The Photoreceptor Molecules in Xenopus Tadpole Tail Fin, in which Melanophores Exist

Authors: Miyashita, Yoko, Moriya, Tsuneo, Yamada, Keiko, Kubota, Toru, Shirakawa, Sachiko, et al.

Source: Zoological Science, 18(5) : 671-674

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.671

ZOOLOGICAL SCIENCE **18**: 671–674 (2001)

The Photoreceptor Molecules in *Xenopus* Tadpole Tail Fin, in which Melanophores Exist

Yoko Miyashita[1*], Tsuneo Moriya[1], Keiko Yamada[2], Toru Kubota[3], Sachiko Shirakawa[3], Nobuhiro Fujii[3] and Kouichi Asami[1]

[1]*Department of Biology, School of Medicine, Sapporo Medical University, Sapporo, Hokkaido 060-8556, Japan*
[2]*Department of Biology, School of Health Science, Sapporo Medical University, Sapporo, Hokkaido 060-8556, Japan*
[3]*Department of Microbiology, School of Medicine, Sapporo Medical Medical University, Sapporo, Hokkaido 060-8556, Japan*

Abstract—Melanophores of the isolated tail fin of the *Xenopus* tadpole respond to light, resulting in melanin aggregation in the melanophores.

Western blot analysis showed that a protein in the *Xenopus* tail fins, in which photosensitive melanophores exist, had reacted with the antibody against bovine rhodopsin.

RT-PCR and nested-PCR using rhodopsin-specific primers showed the expression of rhodopsin mRNA in the tail fins. The amino acid sequences deduced from the PCR products were completely identical to those of rhodopsin.

We also detected the mRNA of melanopsin in the tail fin, another opsin originally described in cultured melanophores of *Xenopus*.

These results indicate that these two types of opsin molecules exist in *Xenopus* tail fin and may take part in the photo-response in melanophores of the *Xenopus* tadpole.

INTRODUCTION

Many lower vertebrates are able to change body color to adapt to their ambient coloration. These changes depend upon the activities of chromatophores in the body skin. Generally, the motility of chromatophores is controlled by the nervous and/or hormonal system on the basis of visual information, but some of the chromatophores are directly controlled by light. The photo-response of melanophores in the tail fin of *Xenopus* tadpole is well known (Bagnara, 1957; Lythgoe and Thompson, 1984; Daniolos *et al*., 1990; Moriya *et al*., 1996). The tail fins isolated from the *Xenopus* tadpole change skin color in response to light without the aid of vision and/or a central nervous system. Under illumination, the fin becomes pale as a result of melanosome aggregation in melanophores; in contrast, the fin becomes dark after interruption of light, due to melanosome dispersion (Moriya *et al*., 1996). These responses to light were observed in tadpoles of the later-stage (stages 51–56). Interestingly, quite the opposite responses occur in melanophores derived from the *Xenopus* embryo; that is, melanosomes disperse under illumination and aggregate in darkness (Bagnara and Obika, 1967; Daniolos *et al*., 1990; Rollag, 1996). The mechanism and the switch of the photo-response during the development are not yet clearly understood. Our previous observations using the later-stage of tadpole suggest that the photo-response in the *Xenopus* melanophores seems to be close to the visual response in retinal cells, based on the following arguments: 1) The action spectrum (maximum 500 nm) of the response is similar to the absorption spectrum of rhodopsin (Moriya *et al*., 1996). 2) The involvement of Gi/Gt and small G protein Rho in the photo-response is suggested, since this response was blocked by pertussis toxin or botulinum exoenzyme C3 and since the ADP-ribosylation of the G proteins was dependent on light (Miyashita *et al*., 1996). 3) Cyclic-GMP caused dispersion of melanophores under illumination (Moriya *et al*., 1996).

We demonstrate here that in addition to melanopsin, which has been reported in cultured melanophores derived from *Xenopus* embryo (Provencio *et al*., 1998), a rhodopsin-like molecule exists in *Xenopus* tadpole tail fin, where photosensitive melanophores exist.

* Corresponding auther: Tel. +81-11-611-2111;
FAX. +81-11-642-7010.
E-mail: miyasita@sapmed.ac.jp

MATERIALS AND METHODS

Materials

Tadpoles at stages 45–54 of *Xenopus laevis* were used. They were purchased from a commercial source or were bred in our laboratory and kept at 25°C under natural light conditions.

The tail fins (stage 54) were isolated from body trunks and were separated into the anterior and the posterior parts, since melanophores are found only in the posterior part. In the case of younger tadpole (stage 45), the whole tail fin was used.

Western blotting

The whole protein extract was prepared from the *Xenopus* tail fin according to the method described by Kikuchi *et al.* (1994). After treatment with 10% (V/V) trichloroacetic acid for 60 min on ice, the isolated tail fins (n=30) were homogenized in 160 μl of solubilizing solution (9 M urea, 2% (W/V) Triton X-100, and 1%(W/V) dithiothreitol) with a small metal homogenizer (Physcotoron, Tokyo, Japan). After adding 40 μl of 10%(W/V) lithium dodecyl sulfate, the homogenate was sonicated again. The solubilized proteins were separated on 12% sodium dodecyl sulfate-polyacrylamide gels and then transblotted onto nitrocellulose membranes (Pharmacia, Uppsala, Sweden). After blocking with 5% skim milk and 0.5% Tween-20 in PBS at 37°C for 1 hr and with Block Ace (Dainippon Pharmaceutical, Osaka, Japan) overnight at 5°C, the membranes were probed with polyclonal antibodies against both bovine rhodopsin (1:5000) and octopus rhodopsin (1:500). Normal rabbit serum was used as control. Subsequent visualization of antibody binding was carried out with an ABC peroxidase system (VECTASTAIN ABC kit) (Vector Laboratories, Burlingame, CA).

Reverse transcription (RT)-PCR

Total RNA of the *Xenopus* tail fin was extracted using ISOGEN (NIPPON GENE, Toyama, Japan). After treatment with deoxyribonuclease (NIPPON GENE, Toyama, Japan), 5 μg of total RNA was reverse transcribed using oligo (dT) as a primer and a SuperScript™ Preamplification System (GIBCO-BRL, Life Technologies, Grand Island, NY). PCR was performed using an Expand™ High Fidelity PCR System (Boehringer, Indianapolis, IN) with 2 μl of the cDNA corresponding to 0.5 μg of total RNA and 10 pmol each of the appropriate sense and antisense primers. The final reaction volume was 25 μl. The primers used for detection of rhodopsin mRNA were synthesized (ESPEC OLIGO SERVICE CORP, Tsukuba, Japan) to correspond to the nucleotide position (N.P.) of rhodopsin gene DNA of *Xenopus*, as previously described (Batni *et al*, 1996), R1: 5'primer, sense 5'-CAGCACAAGAAACTCAGAACA (N.P.289–319), R2: 3'primer,anti-sense 3'-CGACTCTTTCTCCAGTGGTCT (N.P.1944-1964), R3:3'primer,anti-sense 3'-CGATAGATGTTAGGACAGTAGATG (N.P. 2103–2126) and R4: 5'primer, sense 5'-GCTGAGAAAGAGGTCACCAGA (N.P.1944–1964). Primers (R1, R2 and R3) were chosen so that the expected PCR products would span introns 1,2 and 3, thereby eliminating the possibility of genomic DNA contamination. For detection of melanopsin mRNA, we referred to the report of Provencio *et al.* (1998) on melanopsin and designed the primers; M1: 5'primer, sense 5'-TTGGGCTGGGCATGGTAAATCTTT (N.P. 957-980) and M2: 3'primer,anti-sense 3'-ACGATGGAATGGAGCTTAACAGTA (N.P. 1530–1553).

The cycling protocol used was 94°C for 1 min, 60.5°C for 1 min and 72°C for 2 min for the designated number of cycles.

Sequences were compared with the GenBank database by using BLAST and BLASTIN algorithms (Altschul *et al.*, 1990)

RESULTS AND DISCUSSION

Anti-bovine rhodopsin antibody reacted with a protein of about 35 kDa in the extract of *Xenopus* tail fin (Fig. 1, lane 2). The size of the protein was identical to bovine rhodopsin and was compatible with amphibian rhodopsin reported previously (Okano *et al.*, 2000). We detected another immunoreacted protein with a higher molecular mass (about 78 kDa), shown in lane 2, which conceivably corresponds to a dimmer of rhodopsin.

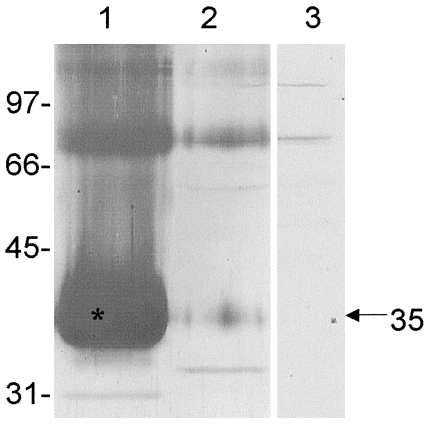

Fig. 1. Western blotting of the posterior tail fin extract with anti-bovine rhodopsin antibody. Lane 1: Bovine ROS membrane (*: bovine rhodopsin as standard), Lane 2: Posterior tail fin, Lane 3: Control. The numbers indicate the size (kDa) of standard protein (left) or of the immunoreacted protein (right).

On the other hand, anti-octopus rhodopsin antibody reacted with a protein of 50 kDa in the fin extract (Fig. 2). This 50 kDa protein seems to be different from rhodopsin from the bovine eye or the *Xenopus* tail fin and its size is similar to that of octopus rhodopsin. This protein is likely to be melanopsin, since melanopsin is strongly related to octopus rhodopsin (Provencio *et al.*, 1998). These results suggest the existence of two kinds of rhodopsin family protein in the tail fin. To confirm this possibility, expression of mRNAs for rhodopsin and melanopsin was examined.

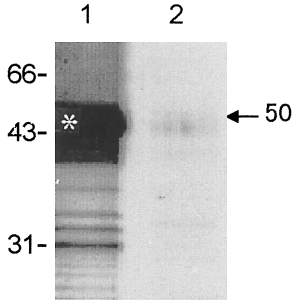

Fig. 2. Western blotting of the posterior tail fin extract with anti-octopus rhodopsin antibody. Lane 1: octopus rhodopsin (*: octopus rhodopsin as standard), Lane 2 : posterior tail fin. The numbers indicate the size (kDa) of standard protein (left) or of the immunoreacted protein (right).

We performed RT-PCR in *Xenopus* tail fin using a variety of primers for rhodopsin. Fig. 3A shows the results of RT-PCR, where three sets of the primers, R1/R3, R1/R2 and R4/R3, were used to amplify cDNA obtained from the tail fin. All three sets of primers synthesized the expected

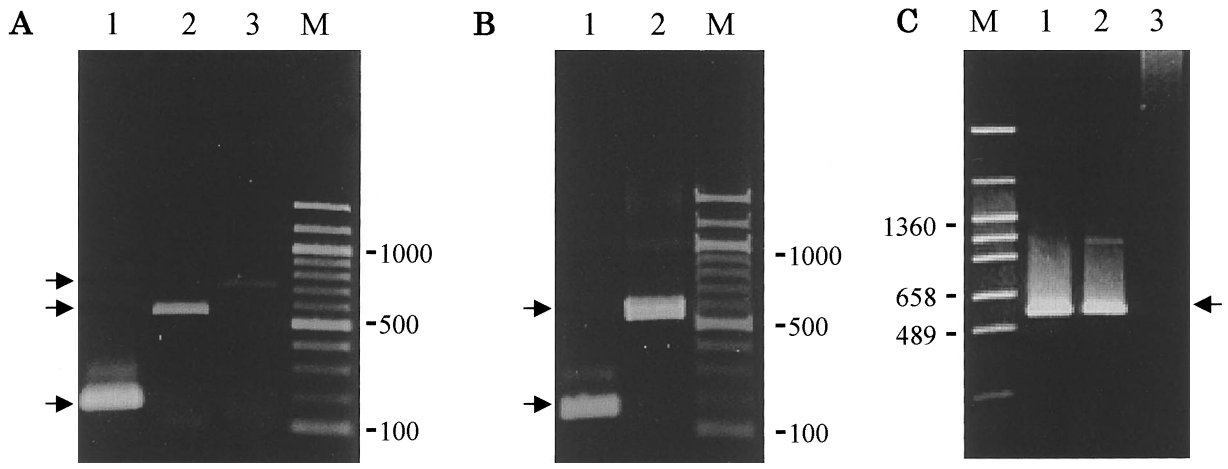

Fig. 3. Agarose gel electrophoresis of the PCR products amplified with the primers for rhodopsin mRNA.
A: The first PCR analyses using cDNAs of the posterior tail fin as the template. 1: R4/R3, 2: R1/R2, 3: R1/R3, M: marker, 60.5°C, 37 cycles. The numbers indicate the size (bp) of standard DNA (right). The arrows indicate the PCR products (expected sizes; 729, 567 and 183 bp, respectively).
B: The second (semi-nested) PCR analyses. The template was the first PCR product (R1/R3) in A. 1: R4/R3, 2: R1/R2, M: Marker, 60.5°C, 35 cycles. The numbers indicate the size (bp) of standard DNA (right). The arrows indicate the PCR products (expected sizes; 567 and 183 bp, respectively).
C: The second (semi-nested) PCR analyses using R1/R2 primer set. The template was the first PCR product (R1/R3). M: marker, 1: brain, 2: Posterior tail fin, 3: Anterior tail fin, 60°C, 35 cycles. The numbers indicate the size (bp) of standard DNA (left). The arrow indicates the PCR product (expected size; 567 bp).

sizes of DNAs, namely: 183bp for R4/R3, 567bp for R1/R2 and 729bp for R1/R3. Since the band of the R1/R3 product was very faint, a second (semi-nested) PCR analysis was performed using the first PCR product as the template. Two predicted PCR products were detected using R1/R2 and R4/R3 respectively as the primer sets (Fig. 3B).

The tail fin was separated into two parts before extraction of RNA, since the anterior part contains no melanophores while the posterior part does. Using the primers described above, the PCR products were found only in the posterior part and not in the anterior; as was expected (Fig. 3C). This result suggests that rhodopsin mRNA may be expressed within melanophore cells. This concurred with results showing that melanophores in the isolated tail fin aggregated in response to a local application of light (Moriya *et al*., 1996). The PCR products were also detected in the brain of *Xenopus* tadpole (Fig. 3C). In previous studies rhodopsin molecules were detected in extraretinal tissues including in the brain of non mammalian vertebrates (Wada *et al*., 1998; Okano *et al*., 2000).

The deduced amino acid sequence of the PCR products (R1/R2) and (R4/R3) were completely identical to Gln_{64}-Arg_{252} and Ala_{246}-Tyr_{306}, their corresponding parts of *Xenopus* rhodopsin, respectively; on the other hand, these sequences showed a low identity with the corresponding parts (Arg_{54}-Lys_{250} and Asn_{244}-Tyr_{304}) of melanopsin (30% and 45%, respectively). Thus the RT-PCR results showing rhodopsin mRNA expression, in combination with the results of the Western blotting (Fig. 1), strongly support the existence of rhodopsin molecules in the *Xenopus* tail fin. This is coincident with our former results showing that the photoresponse of the melanophores in the tail fin is likely to be closely related to that of the visual system in eyes (Moriya *et al*., 1996; Miyashita *et al*., 1996).

To better understand the expression of opsin molecules in *Xenopus* tail fin, RT-PCR was performed using melanopsin specific primers. When RT-PCR was performed on mRNA extracted from the posterior part of the tail fin of later stage tadpoles (stage 54), a very faint band was obtained (data not shown). The RNA obtained from the tail of younger

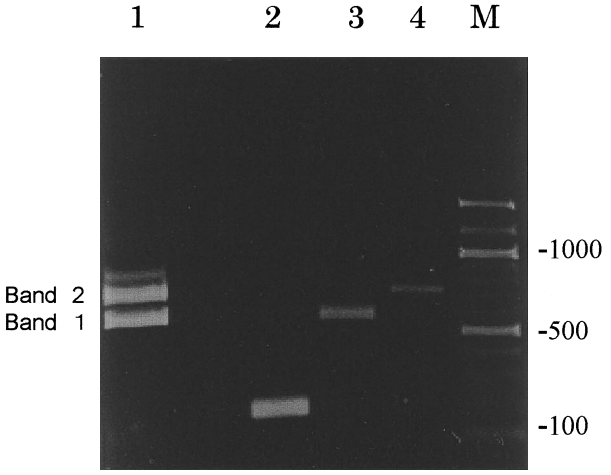

Fig. 4. Agarose gel electrophoresis of the PCR products amplified with the primers for melanopsin mRNA using cDNAs of the *Xenopus* skin as the template.
1: M1/M2, 2: R4/R3, 3: R1/R2, 4: R1/R3, M: Marker, 60.5°C, 35 cycles. The products in lanes 2–4 show the expression of rhodopsin mRNA as described in Fig. 3. The numbers indicate the size (bp) of standard DNA (right).

tadpole (stage 45) produced two clear bands (Fig. 4). One of them (band 1) was close in size to the expected size of 597 bp. The deduced amino acid sequence of band 1 was identical with a corresponding part (Gly_{276}-Arg_{469}) of *Xenopus* melanopsin. The deduced amino acid sequence of band 2 was identical to melanopsin except for an additional 47 amino acids between Gln_{396} and Asp_{397} of melanopsin. It is possible that band 2 is a variant mRNA caused by alternative RNA splicing.

These results showing that melanopsin mRNA is expressed in the tail fin where melanophores are present concur with the results shown by Provencio *et al.* (1998). The combined results suggest that the *Xenopus* tail fin contains both rhodopsin and melanopsin. Melanopsin was isolated from cultured melanophores, which were originally obtained from the early stages of embryo (stage 30-35) and responded to light by activating melanosome dispersion (Daniolos *et al*., 1990; Rollag, 1996 ; Provencio *et al*.,1998). On the other hand, cultured melanophores derived from tadpoles at stage 50–54 aggregated melanosomes on illumination (Seldenrijk *et al*., 1979). We consider that melanopsin and rhodopsin play different roles in the process of photoresponse: rhodopsin is probably involved in the aggregation of melanosomes whereas melanopsin is active in the dispersion. In fact, Rollag *et al.* (2000) reported recently that the melanophores expressing the melanopsin transgene was more sensitive to light and induced remarkable melanin dispersion. During embryonic development, rhodopsin, was first detectable as a visual pigment at stage 35, but the level was very low (Saha and Grainger, 1993). It is likely that in the early stage of development of *Xenopus*, there is a quantitative difference in the expression of the two opsins. The expression of rhodopsin increases as development takes place and this may explain the reversal of the photoresponse in the *Xenopus* melanophores.

We found that vertebrate type rhodopsin mRNA was expressed in cultured murine melanocytes (Miyashita *et al*., 1999). We are interested to know whether all pigment cells inherently carry the two types of opsin and are able to respond to light through these photoreceptor molecules.

AKNOWLEGEMENT

This research was supported by a grant from the Ministry of Education, Science and Culture of Japan (No.11640683).

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410

Batni S, Scalzetti LC, Moody SA, Knox BE (1996) Characterization of the *Xenopus* rhodopsin gene. J Biol Chem 271: 3179–3186

Bagnara JT (1957) Hypophysectomy and the tail darkening reaction in *Xenopus*. Proc Soc Exp Biol Med 94: 572–575

Bagnara JT, Obika M (1967) Light sensitivity of melanophores in neural crest explants. Experientia 23: 155–157

Daniolos A, Lerner AB, Lerner MR (1990) Action of light on frog pigment cells in culture. Pigment Cell Res 3: 38–43

Kikuchi H, Fujinawa T, Kuribayashi F, Nakanishi A, Imajoh-Ohmi S, Goto M, Kanegasaki S (1994) Induction of essential components of the superoxide generating system in human monoblastic leukemia U937 cells. J Biochem 116: 742–746

Lythgoe JN, Thompson M (1984) A porphyropsin-like action spectrum from *Xenopus* melanophores. Photochemistry and Photobiology 40: 411–412

Miyashita Y, Moriya T, Yamada K, Asami K (1999) The expression of rhodopsin in melanophores and melanocytes. Zool Sci 16: 110

Miyashita Y, Moriya T, Yokosawa N, Hatta S, Arai J, Kusunoki S, Toratani S, Yokosawa H, Fujii N, Asami K (1996) Light-sensitive response in melanophores of *Xenopus laevis*: II. Rho is involved in light-induced melanin aggregation. J Exp Zool 276: 125–131

Moriya T, Miyashita Y, Arai J, Kusunoki S, Abe M, Asami K (1996) Light-sensitive response in melanophores of *Xenopus laevis*: I. Spectral characteristics of melanophore response in isolated tail fin of *Xenopus* tadpole. J Exp Zool 276: 11–18

Okano K, Okano T, Yoshikawa T, Masuda A, Fukada Y, Oishi T (2000) Diversity of opsin immunoreactivities in the extraretinal tissues of four anuran amphibians. J Exp Zool 286: 136–142

Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD (1998) Melanopsin: An opsin in melanophores, brain, and eye. Proc Natl Sci 95: 340–345

Rollag MD (1996) Amphibian melanophores become photosensitive when treated with retinal. J Exp Zool 275: 20–26

Rollag MD, Provencio I, Sugden D, Green CB (2000) Cultured amphibian melanophores: a model system to study melanopsin photobiology. Methods in Enzymology 316: 291–309

Saha MS, Grainger RM (1993) Early expression in *Xenopus* embryos precedes photoreceptor differentiation. Mol Brain Res 17: 307–318

Seldenrijk R, Hup DRW, de Graan PNE, van de Veerdonk FCG (1979) Morphological and physiological aspects of melanophores in primary culture from tadpoles of *Xenopus laevis*. Cell Tissue Res 198: 397–409

Wada Y, Okano T, Adachi A, Ebihara S, Fukada Y (1998) Identification of rhodopsin in the pigeon deep brain. FEBS Letters 424: 53–56

(Received February 23, 2001/Accepted March 23, 2001)