Pinopsin expressed in the retinal photoreceptors of a diurnal gecko

Yuki Taniguchi[a], Osamu Hisatomi[a,*], Masao Yoshida[b], Fumio Tokunaga[a]

[a] *Department of Earth and Space Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan*
[b] *Oita University, Oita, Japan*

Received 4 January 2001; revised 26 February 2001; accepted 1 March 2001

First published online 20 April 2001

Edited by Richard Cogdell

Abstract Retinal cDNAs encoding the putative opsins, dg3 and dg4, were isolated from a diurnal gecko, *Phelsuma madagascariensis longinsulae*. dg3 mRNA is localized in about 20% of the thin members of type C double cones, and likely encodes an opsin of the ultraviolet-sensitive pigment. Surprisingly, dg4 is very similar to chicken pinopsin, a pineal-specific photoreceptive molecule. An anti-dg4 antiserum recognized a small population of photoreceptor outer segments in the retina and a large number of pinealocytes. Our results suggest that *P. m. longinsulae* expresses pinopsin in its retina, which usually plays a role as a photoreceptive molecule in the pineal organ.

Key words: Opsin; Pinopsin; Immunohistochemistry; In situ hybridization; Diurnal gecko (*Phelsuma madagascariensis longinsulae*)

1. Introduction

Visual pigments, consisting of a protein moiety (opsin) and a chromophore (11-*cis*-retinal or its derivatives), absorb light as the first step of visual excitation. The spectral sensitivity of visual pigments is mainly responsible for that of photoreceptor cells, and is tuned by special amino acid residues in opsins. Vertebrate opsins have evolved along five lines, which consist of rhodopsins expressed in rods and four types of cone opsins [1–3]. In addition to these opsins, several photoreceptive molecules, such as pinopsin found in chicken pineal, have recently been identified in extra-retinal photoreceptors [4].

Since the photic environment of an animal probably provides strong selective pressure for the evolution of the visual system, a comparison of retinal organization among vertebrates can provide an essential insight into the adaptive evolution. The retinal organization of Gekkonidae has been studied intensively, because they have both nocturnal and diurnal genera. Most geckos are nocturnal and possess retinas which consist of only rod-like photoreceptors [5,6]. It has been proposed that gecko rods have been transmuted from the cones of an ancestral diurnal lizard, as a consequence of adaptation for the nocturnal habit (the transmutation theory) [7].

All geckos investigated so far possess three morphological types of photoreceptor cells: (1) 'type A single', (2) 'type B double' composed of a principal and an accessory member, and (3) 'type C double' consisting of a thin and a thick member [8]. In a nocturnal gecko, *Gekko gekko*, three visual pigments have been identified by microspectrophotometry: they are P521, P467 and P364 with absorption maxima (λ_{max}) at 521 nm, 467 nm and 364 nm, respectively [9,10]. The cDNAs encoding P521 and P467 have been isolated, and their deduced amino acid sequences show high similarities to those of, respectively, the LWS and RH2 pigments of a diurnal lizard, *Anolis carolinensis* [11–13]. These observations indicate that *G. gekko* rods express cone visual pigments, and provide strong support for the transmutation theory from a molecular point of view.

Among geckos, there are a few diurnal genera (such as *Phelsuma*) that have pure-cone retinas. Because cones of diurnal geckos can also be classified into three morphological types, it has been suggested that the photoreceptors of an ancestral nocturnal gecko have reverted to the cones of diurnal geckos as a consequence of a change of their habitat (the reverse transmutation) [7]. That is, the diurnal geckos, descended from a nocturnal ancestor which further evolved from a diurnal lizard, presumably re-evolved their present photopic visual capability [14]. What has occurred in the diurnal gecko retinas, in addition to the morphological change of photoreceptors, during the re-adaptation process to the photopic environment?

We have carried out detailed investigations on retinal organization of a diurnal gecko (*Phelsuma madagascariensis longinsulae*), and have previously reported that *P. m. longinsulae* has two opsins, dg1 and dg2, very similar to *G. gekko* P521 and P467, respectively [15]. In situ hybridization studies showed that dg1 is expressed in most photoreceptor cells except the thin members of type C double. Most of the type C double thin members express dg2, but a small population of the thin members is negative against either dg1 or dg2 cRNA probes. It was suggestive that another type of opsin is expressed in these cells.

In the present study, we isolated and characterized two additional retinal cDNAs, encoding a putative ultraviolet (UV)/violet-sensitive pigment (dg3) and a pinopsin (dg4) of *P. m. longinsulae*. In situ hybridization and immunocytochemical studies suggest that dg3 is expressed in a small population of type C double thin members, and that dg4 is expressed in only a few accessory members of type B double in the central region of the retina. Pinopsins have been reported to be expressed only in the pineal and brain [4,16], and our findings are, therefore, the first report that shows the existence of pinopsin in retinal photoreceptor cells. These data may give us new insights into the evolution of photoreceptor organizations and into the regulation of opsin expression.

*Corresponding author. Fax: (81)-6-6850 5480.
E-mail: hisatomi@ess.sci.osaka-u.ac.jp

0014-5793/01/$20.00
PII: S0014-5793(01)02395-X

2. Materials and methods

2.1. Isolation of dg3 cDNA fragments by rapid amplification of cDNA ends (RACE PCR)

The preparation of retinal cDNAs of a diurnal gecko (*P. m. longinsulae*) and addition of a poly(G) tail to the cDNAs have been described previously [17]. The partial cDNA fragments of dg3 were amplified with RED-F2 and VVP-R2′ degenerate primers by polymerase chain reactions (PCRs), as described previously [3,18]. The 3′ end of the dg3 cDNA fragment with a poly(A) tail was amplified using T-amp and DG3-F1 (5′-CGCAAGCTTGCAGATGACGATGTA-3′) primers on annealing at 50°C [19]. To amplify the 5′ end of dg3 fragments, amplifications were carried out using C-amp and DG3-R1 (5′-TGTAAGCTTACCGCAACCACGGCA-3′) primers on annealing at 50°C [17].

2.2. Isolation of dg4 cDNA fragments by RACE PCR

The partial cDNA fragments of dg4 were amplified using degenerate primers, VVP-R2′ and VVP-F5 (corresponding to amino acid sequences LN(Y/W)ILVN) [3,18]. The 3′ and 5′ ends of dg4 cDNA fragments were obtained by RACE PCR as described in the isolation of dg3 cDNA, using gene-specific primers DG4-F1 (5′-GCGGGATCCTTTCTCGTCTGCTGGC-3′) and DG4-R1 (5′-AGAAAGCTTGGAGAAATAGGACGG-3′).

2.3. Sequencing and data analysis

All sequences were determined for at least three independent clones to avoid PCR errors. Sequence analyses were carried out as described previously [20–22]. Sequence data used in the present analyses were taken from EMBL, GenBank and DDBJ databases, with the following accession numbers: chicken (*Gallus gallus*) red (P22329), violet (M92039) and pinopsin (P51475); bullfrog (*Rana catesbeiana*) FCV (AB001983); toad (*Bufo japonicus*) pinopsin (AF200433); *Xenopus laevis* VCOP (U23463); medaka (*Oryzias latipes*) KFH-V (AB001605); goldfish (*Carassius auratus*) UV (Q90309); American chameleon (*Anolis carolinensis*) SWS1 (AF134192–4) and P-opsin (AF134767–71); pigeon (*Columba livia*) P-opsin (P51476).

2.4. In situ hybridization

A cDNA fragment (820 bases) of dg3 was cloned into a pGEM-3Zf(+) plasmid vector (Promega), and digoxigenin-labeled antisense cRNA riboprobes were synthesized as recommended in the manufacturer's protocol (Boehringer Mannheim). The preparation of retinal cryosections of *P. m longinsulae* and methods for in situ hybridization were as described previously [15,17,18].

2.5. Immunocytochemistry

A cDNA fragment encoding the C-terminal region of dg4 was amplified using DG4-F1 and DG4-BglII (5′-AGGAGATCTGCTATGCAGTTCTGT) as primers, and the fusion protein (mouse dihydrofolate reductase (DHFR), helix VI–VII with the C-terminal region of dg4 and a histidine hexamer tail) was isolated and used to immunize mice as described previously [22]. The antiserum was absorbed with DHFR and used for the immunohistochemical experiments. We confirmed that the anti-dg4 antiserum selectively recognized the recombinant dg4 in *Escherichia coli* homogenates (data not shown).

The eyes of the diurnal gecko (*P. m longinsulae*), a nocturnal gecko (*Gekko japonicus*), and a diurnal lizard (*Takydromus tachydromoides*) were enucleated and dissected at their equator. Posteric halves of the retinas were stripped from the pigment epithelium and fixed with 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.4) overnight at 4°C. The retinas were treated with 1% bovine serum albumin in PBS for 30 min, incubated with the primary antiserum (1:1000 in PBS) for 1 h, and reacted with biotinylated anti-mouse IgG+IgA+IgM (Nichirei Histofine-SAB-PO reaction kit) for 30 min at room temperature. Then, the retinas were incubated with peroxidase-conjugated streptavidin (Nichirei Histofine-SAB-PO reaction kit), and antibody binding was visualized using 3,3′-diaminobenzidine tetrahydrochloride.

For immunofluorescent observations, paraffin sections (10 μm) of retinas and pineal organs were incubated with 3% normal serum diluted in PBS, and incubated with the primary antiserum (1:1000 in PBS) for overnight. Sections were incubated with FITC-conjugated goat anti-mouse antibodies (1:200 in PBS) for 1 h at 4°C, mounted in Permafluor, and analyzed with a confocal microscope (Olympus Fluoview).

3. Results

3.1. The deduced amino acid sequences of dg3 and dg4

Two kinds of cDNAs encoding the putative opsins dg3 and dg4 were isolated from the retinal cDNAs of a diurnal gecko, *P. m. longinsulae*. dg3 and dg4 appear to consist of 346 and 350 amino acids, respectively, and functionally important residues for vertebrate opsins are conserved in their sequences (Fig. 1). The deduced amino acid sequence of dg3 shows high identity with those of the SWS1 pigment (89.3%) of a diurnal lizard (*A. carolinensis*) and of the violet-sensitive pigment (83.1%) of chicken. A phylogenetic tree constructed by the

```
                                                                      I                     II
Phelsuma dg3      MSGE-EDFYLFTNISSVGPFDGPQYHIAPMWAFYIQTAFMGFVFFAGTPLNGIILIAIVKYKKLRQPLNYILVNISAAGFL   80
Anolis   SWS1     MSGQ-EDFYLFENISSVGPWDGPQYHIAPMWAFYFQTAFMGFVFFAGTPLNAIILIVTVKYKKLRQPLNYILVNISFAGFL   80
Phelsuma dg4    MHVQMANASQASLKNGTLSPFDGPQWPHRASRRVYTSLAALMGVVVLSASLANGLVIAVSVRFKRLRSPLNYILVNLATADLL   83
Anolis   P                 MLNGTPGPFEGPQWPFLAPRGTYTSVAVLMGLVVVLATVVNGLVIVVSVRYKRLRSPLNYINVNLNAVADLL   71
                      *       *

                                 III                             IV
FCTFSVFTVFVSSAQGYFVFGKHICALEAFLGSLAGLVTGWSLAFLAMERYIVICKPFGNFRFNAKHASLVVAATWVIGIGVSVPPFFGWSRYIPEGLGC  180
FCTFSVFTVFMASSQGYFFFGRHVCAMEAFLGSVAGLVTGWSLAFLAFERYIVICKPFGNFRFNSKHALLVVAATWFIGIGVSIPPFFGWSRYIPEGLQC  180
VTFFGSIISFVNNAVGFFVFGKTACRFEGFMVSLTGIVGLWSLAILAFERYLVICKPVGDFQFQRRHAVIGCLYTWGWSLIWTVPPLFGWSSYVPEGLGT  183
VTSFGSTISFANNIYGFFVFGPTACEFEGFMVSLTGIVGLWSLAILAFERYLVICKPVGDFRFQQRHAVIGCAFTWLWSLLWTLPPLFGWSSYIPEGLRT  171
                         * *
                    V                                        VI
SCGPDWYTVGTKYRSEYYTWFLFIFCFMVPLTIIIFSYSQLLSALRAVAAQQQESATTQKAEREVSRMVVVMVGSFCTCYVPYAALAMYMVNYRNHGIDL  280
SCGPDWYTVGTKYKSEYYTWFLFIFCFIVPLTLIIFSYSQLLGALRAVAAQQQESATTQKAEREVSRMVVVMVGSFCLCYVPYASLAMYMVNNRDHGLDL  280
SCGPNWYMGGT--NNNSYIVALFVTCFALPLSMILFSYANLLLTLRAVAAQQKEQETTQRAEKEVTRMVITMVMAFLVCWLPYATFAMVVATTKDLSIQP  281
SCGPNWYTGGN--DNNSYIMTLFVTCFITPLAMIIFSYANLLLTLRAVAAQQKEMATTQQAEREVTRMVVTMVMAFLVCWLPYASFAMVVATNKDLLIQP  269
 *
       VII
RMVTIPAFFSKSACVYNPIIYCFMNKQFRGCIMEMVCGKPMSDDSEASTS-QKTEVSSVSSSQVSPS                                   346
RLVTIPAFFSKSSCVYNPIIYCFMNKQFRACILETVCGKPMSDESDVSSSAQKTEVSSVSSSQVSPS                                   347
GLASLPSYFSKTATVYNPIIYVFMNKQFRSCLLNTVSCGRIPQTMPGTPATTAVRGGFVLTSEGRGNKVASTELHS                          350
ALASLPSYFSKTATVYNPIIYVFMNKQFRSCLLSTLSCGRRPQAAQGTTPAAISSPRGRTLEGSRNKVVPSASEGSGNDAMTS                   352
          *
```

Fig. 1. Alignment of the deduced amino acid sequences of *P. m. longinsulae* opsins (dg3 and dg4) and *A. carolinensis* opsins (SWS1 and P). Gaps, denoted by dashes, were introduced to optimize sequence similarity. Asterisks indicate the amino acids typical for vertebrate opsins; asparagine residues serving as sites for *N*-glycosylation, cysteine residues forming a disulfide bond, a lysine residue making a Schiff base linkage for the chromophore, and a glutamate residue serving as a counter-ion for the protonated Schiff base. Putative transmembrane domains I–VII are represented by horizontal lines. The sequences of dg3 and dg4 have been deposited in the EMBL nucleotide database with accession numbers AF074045 and AB022881, respectively.

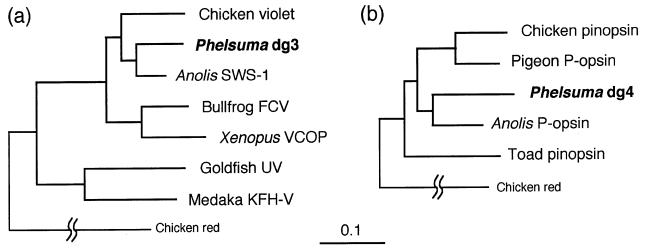

Fig. 2. NJ trees of (a) group S (SWS1) and (b) group P opsins calculated from the deduced amino acid sequences, using the chicken red sequence as an outgroup. Bar represents 10% substitutions per site.

neighbor-joining (NJ) method indicates that dg3 belongs to group S (Fig. 2a). Surprisingly, dg4 is very similar to *A. carolinensis* P opsin and chicken pinopsin with 82.3% and 76.7% amino acid identity, respectively. Judging from the primary structure, it seems reasonable to assume that dg4 is the pinopsin of *P. m. longinsulae* (Fig. 2b).

3.2. Localization of dg3 mRNAs in P. m. longinsulae retina

The retina of *P. m. longinsulae* has three types of cone cells: (1) type A single cones; (2) type B double cones, consisting of a principal member with a colorless oil droplet and an accessory member without oil droplets but with a prominent paraboloid; and (3) type C double cones, consisting of thin and thick members [8]. Fig. 3 shows the distribution of dg3 mRNA in radial sections of *P. m. longinsulae* retina. Hybridization signals are found around the outer limiting membrane, suggesting that dg3 mRNA is localized in the cell bodies and myoids of a small population of type C double thin members (Fig. 3a). We have reported that most thin members express dg2, a group ML opsin similar to gecko P467, but a subpopulation of thin members does not express dg2 [15]. dg2-negative cells likely express dg3, which are about 20–25% of the thin members in our sections (Fig. 3b,c).

3.3. Localization of dg4 in P. m. longinsulae retina

To investigate the localization of dg4, we raised an anti-dg4 antiserum against its C-terminal region whose amino acid sequence is significantly diverged from those of the other gecko opsins. Fig. 4a shows the distribution of anti-dg4 positive cells in the whole-mount preparation of *P. m. longinsulae* retina, and a small population of outer segments were selectively recognized with this serum. The distribution of immunopositive cells was, however, significantly different from those of the other opsins [15]. In a radial section of the central region of *P. m. longinsulae* retina, the antiserum recognized a few outer segments of accessory members of type B double cones (Fig. 4b–d). Distribution of positive cells is not uniform in the retina, but exclusively restricted to the central region. No signals were observed in the control experiments, using either the anti-dg4 antiserum pre-absorbed with the dg4 antigen or a normal mouse serum instead of the anti-dg4 antiserum (data not shown).

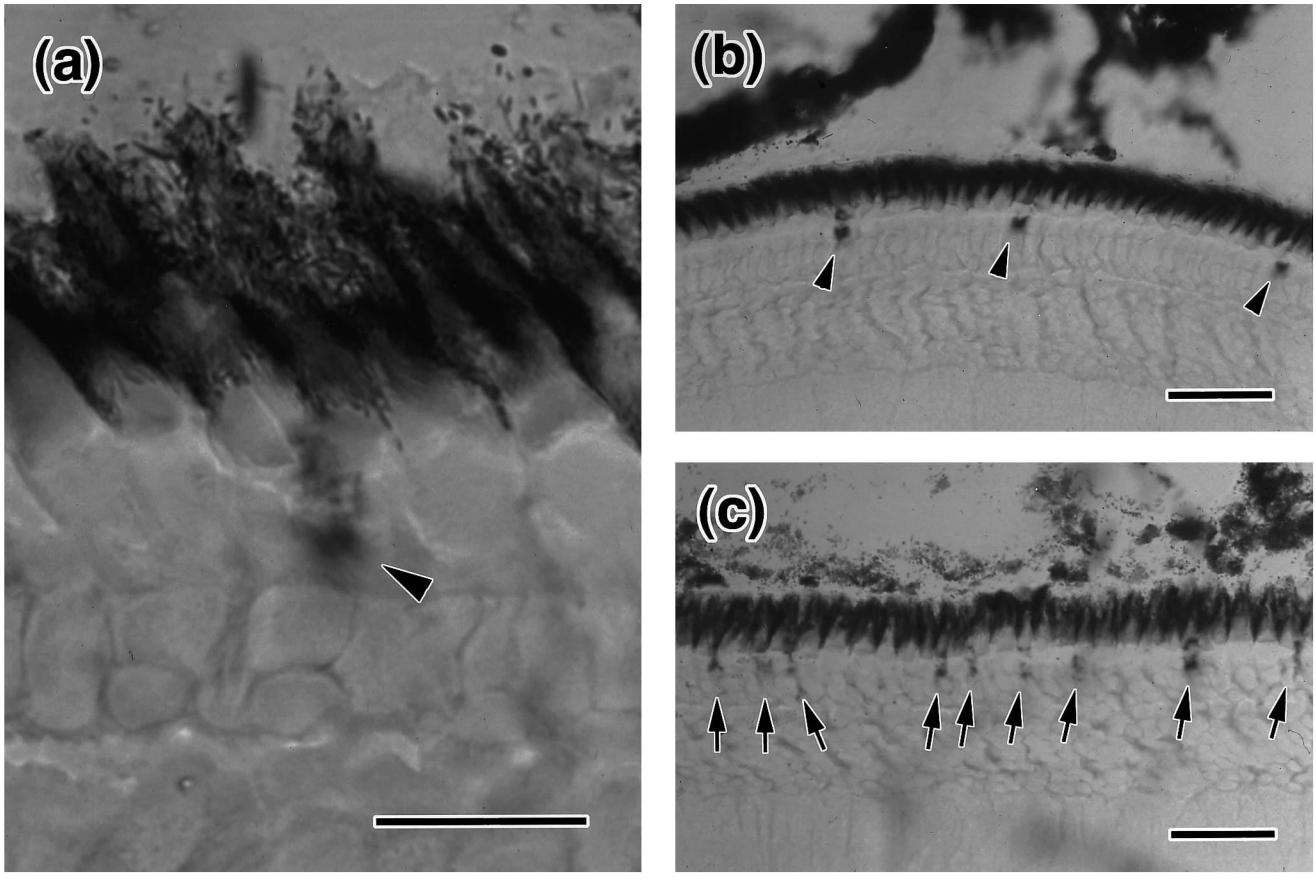

Fig. 3. a: Localization of dg3 mRNA in *P. m. longinsulae* retina. Arrowhead indicates the hybridization signal at the thin member of type C double cone. Distribution of (b) dg3 mRNA (arrowheads) and (c) dg2 mRNA (arrows). OLM, outer limiting membrane. Scale bars = 20 μm.

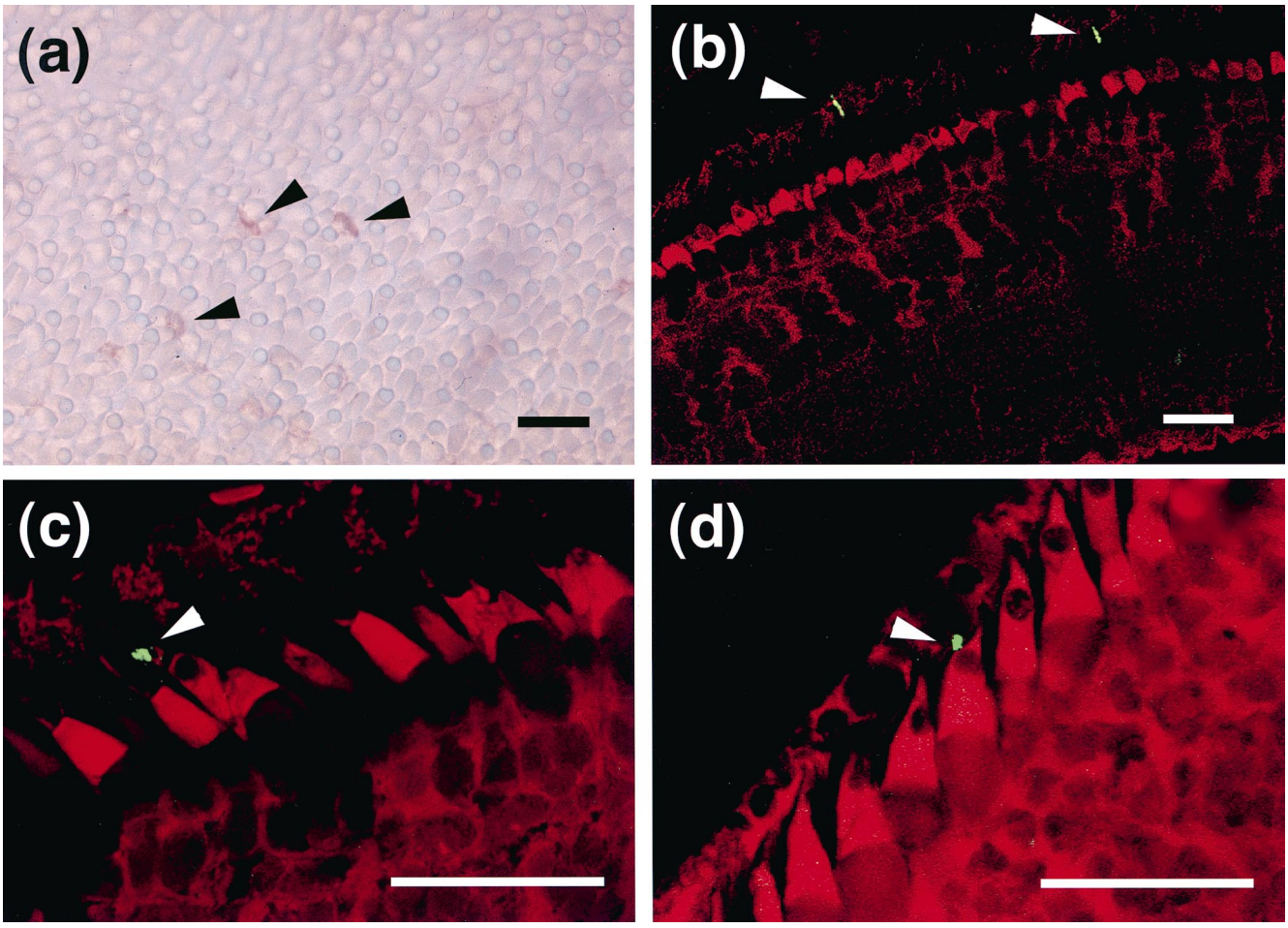

Fig. 4. a: Reactivity of the anti-dg4 antiserum in the whole-mount retina of *P. m. longinsulae*. b: Immunofluorescent analysis of the anti-dg4 antiserum in a radial section of the central region of the retina, and (c, d) magnified images. Signals (shown as green fluorescence, arrowheads) were detected at the outer segments of a subpopulation of accessory members of type B double cones. Scale bars = 20 μm.

3.4. Distribution of dg4-like pigments in Lacertilia

Since pinopsin was originally discovered in the chicken pineal, we examined dg4 expression in the pineal organ. The pineal organ of *P. m. longinsulae* has a large lumen in the central region, and well-developed outer segments of photoreceptor cells protruding into the lumen. Some outer segments of pinealocytes show immunoreactivity against the dg4 antiserum (Fig. 5a). We isolated mRNAs from pineal organ and

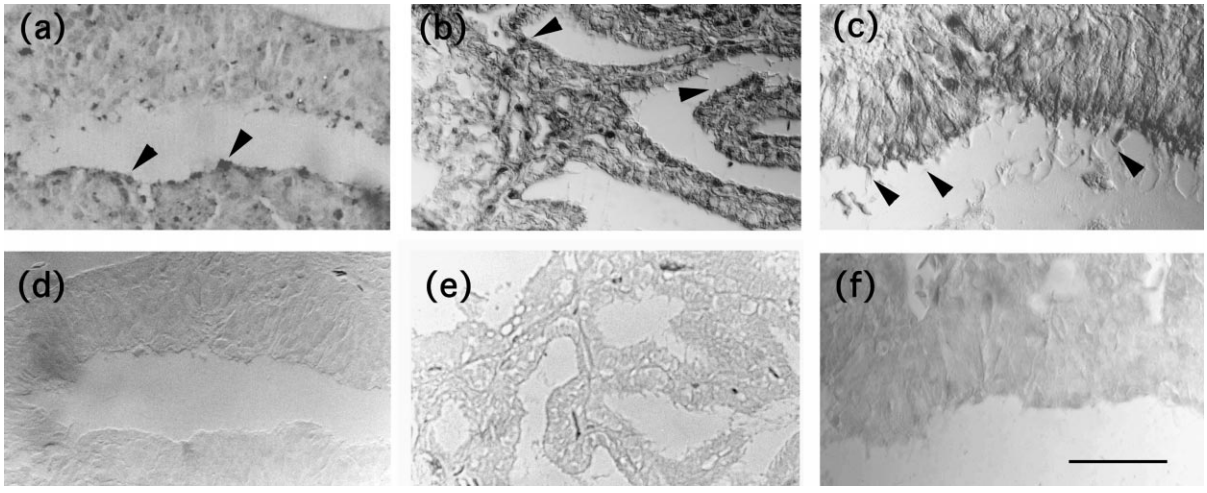

Fig. 5. Reactivities of the anti-dg4 antiserum in the pineal sections of (a, d) *P. m. longinsulae*; (b, e) a nocturnal gecko, *G. japonicus*; and (c, f) a diurnal lizard, *T. tachydromoides*. Arrowheads indicate signals in the outer segments of pinealocytes. d–f: Control experiments using the anti-dg4 antiserum pre-absorbed with the dg4 antigen. Scale bar = 20 μm.

carried out RT-PCR, to confirm whether dg4 is, in fact, expressed in pineal organ or not. The nucleotide sequence of amplified cDNA fragments was completely identical to that of dg4 cDNA, suggesting that dg4 is expressed in both retina and pineal organ in *P. m. longinsulae*.

Immunocytochemical studies were carried out to investigate the distribution of dg4-like pigments in the retina and pineal organ of a diurnal lizard (*T. tachydromoides*) and a nocturnal gecko (*G. japonicus*). In the retina, immunopositive cells were not found even in the whole-mount preparations (data not shown). However, the outer segments of some pinealocytes were immunoreactive (Fig. 5b,c), suggesting that photoreceptive pigments similar to dg4 exist in these pinealocytes but not in the retinas of these Lacertilia. No signals were observed in the control experiments, using the anti-dg4 antiserum pre-absorbed with the dg4 antigen (Fig. 5d–f).

4. Discussion

4.1. The role of dg3 opsin in P. m. longinsulae retina

In the spectral sensitivity curve of *Phelsuma* eyes, measured by electroretinograms, two peaks were observed at the red and blue regions at approximately 560 nm and 470 nm, respectively [8]. *P. m. longinsulae* has only retinal$_1$ in the retina (our unpublished results), and dg1 and dg2, we have previously reported, are likely responsible for these peaks [15]. In contrast, dg3 opsin belongs to group S which would form short wavelength-sensitive pigments with λ_{max} from UV to the violet/blue region. Microspectrophotometric studies have revealed that a small population of type C double thin members are UV-sensitive in gecko retinas [8,23,24]. The distribution of UV-sensitive photoreceptors is similar to that of dg3 mRNA, suggesting that dg3 is the UV-sensitive opsin.

In anolin lizards, it is postulated that their UV vision is important for visual communication, because only species that live in UV-rich habitats possess UV-reflective dewlaps [25]. The ratio (20–25%) of type C double thin members expressing dg3 is slightly higher than the population of UV-sensitive photoreceptors in other geckos [23,24]. UV vision in vertebrates is not well understood, and the population of UV photoreceptors possibly varies among gecko species.

4.2. Expression of pinopsin in the retina

Our immunohistochemical study demonstrated that the anti-dg4 antiserum recognized a few outer segments (only 50–100 per retina, in our estimation) of type B double accessory members. In our Western blot analysis, no positive band was detected in the retinal homogenates of *P. m. longinsulae*, probably due to the extremely low amount of dg4 in the retina. However, the reactivity of the pineal organ strongly suggests that the anti-dg4 antiserum selectively recognized dg4 in our immunohistochemical studies.

Our study suggests that a pinopsin is expressed in *P. m. longinsulae* retina. In chicken, a diurnal lizard (*A. carolinensis*) and toad, pinopsins are expressed only in the extra-retinal tissues (brain and pineal). It is, therefore, called a non-visual pigment [4,16,26,27]. The pineal and retinal photoreceptors have developmentally the same origin, and pineal photoreceptors are morphologically similar to retinal cones in lower vertebrates [28]. Moreover, the pinealocytes express a set of genes involved in phototransduction of retinal photoreceptors [29]. This cross-tissue expression suggests the existence of a common pineal/retina-specific transcriptional apparatus. Recently, a transcription factor (CRX) and its target, a *cis*-regulatory element (PIRE), were identified as pineal/retinal photoreceptor-specific transcription factors [30,31]. The pinopsin transcription in retinal photoreceptors of *P. m. longinsulae* is possibly regulated by a common pineal/retina-specific transcriptional factor and *cis*-regulatory element similar to CRX and PIRE.

4.3. Evolution of retinal organization in geckos

Microspectrophotometric and immunocytochemical studies revealed that all geckos investigated so far possess three types of visual pigments, 'red/green-', 'blue-' and 'UV'-sensitive [9,10,23,24]. These three types are likely to be phylogenetically closely related to, respectively, dg1 (group L/LWS), dg2 (group ML/RH2) and dg3 (group S/SWS1) of the diurnal gecko [11,15]. On the other hand, a diurnal lizard, *A. carolinensis*, is known to have four kinds of cone pigments with λ_{max} at 560, 495, 437 and 358 nm (consisting of group L, group ML, group MS/SWS2 and group S opsins, respectively) in its pure-cone retina [27,32]. During evolution to a nocturnal lizard, an ancestral gecko might lose the group MS opsin. The diurnal geckos descended from a nocturnal ancestor may have re-evolved their retinal organization, and expressed pinopsin as a fourth opsin (instead of the group MS opsin) in the retina.

Acknowledgements: This work was supported by Special Coordination Funds for Promoting Science and Technology (SCF), by SUNBOR, and by a Grant-in-Aid from the Japanese Ministry of Education, Culture and Welfare.

References

[1] Okano, T., Kojima, D., Fukada, Y., Shichida, Y. and Yoshizawa, T. (1992) Proc. Natl. Acad. Sci. USA 89, 5932–5936.
[2] Yokoyama, S. (1994) Mol. Biol. Evol. 1, 32–39.
[3] Hisatomi, O., Kayada, S., Aoki, Y., Iwasa, T. and Tokunaga, F. (1994) Vision Res. 34, 3097–3102.
[4] Okano, T., Yoshizawa, T. and Fukada, Y. (1994) Nature 372, 94–97.
[5] Dunn, R.F. (1966) J. Ultrastruct. Res. 16, 651–671.
[6] Yoshida, M. (1978) Vision Res. 18, 137–143.
[7] Walls, G.L. (1934) Am. J. Ophthalmol. 17, 892–915.
[8] Tansley, K. (1961) Arch. Gen. Physiol. 272, 262–269.
[9] Crescitelli, F. (1977) in: Handbook of Sensory Physiology, 7/5 (Crescitelli, F., Ed.), pp. 391–450, Springer, Berlin.
[10] Loew, E.R. (1994) Vision Res. 34, 1427–1431.
[11] Kojima, D., Okano, T., Fukada, Y., Shichida, Y., Yoshizawa, T. and Ebrey, T.G. (1992) Proc. Natl. Acad. Sci. USA 89, 6841–6845.
[12] Kawamura, S. and Yokoyama, S. (1993) FEBS Lett. 323, 247–251.
[13] Kawamura, S. and Yokoyama, S. (1995) J. Mol. Evol. 40, 594–600.
[14] Fleishman, L.J., Bowman, M., Saunders, D., Miller, W.E., Rury, M.J. and Loew, E.R. (1997) J. Comp. Physiol. A 181, 446–460.
[15] Taniguchi, Y., Hisatomi, O., Yoshida, M. and Tokunaga, F. (1999) FEBS Lett. 445, 36–40.
[16] Yoshikawa, T., Okano, T., Oishi, T. and Fukata, Y. (1998) FEBS Lett. 424, 69–72.
[17] Hisatomi, O., Satoh, T., Barthel, L.K., Stenkamp, D.L., Raymond, P.A. and Tokunaga, F. (1996) Vision Res. 36, 933–939.
[18] Hisatomi, O., Satoh, T. and Tokunaga, F. (1997) Vision Res. 37, 3089–3096.
[19] Hisatomi, O., Iwasa, T., Tokunaga, F. and Yasui, A. (1991) Biochem. Biophys. Res. Commun. 174, 1125–1132.
[20] Saitou, N. and Nei, M. (1987) Mol. Biol. Evol. 4, 406–425.

[21] Hisatomi, O., Imanishi, Y., Satoh, T. and Tokunaga, F. (1997) FEBS Lett. 411, 12–18.
[22] Hisatomi, O., Kayada, S., Taniguchi, Y., Kobayashi, Y., Takunori, S. and Tokunaga, F. (1998) Comp. Biochem. Physiol. 199B, 585–591.
[23] Loew, E.R., Govardovskii, V.I., Rohlich, P. and Szel, A. (1996) Visual Neurosci. 13, 247–256.
[24] Ellingson, J.M., Fleishman, L.J. and Loew, E.R. (1995) J. Comp. Physiol. A. 177, 559–567.
[25] Fleishman, L.J., Loew, E.R. and Leal, M. (1993) Nature 365, 397.
[26] Okano, T., Takanaka, Y., Nakamura, A., Hirunagi, K., Adachi, A., Ebihara, S. and Fukada, Y. (1997) Mol. Brain Res. 50, 190–1996.
[27] Kawamura, S. and Yokoyama, S. (1997) Vision Res. 37, 1867–1871.
[28] Korf, H.W. (1994) Ann. NY Acad. Sci. 719, 13–42.
[29] Blackshaw, S. and Snyder, S.H. (1997) J. Neurosci. 17, 8074–8082.
[30] Furukawa, T., Morrow, E.M. and Cepko, C.L. (1997) Cell 91, 521–530.
[31] Li, X., Chem, S., Wang, Q., Zack, D.J., Snyder, S.H. and Borjigin, J. (1998) Proc. Natl. Acad. Sci. USA. 95, 1876–1881.
[32] Kawamura, S. and Yokoyama, S. (1998) Vision Res. 38, 37–44.