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Whole Structure of the Photoreceptors in the Ascidian Larva Visualized by an Antibody Against Arrestin (Ci-Arr)

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The anterior brain vesicle of ascidian larvae contains two distinct pigment cells. Ultrastructure of these pigment cells has been shown that the anterior pigment cell is an otolith for perception of gravity and the posterior pigment cell is an ocellus for light reception. The larva has remarkably simple central nervous system (CNS) composed of about 330 cells. We focused to study neural networks of visual systems. In the present paper, we report the whole structure of the photoreceptors of the ascidian larva visualized by an antibody against arrestin.

Visual arrestin is the key protein for the termination of phototransduction and one of the abundant proteins in photoreceptors. Recently, we cloned an arrestin homologue gene, *Ci-arr* and the expression of *Ci-arr* was found to be restricted to the photoreceptors in the ocellus. To study the whole structure of the photoreceptors in the larva, we prepared an antibody against Ci-Arr. It is found that anti Ci-Arr antibody specifically stains the photoreceptors, including the cell bodies, the axons, and the nerve terminals. The photoreceptor cell bodies lies in row outside the pigment cup which penetrate the pigment cell and is continuous with the outer segments of the photoreceptor cell, inside the concavity of the pigments. The axons form bundle into a single tract. The tract extends toward the midline, where the nerve terminals diverge and seem to form synapses

Key words: ascidian, arrestin, immunohistochemistry, photoreceptor, opsin, morphology

INTRODUCTION

Ascidians (sea squirts) belong to the phylum Chordate and are though to be a prototype of the vertebrate group. Unlike the sessile adult, the larva is tadpole shaped and possesses some typical features of vertebrates, such as a dorsal tubular central nervous system (CNS) and a notochord underlying the caudal neural tube [1,2]. Since the CNS is composed of only about 330 cells, it is regarded as a simple model for understanding CNS of the vertebrate [2,3].

The brain vesicle of ascidian larvae contains a single large vesicle in which lie two distinct pigment cells, anterior and posterior pigment cells. These pigment cells have been shown that anterior pigment cell is an otolith for perception of gravity and posterior pigment cell is an ocellus for light reception. Ultrastructure of these pigment cells has been described by Dilly [4,5] Eakin et al [6] Torrence [7] and Ohtsuki [8,9]. Ocellus is composed of three parts: a V-shaped single pigment cup; three lens cells aligned partly within the pigment cup; and more than ten photoreceptor cells. The photoreceptors are cilia-type like those of vertebrates and the outer segments protrude through the pigment cup into the concavity. However, the whole structure of the photoreceptors, especially the axons and nerve terminal, remains to be elucidated. Furthermore, the cell number of photoreceptors is still controversial. Specific marker for the photoreceptor of the larvae may enable us to make clear the structure of photoreceptor cells.

Recently, we cloned two photoreceptor specific genes, *Ci-opsin1* [10] and *Ci-arr* (Nakagawa et al., submitted) from the *Ciona intestinalis* larva cDNA. The deduced amino acid sequences of *Ci-opsin1* and *Ci-arr* were closely related to the vertebrate type. *In situ* hybridization shows that both genes were expressed in the photoreceptors of the ocellus. It is expected that opsin is restrictively localized in the outer segment of the photoreceptors, whereas arrestin distributes throughout the photoreceptors. Moreover, the amount of the expression of *Ci-arr* in photoreceptor is more than that of

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Ci-opsin1. Thus, we prepared an antibody against Ci-Arr to examine the whole structure of the photoreceptors in the larva.

MATERIALS AND METHODS

Biological materials. Mature adults of the ascidian *Ciona intestinalis* were collected in Murotsu, Hyogo, Japan. The embryos were prepared using gametes obtained from the gonoducts, as described previously [11]. Given period after hatching, the larvae were collected and fixed with 10 % formalin in artificial sea water at 4℃ for 3 hours.

Production of Antibody. A C-terminal peptide of Ci-Arr was over expressed in *E. coli* as a fusion protein with 6xHis-tagged dihydrofolate reductase protein employing a QIAexpression system, and purified according to the recommended protocol. Three mice were immunized every 2 weeks.

Whole-mount Immunostainning. The fixed samples were washed with phosphate-buffered saline (PBS) containing 0.1 % TritonX-100 (T-PBS) several times. They were incubated with blocking solution (10% goat serum in T-PBS) at 4℃ for 3 hours, and then incubated with the anti Ci-Arr antibody diluted 1:1000 in the blocking solution at 4℃ overnight. After washed with T-PBS at 4℃ for 8 hours, they were incubated with Alexa 488-conjugated anti-mouse IgG goat antibody diluted 1:1000 in the blocking solution at 4℃ overnight. After rinsing several times, the specimens were mounted in PBS containing 50% glycerol and observed under a fluorescence microscope (Zeiss, Axioplan2).

RESULTS AND DISCUSSIN

Figure 1 shows immunofluorescence images of *Ciona intestinalis* larva stained with the anti Ci-Arr antibody. Immunopositive cells were located around the posterior pigment, ocellus (Fig. 1A). A higher magnified image revealed the detailed structure of the photoreceptor including cell bodies, axons, and nerve terminals (Fig.1 B, C). The photoreceptor cell bodies lies in row outside the pigment cup of the ocellus. Anti Ci-Arr antibody stains these cells that penetrate the pigment cell and is continuous with the outer segments of the photoreceptor cells, inside the concavity of the pigments (Horie et al., unpublished). These results were supported by the study using an antibody against Ci-opsin1 that solely stained outer segment in inside the concavity of the pigments (Horie et al., unpublished). The axons from the cell bodies are bundled in the posterior side to form a single axon tract. The tract extends toward the medial side and reach to the internal midline, where the nerve terminals diverge and expected to form synapses. It is well established that the foot processes of vertebrate photoreceptors synapse with adjacent neural elements of the retina [12]. However, electron microscopy observation failed to observe synaptic structure in the basal regions of the photoreceptor cells of ascidian larvae [5,13]. Present results clearly shows the terminal structure of photoreceptor as shown in Figure 1. These results may give an insight into the synaptic structure between the photoreceptor cells and ganglion cells of ascidian. Since there are no layer structure composed of several type visual neurons, which are commonly seen in the vertebrate retinae and the photoreceptors axons directly project to the internal CNS, the ocellus of the ascidian larva is rather similar to the pineal organ of the vertebrate.

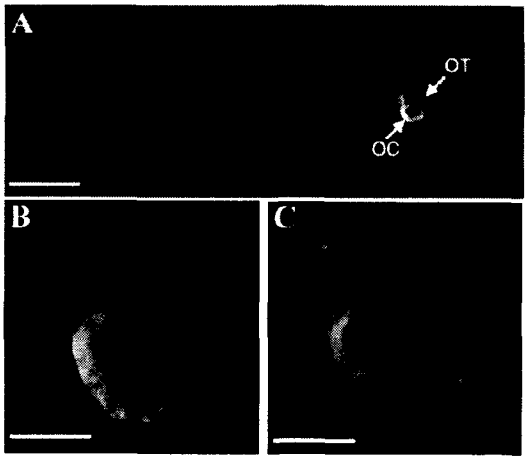

Figure 1. Immunofluorescence images of *Ciona intestinalis* larva using anti Ci-Arr antibody. (A) whole image of the larva in right-dorsal view. (B), (C) higher magnification of photoreceptors. (B) and (C) are focused on the right lateral side and medial side, respectively. OT: otolith, OC: ocellus. Scale bar: (A) 100 μm; (B), (C) 20 μm.

Since the anti Ci-Arr antibody stained not only cell bodies, but also axons, and nerve terminals of the photoreceptors. The antibody is quite useful tool for following the morphological changes of the photoreceptors during development.

Ascidian tadpole larvae change swimming behavior during the course of development. Newly hatched larvae show no response to light stimuli. They were induced to swim more rapidly by a sudden decrease in light intensity 4 h after hatching [11,14]. The maximal speed of swimming behavior increased with time until 8h after hatching and then plateaued. It is expected that ontogenetic changes in these photobehavior of ascidian larvae could relate to the morphological change of neural network from photoreceptor to motor neurons.

Compared with the structure of nerve terminal of photoreceptor 4 h after hatching shown in Figure 1, the nerve terminals 1 h after hatching are immature (Horie et al., unpublished). Like other retinal cell bodies in vertebrates, it is expected that synapses connect the photoreceptor with the rest of the nervous system may not form synapse with ganglion cells. Immature nerve terminals of photoreceptor 1 h after hatching may not formed synapses, which may be one of the reasons why newly hatched larvae show no response to light stimuli. More details of ontogenetic changes in morphology of the photoreceptors are under investigation.

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