Molecular cloning of fresh water and deep-sea rod opsin genes from Japanese eel *Anguilla japonica* and expressional analyses during sexual maturation[1]

Huan Zhang[a,*], Kunihiko Futami[b], Noriyuki Horie[a], Akihiro Okamura[a], Tomoko Utoh[a], Naomi Mikawa[a], Yoshiaki Yamada[a], Satoru Tanaka[a], Nobuaki Okamoto[b]

[a] *IRAGO Institute, Shinden 377-Ehima, Atsumi-cho, Atsumi-gun, Aichi 441-3605, Japan*
[b] *Department of Aquatic Biosciences, Tokyo University of Fisheries, 4-5-7 Konan Minato-ku, Tokyo 108-8477, Japan*

Received 7 December 1999

Edited by Takashi Gojobori

Abstract We have determined the complete cDNA sequences of fresh water rod opsin gene (*fwo*) and deep-sea rod opsin gene (*dso*) from Japanese eel *Anguilla japonica*. The cDNA clones of *fwo* and *dso* consisted of 1437 and 1497 nucleotides, respectively. The predicted opsins of both genes consisted of 352 amino acid residues. Southern blot and PCR analyses of genomic DNA indicated that the Japanese eel genome contains only one *fwo* and one *dso* and they are intronless. Quantitative RT-PCR analyses revealed that the expression of *fwo* decreases with sexual maturation while that of *dso* increases.

Key words: Japanese eel; Rod opsin; Intronless; Expression; Maturation

1. Introduction

Vertebrate photoreceptor cells, rods and cones, contain visual pigments which absorb light as the first step in the process of vision. A visual pigment is composed of a protein, opsin, and a chromophore, aldehyde of either vitamin A1 or A2. The electrostatic interaction between the opsin and the chromophore determines the spectral sensitivity of the visual pigment [1–3]. Generally A2-based pigments (porphyropsins) have a longer wavelength of maximal light absorption (λ_{max}) than A1-based pigments (rhodopsins) [4].

During the process of evolution, animals have developed their special visual systems to adapt to the photic environments they live in. Catadromous fishes, such as eels of the genus *Anguilla*, are good models for studying the process of adaptive evolution of vision, since they undergo dramatic environmental changes during life cycles. The juvenile eels (yellow eels) live in fresh water, while the adult eels (silver eels) migrate into the open sea and spawn there. The hatched leptocephalus larvae disperse through passive transport toward coastal regions, metamorphose into glass eels which enter fresh water and grow up there [5–8]. Therefore, in their life cycles, the eels experience three different aquatic habitats: the yellow/red stained fresh water, the green coastal water and the blue water of the open sea.

Several studies have been carried out on the visual pigments of the Atlantic eel species, European eel *Anguilla anguilla* and American eel *Anguilla rostrata* [5,6,9–13]. The studies indicated that eels adapt their visual system to the photic environments by using different opsin genes (fresh water form or deep water form) and different chromophores (A1 or A2) in different stages of their lives. As nocturnal fish, eels contain mainly rods in their retinas. During the juvenile stage in fresh water, the opsin gene expressed is the fresh water rod opsin gene (*fwo*). Both rhodopsin (λ_{max} 501 nm) and porphyropsin (λ_{max} 523 nm) exist in rods with the latter one dominated. The λ_{max} of the pigment mixture is close to 520 nm, similar to the λ_{max} of rod pigments of many other fresh water fishes which usually have only porphyropsins [14]. As reaching maturity, the eels start their downstream migration, the ratio of pigments changes with rhodopsin being dominant and the λ_{max} shifting towards 500 nm. In addition, a new opsin gene, deep-sea rod opsin gene (*dso*), starts to express in the existing rods. This new opsin forms a rhodopsin with λ_{max} of 482 nm, which shifts λ_{max} of the rod pigment mixture to below 500 nm. The substitution from longwave- to shortwave-sensitive rhodopsins mirrors the difference of rod pigments typically observed among freshwater, coastal and deep-sea water fishes [15].

So far studies on eel opsin genes are limited to European eel. The cDNAs of *fwo* and *dso*, which were incomplete in the 5′ untranslated regions (UTRs), have been isolated from this species [12]. The genomic structures of eel opsin genes are unknown. In addition, the quantitative analyses of the expression of *fwo* and *dso* have not been successful [13]. The relationship between sexual maturation and the expression patterns of *fwo* and *dso* is not clear.

In the present study, we report the complete cDNAs of *fwo* and *dso*, and their genomic structures of a Pacific eel species, Japanese eel *Anguilla japonica*. We also report the expression patterns of *fwo* and *dso* during the process of sexual maturation by quantitative RT-PCR analysis. Our findings provide a better understanding of eel visual adaptation by revealing details on the relationship between sexual maturation and opsin gene expression. For Japanese eel, this is the first study on the

*Corresponding author. Fax: (81)-5313-4 3111.
E-mail: hazhang@irago.co.jp

[1] IRAGO Institute contribution No. 004.

Abbreviations: fwo, fresh water rod opsin gene; *dso*, deep-sea rod opsin gene; FWO, fresh water rod opsin; DSO, deep-sea rod opsin; λ_{max}, wavelength of maximal light absorption; pBS, pBluescript II vector; ORF, open reading frame; nt, nucleotide; aa, amino acid; UTR, untranslated region; od, oocyte diameter; Pref., Prefecture

PII: S0014-5793(00)01233-3

changes of visual system at molecular level during spawning migration.

2. Materials and methods

2.1. Construction of a retinal cDNA library

A female Japanese silver eel (66 cm in length) was collected in Mikawa Bay in Aichi Prefecture (Pref.) by fixed shore net in January 1999. The eel was anesthetized in 0.08% 2-phenoxyethanol and anatomized. Total RNA was isolated from retina by acid guanidine thiocyanate-phenol-chloroform extraction [16] and poly(A)$^+$ RNA was extracted using the mRNA isolation kit (Boehringer Mannheim) and reverse transcribed using the TimeSaver™ cDNA synthesis kit (Amersham Pharmacia Biotech). The obtained cDNA with *Eco*RI–*Not*I adapter was cloned into the *Eco*RI site of pBluescript II vector (pBS) (Stratagene) to construct a library.

2.2. Isolation and sequencing of the full length cDNAs of fwo and dso

Using the cDNA library as template, the open reading frames (ORFs) of *fwo* and *dso* were amplified by PCR. The primer sets P1-P2 or P1-P3 (P1: 5′-ATGAATGGCACAGAGGGACCTAAYTTCT-3′, P2: 5′-GTGTCCTGTGAGACAAGGTTTATGC-3′ and P3: 5′-TTTGTGGTGGGTCGGTGCCTTATGC-3′; Y = C/T) were used (Fig. 1). These primers were designed based on the reported sequences of *fwo* and *dso* cDNAs of European eel [12]. PCR was performed as follows: 94°C for 5 min once, followed by thermal cycling at 94°C for 30 s, 62°C for 30 s, 72°C for 45 s for 35 cycles, and finally one cycle at 72°C for 7 min. Reaction was performed in a total volume of 25 μl containing 50 pmol of each primer, 0.2 mM deoxynucleotides and 0.5 unit of *Taq* polymerase (Boehringer Mannheim). The amplified ~1.1 kb fragments, named FO and DO (Fig. 1), were subcloned and sequenced on both strands by the standard method [17].

The 5′ ends cDNA sequences of *fwo* and *dso* were determined by the following protocol. Three primers, Pa, P4 and P5 were used (Fig. 1). Pa (5′-TTGATATCGAATTCGCGGCCGCT-3′) corresponded to the junctional region of pBS and the *Eco*RI–*Not*I adapter. P4 (5′-ACCTTCGATGTTGCAGCCGGTGG-3′) was the *fwo* specific primer and P5 (5′-TCTCGATAGCCAGGACAACCAGAG-3′) was the *dso* specific primer, which were designed based on the sequences of the ORFs of *fwo* and *dso*, respectively. Using the cDNA library as template, PCR was performed by the same protocol as described above. Only one fragment of ~430 bp, named F5, was obtained for the primer set Pa-P4 and one fragment of ~500 bp, named D5, for Pa-P5 (Fig. 1). These PCR fragments were subcloned and sequenced.

The 3′ ends cDNA sequences of *fwo* and *dso* were determined by a similar method as that of 5′ ends. Three primers, P6 (5′-CTTCAC-CATCCCGCTCACCGTC-3′, *fwo* specific), P7 (5′-ACTTCTC-CATCCCCCTCACCATT-3′, *dso* specific) and Pb (5′-CTGCAG-GAATTCGCGGCCGCT-3′, corresponding to another junction of pBS and the *Eco*RI–*Not*I adapter), were used (Fig. 1). Only one fragment of ~760 bp, named F3, was obtained for the primer set P6-Pb and one fragment of ~810 bp, named D3, for P7-Pb (Fig. 1).

2.3. Southern blot analyses and genomic DNA PCR amplification

Genomic DNA was isolated from the muscle tissue of the same individual used for cDNA library construction and Southern blot analyses were performed according to the standard protocol [17]. Probes used were PF5, PFO and PF3 for *fwo* and PD5, PDO and PD3 for *dso* (Fig. 1), which corresponded to the exon 1, ORF and exon 5 regions of the rod opsin genes of other vertebrates. Hybridized membranes were washed in 1×SSC/0.1% SDS for 20 min four times at 55°C, 65°C or 70°C, which allows about 30%, 20% and 15% mismatches, respectively [18].

To obtain the genomic clones of *fwo* and *dso*, PCR was performed using the genomic DNA as template and P1-P2 and P1-P3 as primers (Fig. 1) by the same protocol as described in Section 2.2. The products were subcloned and sequenced.

2.4. RT-PCR of fwo and dso in different sexual maturation stages

Eel individuals used in RT-PCR experiment were collected in Yodogawa River in Osaka Pref., Shinjiko Lake in Shimane Pref., Hamanako Lake in Shizuoka Pref. and Mikawa Bay in Aichi Pref. by fixed shore net or trap pot from August 1998 to August 1999. Only females were used because it was easy to describe the maturation process explicitly by measuring the oocyte diameter (od) which increases with maturation [19]. In the present study, sexual maturation level of Japanese eel was divided into five stages: stage I: od < 0.10 mm (yellow eels with oocytes before oil droplet stage); stage II: od 0.10–0.18 mm (yellow eels with oocytes in oil droplet to primary yolk globule stage); stage III: od 0.18–0.30 mm (silver eels with oocytes in primary yolk globule stage); stage IV: od 0.30–0.50 mm (oocytes in secondary yolk globule stage) and stage V: od > 0.50 mm (oocyte after secondary yolk globule stage [19]). For each stage 10–30 individuals were examined. Total retinal RNA of eels in stages I, II and III was isolated as described above immediately after the collection and used for the following RT-PCR template. Since it is impossible to catch naturally matured eels in stages IV and V, those eels were obtained by injecting the saline suspension of chum salmon pituitary for 4–14 times (20 mg/kg body weight, once/week) [20].

The expression of *fwo* and *dso* of Japanese eel in different sexual maturation stages was evaluated by RT-PCR. Primer set P1-P4 was used for *fwo* and P1-P5 for *dso* (Fig. 1). The expression of cytoskeletal β-actin gene was evaluated to standardize expression levels of opsin genes. The PCR primers for β-actin gene (forward primer, 5′-ACA-GACTACCTCATGAAGATCCTG-3′; reverse primer, 5′-CTGCT-TGCTGATCCACATCTGCTG-3′) were designed based on the reported sequences [21]. RT-PCR was performed using the following protocol, RNA denaturing: 4 μl total retinal RNA (100–300 ng) and 16 μl master mix A (prepared on ice, containing 62.5 nM of each primer, 0.84× M-MLV reaction buffer (Promega)) were mixed, incubated at 94°C for 2 min, and then cooled immediately on ice. First strand cDNA synthesis: 5 μl master mix B (prepared on ice, containing 20 unit/μl M-MLV reverse transcriptase (Promega), 1.6 unit/μl RNasin ribonuclease inhibitor (Promega), 0.2 unit/μl *Taq* polymerase (Promega), 1 mM of each dNTP (TaKaRa) and 0.67×

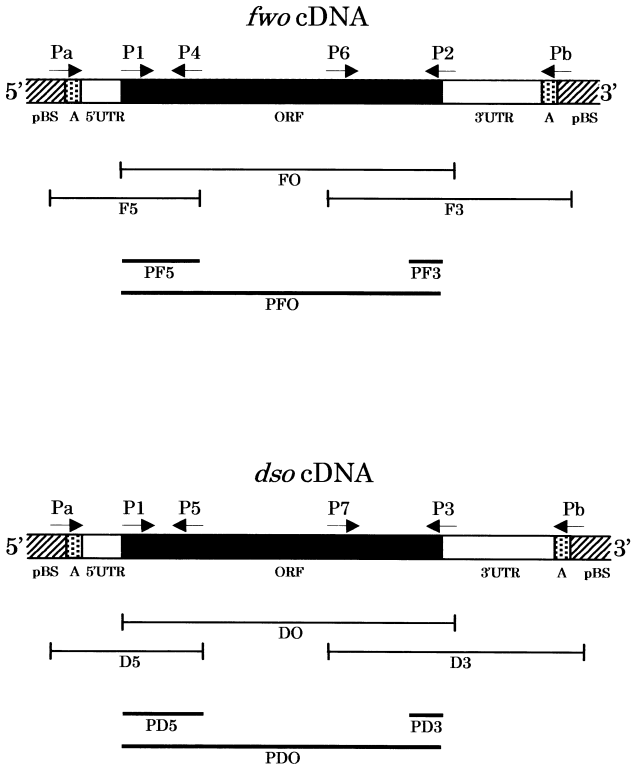

Fig. 1. Strategy of cloning the full length cDNAs of *fwo* and *dso* of Japanese eel. The full length cDNAs of *fwo* (1437 bp) and *dso* (1497 bp) were determined by six overlapped fragments, F5, FO, F3 for *fwo*, and D5, DO, D3 for *dso*. Pa, Pb, P1-P7, primers; A, adapter; 5′UTR, 5′ untranslated region; 3′UTR, 3′ untranslated region. PF5, PFO and PF3, probes used for genomic Southern hybridization of *fwo*; PD5, PDO and PD3, probes used for Southern hybridization of *dso*.

M-MLV reaction buffer (Promega) was added to the above mixture and incubated at 42°C for 30 min. PCR amplification: The reaction mixture was incubated at 94°C for 5 min once, followed by thermal cycling at 94°C for 30 s, 60°C for 30 s, 72°C for 45 s for 30 cycles, and finally one cycle at 72°C for 7 min. By this protocol, the PCR amplification was in linear phase when the reaction cycle number was between 25 and 35 (data not shown). The RT-PCR products were run in 1.8% agarose gels, stained with Vistra green (Amersham) and quantified by using FluorImager 595 (Molecular Dynamics).

3. Results

3.1. Nucleotide sequence analyses of the cDNAs of fwo and dso

The full length cDNAs of *fwo* and *dso* were 1437 bp (EMBL accession number AJ249202) and 1497 bp (AJ249203), respectively. These sizes matched well with the band sizes in the Southern blot of the total retinal cDNA, which showed a ~1.5 kb single band when the entire ORF portion of either *fwo* or *dso* was used as the probe (data not shown). The ORFs of *fwo* and *dso* were both 1056 bp and the deduced fresh water rod opsin (FWO) and deep-sea rod opsin (DSO) were both 352 amino acid (aa) residues. The 5′ and 3′ UTRs were 67 bp and 311 bp for *fwo*, and 74 bp and 364 bp for *dso*, respectively. These two opsin cDNAs were 83.0% identical in ORF, 62.7% in 5′ UTR and no identity in 3′ UTR. The aa identity between FWO and DSO was 88.4%.

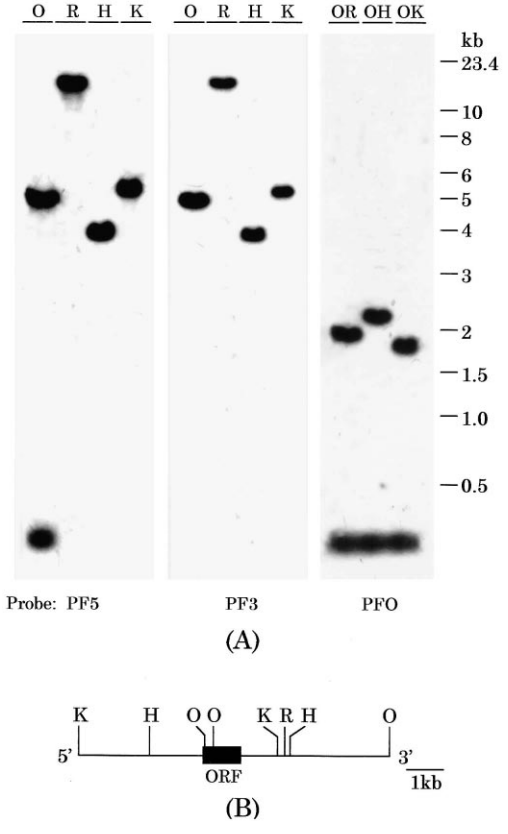

Fig. 2. A: Genomic DNA Southern hybridization of *fwo* of Japanese eel. PF5, PFO and PF3 (see Fig. 1), which corresponded to the exon 1, ORF and exon 5 regions of the rod opsin genes of other vertebrates, were PCR amplified using *fwo* cDNA as template and used as probes. Each lane contains 5 μg genomic DNA single digested with *Eco*O109I (O), *Eco*RI (R), *Hin*dIII (H) and *Kpn*I (K), or double digested with *Eco*O109I–*Eco*RI (OR), *Eco*O109I–*Hin*dIII (OH) and *Eco*O109I–*Kpn*I (OK). B: Restriction map of *fwo*. Constructed based on Southern hybridization.

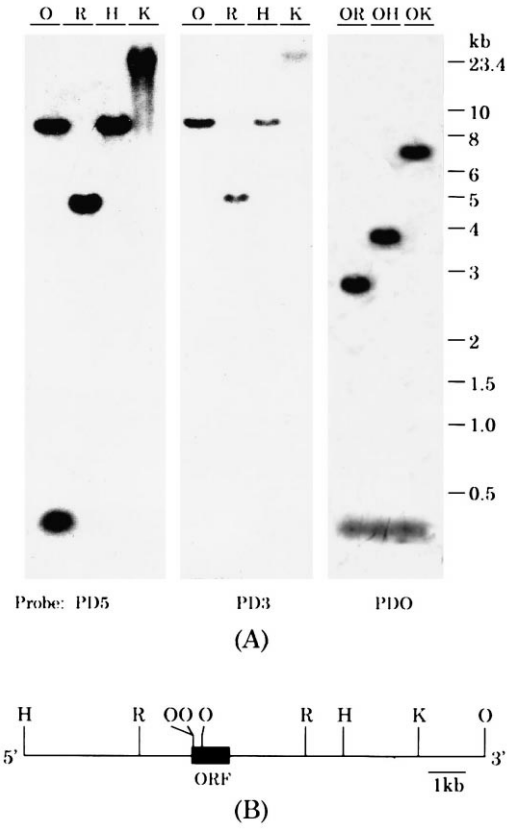

Fig. 3. A: Genomic DNA Southern hybridization of *dso* of Japanese eel. PD5, PDO and PD3 (see Fig. 1), which corresponded to the exon 1, ORF and exon 5 regions of the rod opsin genes of other vertebrates, were PCR amplified using *dso* cDNA as template and used as probes. Each lane contains 5 μg genomic DNA single digested with *Eco*O109I (O), *Eco*RI (R), *Hin*dIII (H) and *Kpn*I (K), or double digested with *Eco*O109I–*Eco*RI (OR), *Eco*O109I–*Hin*dIII (OH) and *Eco*O109I–*Kpn*I (OK). B: Restriction map of *dso*. Constructed based on Southern hybridization.

The rod opsins of Japanese eel are very similar to those of European eel [12]. The nucleotide (nt) identity in ORF is 98.1% for *fwo* and 98.4% for *dso*, and the aa identity is 96.3% for FWO and 98.6% for DSO. Rod opsin genes have been characterized in various vertebrates, including lampreys, bony fish (teleosts), coelacanth, amphibia, reptiles, birds and mammals [22–29]. The nt identities in ORF between Japanese eel rod opsin genes and other reported vertebrate ones are 74–89%, and the aa identities are 77–91%.

3.2. Genomic structures of fwo and dso

To estimate the copy numbers of *fwo* and *dso*, and to construct their restriction maps in the genome of Japanese eel, Southern blot analyses were carried out. When the genomic DNA was digested with *Eco*RI, *Hin*dIII or *Kpn*I and hybridized with the *fwo* or *dso* probes, only one band was detected under stringently washing condition (1× SSC/0.1% SDS, 70°C) (see Fig. 2 for *fwo* and Fig. 3 for *dso*). When the washing condition was less stringent (1× SSC/0.1% SDS, 55°C or 65°C) which allows about 20–30% mismatches [18], the two rod opsin genes cross-hybridized, but no other band was detected (data not shown). Since the nt identities in ORF be-

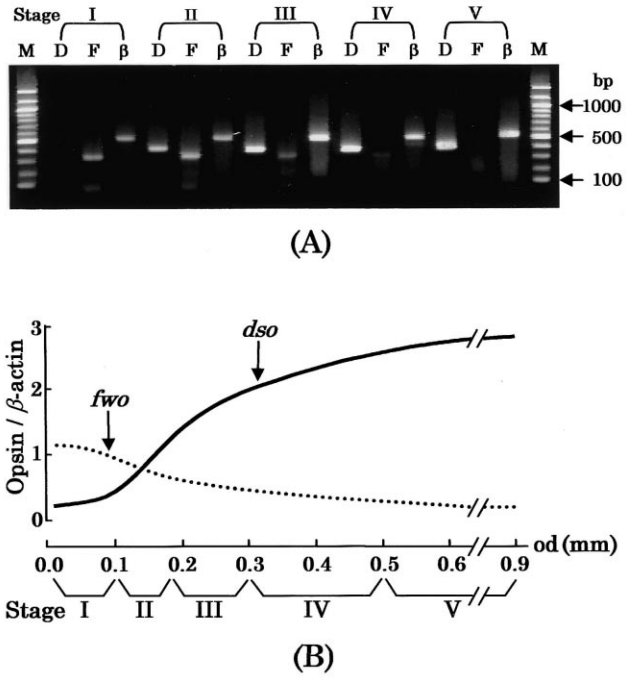

Fig. 4. A: An example of agarose gel analysis of RT-PCR products of *fwo*, *dso* and cytoskeletal β-actin gene of Japanese eel in different sexual maturation stages. D, product of *dso* (403 bp); F, product of *fwo* (342 bp); β, product of β-actin (510 bp); M, 100 bp ladder DNA size marker. B: Expression patterns of *fwo* and *dso* during sexual maturation. *x*-Axis: od which is used as the indicator to divide maturation levels into five stages. *y*-Axis: ratio of band intensity of RT-PCR product of *fwo* or *dso* to that of β-actin.

tween rod opsin genes of Japanese eel and other vertebrates [12,22–29] are 74–98%, it is reasonable to believe that *fwo* and *dso* are the only rod opsin genes in the genome of Japanese eel and they are single copy genes.

For both *fwo* and *dso*, the PCR products of genomic DNA had the size of ~1.1 kb. Sequencing results revealed that they completely matched the nt sequences of the cDNA clones of *fwo* and *dso*. These results indicated that *fwo* and *dso* of Japanese eel lack introns.

3.3. Expression of fwo and dso during sexual maturation

Expression of *fwo* and *dso* of Japanese eel in different sexual maturation stages was evaluated by RT-PCR (Fig. 4A). The ratio of band intensity of *fwo* to that of β-actin (mean ± standard deviation) was 1.01 ± 0.33 in stage I, 0.71 ± 0.47 in stage II, 0.50 ± 0.11 in stage III, 0.32 ± 0.10 in stage IV and 0.15 ± 0.01 in stage V. For *dso*, the ratio of *dso* to β-actin was 0.32 ± 0.22 in stage I, 0.73 ± 0.52 in stage II, 1.67 ± 0.60 in stage III, 2.36 ± 0.12 in stage IV and 2.81 ± 0.27 in stage V. As summarized in Fig. 4B), the expression of *fwo* decreased gradually with sexual maturation while that of *dso* increased remarkably in stages II and III. The expression of both genes became stable in stage V (the ratio of *fwo*:*dso* was about 1:18).

4. Discussion

All rod opsin genes characterized in various vertebrates, except those of teleosts, consist of five exons separated by four introns at the identical positions [25]. On the basis of PCR analysis of genomic DNA, it has been reported that the rod opsin genes of teleosts lack introns [23]. However, it has not been demonstrated whether there are additional rod opsin genes in teleost genomes that contain introns. In the present study, we revealed that Japanese eel, a teleost, contains no other rod opsin genes than *fwo* and *dso* in its genome, and that the two genes are intronless.

The deduced aa sequences of the opsins of Japanese eel are very similar to those of European eel [12]. The identity is 96.3% for FWO and 98.6% for DSO. The rhodopsins of FWO and DSO of European eel have a λ_{max} at 501 nm and 482 nm, respectively. Three aa positions, 83 (aspartate in FWO and asparagine in DSO), 124 (alanine in FWO and serine in DSO) and 292 (alanine in FWO and serine in DSO), have been suggested to be important to differentiate their λ_{max} values [12]. These aa are observed in the corresponding genes of Japanese eel, suggesting that FWO and DSO of Japanese eels have a similar λ_{max} to their European eel's counterparts.

The eel individuals used for RT-PCR in the present study were collected from fresh water (Yodogawa River, Shinjiko Lake), brackish water (Hamanako Lake) or sea water (Mikawa Bay). The individuals from fresh and brackish water were in maturation stages I and II. The ones collected from sea water during spawning migration season (October to January) were in stage III. The seawater individuals collected during late spring to early autumn were in stages I and II. No differences of *fwo* and *dso* expression were observed among the individuals collected from different salinity, as long as their maturation levels were similar, indicating that the environmental salinity is not relevant to the regulation of the expression of *fwo* and *dso* of Japanese eel.

Hope et al. [13] reported the expressional switch of *fwo* to *dso* in European eel during artificial maturation by gonadotropin administration. In their investigation the eel individuals used were male and the sexual maturation process was not described. Furthermore, their expressional analysis was not quantitative. All of this makes it difficult to compare their results to those of our study.

We aim to reveal the relationship between sexual maturation and visual adaptation in terms of expression level of rod opsin genes of Japanese eel in nature. We found that the expression of *dso* of Japanese eel increased remarkably in early maturation stages, indicating that the eels adapt their visual system to the open sea photic environment even before their spawning migration. So far there is little information on the migration of Japanese eels once they leave coastal water. Our data showed that in maturation stage V, the expression of *fwo* decreased to a very low level, while that of *dso* became dominant and stable. These results suggest that Japanese eels in stage V live in deeper water of the open sea (under the depth of 100 m, where blue light 480 nm is dominant [30]). According to our experiment (Yamada et al., unpublished), Japanese eels injected with saline suspension of chum salmon pituitary could not mature and spawn normally at temperatures lower than 15°C. In the supposed spawning area of Japanese eel in the North Equatorial Current west of the Mariana Islands [7,8], the water temperature under 300 m is lower than 15°C [31]. Therefore, the depth where matured Japanese eels stay and spawn is probably between 100 and 300 m in their supposed spawning site.

Acknowledgements: We thank Dr. Shoji Kawamura, the University of Tokyo, for his valuable advice. We also thank Mr. Hideo P. Oka, Director of IRAGO Institute Co., Ltd., for critically reading the manuscript.

References

[1] Nathans, J. (1992) Biochemistry 31, 4923–4929.
[2] Merbs, S.L. and Nathans, J. (1992) Nature 356, 433–435.
[3] Hunt, D.M., Williams, A.J., Bowmaker, J.K. and Mollon, J.D. (1993) Vision Res. 33, 147–154.
[4] Ebrey, T.G. (1977) Vision Res. 17, 147–151.
[5] Schmidt, J. (1923) Nature 111, 51–54.
[6] Beatty, D.D. (1975) Vision Res. 15, 771–776.
[7] Tsukamoto, K. (1992) Nature 356, 789–791.
[8] Tsukamoto, K. (1996) in: Early Life History and Prospects of Seed Production of the Japanese Eel *Anguilla japonica* (Tabeta, O., Ed.), pp. 11–21, Koseisha, Tokyo (In Japanese).
[9] Pankhurst, N.W. (1982) J. Fish Biol. 21, 127–140.
[10] Pankhurst, N.W. and Lythgoe, J.N. (1983) J. Fish Biol. 23, 229–240.
[11] Wood, P. and Partridge, J.C. (1993) Proc. R. Soc. Lond. B 254, 227–232.
[12] Archer, S., Hope, A. and Partridge, J.C. (1995) Proc. R. Soc. Lond. B 262, 289–295.
[13] Hope, A.J., Partridge, J.C. and Hayes, P.K. (1998) Proc. R. Soc. Lond. B 265, 869–874.
[14] Bowmaker, J.K. (1995) Prog. Retin. Eye Res. 15, 1–31.
[15] Lythgoe, J.N. (1988) in: Sensory biology of aquatic animals (Atema, J., Fay, R.R., Popper, A.N. and Tavolga, W.N. Eds.), pp. 57–82, Springer-Verlag, New York.
[16] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[17] Sambrook, J., Fritsch, E.F. and Maniatus, T. (1989) Molecular Cloning. A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[18] Meinkoth, J. and Wahl, G. (1984) Anal. Biochem. 138, 267–284.
[19] Todo, T., Adachi, S., Saeki, F. and Yamauchi, K. (1995) Zool. Sci. 12, 789–794.
[20] Yamamoto, K., Morioka, T., Hiroi, O. and Omori, M. (1974) Bull. Japn. Soc. Sci. Fish (in Japanese) 40, 1–7.
[21] Katagiri, T., Hirono, I. and Aoki, T. (1997) Fish. Sci. 63, 73–76.
[22] Zhang, H. and Yokoyama, S. (1997) Gene 191, 1–6.
[23] Fitzgibbon, J., Hope, A., Slobodyanyuk, S.J., Bellingham, J., Bowmaker, J.K. and Hunt, D.M. (1995) Gene 164, 273–277.
[24] Archer, S. and Hirano, J. (1996) Proc. R. Soc. Lond. B 263, 761–767.
[25] Yokoyama, S., Zhang, H., Radlwimmer, F.B. and Blow, N.S. (1999) Proc. Natl. Acad. Sci. USA 96, 6279–6284.
[26] Batni, S., Scalzetti, L., Moody, S.A. and Knox, B.E. (1996) J. Biol. Chem. 271, 3179–3186.
[27] Kawamura, S. (1994) Gene 149, 267–270.
[28] Takao, M., Yasui, A. and Tokunaga, F. (1988) Vision Res. 28, 471–480.
[29] Nathans, J. and Hogness, D.S. (1983) Cell 34, 807–814.
[30] Levine, J.S. and MacNichol, E.F. (1982) Sci. Am. 246, 140–149.
[31] Inagaki, T., Kimura, S. and Hasumoto, H (1996) in: Early life history and prospects of seed production of the Japanese eel *Anguilla japonica* (Tabeta, O., Ed.), pp. 44–55, Koseisha, Tokyo (in Japanese).