Gene Expression Patterns 7 (2007) 746–753

www.elsevier.com/locate/modgep

Expression pattern of the *melanopsin-like* (*cOpn4m*) and *VA opsin-like* genes in the developing chicken retina and neural tissues

Sayuri Tomonari, Akira Takagi, Sumihare Noji, Hideyo Ohuchi *

Department of Life Systems, Institute of Technology and Science, University of Tokushima, 2-1 Minami-Jyosanjima, Tokushima 770-8506, Japan

Received 23 February 2007; received in revised form 6 June 2007; accepted 8 June 2007
Available online 13 June 2007

Abstract

We examined the expression pattern of *melanopsin-like* (*cOpn4m*) and *VA opsin-like* (*cVAL*) genes during chicken development. Two types of *cOpn4m* transcripts, distinct in their carboxyl terminals were found, as is the case for the chicken *melanopsin* (*cOpn4*) reported previously. The expression of *cOpn4m* was restricted to the developing retina, specifically to a subset of developing amacrine cells from embryonic day 10. VA opsin is one of the non-canonical opsins, reported to exist in fish so far. In this study, an aberrant type of *VA opsin-like* (*cVAL*) cDNA was isolated from chicken embryonic neural tissues. The expression of *cVAL* was observed in the ventral region of the developing brain and neural tube; however, specific signals for *cVAL* could not be detected in the developing retina. These results indicate that the additional melanopsin in avian identifies a subset of developing amacrine cells in the retina and that the aberrant transcript of the *VA opsin-like* gene are present during neural tube development in the chicken.

Keywords: Chicken; Retina; Development; *cOpn4m*; Melanopsin-like; VA opsin-like; Vertebrate ancient opsin; Amacrine cells; Basal plate; Neural tube; Brain

1. Results and discussion

1.1. Two types of chicken Opn4m transcripts are expressed during retinal development

Melanopsin (Opsin 4) is a non-canonical opsin localized in non-rod, non-cone cells in the retina. In rodents, *melanopsin* is expressed in a small subset of retinal ganglion cells (RGCs), called intrinsically photosensitive RGCs (ipRGCs) (reviewed by Kumbalasiri and Provencio, 2005). In other vertebrates, *melanopsin*-expressing cells in the retina are diversified as horizontal cells (*Xenopus*, teleost) (Provencio et al., 1998; Bellingham et al., 2002; Jenkins et al., 2003; Drivenes et al., 2003), or subsets of cells in the ganglion and amacrine cell layers (teleost) (Jenkins et al., 2003; Drivenes et al., 2003).

Creation of *melanopsin*-null mice and crossing the mice with those lacking functional rods and cones have revealed that melanopsin-containing ipRGCs play a role in circadian photoentrainment and the papillary light reflex (Kumbalasiri and Provencio, 2005; for a review). Furthermore, it has been shown that mammalian melanopsin and other non-canonical opsins are expressed early in ocular development (Tarttelin et al., 2003a), suggesting their distinct roles during development.

We previously reported that a chicken *melanopsin* gene, *cOpn4* (Chaurasia et al., 2005; the same as *cOpn4x* in Bellingham et al., 2006) is expressed in a portion of developing brain and subsets of differentiating retinal cells (Tomonari et al., 2005). Through a further in silico search, another *melanopsin-like* gene was found in the chicken genome located in the chromosome 6, which has turned out to be the same as *cOpn4m* reported by Bellingham et al. (2006). To investigate the expression of *cOpn4m* in chicken embryos, we first performed reverse transcription (RT)-PCR on samples from the neural retina (NR), retinal

* Corresponding author. Tel.: +81 88 656 7529; fax: +81 88 656 9074.
E-mail address: hohuchi@bio.tokushima-u.ac.jp (H. Ohuchi).

1567-133X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.modgep.2007.06.001

pigment epithelium (RPE), and brain including the pineal gland, at embryonic days 9 (E9) and 17 (E17). At both stages, *cOpn4m* was expressed in the three tissues examined, while it was not expressed in the liver (data not shown).

Sequence analysis of the amplified DNA fragments has demonstrated that the *cOpn4m* gene produces multiple types of *cOpn4m* transcripts. Further analysis through 3′ RACE and RT-PCR has identified at least two forms of transcripts, designated *cOpn4m-a* and *cOpn4m-b*. The nucleotide sequence of *cOpn4m-a* was essentially identical to the one deposited in GenBank (Accession No. AY882944; *cOpn4m*) (Bellingham et al., 2006), encoding a deduced 528 amino acid-protein (Fig. 1A). On the other hand, another transcript of *cOpn4m-b* encodes a deduced 422 amino acid-protein. Sequence similarity searching on the chicken genome has revealed that the *cOpn4m* gene consists of 12 exons (Gene ID: 423609). We deduced the putative protein structure of cOpn4m isoforms (Fig. 1A) and the splicing pattern of the *cOpn4m* gene (Fig. 1B). We found an additional 140 bp-nucleotides in the *cOpn4m-b* transcript, which corresponds to a portion of sequences in the intron 8–9 (nucleotides 1819262–1819401 in contig NW_060392, chromosome 6). Since this sequence is flanked by AG at the 5′ site and GT at the 3′ site in the genomic DNA (data not shown), it could be an additional exon. Therefore, it is designated exon 8b here, while the authentic exon 8 is designated exon 8a. The additional nucleotides cause a frame shift in the subsequent sequence, resulting in the C-terminal truncated protein of cOpn4m-b.

To precisely characterize the kinetics of expression and the relative abundance of each transcript, we performed quantitative PCR analysis. We measured the amount of *cOpn4m* transcripts relative to the ubiquitous *β-actin* mRNA, taken as an internal standard, using total RNA from the NR, RPE, as well as pineal gland, and liver as a negative control (Fig. 1C). With the level of *cOpn4m* in E10 NR referred to as 1, the relative mRNA level of *cOpn4m* in the NR increased to 9.6 at E14 and 59.7 at E17. In the pineal gland, the relative mRNA level of *cOpn4m* also increased from 0.17 at E10 and 0.46 at E14 to 1.39 at E17. On the contrary, in the RPE, the relative mRNA level of *cOpn4m* appeared unchanged from E10 to E17. We further examined relative amount of *cOpn4m-b* transcripts compared to the total amount of *cOpn4m* transcripts, finding that a considerable amount of *cOpn4m* mRNA was derived from *cOpn4m-b* (Fig. 1D). This implies that the *cOpn4m* gene may give rise to relatively high amount of cOpn4m-b, which is a similar truncated protein to cOpn4-b (Tomonari et al., 2005) (Fig. 1D). In the developing pineal gland, *cOpn4m* was expressed at a relatively low level (Fig. 1C and D).

cOpn4m shares structural similarities with all known opsins including an extracellular amino acid terminus, 7 transmembrane domains, and an intracellular carboxyl terminus. It has been postulated that melanopsin's conspicuously long cytoplasmic tail contains potential phosphorylation sites (serines and threonines) and that its activation state may be regulated by kinases (Provencio et al., 1998). The long form of cOpn4m (cOpn4m-a) has an increased number of serines and threonines in the cytoplasmic tail (36 in total), whereas only 6 are found in the short cOpn4m (cOpn4m-b) (analyzed by NetPhos 2.0: http://www.cbs.dtu.dk/services/NetPhos/). Therefore, as is proposed in cOpn4 (Tomonari et al., 2005), cOpn4m-a and cOpn4m-b may be differentially regulated by phosphorylation in their carboxyl termini.

1.2. Expression pattern of cOpn4m in the developing retina

To determine *cOpn4m*-expressing cells in the developing retina, in situ hybridization (ISH) was performed using chicken retinal sections from E3.5 to post-hatching day 30 (P30). At E3.5, E5, or E7, when the early-born cell types, such as retinal ganglion cells are emerging, *cOpn4m* mRNA was not detected in the developing retina (data not shown). In contrast to *cOpn4*, *cOpn4m* was not expressed in the developing brain or other organ anlagen at these stages (not shown). By E10, when the three-nuclear layered structure has formed in the neural retina, *cOpn4m* became expressed in the retina, by a small subset of retinal cells in the inner nuclear layer (INL) (Fig. 2A). At E12 and E15, *cOpn4m* was expressed by a subset of cells in the INL (Fig. 2B and B′ and not shown). At E17, when the outer segment layer of photoreceptors has developed in the central retina and the three-nuclear layered structure has formed in the peripheral retina, the expression of *cOpn4m* also became observed in the outer half of the INL at a low level (Fig. 2C and C′). Co-labeling of *cOpn4m* with the pan-amacrine cell marker Pax6 demonstrated that *cOpn4m*-positive cells were Pax6-positive (Fig. 2E–G), implying their amacrine identity (Feng et al., 2006). On the other hand, none of the *cOpn4m*-positive amacrine cells expressed Islet1 (Fig. 2H), a marker for cholinergic neurons (Feng et al., 2006).

At P1, *cOpn4m* was expressed in the outer half of the INL, where bipolar and horizontal cells reside (not shown). In the P13 and P30 retinae, *cOpn4m* continued to be expressed in the outer half of the INL (Fig. 2D and D′ and not shown). The expression pattern of *cOpn4m* in the 2-week-old chicken retina was documented previously (Bellingham et al., 2006), and our findings are consistent with the report of its strongest signal in the inner nuclear layer.

These results indicate that *cOpn4m*-expressing cells in the developing retina are partly overlapping with those of *cOpn4*, but that the major cell types are different between these two *melanopsin*-related genes: *cOpn4m* is expressed by a subset of amacrine cells, while *cOpn4* is expressed by a subset of ganglion and horizontal cells (Tomonari et al., 2005). In contrast to *cOpn4, cOpn4m* is not expressed in the developing brain and other tissues.