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Expression of Membrane-Bound and Soluble Guanylyl Cyclase mRNAs in Embryonic and Adult Retina of the Medaka Fish *Oryzias latipes*

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ABSTRACT—Localization of mRNAs for four membrane-bound guanylyl cyclases (membrane GCs; OlGC3, OlGC4, OlGC5, and OlGC-R2), three soluble guanylyl cyclase subunits (soluble GC; OlGCS-α_1, OlGCS-α_2, and OlGCS-β_1), neuronal nitric oxide synthase (nNOS), and cGMP-dependent protein kinase I (cGK I) was examined in the embryonic and adult retinas of the medaka fish *Oryzias latipes* by *in situ* hybridization. All of the membrane GC mRNAs were detected in the photoreceptor cells of the adult and embryonic retinas, but in different parts; the *OlGC3* and *OlGC5* mRNAs were expressed in the proximal part and the *OlGC4* and *OlGC-R2* mRNAs were expressed in the outer nuclear layer. The mRNA for nNOS was expressed in a scattered fashion on the inner side of the inner nuclear layer in the adult and embryonic retinas. The mRNAs (*OlGCS-α_2* and *OlGCS-β_1*) of two soluble GC subunits (α_2 and β_1) were expressed mainly in the inner nuclear layer and the ganglion cell layer of the embryonic retina while the mRNAs of the soluble GC α_1 subunit and cGK I were not detected in either the adult or embryonic retina. These results suggest that NO itself and/or the cGMP generated by soluble GC (α_2/β_1 heterodimer) play a novel role in the neuronal signaling and neuronal development in the medaka fish embryonic retina in addition to the role played by phototransduction through membrane GCs in the adult and embryonic retinas.

Key words: membrane guanylyl cyclase, soluble guanylyl cyclase, nitric oxide synthase, retina, medaka fish

INTRODUCTION

In the photoreceptor cells of the vertebrate retina, cGMP plays an important role. The decrease of the cGMP level due to hydrolysis by phosphodiesterase (PDE) triggers the signal transduction from photoreception to neuronal signal output (Koch *et al*., 2002). The decreased cGMP level is restored to its initial level by the activation of guanylyl cyclases (GCs), which are enzymes that convert GTP to cGMP. GCs in living organisms are grouped into two major forms, those found membrane-bound in the plasma (membrane GCs) and those found in the cytoplasm (soluble GCs) (Lucas *et al*., 2000).

The membrane GCs responsible for phototransduction have been obtained from human (Shyjan *et al*., 1992; Lowe *et al*., 1995), rat (Yang *et al*., 1995), bovine (Goraczniak *et al*., 1994, 1997) and medaka fish retinas (Seimiya *et al*., 1997; Hisatomi *et al*., 1999). From a retinal cDNA library of the medaka fish *Oryzias latipes*, Seimiya *et al*. (1997) and Hisatomi *et al*. (1999) each independently isolated cDNA clones for a set of three membrane GCs, and named them *OlGC3*, *OlGC4* and *OlGC5*, and *OlGC-R1*, *OlGC-R2* and *OlGC-C*, respectively. A comparison of the nucleotide and deduced amino acid sequences of these six cDNAs indicates that *OlGC-R1* and *OlGC-C* correspond to *OlGC4* and *OlGC5*, respectively. Since *OlGC-R2* is distinct from *OlGC3*, *OlGC4* and *OlGC5*, it is assumed that four different membrane GCs, namely *OlGC3*, *OlGC4* (*OlGC-R1*), *OlGC5* (*OlGC-C*), and *OlGC-R2*, are expressed in the medaka fish retina (Kusakabe and Suzuki, 2000a).

Soluble GC is a heme-containing heterodimer composed of α and β subunits (Kamisaki *et al*., 1986). Soluble GC is activated primarily by nitric oxide (NO) generated from L-arginine by nitric oxide synthase (NOS) (Denninger and Marletta, 1999; Alderton, *et al*., 2001; Bellamy and Garth-

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waite, 2002), which is grouped into three subfamilies: neural NOS (n*NOS*), endothelial NOS (*eNOS*) and inducible NOS (*iNOS*) (Hall *et al*., 1994; Chartrain *et al*., 1994; Marsden *et al*., 1993). Two α (α_1 and α_2) and two β (β_1 and β_2) subunits of the soluble GC mRNAs are found in mammals (Wedel and Garbers, 1997; Lucas *et al*., 2000). In the medaka fish, mRNAs for soluble GC α_1 and β_1 subunits have been isolated and named *OlGCS-*α_1 and *OlGCS-*β_1, respectively (Mikami *et al*., 1998). The primary structure of the medaka fish α_1 and β_1 subunits is closely related to the structure of the respective mammalian subunits. Recently, we also obtained the α_2 subunit gene (*OlGCS-*α_2) of medaka fish soluble GC (Yao, Y., Yamamoto, T. and Suzuki, N., unpublished data). Several papers have demonstrated on the basis of immunohistochemistry and GC activation induced by NOS that some of the soluble GC subunits exist in the mammalian retina (Haberecht *et al*., 1998; Margulis *et al*., 1998; Sitaramayya, 2002). However, as far as we know, no identification of the type of the soluble GC subunit expressed in the retina has been performed yet. In addition, none of the papers dealing with the expression of the soluble GC subunit mRNA in the teleost retina have been published yet.

It has been reported that the NO/cGMP signaling pathway comprising NOS, soluble GC, cGMP-dependent protein kinase (cGK), and PDE plays an important role in smooth muscle contraction in the vascular system and in immune response (Lucas *et al*., 2000). In a study, we obtained a cDNA fragment of *nNOS* by RT-PCR from the whole body of a medaka fish (Yamamoto *et al*., 2003). Downstream of the cGMP signaling pathway, cGK functions similarly to cAMP-dependent protein kinase in the cAMP signaling pathway. Two distinct isoforms (*cGK I* and *cGK II*) of cGK have been identified in mammals (Wernet *et al*., 1989; Tamura *et al*., 1996; Ørstavik *et al*., 1996). Recently, we also obtained cDNA fragments of the medaka fish *cGK I* and *II* (Yamamoto *et al*., 2003). In the present study, we examined the localization of mRNAs for four membrane GCs, three soluble GC subunits, *nNOS*, and *cGK I* in the adult and embryonic retinas of the medaka fish *Oryzias latipes* by *in situ* hybridization (ISH). Here, we report that *OlGC3* and *OlGC5* are expressed in the proximal part of the adult and embryonic retinas and *OlGC4* and *OlGC-R2* are expressed in the outer nuclear layer of the adult and embryonic retinas, while *OlGCS-*α_2 and *OlGCS-*β_1 are expressed mainly in the inner nuclear layer and ganglion cell layer of the embryonic retina.

MATERIALS AND METHODS

Animals and embryos

Adult individuals and embryos of the orange-red variety of the medaka fish *O. latipes* were purchased from a dealer. They were kept in indoor tanks under artificial reproductive conditions (14 hr light, 10 hr dark, 27°C) and fed on Otohime B2 (Nisshin Seifun Group Inc., Tokyo, Japan). Naturally spawned and fertilized eggs were collected, and the embryos were cultured in distilled water containing 0.6 ppm methylene blue at 27°C. The developmental stage of the embryos was expressed in days, with the day of fertilization referred to as day 0.

The eyes of embryonic and adult medaka fish were fixed with 4% paraformaldehyde in 70 mM phosphate buffered saline (PBS), pH 7.3. After being rinsed in PBS, the samples were treated with 0.1% diethylenepyrocarbonate (DEPC) overnight at 4°C, and then left in DEPC-treated 30% sucrose-PBS at 4°C. The tissue was sliced into 15 μm-sections on a cryostat microtorm (LEICA, CM3000) and mounted on a glass slide coated with poly-L-lysin (Sigma).

Isolation of cDNA fragment encoding medaka fish *nNOS* and *cGK-I*

The medaka fish *nNOS* and *cGK-I* cDNA fragments were obtained by RT-PCR using degenerate primers designed using zebrafish and salmon sequences (Holmqvist *et al*., 2000; Øyan *et al*., 2000; Yamamoto *et al*., 2002). The primer pairs used for the first PCR were: *nNOS*, 5'-CCYGTBTTCCAYCAGGAGATG-3' and 5'-RAAGGCRCARAASTGRGGGTA-3'. The primer pairs used for nested PCR were as follows: *nNOS*, 5'-CAGGAGATGCTCAACTATC-3' and 5'-TCCAGTGCTCTCGAAGTTG-3'; *cGK-I*, 5'-ATCATCGACACCTTTGGAGTTGG-3' and 5'-CACATTGTAAATGCTTTATCCAGAG-3'. The amplified PCR products of *nNOS* (502 bp) and *cGK-I* (1145 bp) were purified and subcloned into the plasmid vector pBluescript II KS (+) (Stratagene).

In situ hybridization (ISH)

The pBluescript II vector containing a cDNA fragment for the coding region of *OlGC3* (nucleotides 431–1257), *OlGC4* (nucleotides 5–1811), *OlGC5* (nucleotides 283-1071), *OlGC-R2* (nucleotides 1–1389), *OlGCS-*α_1 (nucleotides 195–953), *OlGCS-*α_2 (nucleotide 1356-2894), *OlGCS-*β_1 (nucleotides 252-953), *nNOS* (nucleotides 1–502) or *cGK I* (nucleotides 1–1145) was used as a template to generate a digoxigenin-labeled cRNA probe using a DIG RNA Labeling Kit (Boehringer Mannheim) according to the manufacturer's protocol (Yamamoto *et al*., 2003).

ISH was carried out according to the protocol described by Yoshida *et al*. (1994) with the following modifications. The section was postfixed with 0.1 M phosphate buffer (PB, pH 7.4) containing 4% formalin for 20 min. The fixed section was treated with 10 μg/ml proteinase K (Boehringer Mannheim) in TE, and fixed again with PB containing 4% formalin. The acetylation of the section was carried out with 0.1 M triethanolamine containing 0.25% acetic acid. After being rinsed in PB, the section was treated with ethanol and chloroform. Prehybridization was carried out at 55°C for 1 hr in a hybridization buffer containing 50% formamide, 20 mM Tris-HCl (pH 8.0), 0.3 M NaCl, 10% dextran sulfate, 0.2% sarcosyl, 0.02% salmon sperm DNA, and 1xDenhardt's solution. After an overnight hybridization with about 2 ng/μl antisense or sense probe in the hybridization buffer at 55°C, the section was washed with 4xSSC at 60°C for 20 min and then with a formamide buffer (50% formamide in 2xSSC) at 60°C for 30 min. The section was incubated in 1 μg/μl RNase A (Sigma) in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA and 500 mM NaCl at 37°C for 30 min. After the RNase A was rinsed out of the section using the same buffer, the section was treated with the formamide buffer again at 60°C for 30 min. The section was further rinsed with Buffer 1 containing 100 mM Tris-HCl (pH 7.5) and 150 mM NaCl, after which it was rinsed with a blocking buffer containing 1.5% blocking reagent (Boehringer Mannheim) in Buffer 1 for 1 hr at room temperature. The section was then incubated with alkaline phosphatase-conjugated anti-digoxigenin sheep antibody (1:500 dilution in the blocking buffer) overnight at room temperature and rinsed with the Buffer 1 and then Buffer 2 containing 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, and 50 mM $MgCl_2$. The hybridization signal on the section was visualized by a treatment with a solution containing 340 μg/ml nitroblue tetrazolium and 170 μg/ml 5-bromo-4-chloro-3-indolyl phosphate in Buffer 2 and 10% polyvinyl

alcohol 2000 (Sigma). The section was then rinsed with TE to terminate the coloring reaction, mounted under coverslips with TE containing 50% glycerol and observed under Nomarski optics.

RESULTS

Expression of membrane GC genes in medaka fish embryonic retina

The most external layer of the medaka fish retina is a pigment epithelium containing a large number of melanin granules (Fig. 1). Photoreceptor cells consisting of rod and cone cells are arranged inside of the pigment epithelium. The cell bodies containing nuclei of the photoreceptor cells are located in one layer known as the outer nuclear layer. The outer layer of the outer nuclear layer in the photoreceptor cells can be further divided into two layers, the inner segment containing ellipsoid and myoid and the outer segment containing rod and cone cells. Under light-microscopic observations, the outer nuclear layer and the proximal part of the photoreceptor cells are delimited with an outer limiting membrane. The expression of four membrane GC genes, *OlGC3*, *OlGC4*, *OlGC5* and *OlGC-R2*, in the adult medaka fish retina was examined by ISH, which demonstrated that the *OlGC3* and *OlGC5* genes are expressed in the proximal part of the photoreceptor cells and that the *OlGC4* and *OlGC-R2* genes are expressed in the outer nuclear layer and around the outer limiting membrane (Fig. 1). Unfortunately, the difference in the gene expression of the four GCs between the cone and rod cells could not be recognized clearly in this study. However, the present observations are almost consistent with the previous findings on the *OlGC-C* (*OlGC5*), *OlGC-R1* (*OlGC4*) and *OlGC-R2* expression in the medaka fish retina; *OlGC-C* is expressed in both the cones and myoids, while *OlGC-R1* and *OlGC-R2* are expressed in the cell bodies and myoids in the rod cells (Hisatomi *et al*., 1999). In addition to these findings, we showed that *OlGC3* and *OlGC5* are expressed in the proximal part of the photoreceptor cells (Fig. 1). The results obtained by ISH of the membrane GCs with the medaka fish embryonic retina are shown in Fig. 2. Although the expression of *OlGC3*, *OlGC4*, and *OlGC5* was detected in the retinas of 5-day-old embryos, *OlGC4* was expressed only in a part of the retinas of 4-day-old embryos. Moreover, the expression of *OlGC4*

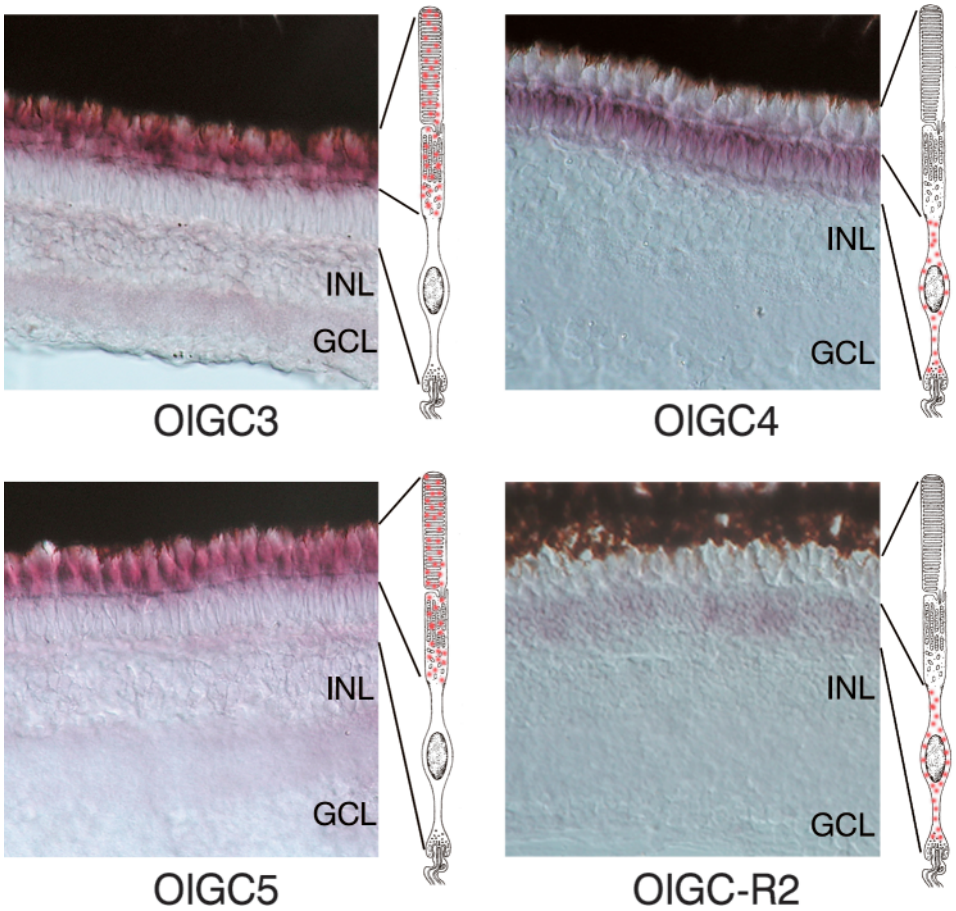

Fig. 1. Localization of the *OlGC3, OlGC4, OlGC5* and *OlGC-R2* mRNA in the adult medaka fish retina. Schematic drawing of a rod cell is attached at the right side of each photograph. The red dots in the drawings depict the localization of mRNA hybridized with the respective cRNA probes of the membrane GCs. The three black lines in each figure indicate the position of the outer and inner edges and the outer-limiting membrane in the rod cell. The hybridization and cRNA probes used are described in **MATERIALS AND METHODS**. The abbreviations used are INL, inner nuclear layer; GCL, ganglion cell layer. The side-to-side distance in each photograph is 0.1 mm.

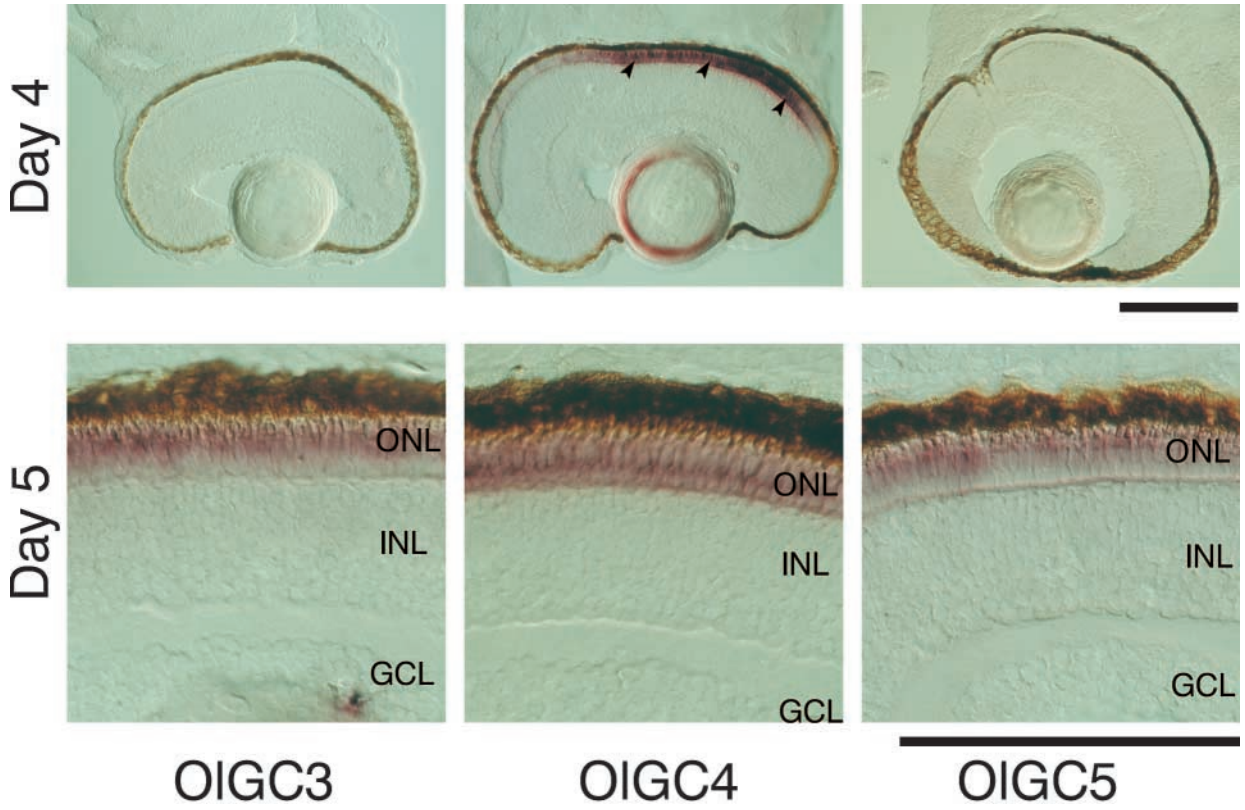

Fig. 2. Localization of the *OlGC3, OlGC4* and *OlGC5* mRNA in the retina of medaka fish embryos at days 4 and 5. The hybridization and cRNA probes used are described in **MATERIALS AND METHODS**. Arrowheads indicate the hybridization signal of *OlGC4* found in the embryo at day 4. The abbreviations used are ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Each bar indicates 0.1 mm.

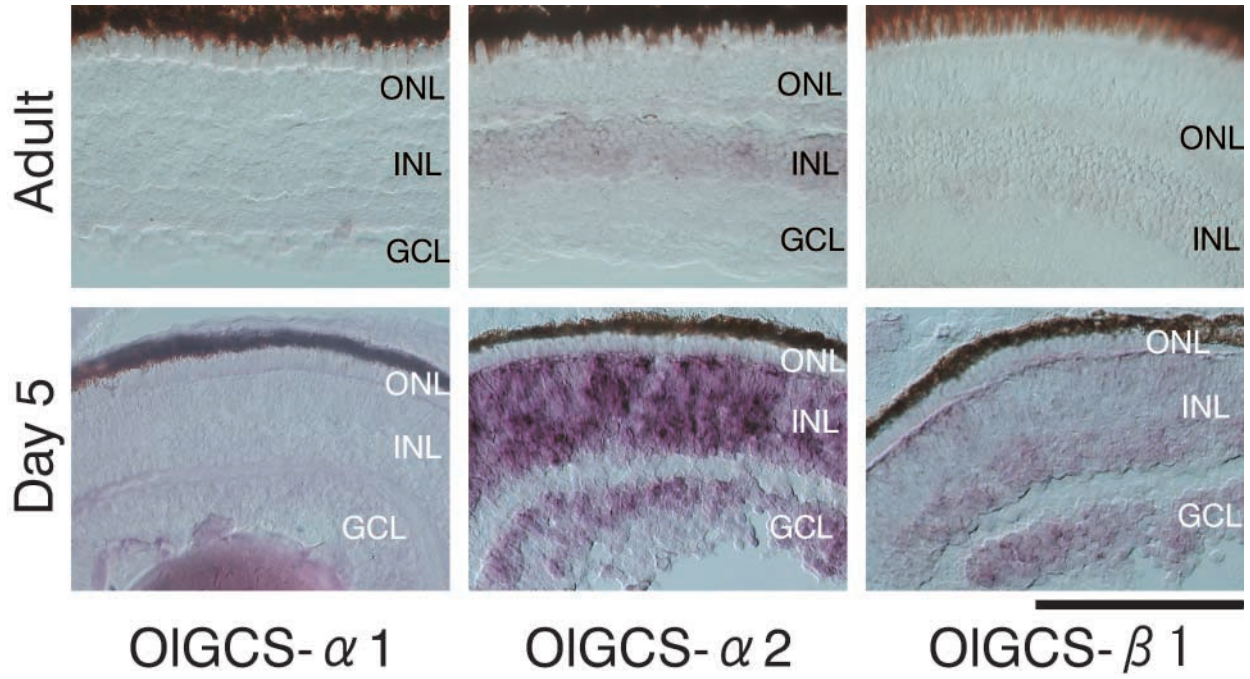

Fig. 3. Localization of the *OlGCS-α1, OlGCS-α2* and *OlGCS-β1* mRNA in the retina of the adult and 5-day-old embryo of the medaka fish. The hybridization and cRNA probes used are described in **MATERIALS AND METHODS**. The abbreviations used are ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. The bar indicates 0.1 mm.

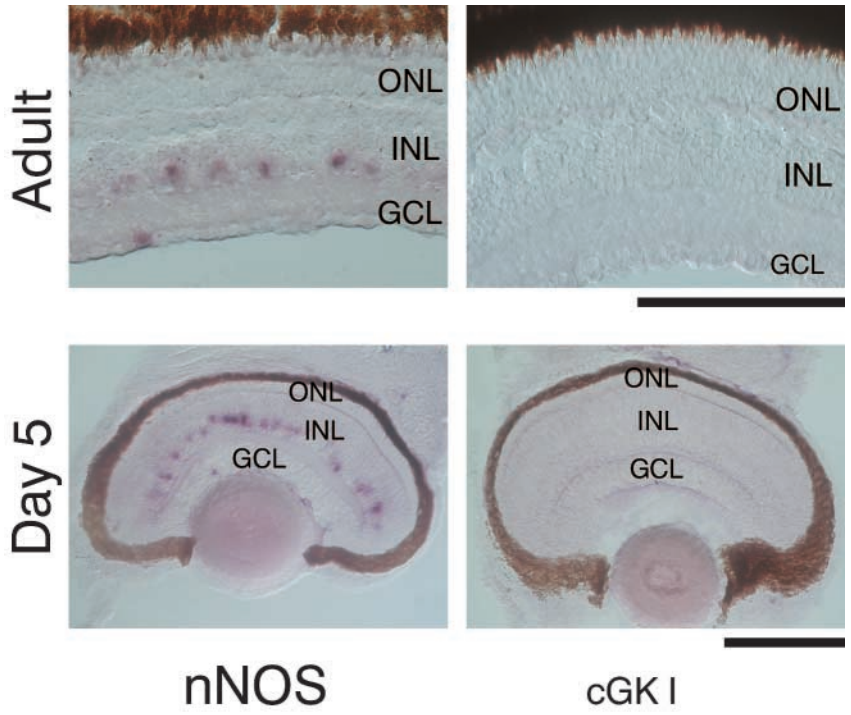

Fig. 4. Localization of the *nNOS* and *cGKI* mRNA in the retina of adult and 5-day-old embryo of the medaka fish. The hybridization and cRNA probes used are described in **MATERIALS AND METHODS**. The abbreviations used are ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Each bar indicates 0.1 mm.

gene was also detected even in the retinas of 3-day-old embryos. On the other hand, the expression of *OlGC-R2* could not be detected in the retinas of 3-day- to 5-day-old embryos.

Expression of soluble GC subunit genes in medaka fish adult and embryonic retinas

The inner nuclear layer is a nuclear layer located between the outer nuclear layer and the ganglion cell layer of the photoreceptor cells. In the medaka fish adult and embryonic retinas, the expression of the soluble GC subunit genes (*OlGCS-*α_1, *OlGCS-*α_2, and *OlGCS-*β_1) was examined with ISH (Fig. 3). A weak signal of *OlGCS-*α_2 was detected in the inner nuclear layer of the adult retina, although *OlGCS-*α_1 and *OlGCS-*β_1 transcripts could not be detected in it. In the retinas of 5-day-old embryos, *OlGCS-*α_2 and *OlGCS-*β_1 were expressed extensively in the inner nuclear layer and the ganglion cell layer (Fig. 3). On the other hand, the expression of *OlGCS-*α_1 could not be detected in the embryonic and adult retinas.

Expression of *nNOS* and *cGK I* genes in the medaka fish adult and embryonic retinas

In the adult and embryonic medaka fish retinas, the expression of *nNOS* and *cGKI*, both of which are present in the NO/cGMP signaling pathway, was examined by ISH. The transcript of *nNOS* was detected in the inner nuclear layer and the ganglion cell layer in the adult retina (Fig. 4). In contrast with the localization pattern of the *OlGCS-*α_2 mRNA, the cells expressing the *nNOS* gene are scattered in both the ganglion cell layer and the inner layer of the inner nuclear layer of the adult retina. The expression of the *nNOS* gene was also detected in the retinas of 5-day-old embryos and adult fish with the same pattern. The identification of the cells expressing *nNOS* remains to be resolved. However, since it is known that amacrine cells are located in the innermost layer of the inner nuclear layer (Haverkamp *et al*., 2000), it is possible that the amacrine cells express the *nNOS* gene and synthesize nitric oxide. The ganglion cell layer contains cell bodies of midget and polysynaptic ganglion cells and astrocytes. The cell expressing the *nNOS* gene in the ganglion cell layer is not yet clear. The expression of the *cGKI* gene could not be detected in either the adult or the embryonic retina (Fig. 4).

DISCUSSION

It has been reported that four membrane GC genes, *OlGC3*, *OlGC4* (*OlGC-R1*), *OlGC5* (*OlGC-C*), and *OlGC-R2*, are expressed in the medaka fish adult and embryonic retina (Seimiya *et al*., 1997; Hisatomi *et al*., 1999; Kusakabe and Suzuki, 2000b, 2001). *OlGC-R1* and *OlGC-R2* are expressed around the nuclei of photoreceptor cells while *OlGC-C* is expressed proximal to the nuclei (Hisatomi *et al*., 1999). Our present results are consistent with the previous observations on the expression pattern of these membrane GCs in the adult medaka fish tissues. Furthermore, we showed in this study that the *OlGC3* gene is expressed near the site

where *OlGC5* is expressed, indicating that the membrane GCs expressed in the medaka fish retina may be grouped into two groups: one comprising *OlGC3* and *OlGC5*, which are expressed outside of the outer limiting membrane, and the other comprising *OlGC4* and *OlGC-R2*, which are expressed in the cell bodies containing the nuclei of the photoreceptor cells. This classification based on the expression pattern of the retinal membrane GC genes coincides with the evolutionary distance of these membrane GCs as estimated by molecular phylogenetic analysis (Kusakabe and Suzuki, 2000a). However, the relationship between the functional difference between these membrane GCs and their localization is not clear at this point. In mammals, two retina-specific membrane GCs have been cloned in humans (*RetGC-1* and *Ret GC-2*; Shyjan *et al*., 1992; Lowe *et al*., 1995), rats (*GC-E* and *GC-F*; Yang *et al*., 1995) and bovines (*ROS-GC1* and *ROS-GC2*; Goraczniak *et al*., 1994, 1997). It has been reported that a *GC-E*-deficient mouse shows no abnormality in its apparent rod morphology and its behavior toward light, although its cones had disappeared completely within five weeks after birth (Yang *et al*., 1999), suggesting that the other retinal membrane GC (*GC-F*) compensates for the *GC-E*-deficient effects in the mouse. On the other hand, it is known that membrane GCs form homo-oligomers (Lowe, 1992; Chinkers and Wilson, 1992; Vaandrager *et al*., 1994). Yang and Garbers (1997) showed that the two retinal GCs, *GC-E* and *GC-F*, form homodimers preferentially, but a limited number of heterodimers are also formed. However, it remains to be solved whether the membrane GCs in the medaka fish retina compensate each other and form a homodimer and/or a heterodimer. In the present study, we demonstrated that *OlGC4* is expressed in the retinas of 3-day- and 4-day-old embryos while *OlGC3* and *OlGC5* are expressed in the retinas of 5-day-old embryos, suggesting that *OlGC3* and *OlGC5* compensate for each other if necessary, and that *OlGC4* may play a novel role in the developing retina.

The evidence showing that soluble GC exists in different types of retinal cells such as photoreceptor cells, horizontal cells, bipolar cells, amacrine cells, ganglion cells, and Müller cells has been presented, based on biochemical and immunohistochemical experiments (Sitaramayya, 2002). However, there has been no study dealing with the types of subunits expressing and functioning in these cells. In the present study, we showed the strong signals in the embryonic retina due to the mRNAs of *OlGCS-α_2* and *OlGCS-β_1*, both of whose translation products form a NO-activating heterodimer, and the weak signals due to them in the adult retina. We could not detect any signal due to the α_1 subunit gene in ither the adult or embryonic retina, which suggests that the soluble GC expressed in the medaka fish embryonic retina is a α_2/β_1 heterodimer. In previous papers, we demonstrated that the medaka fish soluble GC subunit genes, *OlGCS-α_1* and *OlGCS-β_1*, are organized in tandem on the medaka fish genome and that their transcriptions seem to be coordinated (Mikami *et al*., 1999; Yamamoto and Suzuki, 2002). Recently, we showed that the transcripts of *OlGCS-α_1* and *OlGCS-β_1* are colocalized in the embryonic whole brain and kidney (Yamamoto *et al*., 2003). The genes of the human soluble GC α_1 and β_1 subunits are mapped on the same chromosome 4q32 (Giuili *et al*., 1993; Lucas *et al*., 2000), while the human soluble GC α_2 gene is located on chromosome 11 (Yu *et al*., 1996). It has been reported that the human soluble GC α_2 subunit forms an enzymatically active heterodimer with the β_1 subunit when both subunit genes are co-expressed in the cultured cells (Yu *et al*., 1996), and the physiological existence of the α_2/β_1 heterodimer has been found in the human placenta (Russwurm *et al*., 1998) and rat brain synaptosomes (Russwurm *et al*., 2001). Moreover, it has been reported that the α_2/β_1 heterodimer interacts with postsynaptic density-95 protein (PSD-95), which is a synaptic protein containing three PDZ protein motifs that also interact with nNOS and NMDA receptor and which contributes to anchoring the heterodimer to the plasma membrane (Brenman *et al*., 1996; Russwurm *et al*., 2001; Russwurm and Koesling, 2002). At the present time, we do not know whether the transcription of the *OlGCS-α_2* gene is coordinated with that of the *OlGCS-β_1* gene in the medaka fish. However, we demonstrated that the expression site of the *OlGCS-α_2* and *OlGCS-β_1* genes is rather restricted in the embryonic retina (Fig. 3). Recently, we demonstrated that the knock-down of the soluble GC α_2 subunit gene using morpholino antisense oligonucleotide results in abnormal development of the eye in the medaka fish (Yamamoto *et al*., 2003). In this regard, it should be mentioned that the diminishment of the soluble GC activity due to the mutation in the α subunit gene causes defects in the development of the *Drosophila* visual system leading to defects in the visual system function and in visually mediated behavior (Gibbs *et al*., 2001). Taking account of these reported facts and our results presented here, we presume that a soluble GC consisting of α_2 and β_1 subunits plays a novel role in the retinal development in medaka fish embryos, although the downstream of the cGMP signaling pathway in the medaka fish retina after soluble GC remains to be determined.

There have been many papers demonstrating that by using immunohistochemical and NADPH diaphorase histochemical methods, NOS, not being specified either as *nNOS*, *eNOS* or *iNOS*, is detected in amacrine cells in the inner nuclear layer and ganglion cell layer of rat, guinea pig, and turtle retinas (Chun *et al*., 1999; Oh *et al*., 1999; Haverkamp *et al*., 2000), in amacrine and ganglion cells as well as horizontal and photoreceptor cells in zebrafish retinas (Devadas *et al*., 2001), and in amacrine and ganglion cells as well as Mülar cells in goldfish and catfish retinas (Liepe *et al*., 1994). As shown in Fig. 4, we showed that *nNOS* is expressed in the inner nuclear layer and ganglion cell layer in both the adult and embryonic retinas as a series of spots, suggesting that in the medaka fish, *nNOS* is also expressed in amacrine cells in the inner nuclear layer as well as in ganglion cells in the adult and embryonic retinas.

It is known that the major target molecule of NO generated by NOS is soluble GC (α_1/β_1 and/or α_2/β_1 heterodimer) (Russwurm and Koesling, 2002), a key enzyme in the NO/cGMP signaling pathway (Denninger and Marletta, 1999; Kusakabe and Suzuki, 2000a). Several investigators reported that activation of soluble GCs or cGMP elevation by NO or NO donors occurs in bipolar cells but not in amacrine cells in the mammalian retina (Shiells and Falk, 1992; Koistinaho *et al*., 1993). On the other hand, there have been several papers reporting that NO is involved in many optic systems such as the control of retinal flow (Deussen *et al*., 1993; Toda *et al*., 1994; Wiencke *et al*., 1994) and the induction of neurotoxic effects leading to retinal destruction (Lipton and Rosemberg, 1994; Cui and Harvey, 1995; Goureau *et al*., 1995), although the relationship with the cGMP signaling pathway is unknown. Inconsistent with the above papers, it has been reported that NO exhibits a neuroprotective effect in retinal development, and its effect is partially mediated by soluble GC (Guimaraes *et al*., 2001). Moreover, the NO in the retina is also reported to have cGMP-independent effects on the phototransduction and synaptic output from photoreceptor cells (Kurenny *et al*., 1994). Although there are several apparent inconsistencies and points of confusion among the reported results and explanations of experimentally obtained facts regarding the NO/cGMP signaling pathway, the most plausible explanation is that NO and soluble GC are required to regulate the appropriate development of the retina (Ernst *et al*., 1998; Guimarases *et al*., 2001; Gibbs *et al*., 2001). In this sense, we postulate here that in the medaka fish, NO itself or NO with a soluble GC (α_2/β_1 heterodimer) plays a role as a regulator of retinal development.

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