



# Sequence and structure of the mouse gene for RPE65

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**Purpose:** To determine the genomic organization of the mouse gene for the retinal pigment epithelium (RPE) specific protein RPE65.

**Methods:** A genomic clone containing the entire *Rpe65* gene was isolated from a mouse genomic P1 library. Fragments of this clone were subcloned and sequenced by automated fluorescent dideoxy DNA sequencing and analyzed. Direct sequencing of PCR amplification products was used to complete the structure. Primer extension analysis was used to determine the transcription start site.

**Results:** Southern hybridization of restriction digests of mouse genomic DNA reveals a likely single autosomal gene for *Rpe65* with no evidence of pseudogenes. Sequence analysis of the mouse P1 clone for *Rpe65* and fragments thereof reveals 14 exons distributed over 27 kbp. The transcription start site is located 57 bp upstream of the initiation codon. The protein encoded by the mouse *Rpe65* gene is highly conserved when compared with RPE65s from other species.

**Conclusions:** RPE65 is a highly conserved protein and it appears that the genes for the mouse and human RPE65s, at least, are also conserved in overall structure.

A major function of the retinal pigment epithelium (RPE) is the production of 11-*cis* retinal chromophore required for the regeneration of photoreceptor opsins, in a process termed the visual cycle [1]. A series of enzymes and retinoid binding proteins is known to be involved in the successive steps of the visual cycle. Most of the steps have been identified and many have been characterized. Despite this, the identity of the protein(s) catalyzing the central all-*trans* to 11-*cis* isomerization reaction is still not known though its enzymology has been well studied [2-4]. Most of the proteins known to be associated with the visual cycle are highly preferentially expressed in the RPE. One such protein is RPE65 [5,6]. Though its precise role in the visual cycle is still debated, its absence, such as in the *Rpe65*-deficient mouse [7], the Briard dog model of congenital stationary night-blindness [8-10], and in presumed null human RPE65 gene mutations [11-13], results in severe blindness. Retinoid analysis of the retinae of the *Rpe65*-deficient mouse reveals severe depletion of 11-*cis* retinoids (no or very little rhodopsin) and over-accumulation of all-*trans*-retinyl esters in the RPE [7]. This is suggestive of a role for RPE65 in the all-*trans* to 11-*cis* isomerization of vitamin A.

The human gene for RPE65 has been cloned and sequenced [14]. It consists of 14 exons spread over about 25 kb pairs of genomic DNA. The chromosomal localizations of both the human and mouse genes have been identified, at 1p31 [14,15] and distal 3 [15], respectively. Though certain aspects of the mouse gene have been reported upon, including the targeted disruption of the mouse *Rpe65* gene [7], and the analysis of the promoter function of the 5' flanking region of the

mouse *Rpe65* gene [16], the structure of the gene itself has not.

In this brief report we present the structure of the mouse *Rpe65* gene, the sequence of the intron/exon boundaries, and the determination of the start site of the gene. In general, it is quite similar to that observed for the human gene [14], further extending the high degree of conservation of RPE65 to the organization of the homologous genes.

## METHODS

**Genomic Southern blot analysis of the mouse *Rpe65* gene:** A mouse Genoblot containing mouse genomic DNA digested with *EcoRI*, *Hind III*, *BamHI*, *Pst I* and *Bgl II* was purchased from Clontech (Palo Alto, CA). This blot was prehybridized with QuikHyb (Stratagene, La Jolla, CA) and hybridized with a random primed [17] bovine cDNA probe [6]. The blot was washed to a final stringency of 0.1 X SSC+ 0.1% SDS at 63 °C.

**Cloning and Sequencing of mouse *Rpe65* gene:**

A P1 clone containing the entire mouse *Rpe65* gene was isolated employing a PCR screening method (Incyte Genomics, St. Louis, MO). Restriction fragments containing the 5' region of the mouse *Rpe65* gene were identified by Southern blot hybridization to a random-primed <sup>32</sup>P-labeled bovine cDNA 5' end probe [6]. *EcoRI* and *BamHI* restriction fragments thereof were separated on agarose gels, excised, purified by binding to GeneClean II (Bio101/Qbiogene, Carlsbad, CA) and subcloned into pBluescript II SK- (Stratagene, La Jolla, CA). pBluescript II subclones containing the 5' region of the RPE65 gene were sequenced. Double-stranded dideoxy sequencing was performed using the Dye Terminator cycle sequencing protocol on a model 373A automated fluorescent DNA sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA). A primer-walking sequencing strategy was em-

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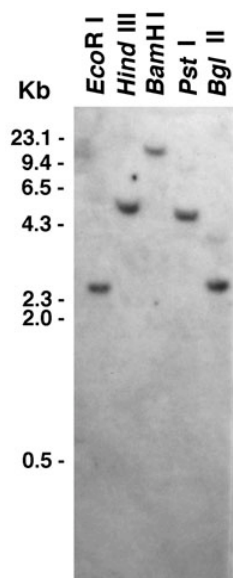


Figure 1. Genomic Southern blot analysis of mouse *Rpe65* gene. Mouse genomic DNA digested with several restriction enzymes was blotted onto nylon and hybridized to a random-primed bovine RPE65 cDNA probe. The blot was washed to a final stringency of 0.1 X SSC+ 0.1% SDS at 63 °C.

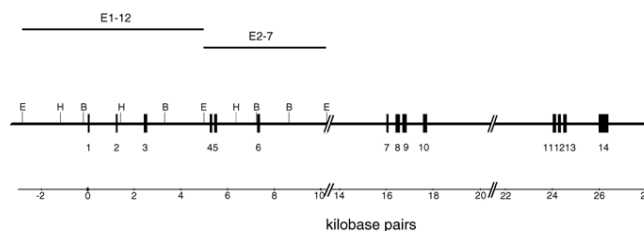


Figure 2. Organization of the mouse *Rpe65* gene. A partial restriction map of the 129/Sv mouse *Rpe65* gene was derived from sequencing of 2 contiguous subclones (E1-12 and E2-8) of the original P1 clone. *Hind III*, *BamHI* and *EcoR I* sites are shown. No such sites were detected in exons 7-14 or in the sequenced introns beyond exon 7. The large introns between exons 10 and 11 and 13 and 14 were not sequenced. Only the 5' half of the 8 kb intron between exons 6 and 7 was sequenced. The position of exons 1-14 are indicated by the solid boxes, numbered below, with the intervening introns designated by capital letters beneath this. Sequences of exons 7-14 were derived from direct sequencing of PCR products amplified from this P1 clone.

| Exon No. | bp  | Exon Sequence                | Intron (No.; bp) | Intron Sequence                                       | Exon No. |
|----------|-----|------------------------------|------------------|---|----------|
| 1        | 65  | AAA ATG TCT ATC<br>M S I Q   | (A; 1072)        | CA/gtaagtatct--(A; 1072)-cgaatttcag/A<br>I E H P      | 2        |
| 2        | 83  | GCT CAT GTC ACA<br>A H V T   | (B; 1118)        | G/gttggtctca---(B; 1118)-tttgetgcag/GC<br>G R I P L   | 3        |
| 3        | 151 | ACA TAC CAC AGA<br>T Y H R R | (C; 2680)        | AG/gtaagtccat--(C; 2680)-tattcttcag/A<br>F I R T      | 4        |
| 4        | 108 | AAT ATA TTT TCC<br>N I F S R | (D; 90)          | AG/gttaatgaaa----(D; 90)-gcttctgcag/G<br>F F S Y      | 5        |
| 5        | 142 | GAG ACA ATT AAG<br>E T I K Q | (E; 1685)        | CAG/gtaggatatt-(E; 1685)-cattctacag/GTT<br>V D L C N  | 6        |
| 6        | 148 | CCA CTG AAA GCA<br>P L K A   | (F; ~8500)       | G/gtgaggttgt---(F; ~8500)-tctatcttcag/AC<br>D K E D P | 7        |
| 7        | 82  | TCT TAC GTA CAC<br>S Y V H S | (G; 218)         | AG/gtaattttaa---(G; 218)-ttttgaacag/T<br>F G L T      | 8        |
| 8        | 133 | AAT GAA AGC ATG<br>N E S M G | (H; 95)          | GGG/gtatgtctga---(H; 95)-acttttcag/GTT<br>V W L H V   | 9        |
| 9        | 140 | TGT TGC TGG AAA<br>C C W K G | (I; 746)         | GG/gtaaaaaatt---(I; 746)-gttttcacag/G<br>F E F V      | 10       |
| 10       | 130 | TTG ACA ATT GAC<br>L T I D K | (J; ~6000)       | AAG/gtaactttct-(J; ~6000)-tctttcttag/GTC<br>V D T G R | 11       |
| 11       | 115 | GGG CCT CGT CAA<br>G P R Q   | (K; 88)          | G/gtaagatgat----(K; 88)-tattttaag/CC<br>A F E F P     | 12       |
| 12       | 95  | TTT GTT CCT GAC<br>F V P D K | (L; 103)         | AAG/gtaataagca--(L; 103)-tcataagcag/CTC<br>L C K L N  | 13       |
| 13       | 112 | GAA GAA GAT GAT<br>E E D D   | (M; ~1270)       | G/gtaatggaat---(M; ~1270)-taattaacag/GT<br>G V V L S  | 14       |

Figure 3. Intron/exon boundaries of the mouse *Rpe65* gene. Each exon (numbered from 1 to 14) is listed and the length in base pairs (bp) given on the left hand side. Each intron (given alphabetically A to M) is listed and the length in bp given after the letter designation. Exonic sequence (with translation underneath) is given as uppercase, while intronic sequence is depicted as lowercase.



verse Transcriptase kit (Promega, Madison, WI). The antisense oligonucleotide (5' CAT TTT CTT CCA GTG AAG ATT AGA GAG AG 3') based on mouse RPE65 cDNA was labeled in the presence of 60  $\mu$ Ci  $\gamma^{32}$ P and hybridized with 12  $\mu$ g of total RNA extracted from RPE/choroid of mice eyes. The first strand cDNA was synthesized using AMV reverse transcriptase (Promega) at 42 °C for 30 min and the fragments produced were analyzed by 6% denaturing sequencing gel electrophoresis (Stratagene).

*Sequence analysis:* DNA and protein sequences were analyzed and aligned using the AutoAssembler (v.1.3; Applied Biosystems, Foster City, CA), Sequencher (v. 3.1.1) and MacVector package (v. 6.5.3; Oxford Molecular Group, Madison, WI).

## RESULTS & DISCUSSION

Southern blot analysis of restriction digests of mouse genomic DNA, hybridized to a bovine cDNA probe, revealed a simple restriction pattern (Figure 1), suggestive of a single gene and excluding the existence of pseudogenes. This is consistent with the assignment of the mouse chromosomal locus where no cross-hybridizing locus was seen [15].

The structure of the mouse *Rpe65* gene is shown in Figure 2. The subcloned 5' end fragments of the gene are shown above. One such clone, E1-12, was found to contain the first three exons of mouse *Rpe65* and intervening introns, as well as 2.8 kb of 5' flanking region. Another clone, E2-8, contained exons 4, 5, and 6 and intervening introns. Exons 7 and beyond were sequenced from PCR amplification products using the mouse P1 clone as template. The gene has 14 exons distributed over 27 kb. The intron-exon boundaries, determined by comparison of genomic sequence with the mouse RPE65 cDNA (unpublished data), are presented in Figure 3. Intron length varies from 88 bp to about 8 kbp. The sizes of the longer introns F, J and M were estimated from agarose gel electrophoresis of PCR products amplified using primers flanking these introns (data not shown). In general, the donor/acceptor sites corresponded to the GT/AG rule, though not always perfectly. This organization is generally quite similar to that found for the human *RPE65* gene [14] which also has 14 exons. The exon breaks found for the mouse gene correspond exactly to those seen in the human gene [14]. The sequences have been deposited in GenBank under the accession numbers [AF432266](#), [AF432267](#), and [AF432268](#).

The transcription initiation site was determined by primer extension analysis using a primer complementary to the known 5' end of mouse RPE65 cDNA. One elongation product was identified from the RNA of RPE65-expressing mouse RPE cells (Figure 4), but it was not produced when the primer extension reaction was carried out without RNA (not shown). The transcription start site corresponds to the one deduced from the bovine RPE65 cDNA but differs by one nucleotide from that of the human sequence [14].

The deduced protein sequence for mouse RPE65 is shown in Figure 5 in comparison with the sequences for rat, human, bovine, dog, chicken, and salamander RPE65s. Sequence conservation is, in general very high with mouse RPE65 being

95% identical to its human, dog and cow homologs.

In conclusion, the organization and structure of the mouse *Rpe65* gene is quite similar to that of the homologous human *RPE65* gene. When taken together with the obviously close homology at the protein level and the degree of similarity of the 5' flanking region [16], these data speak to the marked conservation of this gene in all aspects of its organization, regulation, and expression.

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