SHORT COMMUNICATION

The Sequence of the Human Phosducin Gene (*PDC*) and Its 5′-Flanking Region

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Phosducin, a principal protein of the retinal photoreceptor cells, modulates the phototransduction cascade by interacting with transducin. Recently, it has been reported that phosducin is a protein virtually identical to the G-protein inhibitor protein (GIP) in brain. Here, we have sequenced the complete human gene (*PDC*) and 2215 bp of its 5′-flanking region. The gene is 18 kb in length and has four exons and three introns. The splicing sites for donor and acceptor are in good agreement with the GT/AG rule. Comparative studies of human and mouse phosducin revealed highly homologous sequences. Both the human phosducin gene and a mutant gene locus for Usher syndrome type II have been assigned to chromosome 1q25–q32. The association of this gene with a human disease locus suggests that phosducin may be a potential candidate gene for this disorder.

Sequence data from this article have been deposited with the EMBL Database under Accession No. L25260.

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Phosducin (33-kDa protein or MEKA) is a principal water-soluble phosphoprotein in rod and cone photoreceptor cells (1–4) and pinealocytes (5). This protein modulates the phototransduction cascade by binding to the $\beta\gamma$ subunit complexes of transducin (Td$\beta\gamma$) to form phosducin–Td$\beta\gamma$ complexes (6, 7). Phosphorylation of phosducin is catalyzed by cyclic adenosine 3′,5′-monophosphate-dependent protein kinase (A-kinase) (4, 6). Phosphorylated phosducin loses binding affinity to the transducin and consequently loses inhibitory activity (8).

Previously, deduced amino acid sequences as well as cDNA sequences have shown that phosducins are highly conserved among mammalian species (9–11). In addition, the mouse phosducin gene was found to be one of a multiple gene family (12).

While phosducin is a principal protein in the photoreceptor cells and pineal gland, it is also detected at low levels in a wide range of tissues (8, 13). A recent report indicates that the phosducin sequence in retina is virtually identical with that of the G-protein inhibitor protein (GIP) found in bovine brain (8). The GIP is known to inhibit both GTPase activity of G proteins and Gs-

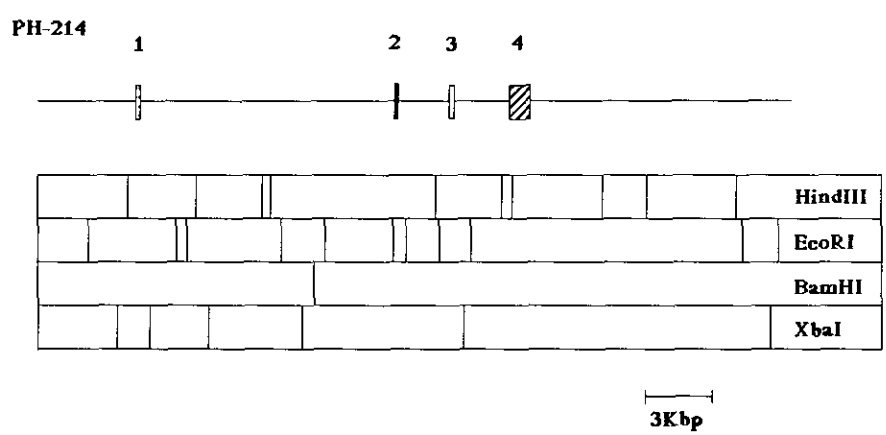

FIG. 1. A diagram of *PDC* and its restriction map. Horizontal bar indicates the introns and the 5′- and 3′-flanking regions. Boxes indicate the first (dotted box), second (black box), third (white box), and fourth (slashed box) exons. Sites for restriction endonucleases *Hin*dIII, *Eco*RI, *Bam*HI, and *Xba*I are indicated below. The human gene was isolated from a genomic P1 phage library (Genome Systems Inc., St. Louis, MO). An approximately 80-kb fragment containing a phosducin clone (PH-214) was partially digested with the restriction enzyme *Sau*3AI. These fragments were subcloned into the *Bam*HI site of λ DASH phage DNA (Stratagene, La Jolla, CA) and screened by the hybridization method (23). The restriction sites of *Hin*dIII, *Eco*RI, *Bam*HI, and *Xba*I were determined by the gel analysis. DNA sequence for both strands was determined by the dideoxy chain termination method (24) in a pBluescript IISK+ vector (Stratagene). The resulting sequences were analyzed using the GCG package (Genetics Computer Group, Inc., Madison, WI).

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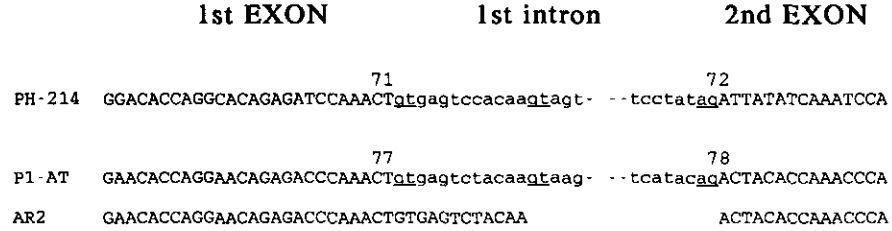

FIG. 2. Sequence comparison of the first intron of phosducin genes between human and mouse. The *PDC* sequence was compared with the mouse gene (P1-AT) sequence. In addition, a mouse cDNA sequence (AR2) was also compared. The nucleotides are numbered from the presumptive transcriptional start site of *PDC*. The exons and introns are indicated by capital and lowercase letters, respectively. Mouse cDNAs were made and isolated as described previously (12).

mediated adenyl cyclase activation in the signal transduction cascades (8).

Both the phosducin gene (14) and a mutant gene locus for a hereditary retinal degenerative disease, Usher syndrome type II (15, 16), are located on the chromosome 1q25–q32. Usher syndrome is an autosomal recessive disorder characterized by deafness and retinitis pigmentosa. Type II is usually distinguished from type I on the basis of severity of hearing loss. Type II patients have a mild hearing loss in the lower frequencies, but hearing

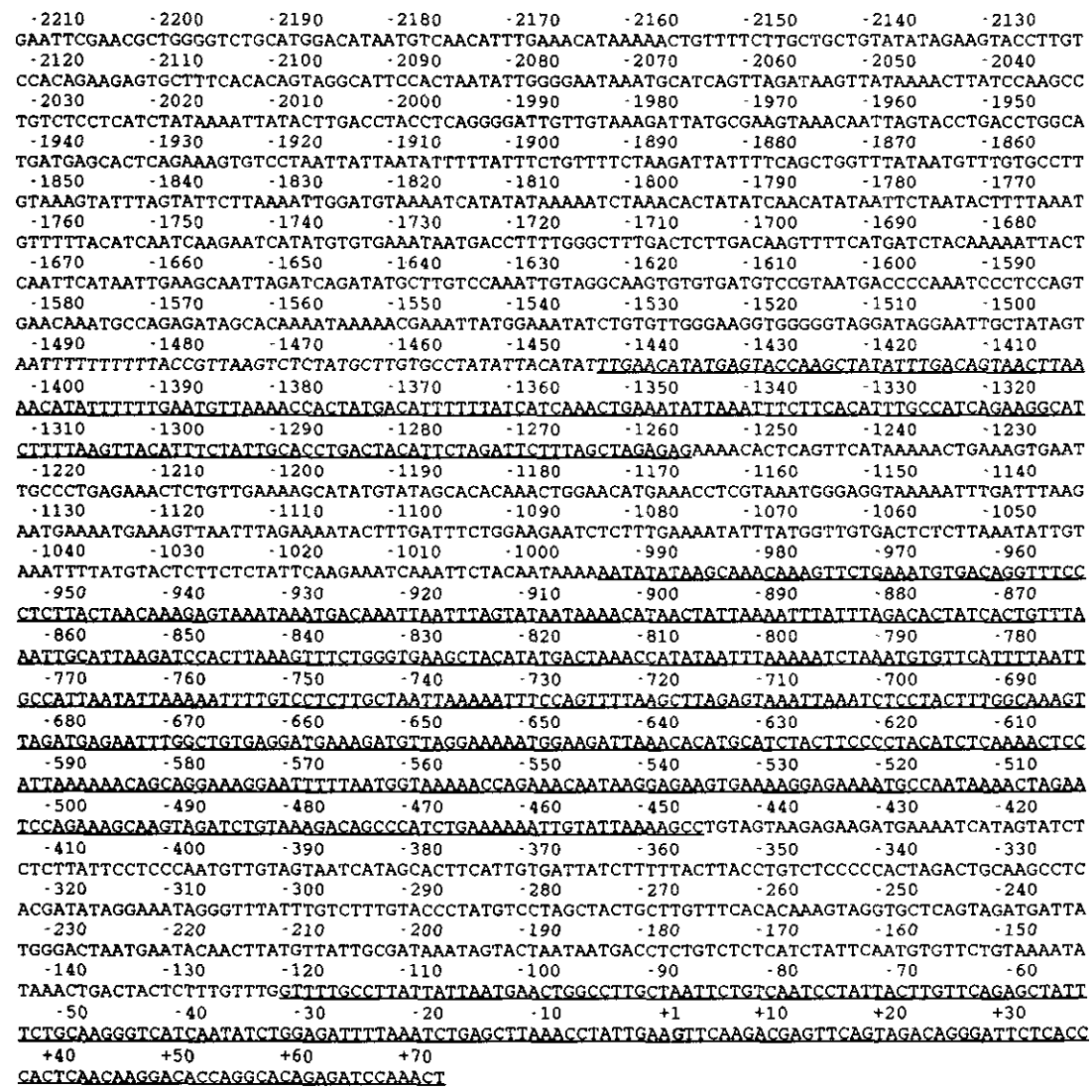

FIG. 3. Sequence of the 5′-flanking and the first exon of the human gene. Highly homologous sequences between human and mouse are indicated by underlines. Basepair positions are numbered from the presumptive transcriptional start site of the human PH-214 clone. The first exon and 5′-flanking region are shown. The human gene sequence is numbered from G at position 1 since the mouse transcriptional start site corresponds to A.

loss is more severe at higher frequencies (16, 17). Thus, human phosducin is of considerable interest with respect to its function and its gene expression in specific tissues and as a candidate gene with respect to linkage studies for specific forms of retinitis pigmentosa. In this report, we present the sequence of a complete human phosducin gene (*PDC*). In addition, we have sequenced 2215 bp of its 5′-flanking region to elucidate potential sequences regulating tissue-restricted *PDC*.

To characterize *PDC*, an independent phage clone (PH-214; 80 kb) was isolated from a human P1 phage library (Genome Systems) with human cDNA primers (10) and was mapped with restriction enzymes *Hin*dIII, *Eco*RI, *Bam*HI, and *Xba*I. The resulting restriction maps are summarized in Fig. 1.

To define the relative positions of the exons and introns, DNA sequences in all exons and part of the introns contained in PH-214 were determined, and the sequence is in complete agreement with corresponding human cDNA sequence (10). The phosducin gene was composed of four exons interrupted by three introns spanning approximately 18 kb in length. Each of the splice junctions is in good agreement with the GT/AG rule (18) (data not shown). As shown in Fig. 2, human *PDC* has a GT at the 5′-end of the first intron; in addition, another GT was found 12 bp downstream of the first intron. Previously, we have found a similar sequence in the corresponding position of the mouse phosducin gene (10). In this study, we isolated seven cDNAs from a mouse retinal cDNA library, and six of the cDNAs had no first intron sequence, as expected. Interestingly, one of the cDNAs had an additional 12 bp in which the sequence is identical with that of the 5′-end of first intron of mouse gene (Fig. 2). The results suggest that the phosducin gene undergoes alternative splicing or multiple phosducin genes are expressed in the retina.

To define the boundary of the gene, sequences in the 5′- and 3′-flanking regions, 2215 bp, were also determined (EMBL Accession No. L25260). There are no TATA, GC, or CAAT boxes in the 5′-flanking region. The closest similarities we have found are TATA-like sequences between −29 and −21 (ATTTTAAAT). Such promoter sequences have been reported in mouse phosducin (12), arrestin (19), and IRBP (20, 21), among the other photoreceptor specific genes. A comparison of sequences between human *PDC* and the mouse P1-AT gene (12) indicates that intron 1 in *PDC* is 3.5 kb larger than that in the mouse (Fig. 1). In the 5′-flanking region, there are three stretches of highly homologous regions between human and mouse genes, as shown (Fig. 3). The highest homology was found between −122 and +70 (base identity 79%; corresponding mouse sequence is between −128 and +77), and the other homologies were found between −996 and −448 (base identity 68%; corresponding mouse sequence is −2326 and −1771) and −1446 and −1259 (base identity 65%; corresponding mouse sequence is −2696 and −2486). The *PDC* sequence was also in agreement with the previously published human partial gene sequence that lacked the first exon, part of the first intron, and the entire 5′-flanking regions (22).

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