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Cloning, Tissue Expression, and Mapping of a Human Photolyase

P. J. VAN DER SPEK,*,† K. KOBAYASHI,‡ D. BOOTSMA,* M. TAKAO,‡
A. P. M. EKER,* AND A. YASUI‡,[1]

*Department of Cell Biology and Genetics, MGC, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands; †Department of Radiotherapy, Division of Clinical Radiobiology, Dr. Daniel den Hoed Center, 3075 EA Rotterdam, The Netherlands; and ‡Institute of Development, Aging and Cancer, Tohoku University, Sendai 980-77, Japan

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Enzymatic photoreactivation is a DNA repair mechanism that removes UV-induced pyrimidine dimer lesions by action of a single enzyme, photolyase, and visible light. Its presence has been demonstrated in a wide variety of organisms, ranging from simple prokaryotes to higher eukaryotes. We have isolated a human gene encoding a 66-kDa protein that shows clear overall homology to known bacterial photolyase genes. The human gene product is more similar to plant blue-light receptors within class I photolyases than to higher eukaryote class II photolyases. Northern blot analysis showed two transcripts with constitutive expression in all tissues examined and an elevated expression in testis. *In situ* hybridization with a cDNA-derived probe localized this human gene to chromosome 12q23–q24.1. Southern analysis of the cloned human gene suggests a wide distribution of the gene family in various species.

INTRODUCTION

Photoreactivation (PHR) is a repair mechanism that removes UV-induced DNA damage by action of a single enzyme: photolyase. These lesions can interfere with processes such as replication and transcription and therefore affect viability and functional integrity of living cells. Illumination with visible light of photolyase bound to a pyrimidine dimer lesion leads to conversion of this lesion into two pyrimidine monomers. Photoreactivation of cyclobutane type pyrimidine dimers (CPD) has been known of for a long time, and at least 15 photolyase genes have been cloned. More recently, the presence of a photolyase specific for (6-4)photoproducts in insects and plants was reported (Todo *et al.*, 1993; Chen *et al.*, 1994).

Most CPD photolyases contain two chromophores, one of which is reduced FAD and essential for repair activity. The second chromophore, either 5,10-methenyltetrahydrofolate (MTHF) or 8-hydroxy-5-deazaflavin (8-HDF), works as an efficient antenna cofactor to gather light as energy supply for the dimer splitting reaction sensitized by the reduced FAD cofactor (Sancar, 1994; Eker *et al.*, 1988). Thus far, two distantly related classes of CPD photolyases, which can be distinguished by their amino acid sequence, have been identified. Class I contains most microbial photolyases as well as several plant blue-light photoreceptors, while in class II higher eukaryote photolyases are found (Yasui *et al.*, 1994).

Photolyases are present in cells from many species, ranging from bacteria and yeasts to aplacental mammals like the opossum (*Monodelphis domesticum*) and rat kangaroo (*Potorous tridactylis*) (Kato *et al.*, 1994; Yasui *et al.*, 1994). In contrast, contradictory results have been obtained for placental mammals (see, e.g., Li *et al.*, 1993; Sutherland and Bennett, 1995), and a corresponding photolyase gene has not been identified. Here we report the cloning, expression analysis, and chromosomal localization of a human gene that has clear homology to class I microbial photolyases.

MATERIALS AND METHODS

Identification of R19031 and cloning of cDNA. The amino acid sequence of *Anacystis nidulans* photolyase was used to perform a sequence comparison in the expressed sequence tag (EST) database. The NCBI TBLASTN program (Altschul *et al.*, 1990) identified a cDNA clone (R19031, Image Consortium) with an open reading frame homologous to the photolyase sequence used as a query. The cloned sequence was extended by PCR using a human testis library (BRL).

Sequencing and analysis of DNA and encoded proteins. DNA sequencing was carried out by the dideoxynucleotide chain-terminating procedure with fluorescent-tag-labeled deoxynucleotides using a SHIMAZU DSQ-1000. Sequences were compared for homology to other sequences in the GenBank, GenEMBL, and dBEST databases by using the BLAST software package from the NCBI server.

Chromosomal localization. In situ hybridization was performed with a biotin-labeled probe derived from the R19031 partial cDNA clone (Pinkel *et al.*, 1986). After incubation with avidin D–FITC (Vector, USA), the probe was visualized by FISH. A representative

Sequence data from this article have been deposited with the DDJB Database under Accession No. D84657.

[1] To whom correspondence should be addressed at Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryomachi Aoba-Ku, Sendai 980-77, Japan. Telephone: 22-717-8562. Fax: 22-717-8567.

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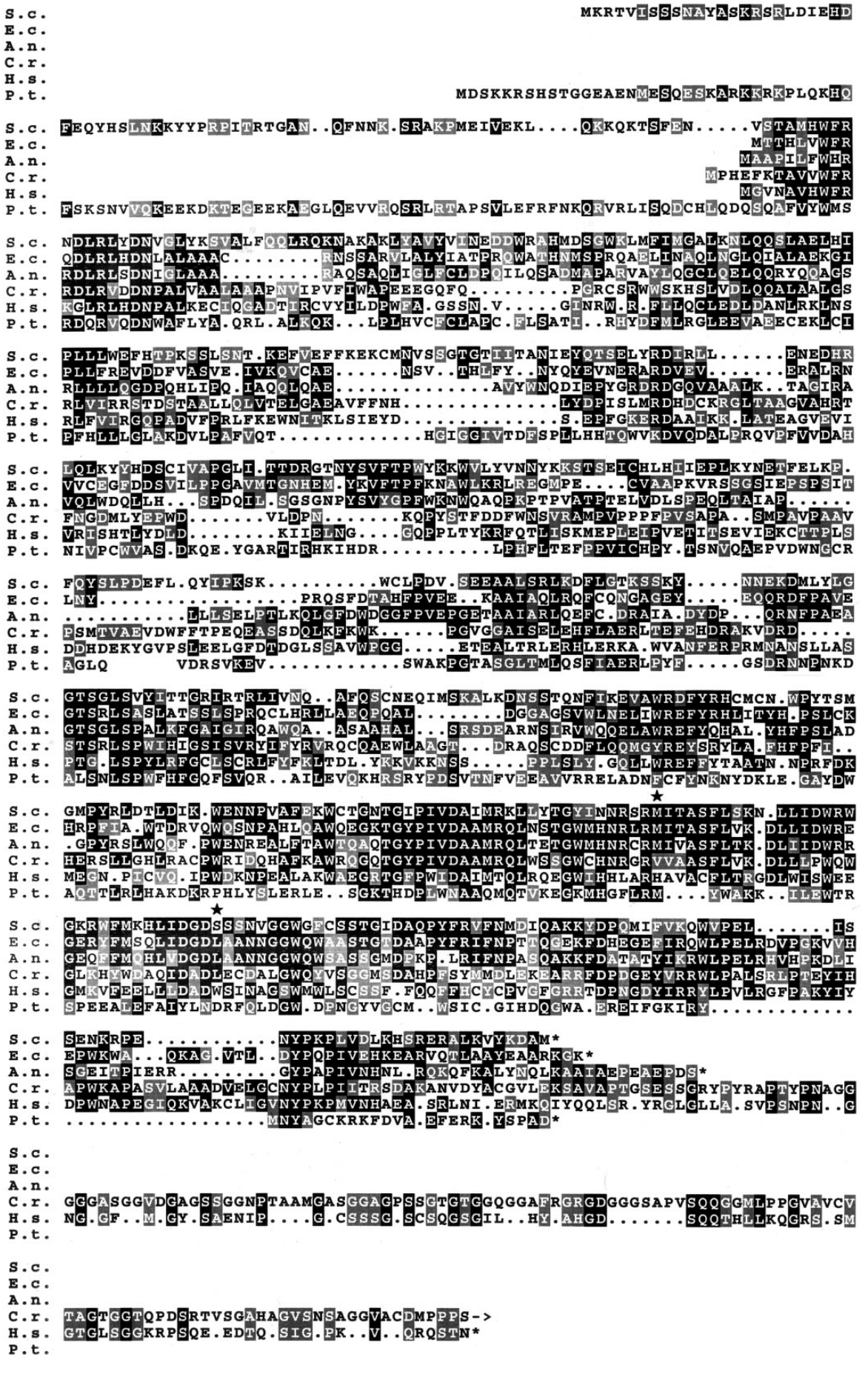

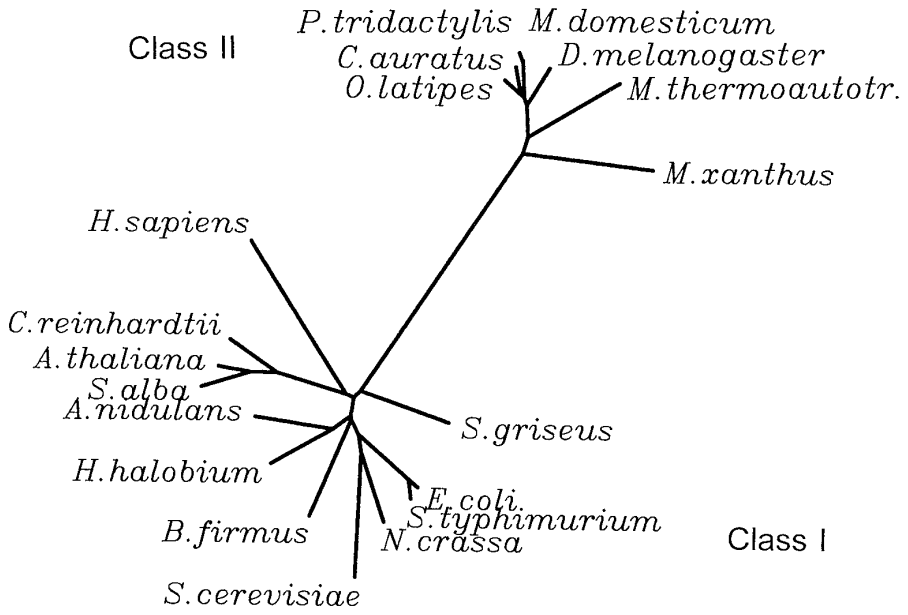

FIG. 2. Unrooted phylogenetic tree for photolyases and photolyase-like blue-light receptors from *Bacillus firmus* (bacterium), *Escherichia coli* (bacterium), *Saccharomyces cerevisiae* (yeast), and *Salmonella typhimurium* (bacterium) (class I MTHF-type photolyases); *Anacystis nidulans* (cyanobacterium), *Halobacterium halobium* (archaeon), and *Streptomyces griseus* (bacterium) (class I 8-HDF-type photolyases); *Arabidopsis thaliana* (plant), *Chlamidomonas reinhardtii* (green alga), and *Sinapis alba* (plant) (class I photolyase-like blue-light receptors); *Carassius auratus* (fish), *Drosophila melanogaster* (insect), *Methanobacterium thermoautotrophicum* (archaeon), *Monodelphis domesticum* (marsupial), *Myxococcus xanthus* (bacterium), *Oryzias latipes* (fish), and *Potorous tridactylis* (marsupial) (Class II photolyase).

set of more than 50 metaphases was analyzed to determine the site of hybridization.

Northern blot analysis. A tissue Northern blot containing 2 μg of poly(A)$^+$ RNA per lane (Clontech human multiple-tissue Northern blot MTN I and II) was hybridized to ^{32}P-labeled DNA probes using hybridization buffer (Clontech Express Hyb) under conditions recommended by the supplier. A 1.2-kb cDNA probe of the cloned human gene and human β-actin cDNA were used as probes.

Southern blot analysis. Genomic DNA (10 μg) from various sources was digested with *Eco*RI and hybridized with a 1.2-kb cDNA probe of the cloned human photolyase-like gene using Quick Hyb hybridization solution (Stratagene). Washing was done with 2× SSC (0.1% SDS) at room temperature for 30 min and subsequently with 0.1× SSC (0.1% SDS) at 68°C (stringent wash) or 60°C (less stringent wash) for 30 min.

Phylogenetic tree. An unrooted phylogenetic tree was obtained by the neighbor-joining method (PHYLIP software package, Felsenstein, 1993) using pairwise distances calculated with the Dayhoff PAM 001 matrix from a stretch of 468 amino acid positions present in all photolyases after alignment with ClustalW. Amino acid sequences of photolyases were taken from Ahmad and Cashmore (1993) (*Arabidopsis thaliana*); Malhotra *et al.* (1994) (*Bacillus firmus*); Kato *et al.* (1994) (*M. domesticum*); Small *et al.* (1995) (*Chlamydomonas reinhardtii*), O'Connor *et al.* (1996) (*Myxococcus xanthus*), and Yasui *et al.* (1994) (other species).

RESULTS AND DISCUSSION

A human photolyase homolog was identified by performing a TBLASTN search of the NCBI expressed sequence tag (cDNA) database using the amino acid sequence of *A. nidulans* photolyase as query. An unpublished EST clone R19031, derived from the Soares infant brain library (Soares *et al.*, 1994), appeared to be homologous to the query sequence. This partial cDNA was cloned from a gridded cDNA library and sequenced. 5′ and 3′ extensions of R19031 were obtained by nested polymerase chain reaction using a human testis library as substrate DNA source. The presence of full-length cDNA was confirmed by amplification using oligonucleotide primers covering the putative start and stop codons.

The cloned DNA fragment contained an open reading frame encoding a protein of 586 amino acids. Figure 1 shows a comparison of the deduced amino acid sequences with those of various photolyases. The human gene has substantial homology (around 25% identical) to class I photolyases. Interestingly, the homology to higher eukaryote photolyases in class II is less pronounced (around 15% identical). Furthermore, several tryptophan residues conserved among photolyases are also present in the human homolog. Among them are W277 of *Escherichia coli* photolyase, which plays a role in substrate binding as well as direct dimer splitting (Kim *et al.*, 1992), and W306 of *E. coli* photolyase, which is necessary for photoreduction of the FAD chromophore (Li *et al.*, 1991). The conser-

FIG. 1. Alignment of amino acid sequences of photolyases from the following species: *Saccharomyces cerevisiae* (S.c.) (class I photolyase, MTHF type); *Escherichia coli* (E.c.) (class I photolyase, MTHF type); *Anacystis nidulans* (A.n.) (class I photolyase, 8-HDF type); *Chlamydomonas reinhardtii* (C.r.) (class I blue-light receptor); *Homo sapiens* (H.s.); and *Potorous tridactylis* (P.t.) (class II photolyase). Identical amino acids are shown as black boxes, whereas similar residues (A, S, T, P; D, E, N, Q; R, K; I, L, M, V; F, Y, W) are indicated by gray boxes. Tryptophan residues 277 and 306 are indicated with stars.

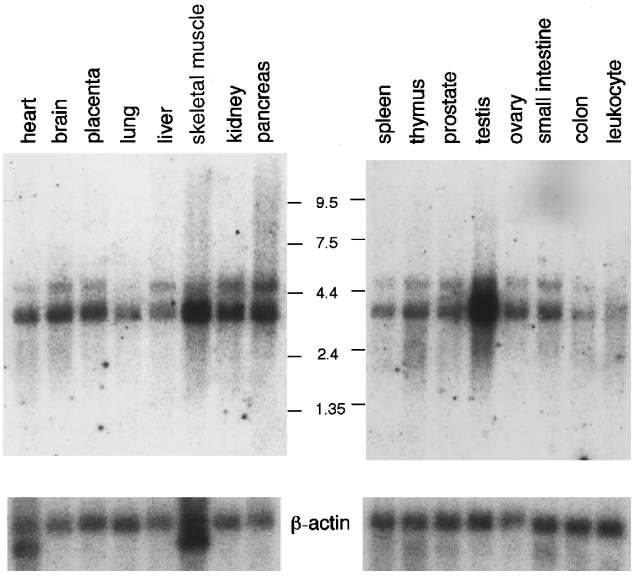

FIG. 3. Northern blot analysis of different human tissues for mRNA expression of the human photolyase homolog.

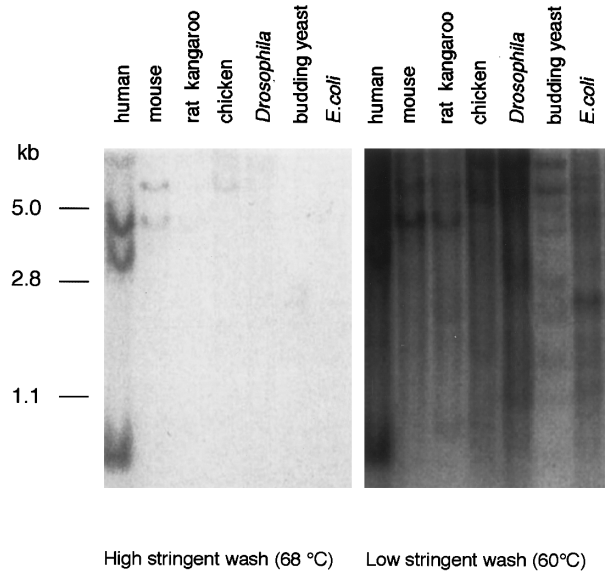

FIG. 5. Southern analysis of genomic DNA from various organisms using human cDNA as probe.

vation of tryptophan residues suggests that the human homolog has FAD binding ability.

Homology to Blue-Light Receptors

Not only the overall similarity but also several features of the deduced amino acid sequence suggest that the cloned human gene might be related to photolyase-like blue-light receptors. Both class I and class II eukaryote photolyases possess an amino-terminal extension, which was shown to be necessary for protein transport into nucleus and mitochondria (Yasui *et al.*, 1992). The putative human protein, however, does not have this extension, as is the case for the plant blue-light receptor family. Instead, a carboxyl-terminal extension is present, which is also found in the *Arabidopsis* blue-light receptor gene *HY4* (Ahmad and Cashmore, 1993) and in the putative blue-light receptor of *C. reinhardtii*

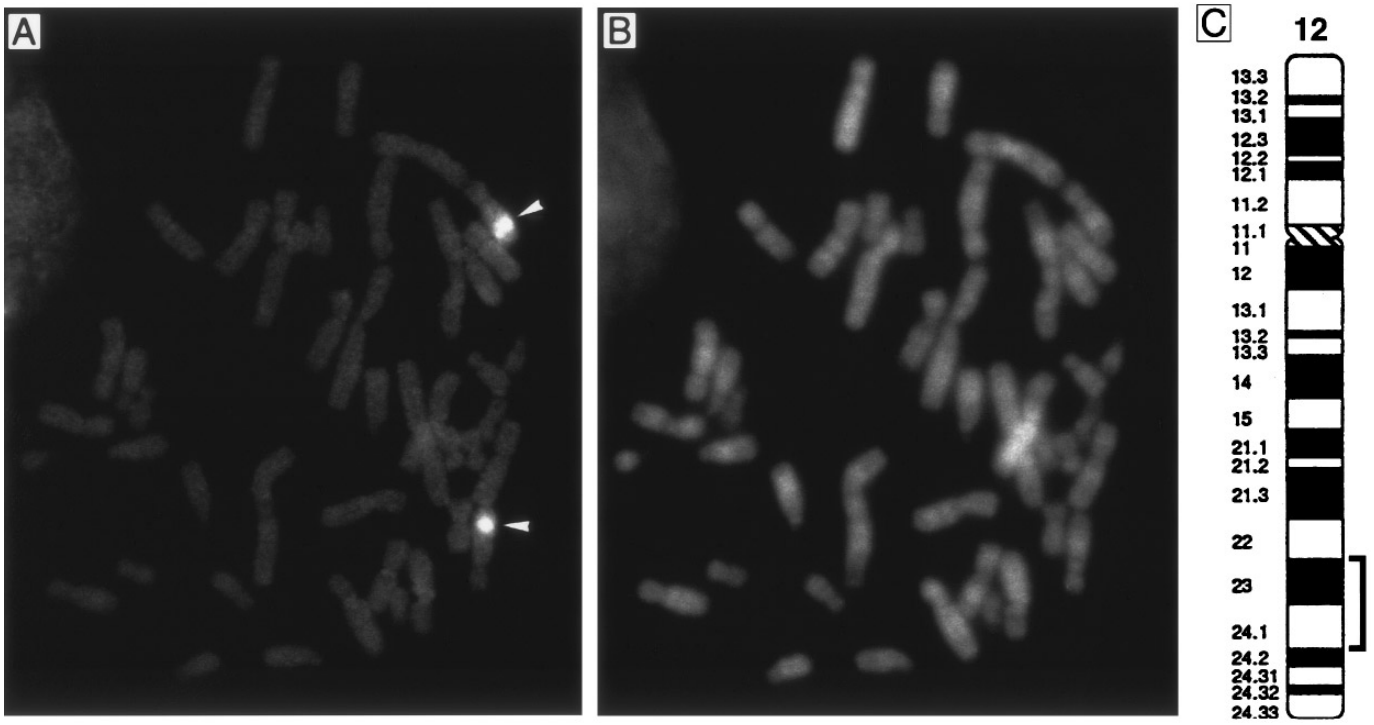

FIG. 4. *In situ* hybridization of metaphase chromosomes with biotinylated human photolyase-like cDNA probe (**A**) compared to DAPI banding of the same metaphase (**B**). The arrowheads indicate the hybridization signal localized on chromosome 12q23–q24.1.

(Small *et al.*, 1995). Interestingly, the amino acid sequence in the C-terminal region of the human protein shows some homology with the C-terminus of the *C. reinhardtii* photolyase-like protein (Fig. 1).

A phylogenetic tree (Fig. 2) was constructed based on amino acid sequences (without the N- or C-terminal extensions) of all known photolyases, confirming the assignment of the cloned human gene as a class I photolyase. Within this class, the human photolyase homolog forms a cluster together with the blue-light receptors, in accordance with the presence of a C-terminal extension.

Tissue Expression

Northern blot analysis of various human tissues was carried out using a radiolabeled PCR fragment from the cloned gene as probe (Fig. 3). Expression of mRNA of the gene is most prominent in testis followed by skeletal muscle, pancreas, and kidney, although constitutive expression was found in all tissues examined. Only a weak signal was found for leukocytes, although photoreactivating activity has been reported for these cells (Sutherland and Bennett, 1995). In additional experiments we found that the level of the gene expression in skin is as high as that in liver or brain (not shown) and less than that in brain or testis. The expression pattern suggests that the gene product may be involved in a function(s) other than repair of UV-damaged DNA. The other function(s) could be related to signal transduction like the plant blue-light photoreceptors. Photolyase activity of the gene product, however, remains to be determined. Two transcripts are observed (Fig. 3) and thought to be the results of alternative polyadenylation or splicing. The relative intensity of the two transcripts differs from tissue to tissue. In pancreas and liver the longer transcripts are relatively more abundant than in skeletal muscle and lung. The meaning of this differentiated expression is not known yet.

Southern analysis of genomic DNA from various organisms (Fig. 4) shows that homologs of the cloned human gene are present in a variety of organisms. This indicates the existence of a gene family related to photolyase-like blue-light receptors.

Chromosomal Localization

In situ hybridization was performed to determine the chromosomal localization of this gene. Chromosome 12q23–q24.1 was unequivocally identified as the site hybridizing to the cloned human gene (Fig. 5). In this region loci for Schinzel syndrome (ulnar-mammary syndrome, MIM No. 181450) and Darier-White disease (keratosis follicularis, MIM No. 124200) that may be related to the cloned gene have been mapped. No known human DNA repair-related syndromes or disorders seem to be associated with this locus as determined by an OMIM search (Pearson *et al.*, 1994).

Very recently, during preparation of the manuscript, the sequence of a (6-4) photoproduct-specific photolyase from *Drosophila melanogaster* was published (Todo *et al.*, 1996). A human homolog that is identical to the gene we cloned from homology to *Anacystis* photolyase was found in a database. It appears that the human sequence is even more similar to (6-4)photoproduct photolyase than to blue-light receptors.

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