Pergamon

PII: S0042-6989(96)00309-4

Vision Res., Vol. 37, No. 14, pp. 1867–1871, 1997

Printed in Great Britain
0042-6989/97 $17.00 + 0.00

Expression of Visual and Nonvisual Opsins in American Chameleon

SHOJI KAWAMURA,* SHOZO YOKOYAMA*†

Received 29 May 1996; in revised form 5 November 1996

We previously characterized five visual opsin genes of American chameleon (*Anolis carolinensis*). Here we report its nonvisual opsin gene orthologous to the chicken pineal gland-specific opsin (P-opsin) gene. In the pure-cone American chameleon retina, all visual opsins including rod opsin are expressed. In both pineal and parietal eye, three visual opsins as well as P-opsin are expressed. Although opsins are detected in the pineal glands of a wide variety of vertebrates, Southern analysis suggests that the P-opsin gene is used mainly by birds and reptiles. © 1997 Elsevier Science Ltd.

P-opsin Retina Pineal Parietal eye Vertebrates

INTRODUCTION

The periodic nature of biological functions is under direct influence of both a daily light/dark cycle and an endogenous clock (Sassone-Corsi, 1994). Biological clocks have been detected in the hypothalamic suprachiasmatic nucleus of mammals (Meijer & Rietveld, 1989), pineal gland of birds, reptiles, and fish (Takahashi *et al.*, 1989; Falcon *et al.*, 1989), and the retina of amphibians, birds, and mammals (Cahill *et al.*, 1991; Tosini & Menaker, 1996). The pineal gland of many non-mammalian vertebrates is believed to regulate photoreception and melatonin synthesis and has been identified as a major component of the circadian system (Underwood, 1990; Korf, 1994). Despite different sites of the clock and variation in the pineal anatomy (Korf, 1994), the core mechanism of the circadian rhythm appears to be fundamentally similar among all vertebrates, involving opsins or opsin-like proteins (Takahashi, 1993). Such proteins have been detected in the pineal gland of lampreys, teleosts, frogs, birds, and reptiles (Vigh-Teichmann *et al.*, 1982; Vigh-Teichmann & Vigh, 1990; Kalsow *et al.*, 1991; Araki *et al.*, 1992; Foster *et al.*, 1993; Tamotsu *et al.*, 1994; Masuda *et al.*, 1994; Yoshikawa *et al.*, 1994). Recently, the gene encoding the pineal gland-specific opsin, named pinopsin (Okano *et al.*, 1994) or P-opsin (Max *et al.*, 1995), has been isolated from the chicken.

The pineal gland of the American chameleon (*Anolis carolinensis*) shows light-dependent daily cycles of melatonin synthesis both *in vitro* and *in vivo* (Menaker & Wisner, 1983; Underwood & Hyde, 1989), where opsin-like proteins have been detected by an immunocytochemical study (Foster *et al.*, 1993). Currently known opsins are classified into six major groups (Yokoyama, 1994, 1995, 1996): (1) the RH1 cluster (consisting of rhodopsins); (2) the RH2 cluster (a mixture of opsins with various absorption sensitivities); (3) the SWS1 cluster (blue, violet, and UV opsins); (4) the SWS2 cluster (blue opsins); (5) the LWS/MWS cluster (a mixture of green and red opsins); and 6) the P-opsin cluster. We have previously characterized visual opsin genes of the American chameleon $rh1_{Ac}$, $rh2_{Ac}$, $sws1_{Ac}$, $sws2_{Ac}$, and lws_{Ac} that encode RH1, RH2, SWS1, SWS2, and LWS, respectively (Kawamura & Yokoyama, 1993, 1994, 1995, 1996). The lizard has an additional simple but highly structured photoreceptor organ, the parietal (third) eye, suspected of enhancing the detection of dawn and dusk (Solessio & Engbretson, 1993). Here we report the cloning and sequencing of the P-opsin gene of American chameleon (denoted P_{Ac}). The isolation of P_{Ac} and the visual opsin genes provides a unique opportunity for the comprehensive study of opsin gene expression in the retina and other photoreceptive organs.

MATERIALS AND METHODS

Genomic library screening

A genomic library was constructed with *Bam*HI-digested lambda EMBL3 vector and *Sau*3AI partially digested genomic DNA of the American chameleon (Kawamura & Yokoyama, 1993). We also constructed another genomic library using *Bam*HI- and *Eco*RI-digested genomic DNA, the long arm of *Bam*HI-digested and the short arm of *Eco*RI-digested EMBL4 lambda vectors. From these libraries, we isolated λAc34 and λAc167 containing exons 1–4 and exons 4 and 5 of P_{Ac}, respectively, using the bovine rhodopsin cDNA (bd20) (Nathans & Hogness, 1983) as the probe. Probe-

*Biological Research Laboratories, Department of Biology, Syracuse University, 130 College Place, Syracuse, NY 13244, U.S.A.
†To whom all correspondence should be addressed [*Tel* 315-443-9166; *Fax* 315-443-2012; *Email* syokoyam@mailbox.syr.edu].

hybridizing restriction fragments were subcloned into the Bluescript SK(−) plasmid vectors. Probe labelling, plaque hybridization, membrane washing and sequencing for both strands were done as described previously (Kawamura & Yokoyama, 1993).

Reverse transcriptase–polymerase chain reaction (RT–PCR) analysis

From four animals, approximately 1, 2, and 20 μg of total RNA were extracted from the parietal eye, pineal gland, and retina, respectively (Chomczynski & Sacchi, 1987). Total RNA was mixed with the PCR reaction mix [10 mM Tris–HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl 0.1% Triton X-100, 200 μM dNTPs, and primers at 1 μM each], MMLV reverse transcriptase, and Taq polymerase in total volume of 25 μl. The samples were placed in a thermal cycler at 50°C for 8 min, followed by 35 cycles of 92°C for 45 sec, 55°C for 60 sec, and 72°C for 90 sec. Five microlitres each of PCR products was electrophoresed on 2.5% agarose gel. PCR was also carried out without reverse transcriptase for each opsin gene, resulting in no amplification in all tissues. Southern hybridization of RT–PCR products was carried out using mixed radioactive probe containing bovine rhodopsin (bd20) (Nathans & Hogness, 1983), human blue (hs37) and red (hs7) (Nathans *et al.*, 1986), and chicken P-opsin cDNAs (Max *et al.*, 1995). Hybridization was carried out at 65°C following the commercial protocol for Hybond-N membrane (Amersham). Hybridized membrane was washed in 1 × SSC/0.1% SDS at 55°C and autoradiographed after 5 hr of exposure. All amplified cDNA fragments were sequenced either directly or after being cloned and were confirmed to match the corresponding genomic DNA sequences.

Zoo blot analysis

High molecular weight DNA were extracted from the blood of the owl (*Bubo virginianus*), turkey (*Meleagris gallopavo*), pigeon (*Columba livia*), human (*Homo sapiens*), and bovine (*Bos taurus*), liver of chicken (*Gallus gallus*), and muscle of lamprey (*Petromyzon marinus*), goldfish (*Carassius auratus*), Mexican cavefish (*Astyanax fasciatus*), and Tokay gecko (*Gekko gekko*) (Blin & Stafford, 1976). Frog (*Xenopus laevis*) and coelacanth (*Latemeria chalumnae*) DNA were gifts from Drs. B. Knox and R. DeSalle, respectively. ten micrograms (5 μg for American chameleon) per lane of genomic DNA was digested with *Sst*I, electrophoresed on 0.5% agarose gel, and transferred to a Hybond-N nylon membrane by using the VacuGene vacuum blotting system (Pharmacia). The last 170 bp portion of the p_{Ac} exon 4 was amplified by PCR using two primers: 5′-TCATGGT(G/C)ATCG(G/C)(T/C)TTCCTI(A/G)T(G/C)TGCTGG(G/C)T-3′ and 5′-CTGTTTGTTCATGAAG(A/C)(A/C)(G/A)TAG-3′ and was used as the hybridization probe.

The probe was labeled with [α-^{32}P] dATP by the random-priming method. Hybridization was carried out at 65°C following the commercial protocol for Hybond-N membrane. The hybridized membrane was washed in 1 × SSC/0.1% SDS at 65°C four times (30 min each), which allows ~20% mismatch (Meinkoth & Wahl, 1984).

RESULTS AND DISCUSSION

Molecular structure of the American chameleon P-opsin gene

The basic structure of P_{Ac} is identical to that of the chicken P-opsin gene. It has five exons. The position of the second intron is displaced 15 nucleotides toward the 3′ direction compared to the equivalent intron in the visual opsin genes (Max *et al.*, 1995). Splice junction signals (GT/AG) are conserved in all introns. The deduced P-opsins of American chameleon (P_{Ac}) and chicken (P_{Gg}) have two less amino acids than visual opsins, located between the fourth and fifth putative transmembrane domains (Okano *et al.*, 1994; Max *et al.*,

```
                                                                                    I                                    II
PAc    M-----------------------------------LNG--TP-GPFEGPQWPFLA-PRGTYTSVAVLMGLVVVLATVVNGLVIVVSVRYKRLRSPLNYILVNLAVADLLVTSFGSTISFANNIYGFFVFGPTACEFEGF 101
PGg    M------S--------S--------NS-SQ-A-P-P-NG--TP-GPFDGPQWPYQA-PQSTYVGVAVLMGTVVACASVVNGLVIVVSICYKKLRSPLNYILVNLAVADLLVTLCGSSVSLSNNINGFFVFGRRMCELEGF 109
RH1Ac  M--N---G-----T--EG----Q--NFYVPMS--NK-T-GVVR-NPFEYPQYYL-ADP-WQFSALAAYMFLLILLGFPINFLTLFVTIQHKKLRTPLNYILLNLAVANLFMVLMGFTTTMYTSMNGYFIFGTVGCNIEGF 115
RH2Ac  M--N---G-----T--EG----I--NFYVPLS--NK-T-GLVR-SPFEYPQYYL-AEP-WKYKVVCCYIFFLIFTGLPINILTLLVTFKHKKLRQPLNYILVNLAVADLFMACFGFTVTFYTAWNGYFIFGPIGCAIEGF 115
SWS1Ac M--SG-----------Q-----E--DFYLF-------E-NISSVGPWDGPQYHI-APM-WAFYFQTAFMGFVFFAGTPLNAIILIVTVKYKKLRQPLNYILVNISFAGFLFCTFSVFTVFMASSQGYFFFGRHVCAMEAF 110
SWS2Ac MTMQKSRPDS----R-DNLP--E--DFFIPVP-LDV-A-NITTLSPFLVPQTHL-GNP-SLFMGMAAFMFILIVLGVPINVLTIFCTFKYKKLRSHLNYILVNLSVSNLLVVCVGSTTAFYSFSNMYFSLGPTACKIEGF 124
LWSAc  M--AGTVTEAWDVAVFAARRRNDEDDT-TRDSLFTYTNSNNTR-GPFEGPNYHI-A-PRWVYNITSVWMIFVVIASIFTNGLVLVATAKFKKLRHPLNWILVNLAIADLGETVIASTISVINQISGYFILGHPMCVLEGY 134
          III                              IV                                          V
PAc    MVSLTGIVGLWSLAILAFERYLVICKPVGDFRFQQRHAVIGCAFTWLWSLLWTLPPLFGWSSYIPEGLRTSCGPNWYTGGN--DNNSYIMTLFVTCFITPLAMIIFSYANLLLTLRAVAAQQKEMATTQQAEREVTRMVV 239
PGg    MVSLTGIVGLWSLAILALERYVVVCRPLGDFQFQRRHAVSGCAFTWGWALLWSTPPLLGWSSYVPEGLRTSCGPNWYTGGS--NNNSYILSLFVTCFVLPLSLILFSYTNLLLTLRAAAAQQKEADTTQRAEREVTRMVI 247
RH1Ac  FATLGGEMGLWSLVVLAVERYVVICKPMSNFRFGETHALIGVSCTWIMALACAGPPLLGWSRYIPEGMQCSCGVDYYTPTPEVHNESFVIYMFLVHFVTPLTIIFFCYGRLVCTVKAAAAQQQESATTQKAEREVTRMVV 255
RH2Ac  FATLGGQVALWSLVVLAIERYIVVCKPMGNFRFSATHALMGISFTWFMSFSCAAPPLLGWSRYIPEGMQCSCGPDYYTLNPDYHNESYVLYMFGVHFVIPVVVIFFSYGRLICKVREAAAQQQESASTQKAEREVTRMVI 255
SWS1Ac LGSVAGLVTGWSLAFLAFERYIVICKPFGNFRFNSKHALLVVAATWFIGIGVSIPPFFGWSRYIPEGLQCSCGPDWYTVGTKYKSEYYTWFLFIFCFIVPLTLIIFSYSQLLGALRAVAAQQQESATTQKAEREVSRMVV 250
SWS2Ac SATLGGMVSLWSLAVVAFERYLVICKPLGNFTFRGTHAIIGCAVTWMFGLAASLPPLFGWSRYIPEGLQCSCGPDWYTTENKWNNESYVIFLFCFCFGVPLSVIIFSYGRLLLTLRAVAKQQEQSATTQKAEREVTKMVV 263
LWSAc  TVSTCGISALWSLAVISWERWVVVCKPFGNVKFDAKLAVAGIVFSWVWSAVWTAPPVFGWSRYWPHGLKTSCGPDVFSGSDDPGVLSYMIVLMITCCFIPLAVILLCYLQVWLAIRAVAAQQKESESTQKAEKEVSRMVV 274
          VI                   VII
PAc    TMVMAFLVCWLPYASFAMVVATNKDLLIQPALASLPSYFSKTATVYNPIIYVFMNKQFRSCLLS-TLSCGR-RP-QAAQGTTPAAISSPRGRTLEGSRNKVVPSASEGSGNDAMTS 352
PGg    VMVMAFLLCWLPYSTFALVVATHKGIIIQPVLASLPSYFSKTATVYNPIIYVFMNKQFQSCLLE-MLCCGY-QP-QRTGKASPGTPGPHADVTAAGLRNKVMPAHPV 351
RH1Ac  IMVISFLVCWVPYASVAFYIFTHQGSDFGPVFMTIPAFFAKSSAIYNPVIYILMNKQFRNCMIM-TLCCGKNPLGDEE--TSAGT---KTETSTVSTSQVSPA 352
RH2Ac  LMVLGFLLAWTPYAMVAFWIFTNKGVDFSATLMSVPAFFSKSSSLYNPIIYVLMNKQFRNCMIT-TICCGKNPFGDED-VSSSVSQS-KTEVSSVSSSQVSPA 355
SWS1Ac VMVGSFCLCYVPYASLAMYMVNNRDHGLDLRLVTIPAFFSKSSCVYNPIIYCFMNKQFRACILE-TV-CGK-PMSDES-D--VSSSAQKTEVSSVSSSQVSPS 347
SWS2Ac VMVMGFLVCWLPYASFALWVVTHRGEPFDVRLASIPSVFSKASTVYNPVIYVLMNKQFRSCMLKLIF-CGKSPFGDED-D--VSGSSQATQVSSVSSSQVSPA 365
LWSAc  VMIIAYCFCWGPYTVFACFAAANPGYAFHPLAAALPAYFAKSATIYNPIIYVFMNRQFRNCIMQ-LF--GK-KV-DDGSE--LSSTS-RTEVSSVSNSSVSPA 369
```

FIGURE 1. Alignment of the deduced amino acid sequences of P_{Ac}, P_{Gg} (Max *et al.*, 1995) and five visual opsins of American chameleon (Kawamura & Yokoyama, 1993, 1994, 1995, 1996). Gaps, denoted by dashes, were introduced to optimize sequence similarity. Putative transmembrane domains I–VII are indicated by horizontal lines.

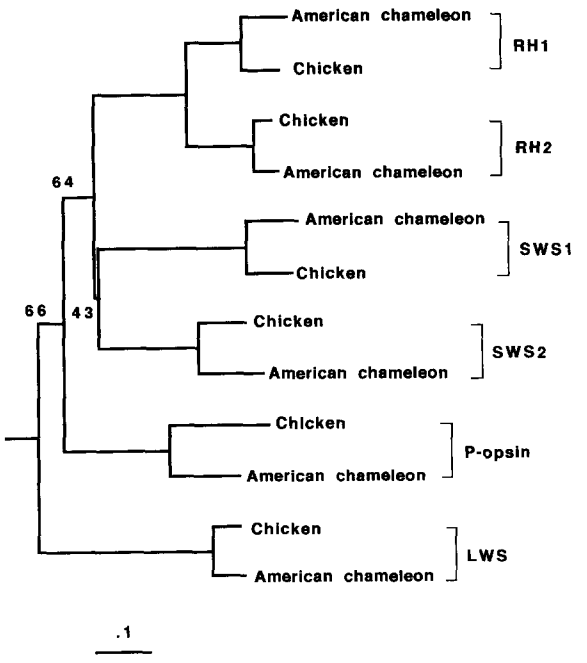

FIGURE 2. The phylogenetic tree for P-opsins and visual opsins of American chameleon and chicken. RH1, RH2, SWS1, SWS2, and LWS opsins of chicken correspond to its rhodopsin, green, violet, blue, and red opsins (Okano *et al.*, 1992). To construct a rooted tree, Rh1 (GnBank K02315), Rh2 (M12896), Rh3 (M17718), and Rh4 (M17719 and M17739) opsins of *Drosophila melanogaster* were used as the outgroup. The topology and branch lengths of the phylogenetic tree were estimated by using the neighbor-joining method (Saitou & Nei, 1987) based on the Poisson-corrected numbers of amino acid substitutions per site. The bootstrap supports were generated by resampling 1000 replications (Higgins *et al.*, 1992) and are indicated beside branch nodes unless they are 100%.

1995) (Fig. 1). P_{Ac} and P_{Gg} have 73% amino acid identity with each other, while they have 45–50% amino acid identity to the visual opsins in vertebrates. A phylogenetic tree constructed from the sequences of a number of bird and reptile opsins clearly shows that P_{Ac} is more closely related to P_{Gg} than to the visual opsins (Fig. 2).

P_{Ac} and P_{Gg} are unique at four sites, containing amino acids F88 (F96 for P_{Gg}), D131 (D139), S163 (S171), and N176 (N184), while those at the corresponding sites of the visual opsins are Y, N, R, and D (Fig. 1). Thus, Fig. 2 suggests that the common ancestor of the two opsins might have achieved amino acid replacements Y88F, N131D, R163S, and D176N, following the site number of P_{Ac}. When the seven transmembrane model of an opsin (Hargrave *et al.*, 1983) is considered, Y88F, R163S, and D176 are located in the cytoplasmic region, while N131D is in the intradiscal region. Furthermore, P_{Gg} shows the chicken P-opsin-specific amino acid replacements K135R and R306Q in the intradiscal region. Since these amino acid changes probably do not interact with the chromophore in the transmembrane region directly, they might have been important in modifying the interaction of the P-opsin with other proteins in the pineal gland. The exact effects of these changes on the function of the P-opsin remain to be examined.

Expression of visual- and P-opsin genes

Using opsin gene-specific primers [Fig. 3(A)], we have examined the expression of $rh1_{Ac}$, $rh2_{Ac}$, $sws1_{Ac}$, $sws2_{Ac}$, lws_{Ac} and p_{Ac} in the retina, pineal gland, and parietal eye of American chameleon by RT–PCR assay. In the retina, all five visual opsin genes are expressed [Fig. 3(B)]. However, the expression of the rod opsin gene, $rh1_{Ac}$, is totally unexpected because not only is rod opsin not detected in the retina by immunocytochemical assay (Foster *et al.*, 1993) but also this species is believed to have a pure-cone retina (Yu & Fager, 1982; Fowlkes *et al.*, 1984; Walter *et al.*, 1986). This suggests that either rod opsin is produced at a low level in some cone photoreceptor cells or this species possesses a small number of rod photoreceptors which might have been overlooked in the previous analyses. In the American chameleon retina, three types of cone pigments with maximal absorption (λmax) at 625 nm, 503 nm, and 462 nm are known to exist (Provencio *et al.*, 1992), which appear to correspond to LWS, RH2, and SWS2 pigments, respectively (Kawamura & Yokoyama, 1993, 1995, 1996). Therefore, expression of two additional visual opsin genes in the retina suggests the presence of ultraviolet-sensitive cones which have been detected in its closely related Puerto Rican *Anolis* species (Fleishman *et al.*, 1993).

(A)

opsin gene	forward	reverse	expected size of cDNA (bp)
p_{Ac}	5'-ccgtcattggctgcgctttc-3'	5'-acagctcgcagggtcaagag-3'	235
$rh1_{Ac}$	5'-cccttattggggtgagttgc-3'	5'-gccgctttcactgtacagac-3'	241
$rh2_{Ac}$	5'-ccttgatgggcatttctttt-3'	5'-gcctctcgaactttgcatat-3'	241
$sws1_{Ac}$	5'-tacacctggttcctcttcat-3'	5'-ctgtttgttcatgaagcagtag-3'	330
$sws2_{Ac}$	5'-ccatcatcggttgtgctgtg-3'	5'-acagctctcagggtgagaag-3'	241
lws_{Ac}	5'-ccgtggctggcattgtcttc-3'	5'-accgcacggatagccaacca-3'	241

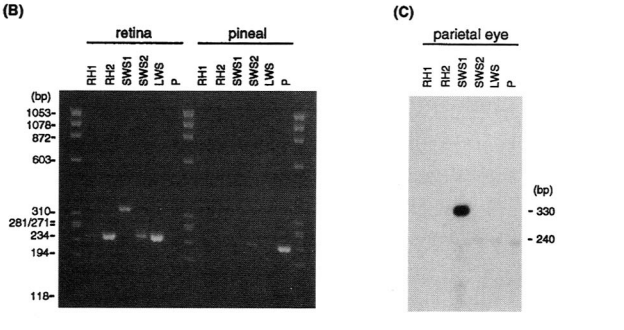

FIGURE 3. RT–PCR assay for the visual- and P-opsin gene expression in the retina, pineal gland and parietal eye of American chameleon. (A) Primers used for the RT–PCR analysis. For $sws1_{Ac}$ and the other opsin genes, codons between Y198-Q307 and those between the second nucleotide of A139 and that of V217 of p_{Ac} are amplified, respectively (see Fig. 1). (B) RT–PCR amplification of opsin cDNAs from the retina and pineal gland. (C) Southern hybridization of RT–PCR products from the parietal eye with the mixed radioactive probe containing bovine rhodopsin (bd20) (Nathans & Hogness, 1983), human blue (hs37) and red (hs7) (Nathans *et al.*, 1986) and chicken P-opsin cDNAs (Max *et al.*, 1995).

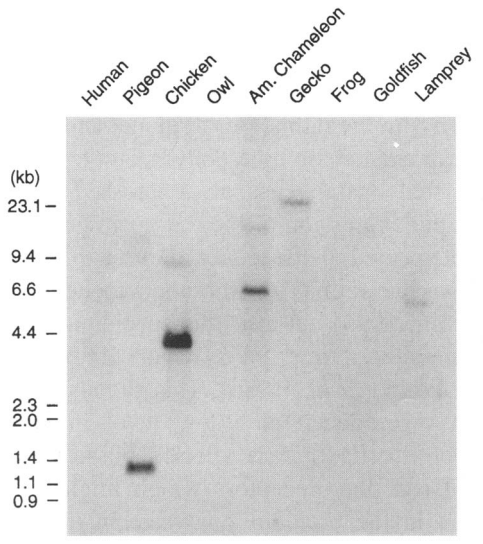

FIGURE 4. Southern hybridization of vertebrate genomic DNA to the exon 4 of P_{Ac}. A mixture of λ *Hind*III and ϕX174RF *Hae*III size standards are indicated in kb. Two hybridizing bands for American chameleon are consistent with those expected from the restriction maps of two allelic forms of P_{Ac} (data not shown).

In the pineal gland, expression of P_{Ac} and lower levels of $sws2_{Ac}$ and lws_{Ac} expression are detected by staining the agarose gel with ethidium bromide [Fig. 3(B)]. When the gel was Southern-blotted and hybridized to mixed radioactive probes (see Materials and Methods), expression of $sws1_{Ac}$ was additionally detected (result not shown). The immunocytochemical study has suggested the presence of RH1 (rod) opsin in the pineal gland of American chameleon (Foster *et al.*, 1993), but we cannot detect it using the RT–PCR assay. In the mouse pineal gland, rhodopsin is actually expressed, but it lacks retinal derivatives, showing that the opsins of the pineal gland are not involved in phototransduction (Kramm *et al.*, 1993). However, using the same HPLC method, retinals have been detected in the directly light-sensitive pineal gland of the trout (Tabata *et al.*, 1985) and the quail (Foster *et al.*, 1989). Similarly, the American chameleon appears to contain retinals in the pineal gland (Provencio & Foster, 1993). Thus, the mechanisms of phototransduction may fundamentally differ between mammalian pinealocytes and the functional photoreceptors of non-mammals (Kramm *et al.*, 1993).

In the parietal eye of the American chameleon, no opsin has been detected by immunocytochemical analysis (Foster *et al.*, 1993). However, hybridization of the RTPCR products to the radioactive probes reveals $sws1_{Ac}$ and a much lesser amount of $sws2_{Ac}$, lws_{Ac}, and P_{Ac} expression [Fig. 3(C)]. The parietal eye is likely to contain photoreceptor cells that are sensitive to blue and green light (Solessio & Engbretson, 1993). Thus, all four different opsins may have an important function in photoreception in this organ.

Phylogenetic distribution of P-opsin gene

To investigate how commonly the P-opsin genes are found among vertebrates, the exon 4 of P_{Ac} was hybridized to genomic DNA from jawless fish (marine lamprey), bony fishes (goldfish, Mexican cavefish, and coelacanth), frog, birds (chicken, pigeon, great horned owl, and turkey), lizards (American chameleon and Tokay gecko), and mammals (human and bovine) (Fig. 4). Hybridization signals were detected only for lizards, chicken, pigeon, turkey and lamprey but not for the other species (the hybridization data for Mexican cavefish, coelacanth, turkey, and bovine are not shown). Curiously, owl DNA showed no hybridization signal. The lack of the P-opsin gene is consistent with the observation that the pineal gland of another nocturnal owl (*Strix uralensis*) is partially degenerated and may not play a physiological circadian oscillatory role in owls (Taniguchi *et al.*, 1993). The hybridization result strongly suggests that the P-opsin gene is found mainly in some birds and reptiles and that teleosts, amphibians, and mammals do not possess the orthologous gene.

Phylogenetic analyses show that P-opsin already existed before the divergence of various vertebrates (Okano *et al.*, 1994; Max *et al.*, 1995). However, Southern analysis suggests that the P-opsin gene disappeared in many vertebrate lineages during evolution. What are the types of opsins used in the pineal glands of teleosts, amphibians, and even in some mammals (Korf, 1994)? Perhaps these species use visual opsins and/or entirely new, yet unknown, pineal gland-specific opsins for pineal photoreception.

REFERENCES

Araki, M., Fukada, Y., Shichida, Y., Yoshizawa, T. & Tokunaga, F. (1992). Differentiation of both rod and cone types of photoreceptors in the *in vivo* and *in vitro* developing pineal glands of the quail. *Developmental Brain Research, 65*, 85–92.

Blin, N. & Stafford, D. W. (1976). A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Research, 3*, 2303–2308.

Cahill, G. M., Grace, M. S. & Besharse, J. C. (1991). Rhythmic regulation of retinal melatonin: metabolic pathways, neurochemical mechanisms, and the ocular circadian clock. *Cellular and Molecular Neurobiology, 11*, 529–560.

Chomczynski, P. & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate phenol–chloroform extraction. *Analytical Biochemistry, 162*, 156–159.

Falcon, J., Brum-Marmillon, J., Claustrat, B. & Collin, J. P. (1989). Regulation of melatonin secretion in a photoreceptive pineal organ: a study in the pike. *Journal of Neuroscience, 9*, 1943–1950.

Fleishman, L. J., Loew, E. R. & Leal, M. (1993). Ultraviolet vision in lizards. *Nature, 365*, 397.

Foster, R. G., Garcia-Fernandes, J. M., Provencio, I. & DeGrip, W. J. (1993). Opsin localization and chromophore retinoids identified within the basal brain of the lizard *Anolis carolinensis*. *Journal of Comparative Physiology A, 172*, 33–45.

Foster, R. G., Schalken, J. J., Timmers, A. M. & DeGrip, W. J. (1989). A comparison of some photoreceptor characteristics in the pineal and retina. I. Japanese quail (*Curnix coturnix*). *Journal of Comparative Physiology A, 165*, 553–563.

Fowlkes, D. H., Karwoski, C. J. & Proenza, L. M. (1984). Endogenous circadian rhythm in electroretinogram of free-moving lizards. *Investigative Ophthalmology and Visual Science, 25*, 121–124.

Hargrave, P. A., McDowell, J. H., Curtis, D. R., Wang, J. K., Juszczak, E., Fong, S. L., Mohanna Rao, J. K., Argos, P. (1983). The structure of bovine rhodopsin. *Biophysics of structure and mechanism, 9*, 235–244.

Higgins, D. G., Bleasby, A. J. & Fuchs, R. (1992). CLUSTAL V:

Improved software for multiple sequence alignment. *Computer Applications in the Biosciences, 8*, 189–191.

Kalsow, C. M., Greenhouse, S. S., Gern, W., Adamus, G., Hargrave, P. A., Lang, L. S., Donoso, L. A. (1991). Photoreceptor cell specific proteins of snake pineal. *Journal of Pineal Research, 11*, 49–56.

Kawamura, S. & Yokoyama, S. (1993). Molecular characterization of the red visual pigment gene of the American chameleon (*Anolis carolinensis*). *FEBS Letters, 323*, 247–251.

Kawamura, S. & Yokoyama, S. (1994). Cloning of the rhodopsin-encoding gene from the rod-less lizard *Anolis carolinensis*. *Gene, 149*, 267–270.

Kawamura, S. & Yokoyama, S. (1995). Paralogous origin of the rhodopsin-like opsin genes in lizards. *Journal of Molecular Evolution, 40*, 594–600.

Kawamura, S. & Yokoyama, S. (1996). Phylogenetic relationships among short wavelength-sensitive opsins of American chameleon (*Anolis carolinensis*) and other vertebrates. *Vision Research, 36*, 2797–2804.

Korf, H.-W. (1994). The pineal organ as a component of the biological clock. In Pierpaoli, W., Regelson, W. & Fabris, N. (Eds), *The aging clock: the pineal gland and other pacemakers in the progression of aging and carcinogenesis* (p. 1342). New York: New York Academy of Sciences.

Kramm, C. M., de Grip, W. J. & Korf, H.-W. (1993). Rod–opsin immunoreaction in the pineal organ of the pigmented mouse does not indicate the presence of a functional photopigment. *Cell and Tissue Research, 274*, 71–78.

Masuda, H., Oishi, T., Ohtani, M., Michinomae, M., Fukada, Y., Shichida, Y. & Yoshizawa, T. (1994). Visual pigments in the pineal complex of the Japanese quail, Japanese grass lizard and bullfrog: immunocytochemistry and HPLC analysis. *Tissue and Cell, 26*, 101–113.

Max, M., McKinnon, P. J., Seidenman, K. J., Barrett, R. K., Applebury, M. L., Takahashi, J. S., Margolskee, R. F. (1995). Pineal opsin: a nonvisual opsin expressed in chick pineal. *Science, 267*, 1502–1506.

Meijer, J. H. & Rietveld, W. J. (1989). Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. *Physiological Review, 69*, 671–707.

Meinkoth, J. & Wahl, G. (1984). Hybridization of nucleic acids immobilized on solid supports. *Analytical Biochemistry, 138*, 267–284.

Menaker, M. & Wisner, S. (1983). Temperature-compensated circadian clock in the pineal of Anolis. *Proceedings of the National Academy of Sciences USA, 80*, 6119–6121.

Nathans, J. & Hogness, D. (1983). Isolation, sequence analysis, and intron–exon arrangement of the gene encoding bovine rhodopsin. *Cell, 34*, 807–814.

Nathans, J., Thomas, D. & Hogness, D. S. (1986). Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. *Science, 232*, 193–202.

Okano, T., Kojima, D., Fukada, Y., Shichida, Y. & Yoshizawa, T. (1992). Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. *Proceedings of the National Academy of Sciences USA, 89*, 5932–5936.

Okano, T., Yoshizawa, T. & Fukada, Y. (1994). Pinopsin is a chicken pineal photoreceptive molecule. *Nature, 372*, 94–97.

Provencio, I. & Foster, R. G. (1993). Vitamin A_2-based photopigments within the pineal gland of a fully terrestrial vertebrate. *Neuroscience Letters, 155*, 223–226.

Provencio, I., Loew, E. R. & Foster, R. G. (1992). Vitamin A_2-based visual pigments in fully terrestrial vertebrates. *Vision Research, 32*, 2201–2208.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic tree. *Molecular Biology and Evolution, 4*, 406–425.

Sassone-Corsi, P. (1994). Rhythmic transcription and autoregulatory loops: winding up the biological clock. *Cell, 78*, 361–364.

Solessio, E. & Engbretson, G. A. (1993). Antagonistic chromatic mechanisms in photoreceptors of the parietal eye of lizards. *Nature, 364*, 442–445.

Tabata, M., Suzuki, T. & Niwa, H. (1985). Chromophores in the extraretinal photoreceptor (pineal organ) of teleosts. *Brain Research, 338*, 173–176.

Takahashi, J. S. (1993). Circadian-clock regulation of gene expression. *Current Opinion in Genetics and Development, 3*, 301–309.

Takahashi, J. S., Murakami, N., Nikaido, S. S., Pratt, B. L. & Robertson, L. M. (1989). The avian pineal, a vertebrate model system of the circadian oscillator: cellular regulation of circadian messengers, and macromolecular synthesis. *Recent Progress in Hormone Research, 45*, 279–352.

Tamotsu, S., Oishi, T., Nakao, K., Fukada, Y., Shichida, Y., Yoshizawa, T. & Morita, Y. (1994). Localization of iodopsin and rod-opsin immunoreactivity in the retina and pineal complex of the river lamprey, *Lampetra japonica*. *Cell and Tissue Research, 278*, 1–10.

Taniguchi, M., Murakami, N., Nakamura, H., Nasu, T., Shinohara, S. & Etoh, T. (1993). Melatonin release from pineal cells of diurnal and nocturnal birds. *Brain Research, 620*, 297–300.

Tosini, G. & Menaker, M. (1996). Circadian rhythms in cultured mammalian retina. *Science, 272*, 419–421.

Underwood, H. (1990). The pineal and melatonin: regulators of circadian function in lower vertebrates. *Experientia, 46*, 120–128.

Underwood, H. & Hyde, L. L. (1989). The effect of daylength on the pineal melatonin rhythm of the lizard *Anolis carolinensis*. *Comparative Biochemistry and Physiology, 94A*, 53–56.

Vigh-Teichmann, I., Korf, H.-W., Oksche, A. & Vigh, B. (1982). Opsin-immunoreactive outer segments and acetylcholinesterase-positive neurons in the pineal complex of *Phoxinus phoxinus* (Teleostei Cyprinidae). *Cell and Tissue Research, 227*, 351–369.

Vigh-Teichmann, I. & Vigh, B. (1990). Opsin immunocytochemical characterization of different types of photoreceptors in the frog pineal organ. *Journal of Pineal Research, 8*, 323–333.

Walter, A. E., Shuster, T. A. & Farber, D. B. (1986). Light-induced phosphorylation of proteins from the all-cone retina of the lizard, *Anolis carolinensis*. *Investigative Ophthalmology and Visual Science, 27*, 1609–1614.

Yokoyama, S. (1994). Gene duplications and evolution of the short wavelength-sensitive visual pigments in vertebrates. *Molecular Biology and Evolution, 11*, 32–39.

Yokoyama, S. (1995). Amino acid replacements and wavelength absorption of visual pigments in vertebrates. *Molecular Biology and Evolution, 12*, 53–61.

Yokoyama, S. (1996). Molecular evolution of retinal and nonretinal opsins. *Genes to Cells, 1*, 787–794.

Yoshikawa, T., Yashiro, Y., Oishi, T., Kokame, K. & Fukada, Y. (1994). Immunoreactivities to rhodopsin and rod/cone transducin antisera in the retina, pineal complex and deep brain of the bullfrog, *Rana catesbeiana*. *Zoological Science, 11*, 675–680.

Yu, L.-W. & Fager, R. S. (1982). Visual pigments and phosphodiesterase of a cone-dominated lizard retina. *Investigative Ophthalmology and Visual Science, 22 Suppl.*, 43.

Acknowledgements—Comments by J. Belote, G. A. Engbretson, S. Laughlin, E. Main, M. Max, R. Yokoyama, and an anonymous reviewer were greatly appreciated. We thank G. A. Engbretson for his help in isolating RNA from American chameleon and J. Nathans and M. Max for their providing cDNA probes. The work was supported by a NIH grant GM42379.