C to 30 μg of human retina total RNA. The extension reaction was carried out at 42°C for another hour using reverse transcriptase in the presence of actinomycin D (20 ng/μl) (22). The extended product was analyzed on an 8% denaturing polyacrylamide gel.

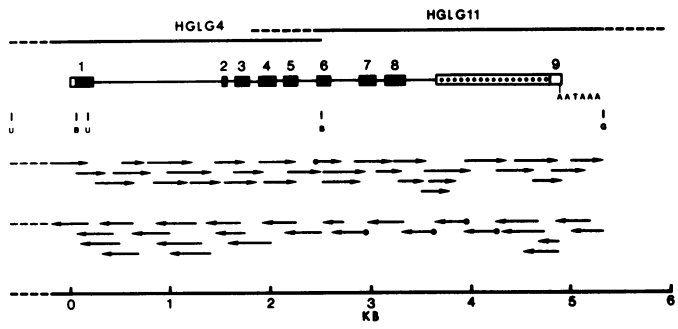

Fig. 1. Sequencing strategy for the human $T_{r\alpha}$ gene. The two overlapping clones (HGLG4 and HGLG11) span the full length of human $T_{r\alpha}$ gene. The exons are labeled 1 through 9 and are shown as bars. The introns are shown as lines. Dashed lines are uncharacterized regions. Blocked exon segments correspond to coding regions, open exon segments correspond to nontranslated regions. The region corresponding to the dotted bar is not present in the 1.3 kb mRNA. Both mRNA species use the same polyadenylation signal, AATAAA. B, *Bam* HI; U, *Stu* I; G, *Bgl* I. Arrows indicate direction (5′ to 3′) and extent of sequencing. Solid circles at the beginning of some arrows show regions where synthetic primers were used. The open circle at the beginning of one arrow indicates that the sequence was obtained from a *Sma* I fragment of the genomic clone HGLG11.

S1 Nuclease Protection Analyses

The method was based on that of Ausubel *et al.* (23). The ^{32}P-labeled synthetic oligonucleotide used for primer extension was hybridized to a single stranded M13 clone carrying a 2.1 kb *Stu* I fragment from the genomic clone HGLG4. The hybrid was extended with Klenow fragment and then digested with *Hpa* II. The single-stranded radiolabeled probe, defined at its 3′-end by *Hpa* II digestion, was isolated on an alkaline agarose gel and hybridized (5×10^4cpm) with 30 μg of human retina total RNA. One thousand units of S1 nuclease were then added and the cleavage reaction was carried out at 15°C for one and half hours. The size of the protected probe was determined on an 8% denaturing polyacrylamide gel.

Computer Analyses and Sequence Comparisons

Sequences were analyzed with Beckman MicroGenie software on an IBM PCXT computer.

RESULTS

Isolation and Characterization of the Human $T_{r\alpha}$ Gene

The cDNA clone HG3 contained 976 nucleotides that were identical with residues 181−1156 of the $T_{r\alpha}$ cDNA sequence published by Van Dop *et al.* (1). The cDNA clone HG14 consisted of 2389 nucleotides that included a 16-base poly (A^+) tail. It differs from Van Dop *et al.*'s (1) sequence in that the clone HG14 contained a 1134-base insertion between residues 1157 and 1158 of the published cDNA (1).

Among the thirteen positive genomic clones obtained by screening a human genomic library with ^{32}P-labeled HG3, two overlapping clones, HGLG4 and HGLG11, encoded the entire $T_{r\alpha}$ gene. The strategy used to sequence those two clones is summarized in Fig. 1. The complete $T_{r\alpha}$ gene sequence is shown in Fig. 2, and the 5′ flanking sequence is shown in Fig. 3.

Table 1. Exons and introns of the Human Tra gene[a]. This table should be read from the left to the right, continuing to the next line. The first column shows the number of the exon, followed by its size, its last six nucleotides, the first six nucleotides of the subsequent intron, the number of this intron, its size, the complete 3′ sequence of the intron between the putative branch point consensus and the first six nucleotides of the next exon. Putative branch sites, conforming to the consensus YNYYRAY (Y, pyrimidine; R, purine; N, any nucleotide) (31), were identified within a region of 18−40 bp upstream of the 3′ intron-exon boundaries. Possible branch sites are underlined.

Exon			Intron				Exon
No.	Size (bp)	5′-splice donor	No.	Size (bp)	Branch site	Polypyrimidine tract	3′-splice acceptor	No.
1	222	TTCTGG gtaggg	1	1301	ctctgag	----------gcgccgcgtctctttcag	GTGCCG	2
2	43	GATGAA gtgagt	2	89	cgctgat	---------tctgctctcctcggcctcag	GATTAT	3
3	142	CGCCAG gtgtgc	3	99	ccgccaccagccac	-----tctcaccctgcccccag	GACGAC	4
4	158	GGGCTA gtgagc	4	85	cacccacctac	--------ggccgggtctcgcgcag	CTACCT	5
5	129	CTTCCG gtacga	5	210	ggcgcaggttcag	-----gccccgcggccccgcag	GATGTT	6
6	130	GAAGTG gtgcgt	6	279	gcccaac	----------agctgctgccctcctcag	AACCGC	7
7	154	ACGATG gtgaga	7	110	ggctgagcagagtgag	---agctcccgcccccgcag	GACCCA	8
8	192	TCTGAG gtaggt	8	310	cagggatgctgac	-----tgggtttccttttcacag	GTGCCT	
9	1249	Poly (A) site						
8	192	TCTGAG gtaggt	8a	1444	tcctcatccagcaccaaatctttgcttatgtcacag		GCCAGG	9
9a	115	Poly (A) site						

[a]Two kinds of mRNA coding for Tra were identified. The differences reside at the 8th intron and 9th exon. The 8th intron and 9th exon for the formation of 1.3 kb mRNA are indicated by 8a and 9a respectively.

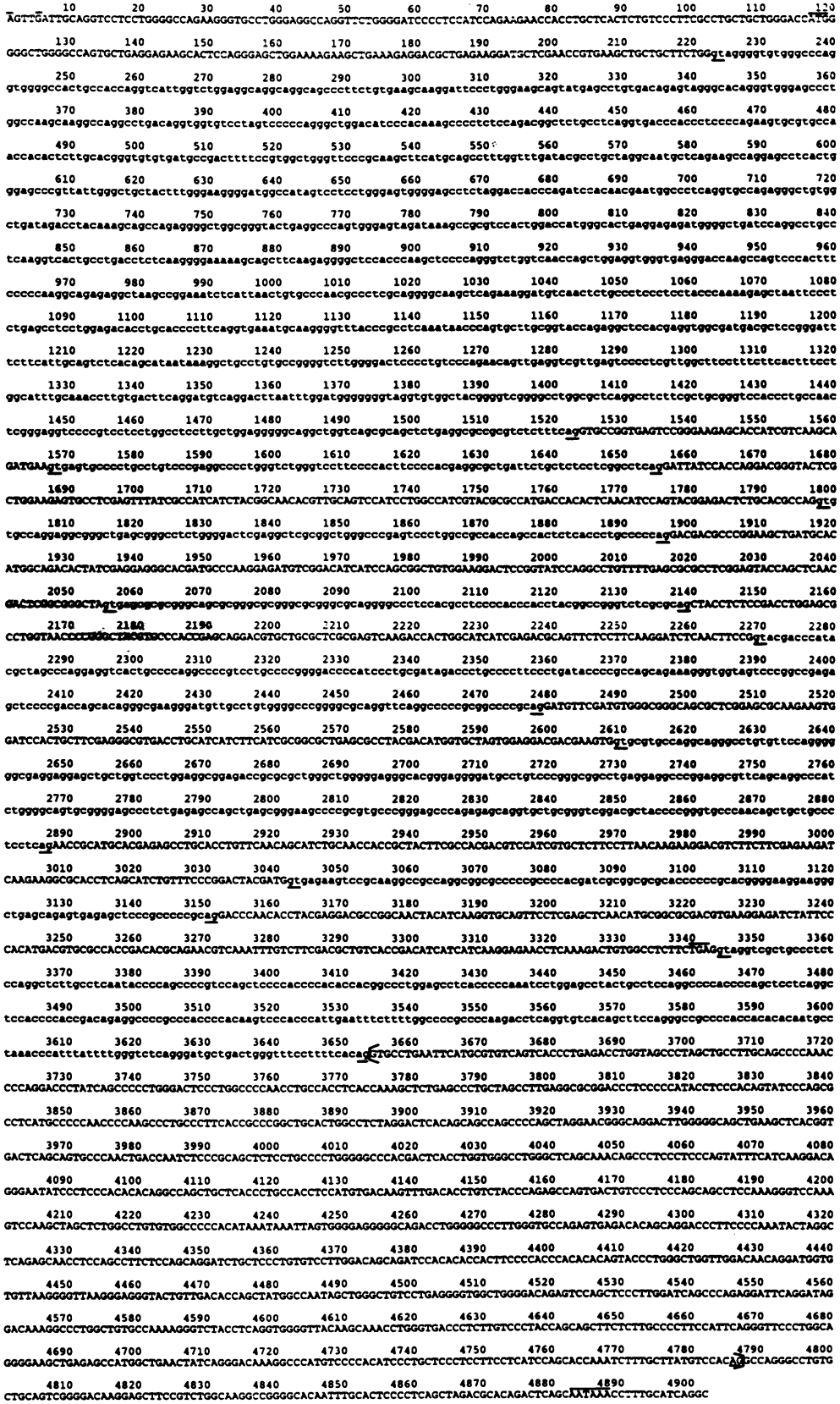

Fig. 2. Complete nucleotide sequence of the human $T_{r\alpha}$ gene. The two putative transcription initiation sites, A and G (at positions 1 and 5); the presumptive translation initiation site, ATG (at positions 117−119); the translation termination codon, TGA (at positions 3340−3342); and the polyadenylation consensus sequence, AATAAA (at positions 4883−4888) are overlined. Exons are denoted by upper-case letters. Introns are denoted by lower-case letters with their consensus gt and ag underlined. The bracketed region is not present in the 1.3kb mRNA. The poly A tail (a stretch of 16 A's followed by a C) for the 2.4 kb mRNA is attached to the nucleotide G at position 4901. The poly A tail for the 1.3 kb mRNA is attached to nucleotide C at position 4902 (1).

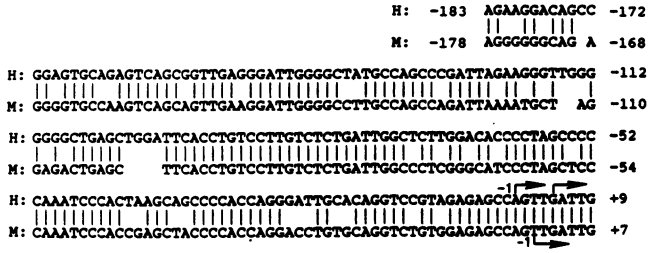

Fig. 3. Comparison of the 5′ upstream region of the human (H) and mouse (M) (2) $T_{r\alpha}$ genes. The human DNA sequence was obtained from a 2.1 kb *Stu* I fragment of the genomic clone HGLG4. The vertical lines indicate identical nucleotides. The bent arrows indicate the transcription initiation sites. The first transcription initiation site in the human sequence is assigned +1.

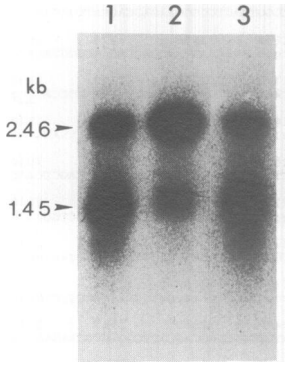

Fig. 4. Northern blot analysis of human mRNA for $T_{r\alpha}$ transcripts. Poly (A^+) RNA equivalent to 50 mg of human retina tissue from individual donors was loaded in each lane. The autoradiogram was obtained after hybridization of the Nitroplus 2000 membranes with the ^{32}P-labeled HG3 cDNA probe. Eyes from all three donors show two sizes of mRNA for $T_{r\alpha}$, but their relative intensities are different.

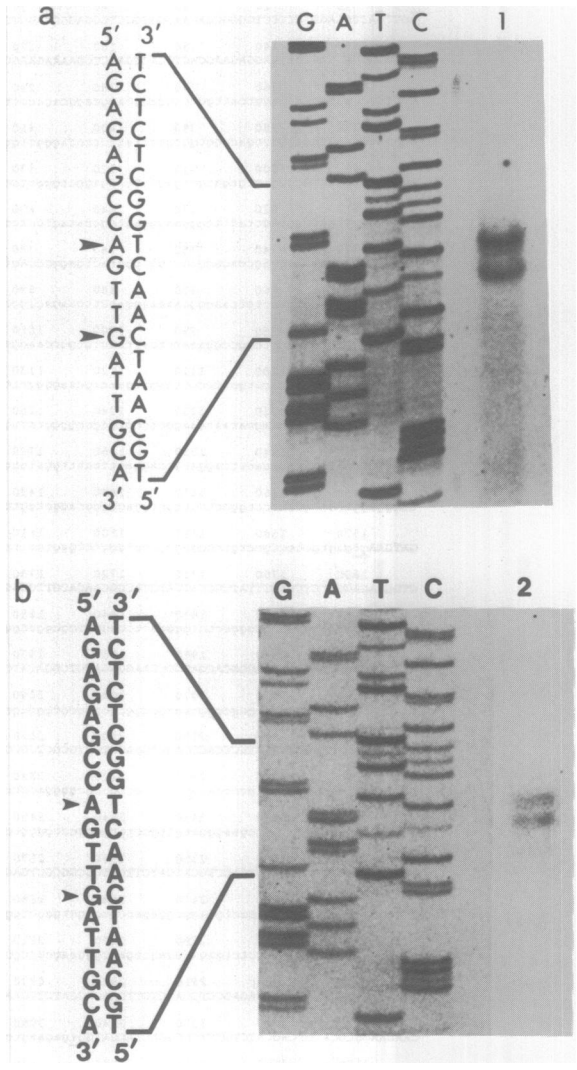

Fig. 5. Mapping the 5′-end of the human $T_{r\alpha}$ gene by primer extension and S1 nuclease protection. Fig.5a, primer extension. Lane 1 contains the primer-extended cDNAs obtained by using 30 μg total RNA from human retina. The primer was a 17-base oligonucleotide complementary to residues 132 to 148 in Fig. 2. Fig. 5b, S1 nuclease protection. Lane 2 shows the products of S1 nuclease digestion of human total RNA (30 μg) hybridized with a probe generated from a *Stu* I fragment of HGLG4 and the same ^{32}P-labeled primer used in the primer extension experiment. The sequencing ladders were obtained from the same labeled primer and *Stu* I genomic fragment used for probe synthesis as in the S1 nuclease protection experiment. The two transcription initiation sites determined are indicated by arrowheads.

Northern blot analysis

Northern blots of poly (A^+) RNA from human retinal tissues were probed with ^{32}P-labeled HG3. Two transcripts with sizes of 1.45 kb and 2.46 kb were found in all three donor retinas but their relative intensities were different (Fig. 4).

Transcription Initiation Site Determination

Total RNA from batches of human retinas was used to map the 5′-end of the human $T_{r\alpha}$ transcript by primer extension and S1 nuclease protection. Two major transcription initiation sites were found in the human $T_{r\alpha}$ gene at nucleotides 1 and 5, respectively (Fig. 5a and 5b, arrowheads). These two sites were clearly seen when the synthetic 17-base primer was extended by reverse transcriptase in the presence of total human RNA (Lane 1, Fig. 5a). These two sites were also seen when the single-stranded probe generated from a Stu I fragment of HGLG4 and the ^{32}P-labeled synthetic 17-base primer hybridized to human retina total RNA was digested with S1 nuclease (Lane 2, Fig. 5b). The smallest sized band in Lane 2 may be an artifact resulting from the exonuclease activity of S1 nuclease which removes three AU base pairs adjacent to the presumed transcription initiation sites (24, 25). A minor band that is 20 and 24 bp larger than the two protected bands seen in lane 2, can also be found in primer extension experiment if the autoradiogram is overexposed.

DISCUSSION

The cDNA clones HG3 and HG14 contain the published human $T_{r\alpha}$ cDNA sequence (1, 13). HG14 differs in that the 3′-end noncoding region is 1134 bp longer. The comparison of the coding regions of bovine, mouse and human $T_{r\alpha}$ genes is shown in Fig. 6. There is a 93.5% identity between human and bovine sequences and an 89.2% identity between the human and mouse sequences.

Many investigators (3, 4, 7, 26) have found that the amino acid sequences of different mammalian G_α subunits are highly conserved. Similarly, in the present study there were only three

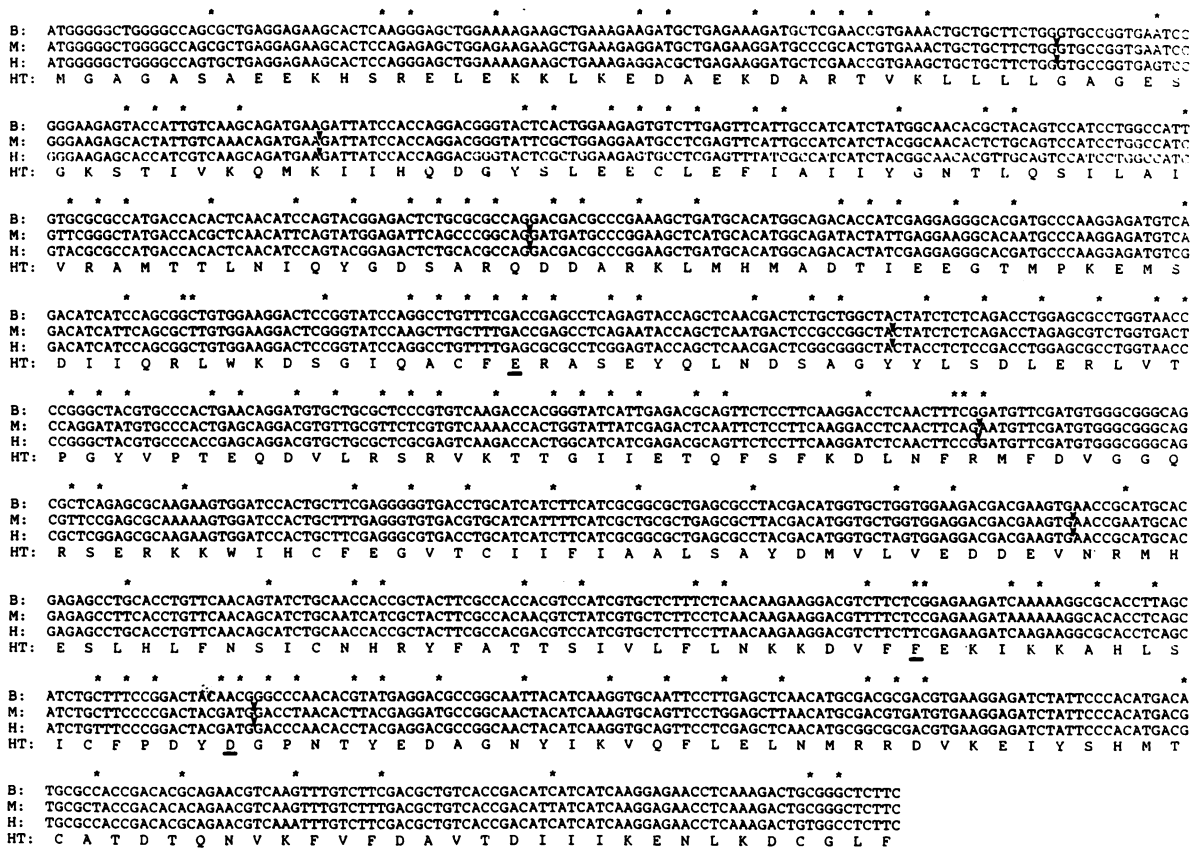

Fig. 6. Homology comparison of the coding regions of bovine, mouse and human (present work) $T_{r\alpha}$ genes and their intron positions. The asterisks indicate the nucleotide mismatches between any two species. Arrowheads indicate the locations for the insertion of seven introns for both mouse and human genes. Underlines show the variation in amino acids between human and bovine or mouse $T_{r\alpha}$. B: bovine, M: mouse, H: human, HT: amino acid sequence of human $T_{r\alpha}$.

Table II. Mismatches between the published $T_{r\alpha}$ cDNA sequences and this work.

Ref. 1		Ref. 13		This Work	
Nucleotides and number	Amino acid involved	Nucleotides and number	Amino acid involved	Nucleotides and number	Amino acid involved
		Coding region			
CG(714−715)	Pro	CG(712−713)	Pro	GC(2511−2512)	Arg
CC(774−775)	Thr	CG(771−772)	Thr	GC(2571−2572)	Ser
G(778)	Ala	C(775)	Ala	C(2575)	Ala
G(923)	Val	A(921)	Ile	A(2999)	Ile
CTG(1093−1095)	Phe,Cys	TGT(1091−1093)	Phe,Val	TGT(3279−3281)	Phe,Val
		Noncoding region			
T(24)		----------[a]		G(35)	
Delete		----------		G,G,G,G(11,33,44,4789)	
A(63)		G(61)		A(76)	
Insert G(1242)		----------		Between 4872−4873	
		Presumed-cloning artifact at 5′end			

[a] Dashed lines indicate that no information is available.

differences out of 350 residues between the human and bovine $T_{r\alpha}$ sequences, corresponding to the substitution of Glu, Phe, and Asp for Asp, Ser, and Asn respectively at residues 137, 271 and 287. There were only two amino acid differences between human and mouse, corresponding to the substitution of Glu and Phe for Asp and Ser at residues 137 and 271 (Fig. 6).

A comparison of the genomic sequence with the cDNA sequence of HG14 reveals that the human $T_{r\alpha}$ gene is composed of nine exons interrupted by eight introns. These nine exons predict an mRNA of 2418 nucleotides, in good agreement with the observed mRNA of 2.46 kb in size (Fig. 4). On the other hand, the cDNA sequence published by Van Dop *et al.* (1) predicts a smaller mRNA of 1,285 bases, corresponding to the 1.45 kb band seen in Fig. 4. The difference is due to the absence

of a non-coding segment of 1,134 bases in Van Dop *et al.*'s sequence that is probably attributable to an alternative splicing mechanism. The segment in question is indicated by the dotted bar in Fig. 1 and by the bracketed region in Fig. 2. The reasons for having two species of mRNA in human and only one species (2.6kb) in bovine and mouse (9, 2), as well as for having a big difference in size of the 3'-noncoding region between the two human mRNAs remain to be elucidated. Both mRNA species were found in all three human donors, but the relative intensities varied from individual to individual (Fig. 4). It can not be determined from present work if the age is a factor for this variability.

The first seven introns of the human $T_{r\alpha}$ gene are located at positions identical to those in mouse $T_{r\alpha}$ (Fig. 6). The intron-exon organization of the mouse $T_{r\alpha}$ gene has been demonstrated to be the same as those of human Goa, Gi2a, and Gi3a genes (2, 4).

Fig. 2 shows the entire DNA sequence for the human $T_{r\alpha}$ gene, starting at the putative cap site and ending at the polyadenylation site. The two mRNA sequences, 1.3 and 2.4 kb, can be generated by alternative splicing (Fig. 2). The exon and intron lengths and their arrangement are summarized in Table 1. Like most of the exons in mammalian genes (27, 28), six of the ten exons (for both mRNA species) are in the size range of 100–170 bp. Intron sizes vary from 85 to 1444 bp, and all have the canonical GT dinucleotide at their 5' ends and an AG dinucleotide at their 3' ends (29,30).

An important element for pre-mRNA splicing is the branch point. In most introns, this is located 18–40 upstream of the 3' intron boundary. Therefore, the branch point consensus YNYYRAY (Y, pyrimidine; R, purine; N, any nucleotide) (31) was searched for in the 3'-ends of the $T_{r\alpha}$ introns. Matches with the consensus sequence are underlined in Table 1. As expected that these branch point consensus sequences may be weakly conserved in higher eukaryotes, only two perfect matches were found in 15 possible branch site sequences. It remains to be determined which of these splice sites may be used during mRNA maturation.

The coding sequence of the human $T_{r\alpha}$ gene is almost identical with the two published human $T_{r\alpha}$ cDNA sequences (1, 13). There are 14 differences from the Van Dop *et al.* (1) sequence. Eight are in the coding region and cause 4 amino acid changes. There are 5 differences from the sequence reported by Lerea *et al.* (13). Four are in the coding region and cause 2 amino acid changes (Table II). In addition, residues 1 through 59 of the cDNA sequence published by Lerea *et al.* (13) are identical with the opposite strand of residues 17 through 75 in Fig. 2 of present work. This is an apparent cloning artifact, and has been observed several times in Nathans' human retina cDNA library (32, 33).

Two major transcription initiation sites were found by primer extension and S1 nuclease protection assays. The first site in the human $T_{r\alpha}$ gene, an A residue, is two nucleotides upstream and the second site, a G residue, is two nucleotides downstream from the single mouse cap site T (Fig. 3). A TATA box (34, 35), a consensus promoter element, is not present in either the human or mouse $T_{r\alpha}$ genes (Fig. 3).

The 5'-end upstream region of the mouse (178 bp) and human (183 bp) $T_{r\alpha}$ genes show 78.1% identity, including seven gaps in mouse (Fig. 3). This high degree of homology indicates that human and mouse may share a common regulatory mechanism for the expression of $T_{r\alpha}$ gene in rod photoreceptors.

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