Cloning of a marsupial DNA photolyase gene and the lack of related nucleotide sequences in placental mammals

Tomohisa Kato Jr, Takeshi Todo¹, Hitoshi Ayaki, Kanji Ishizaki, Takashi Morita², Sankar Mitra³ and Mituo Ikenaga*

Radiation Biology Center, Kyoto University, Yoshida-konoecho, Sakyo-ku, Kyoto 606-01, ¹Department of Radiation Biology, Faculty of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565, ²Department of Microbial Genetics, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565, Japan and ³Sealy Center for Molecular Science, The University of Texas Medical Branch at Galveston, Galveston, TX 77555-1079, USA

Received July 8, 1994; Revised and Accepted September 6, 1994

DDBJ accession no. D31902

ABSTRACT

Photoreactivating enzyme, DNA photolyase, reduces lethal, mutagenic and carcinogenic effects of ultraviolet light (UV) by catalyzing near UV or visible lightdependent repair of cyclobutane pyrimidine dimers (CPDs) in DNA. The enzyme activity has been detected in a wide variety of organisms ranging from bacteria to nonplacental mammals. However, the evidence for photoreactivation in placental mammals, including humans, is controversial. As a first step to identify the presence and activity of the gene in mammalian species, we isolated a cDNA clone of this gene from a marsupial, the South American opossum Monodelphis domestica. Photolyase activity was expressed in Escherichia coli from the cDNA which is predicted to encode a polypeptide of 470 amino acid residues. The deduced amino acid sequence of this protein is strikingly similar to those of photolyases from two metazoans; the opossum photolyase shares 59% and 63% sequence identity with the Drosophila melanogaster and goldfish Carassius auratus enzymes. respectively. However, no closely related nucleotide sequence was detected in higher mammals and a homologous transcript was undetectable in a number of human tissues. These results strongly suggest that humans, as well as other placental mammals, lack the photolyase gene.

INTRODUCTION

Epidemiological as well as experimental evidence is accumulating to indicate that ultraviolet (UV) light is the primary etiologic agent of human skin cancers, because it induces DNA photoproducts (1). Thus, repair of critical UV-induced DNA lesions is an important step toward the prevention of tumor formation (2). Photoreactivation is a simple and efficient DNA repair pathway which reduces lethal, mutagenic and carcinogenic effects of UV (3-6). This process is mediated by DNA photolyase (EC 4.1.99.3) which splits cyclobutane pyrimidine dimers (CPDs), the major UV photoproducts in DNA, in the presence of near UV or visible light (4,7). The enzyme activity has been detected and characterized in a wide variety of organisms ranging from bacteria to nonplacental mammals (3-8), whereas the evidence for photoreactivation in higher mammals, including humans, is controversial (9-12). Thus, the question of whether a DNA photolyase activity is present in humans is of considerable significance to public health (13).

In an earlier study (14), we identified two distinct lightdependent repair enzymes in Drosophila melanogaster; an ordinary DNA photolyase for splitting CPDs and a new photoreactivating enzyme that repairs (6-4)photoproducts. Subsequently, by expressing a cDNA library constructed from Drosophila in a photolyase-deficient (phr⁻) Escherichia coli strain, the Drosophila photolyase gene was cloned and sequenced (15). Comparison of the predicted amino acid sequences of photolyases from the Drosophila (15) and goldfish Carassius auratus (16) revealed extremely conserved sequences throughout the polypeptides. This provided an opportunity to isolate cDNA of a mammalian photolyase gene by using the conserved sequences as probes, and to examine the presence and activity of the gene in various mammalian species. Here, we describe the isolation of a cDNA clone of this gene from a marsupial, the South American opossum Monodelphis domestica. We report the primary structure of the opossum photolyase and suggest a probable absence of the gene from higher mammals.

MATERIALS AND METHODS

Cells and culture conditions

A corneal tumor cell line DG3-3R derived from the opossum *M.domestica* (5) was kindly provided from Dr Ronald D.Ley,

^{*}To whom correspondence should be addressed

Center for Photomedicine, Lovelace Medical Foundation, Albuquerque, New Mexico, USA, and used to isolate cDNA clones for DNA photolyase. *M.domestica* is known to possess active DNA photolyase (5, 17). DG3-3R cells were maintained in Dulbecco's modified minimum essential medium (DMEM) supplemented with L-glutamine, essential and non-essential amino acids, vitamins, and 10% fetal bovine serum (Hyclone). For 'zoo' blot analysis, the following twelve mammalian cell strains were used; PtK2 (rat kangaroo), Tb1Lu (bat), m5S/1M (mouse), V79 (Chinese hamster), MDCK (dog), LLC-PK1 (white rabbit), Mv1Lu (mink), PK(15) (pig), MDBK (bovine), Muntjac (Indian muntjac), CL (chimpanzee) and HeLa S3 (human). They were grown in DMEM supplemented with 10% fetal bovine serum. Other information for these cell lines is given elsewhere (18).

Oligonucleotide primers, PCR-mediated amplification and sequencing

Oligonucleotide primers (K1-K8) were synthesized in a DNA synthesizer (Applied Biosystems, Model 381A). Poly (A)+ RNA was extracted from DG3-3R cells by a standard method (19, 20). Opossum photolyase cDNA was isolated by several steps of amplification from the $poly(A)^+$ RNA using reverse transcriptase-mediated polymerase chain reaction (RT-PCR) (19) with SUPERSCRIPTTM II RNase H-Reverse Transcriptase (GIBCO BRL). The 5' and 3' ends of the cDNA fragment were extended by rapid amplification of cDNA ends (RACE) PCR (21), using 5' and 3' RACE Systems (GIBCO BRL), respectively. Denaturation for all the PCR reactions was carried out at 94°C for 1 min, and other amplification conditions were as follows: K1 and K2 primers, annealing (57°C, 2 min) and extension (72°C, 3 min), 40 cycles; K7 and K8 primers, annealing (58°C, 2 min) and extension (72°C, 4 min), 35 cycles; 5'RACE PCR, K3 and anchor primers, annealing (58°C, 2 min) and extension (72°C, 3 min), 40 cycles; K4 and universal amplification primers, annealing (57°C, 2 min) and extension (72°C, 3 min), 40 cycles; 3' RACE PCR, K5 and d(T)₁₇ primers, annealing (58°C, 2 min) and extension (72°C, 3 min), 30 cycles; K6 and d(T)₁₇ primers, annealing (58°C, 1.5 min) and extension (72°C, 2.5 min), 30 cycles.

Direct DNA sequencing of both strands of purified PCR products was performed by the dideoxynucleotide chain termination method (19) with $BcaBest^{TM}$ Dideoxy Sequencing kit (TAKARA, Japan).

Phenotypic rescue of photolyase-deficient E.coli cells

Escherichia coli strain SY2 (JM107 $\Delta phr::Cm^r \Delta uvrA::Km^r \Delta recA::Tet^r$) (16), which contains a deletion in the DNA photolyase gene (*phr*), was a generous gift from Dr Akira Yasui, Institute of Development, Aging and Cancer, Tohoku University, Japan. All methods were essentially the same as described previously (15,16). Briefly, overnight cultures of SY2 cells harboring either cloned opossum photolyase cDNA, *Drosophila* photolyase cDNA or empty vector, were appropriately diluted, spread on agar plates containing antibiotics and irradiated with 254 nm UV. Half of the irradiated plates were immediately illuminated with visible light (National fluorescent lamps FL15D) for 60 min at a distance of 2 cm through a 10 mm thick glass plate, keeping the other halves in the dark. Colonies were counted after overnight incubation at 37°C to determine survivals.

Assay of *Micrococcus luteus* UV endonuclease-sensitive sites (ESS)

Activity of the opossum cDNA-encoded photolyase to repair CPDs was assayed by disappearance of the sites sensitive to *M.luteus* UV endonuclease, which cleaves DNA specifically at CPDs (ESS assay), as described (14, 15, 22). In brief, *E. coli* SY2 cell extracts containing recombinant opossum photolase were prepared by sonication of cells, mixed with UV-irradiated 86 bp T8-3 DNA probe labeled with ³²P, and illuminated with photoreactivating light. After the treatment, the DNA probes were recovered by phenol extraction, incubated with *M.luteus* UV endonuclease and then electrophoresed on a 6% denaturing polyacrylamide gel. The *M.luteus* UV endonuclease was supplied by Dr Hiroshi Mitani, Zoological Institute, University of Tokyo.

Southern and Northern blot analyses

Genomic DNAs of DG3-3R cells and the mammalian cell lines of twelve different species described above were isolated by established procedures (19). DNAs of *Drosophila* and chicken were isolated from whole larvae and embryos, respectively. Medaka fish DNA was a gift from Dr Hiroshi Mitani. Genomic DNAs were digested with *Bam*HI and subjected to Southern hybridization with ³²P-labeled opossum photolyase cDNA as described (19, 20), except that the filters were washed in $0.5 \times$ SSC containing 0.2% SDS at 60°C for 30 min.

Total RNAs were isolated from DG3-3R cells and *Drosophila* ovary by the methods described previously (14, 20) and then



Figure 1. Nucleotide sequences of 5' (K1) and 3' (K2) primers used to clone the 1.1 kb opossum photolyase cDNA middle fragment and the corresponding conserved sequences in the cloned cDNAs encoding goldfish *Carassius auratus* and *Drosophila melanogaster* photolyases. Lower case letters represent bases in the primer sequences and the linker sequences are underlined; i indicates inosine, and letters in parentheses indicate that two bases are equally present at these positions in the oligonucleotides. Deduced amino acid sequences of *Carassius* (16) and *Drosophila* (15) photolyases are shown in single capital letter codes.

poly(A)⁺ RNAs were purified by standard procedures (19). Two μ g of the poly(A)⁺ RNAs were hybridized with opossum photolyase cDNA probe as described (19,20). Human multiple tissue Northern blots were purchased from CLONETECH (Catalog #7759-1 and #7760-1). Hybridization was carried out as recommended by the supplier and the filters were washed in 1×SSC containing 0.2% SDS at 55°C for 30 min.

RESULTS

Isolation of opossum photolyase cDNA

The presumed opossum photolyase cDNA was amplified from poly(A)⁺ RNA of DG3-3R cells by RT-PCR using two 26 nucleotide primers, K1 and K2. These primers were constructed on the basis of the highly conserved sequences near the aminoand carboxyl-termini, respectively, of the Drosophila and goldfish Carassius enzymes (Fig. 1). The resulting 1.1 kb cDNA middle fragment was extended to both 5' and 3' ends by RACE PCR. To extend the 5' end of the cDNA fragment, DG3-3R cell mRNA was reverse transcribed with a 5' RACE kit using a gene specific primer K3 (positions of the K3 and other primers below are indicated in Fig. 2). The reaction products were used as template for PCR amplification with the K3 primer and the anchor primer supplied with the kit, and further amplified with an internally nested primer K4. The resulting 530 bp PCR product was purified and sequenced, which revealed the presence of an in-frame start codon about 140 bp upstream of the 5' end of the 1.1 kb cDNA fragment. Similarly, 3' end of the mRNA was isolated by 3' RA-CE with a gene specific primer K5, followed by PCR amplification with a nested primer K6. This yielded 1.4 kb PCR products including a stop codon, which was located about 150 bp beyond the 3' end of the 1.1 kb cDNA. Finally, based on the sequence information of the RACE products, additional primers, K7 and K8, were designed for cloning the entire coding sequence by RT-PCR amplification of DG3-3R mRNA. The resulting 1.5 kb PCR product was purified, subcloned into expression vector pCRIITM (Invitrogen), and the sequence was determined. Figure 2 shows the nucleotide sequence of the PCR product combined with those of the 5' and 3' RACE products.

Photoreactivating activity expressed by the opossum cDNA

To test whether the isolated opossum cDNA encodes active photolyase, expression of the enzyme activity was assayed by phenotypic rescue of phr- E.coli SY2 cells following UV exposure. Twelve independent cDNA plasmid clones were transfected into SY2 cells, and tested for expression of the photoreactivating activity. Six identical clones demonstrated efficient photoreactivating activities, and were named pOPC1. Figure 3 shows a typical example of photoreactivation of UVinduced killing in SY2 cells transformed with pOPC1. The opossum cDNA clone conferred the photoreactivating activity to the phr- E. coli cells, as efficiently as the DNA photolyase cDNA of Drosophila. This strongly suggests that pOPC1 encodes an opossum photolyase. To confirm this possibility, cell extracts from SY2 harboring pOPC1 were checked for the activity to repair CPDs by ESS assay with M. luteus UV endonuclease. As shown in Fig. 4, CPDs (measured as ESS) disappeared from UVirradiated DNA on treatment with cell extracts in the presence of photoreactivating light (lane 5), as revealed by the absence of shorter DNA fragments, whereas such activity was neither detected on treatment in the dark (lane 4) nor without cell extracts (lane 3). These results demonstrate that pOPC1 encodes the opossum DNA photolyase.

Comparison of the opossum, *Drosophila* and goldfish sequences, and the occurrence of related sequences in mammals

The sequence of the 1.5 kb insert in pOPC1 shows the presence of an open reading frame of 1,413 bp that is predicted to encode a protein of 470 amino acids with molecular weight of 54,686 daltons. The deduced amino acid sequence of this protein is

CCCTGGACCT CGAGCTGCTG CTGCTGCGTA AAGCGCGGAC ACTGTGTGCT 50 TGGCCTAGCT TGTTGCGAGC TCCGCCGGAC GCGGGGGGCC TCGGGCTTCC 100 AGCACTGTTT ACAACCGCCC CCAGAATAAA AGGAATAAGA CCCACCATGG 150 CCCCCAAAAA GAGGAGCCTT GGTGACGGTG ATGAGCAAGA GAAGACAGAA 200 ACCCGGAGAA TAAAAACCAAG AGGAAGCCTC TCCAGAAACA CCAATTCAGT 250 AAGAGTAACG TGGTTCAGAA AGAAGAGGAA AACAAGAGAG AGGGAGAGAA 300 AAAGGGAGGA CCAGAAGGCT TGCAAGAAGT CGTGATGCAG TCCAGGCTGC 350 AGACAGCTTC CTCTGTGCTG GAATTTCGTT TTAACAAACA ACGAGTGAGA 400 CTCATCTCCC AGGACTGCCA CCTGCAGGAC CATTCCCAGG CCTTTGTGTA 450 CTGGATGTCT CGGGACCAGA GAGTACAAGA TAATTGGGCT TTCCTTTATG 500 CCCAGAGGTT GGCCCTGAAA CAGAAGCTGC CCCTGCATGT CTGTTTCTGT 550 CTCGCACCCT GCTTCCTGGG TGCTACCATT CGCCATTATG ACTTCATGCT 600 TCGTGGTCTG GAAGAAGTAG CCGAGGAGTG TGAGAAGCTG CACATCCCCT 650 TCCACTTGCT TCTGGGCCTG CCAAAGGATG TACTGCCTGC CTTCGTGCAG 700 GCACATAGCA TTGGTGGCAT CGTGACGGAC TTCTCCCCTC TGCTGCACCA 750 TACTCAGTGG GTGAAGGATG TCCAGGACGG GCTGCCAAAA CAGGTGCCTT 800 TTGTCCAGGT AGATGCTCAC AACATTGTTC CGTGCTGGAT AGCCTCAGAC 850 AAGCAGGAAT ATGGTGCTCG CACCATTCGA CACAAGATCC ATGACCGGCT 900 TCCTCATTTT CTTACTGAGT TCCCCCCTGT TATATGTCAT CCGTATCCTT 950 CAAACATTCA GGCTGAGCCT GTGGACTGGA ATGCCTGTCG GGCAGGATTG 1000 CAGGTGGATC GATCAGTGAA GGAAGTGTCC TGGGCCAAGC CAGGAACTGC 1050 CTCAGGCCTG ACCATGTTGC AGTCCTTCAT ATCCCAGAGG CTCCCCTACT 1100 TTGGATCTGA CCGAAACAAC CCCAACAAGG ATGCACTCAG CAATCTCTCT 1150 CCCTGGTTCC ACTTTGGGCA GGTATCAGTT CAACGGGCCA TTCTGGAAGT 1200 GCAGAAACAT CGAAGCCGGT ACCCAGATTC TGTGGCTAAC TTTGTGGAAG 1250 AGGCTGTGGT GAGAAGGGAG CTGGCAGACA ATTTTTGCTT CTACAACAAG 1300 AACTATGACA AATTAGAAGG TGCTTATGAT TGGGCCCAAA CCACTCTGAG 1350 ACTCCATGCC AAGGACAAGA GGCCCCACCT GTACTCACTG GAACAGCTGG 1400 AGTCTGGGAA GACCCATGAC CCCCTCTGGA ATGCCGCGCA GATGCAGATG 1450 GTACAGGAAG GGAAGATGCA TGGATTCCTC CGCATGTATT GGGCCAAGAA 1500 GATTCTGGAG TGGACGCGCT CCCCTGAAGA AGCCCTAGAG TTTGCTATCT 1550 ATCTCAATGA CCGGTTTCAG CTGGATGGCA GGGATCCCAA TGGCTACGTA 1600 GGCTGCATGT GGTCCATCTG TGGCATCCAT GACCAGGGCT GGGCAGAACG 1650 GGAAATCTTT GGAAAGATCC GCTATATGAA TTATGCCGGC TGTAAGAGGA 1700 AGTTTGACGT GGCAGAGTTT GAGCGCAAAT ACAGCCCTGC GGATTAAGGG 1750 GGCCCCAGAG CCTCCCAGCC ACCCACCTCT CCAGGCCTTC CACTTCCAAC 1800 TTCTCTCCCCT ACATCCAGTC TCTTCCCCAC ACAGAGGGTG GTGACTGGGG 1850 GAGTGCCCGA CACCCAGGCC CAGTTTGTCT TGGTTTAAAC GG 1892

Figure 2. Nucleotide sequence of the cDNA of opossum photolyase. Translation initiation and termination sites are boxed. The positions of the 5' and 3' ends of the cDNA clone in pOPC1 are shown by arrows. Primers indicated are those used for amplification of 1.1 kb cDNA middle fragment (K1 and K2) and 1.5 kb cDNA insert in pOPC1 (K7 and K8), for 5'RACE (K3 and K4) and 3'RACE (K5 and K6). The sequence of the 3' distal part, the region beyond the nucleotide position 1,892, is not known.



Figure 3. Photoreactivating activity of the opossum photolyase as assayed by phenotypic rescue of photolyase-deficient *E. coli* SY2 cells. Colony-forming ability of UV-irradiated cells was measured with (open symbols) or without (closed symbols) photoreactivation treatment. SY2 cells harboring opossum cDNA pOPC1 (circles), *Drosophila* photolyase cDNA pDmPR1 (triangles) or empty vector (squares). Each symbol represents a mean of three independent experiments.

strikingly similar to those of Drosophila and goldfish Carassius photolyases (Fig. 5). The opossum protein shares 59% and 63% sequence identity with the Drosophila and Carassius enzymes, respectively. These results suggest a significant sequence conservation of the photolyase polypeptides in both nonmammalian and mammalian metazoans. We, then, searched for sequences related to the opossum photolyase gene in various placental and nonplacental animal species, as well as lower metazoans, by Southern hybridization with ³²P-labeled 1.5 kb insert of pOPC1 as the probe. As shown in Fig. 6, hybridization at moderate stringency revealed distinct bands for DNAs from five photolyase-positive higher organisms, Drosophila (14,15), chicken (23), Medaka fish (24), rat kangaroo (25) and opossum DG3-3R cells. However, no clear band was detected with cell lines derived from nine placental mammals (lane 6 through lane 14). Although faint bands appeared to be seen in DNA blots of bat, mouse and Chinese hamster cells, we regard they are not significant, because such bands were undetectable when other restriction enzyme, HindIII, was used to digest DNA (data not shown). For primates, dark smears of hybridization appeared in the autoradiograms with restricted DNAs from chimpanzee and human HeLa cells (lanes 15 and 16), even after extensive washing. Similar results were also obtained with DNAs from human liver, diploid human fibroblasts and African green monkey CV-1 cells (data not shown). This result suggests the presence of a primate-specific repeated sequence(s) that has some homology with the opossum cDNA. However, a survey of GenBank data did not locate any known repeated sequence related to that of the photolyase cDNA. To further investigate the possible



Figure 4. Photoreactivation of CPDs in an 86 bp T8-3 DNA segment by cellfree extracts of *E. coli* SY2 cells harboring pOPC1, as assayed by disappearance of *Micrococcus luteus* UV endonuclease-sensitive-sites (ESS). Control and UVirradiated DNAs were mixed with *E. coli* extract and exposed to visible light for 45 min or kept in the dark. DNA samples were then treated with UV endonuclease and electrophoresed on a denaturing gel. Removal of ESS by photoreactivation in lane 5 is indicated by the absence of small fragments.

cause of smear hybridization, the photolyase cDNA was digested into three parts of nearly equal size and these were separately used as probes. However, a similar smear pattern was obtained in all cases (data not shown). We decided not to pursue this problem further at this time.

Expression of photolyase gene in human cells was examined by Northern blot analysis for photolyase transcript in various human tissues. The results presented in Fig. 7 revealed mRNA bands of about 3 kb for DG3-3R cells, and 1.8 kb for *Drosophila* as reported (15). However, no appreciable signal was detected in poly(A)⁺ RNAs from sixteen different human tissues (results for eight tissues are shown in Fig. 7). The absence of a human transcript homologous with the opossum photolyase mRNA strongly suggests that human cells lack photolyase activity.

1	SGECMLHIRLFSTNLYIRSTLLRSVEDENTLLHYCSMSENKRELKRORESPDSGGGKOP	C.auratus
1	. KRTKAQ. KAGPSKKAAKNEKAS SEP KSDQESSDEE R STSK. ALLVSKPDYQNFED-	D.melanogaster
۱		M.domestica
61	RLAEGRARESGWILREVNELRRAAQGCEVNKKLRY - DDDKIKQG - DGFLAMMSKAD	С.а.
55	FLTHL-DHDRVCTAANIQEFSFRKKRVEN	D.m.
2	O SRLQTASSVLE-FRF NKDVR- IRQDCHLQDHQA-FVC	M.d.
		_
20		C.a.
07		D.m.
47		M.d.
80		C.a.
67		D.m.
07		ы. м.д.
0,		14.0.
240	CHENCELLE CAREFORMER THE PEF IN THE DITE-HSASRAAEP HEEVLSS EVE	С.а.
227		D.m.
67	TINDECH STATE HOR HE HOR FIFTI OF TICK - YPSNICAEPURA CRASDVD	M.d.
299	RS CEDERAL DISCONTRACTOR DISCONTRACTOR	С.а.
287	MEEDE CERKE YKAACQO YEECSREER HENDKEDETA EAR GEREEL FEHILARD	D.m.
226		M.d.
359	VKQ -K-REKNASE VASII III V SELATA REPERVISENIS AYD KKE QO AT	C.a.
347	ALE ORF GGQHKA ADA CONTINUE STATE STATE STATES AND A REAL	D.m.
286	ILEROK SRY-POWANY AVERAL SAME STRATE AVERAL AVERAL AVERAL	M.d.
117	SOY TK CONAKE COMPANY ISSUED AND AND AND AND AND AND AND AND AND AN	C.a.
107		D.m.
845	Kaphi Bill Cashara a sa	M.d.
77		C.a.
167		D.m.
105	a na skol na se se se se s	M.d.
537	K TAVKENSNK DSKKSSSKN	С.а.
527	R GGKV-HKKK	D.m.
165	K SPAD	M.d.

Figure 5. Comparison of the amino acid sequences of photolyases of goldfish, *Drosophila* and opossum. These sequences were aligned by computer analysis as described (15). Gaps introduced for optimum alignment are shown by dashes. Shaded areas are regions of sequence identity among three species, and two identical sequences out of the three are enclosed by boxes.

DISCUSSION

The isolation and sequence analysis of the opossum photolyase cDNA revealed that the amino acid sequences of photolyases are extremely conserved throughout the polypeptide chains among three different higher organisms, Drosophila, goldfish and opossum (Fig. 5). In particular, long stretches of identical sequence are present near the carboxyl-termini of the enzymes. The DNA photolyase that have been analyzed so far contain two chromophore cofactors; 1,5-dihydroflavin adenine dinucleotide (FAD) which is commonly observed with all the photolyases, and the second, either 5,10-methenyltetrahydrofolate, or 8-hydroxy-5-deazaflavin, depending on the species (7). The carboxyl-terminal half of the molecule is thought to be involved in the association of two substrates common to all enzymes, i.e., FAD and UV-irradiated DNA (7). However, as indicated previously (16), the sequence of goldfish Carassius photolyase is significantly different from those of bacterial and yeast photolyases, although the fish enzyme shows similarity to those of microorganisms at several sites. These results suggest a significant and specific sequence conservation among photolyases



Figure 6. Distribution of the opossum photolyase gene homolog in various animal species as detected by Southern blot analysis. Genomic DNAs from *Drosophila*, Medaka fish, chicken, two marsupials and eleven placental mammals were digested with *Bam*HI and hybridized with the opossum cDNA. The size markers shown on the left-hand side (kb) were fragments of λ DNA cleaved with *Hind*III.



Figure 7. Northern hybridization with the opossum cDNA probe of $poly(A)^+$ RNAs from opossum DG3-3R cells, *Drosophila* ovary and from multiple human tissues (upper panel). 18S and 28S indicate the position of opossum ribosomal RNAs. Numbers on the left-hand side of human multiple tissue Northern blot indicate size markers in kb. Lower panel shows hybridization of the same filters with human β -actin cDNA.

of metazoans that is divergent from those of microorganisms including yeast.

Early studies indicated that placental mammals lacked photolyase (3,4,9,23), while the activity was easily detectable in marsupials (3,17,25). Southern blot analysis with the opossum cDNA as probe revealed distinct bands for photolyase-positive

higher organisms including marsupials, whereas no reliable band was detected for nine placental animals such as rabbit, dog and pig (Fig. 6). With five of these nine placental mammals, photolyase activity was undetectable using conventional assay methods (3,4,23). Hence, the absence of hybridization with the opossum cDNA strongly suggests that at least these mammalian species, other than primates, have lost the photolyase gene during their evolution. The presence of the background smear of hybridization with primate DNA did not allow us to draw a definitive conclusion regarding the absence of the photolyase gene in human cells. This remains to be elucidated in future study.

The issue of whether the photolyase activity is present in humans is of significant concern in public health, if we consider a possible increasing threat of solar UV exposure, caused by atmospheric ozone depletion (13). As is the case for other placental mammals, photolyase activity was undetectable in human cells in earlier studies (3,9,26). In contrast, two decades ago, Sutherland and her coinvestigators reported the presence of photolyase in human leukocytes (11) and cultured fibroblasts (27), and suggested that its expression is tissue-specific (12). Our observation on the lack of mRNA related to the opossum cDNA in a number of different human tissues, including leukocytes, contradicts this conclusion. It is possible that our inability to detect photolyase mRNA in human cells by Northern blot analysis may be due to an inducible nature of a human enzyme. However, it is unlikely in view of the consistent lack of photolyase genespecific signal in both Northern and Southern blots. Obviously, the possibility cannot be ruled out that humans have a photolyase which is very different from the opossum enzyme. However, this is also unlikely, if we consider that the amino acid sequence of this enzyme is extremely conserved in widely divergent species, i.e., Drosophila, fish and marsupials. Recently, Li et al. (10) have also reported the absence of photolyase in human cells by using a very sensitive enzyme assay. In summary, our present results strongly imply that the absence of photolyase activity in placental mammals, including humans, is due to a missing gene. Why this gene has been eliminated during evolution of the placenta remains an intriguing question.

ACKNOWLEDGEMENTS

We are highly indebted to Drs R.D.Ley for providing opossum DG3-3R cell line, A.Yasui for *E. coli* strain SY2 and H.Mitani for Medaka fish DNA and *M.luteus* UV endonuclease. We also thank Drs T.Yagi, H.Ryo, T.Izumi, Mr J.Fujimoto and Miss K.Fujikawa for their kind help. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (M.I.) and grants CA 31721 and CA 53791 from the United States Public Health Service (S.M.).

REFERENCES

- 1. Brash, D. E. (1988) Photochem. Photobiol., 48, 59-66.
- 2. Tornaletti, S. and Pfeifer, G. P. (1994) Science, 263, 1436-1438.
- 3. Cook, J. S. (1970) Photophysiology, 5, 191-223.
- Rupert, C.S. (1975) In Hanawalt, P. C. and Setlow, R. B. (eds.), Molecular Mechanisms for Repair of DNA. Plenum, New York, pp.73-87.
- Ley, R. D., Applegate, L. A., Fry, R. J. M. and Sanchez, A. B. (1991) Cancer Res., 51, 6539-6542.
- Setlow, R. B., Woodhead, A. D. and Grist, E. (1989) Proc. Natl. Acad. Sci. USA, 86, 8922-8926.
- 7. Sancar, G. B. (1990) Mutat. Res., 236, 147-160.

- Ikenaga, M., Kondo, S. and Fujii, T. (1974) Photochem. Photobiol., 19, 109-113.
- 9. Cleaver, J. E., Paterson, M. C. and Friedberg, E. C. (1976) *Biophys. J.*, 16, 185a.
- Li, Y. F., Kim, S.-T. and Sancar, A. (1993) Proc. Natl. Acad. Sci. USA, 90, 4389-4393.
- 11. Sutherland, B. M. (1974) Nature, 248, 109-112.
- Ogut, S. E., D'Ambrosio, S. M., Samuel, M. and Sutherland, B. M. (1989) J. Photochem. Photobiol. B : Biology, 4, 47-56.
- Russel Jones, R. and Wigley, T. (1989) Ozone Depletion : Health and Environmental Consequences. John Wiley & Sons, New York.
- Todo, T., Takemori, H., Ryo, H., Ihara, M., Matsunaga, T., Nikaido, O., Sato, K. and Nomura, T. (1993) *Nature*, 361, 371-374.
- 15. Todo, T., Ryo, H., Takemori, H., Toh, H., Nomura, T. and Kondo, S. (1994) *Mutat. Res.*, (In press).
- 16. Yasuhira, S. and Yasui, A. (1992) J. Biol. Chem., 267, 25644-25647.
- Sabourin, C. L. K. and Ley, R. D. (1988) Photochem. Photobiol., 47, 719-723.
- Ikenaga, M., Tsujimura, T., Chang, H.-R., Fujio, C., Zhang, Y.-P., Ishizaki, K., Kataoka, H. and Shima, A. (1987) *Mutat. Res.*, 184, 161-168.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning : A Laboratory Manual. 2nd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Wang, Y., Kato, T., Ayaki, H., Ishizaki, K., Tano, K., Mitra, S. and Ikenaga, M. (1992) *Mutat. Res.*, 273, 221-230.
- Frohman, M. A., Dush, M. K. and Martin, G. R. (1988) Proc. Natl. Acad. Sci. USA 85, 8998-9002.
- 22. Todo, T. and Ryo, H. (1992) Mutat. Res., 273, 85-93.
- Cook, J. S. and McGrath, J. R. (1967) Proc. Natl. Acad. Sci. USA, 58, 1359-1365.
- Funayama, T. Mitani, H. and Shima, A. (1993) Photochem. Photobiol., 58, 380-385.
- 25. Cook, J. S. and Regan, J. D. (1969) Nature, 223, 1066-1067.
- 26. Trosko, J. E. and Isoun, M. (1970) Int. J. Radiat. Biol., 18, 271-275.
- 27. Sutherland, B. M. and Oliver, R. (1975) Nature, 257, 132-134.