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Photoreceptors of cubozoan jellyfish

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Abstract

The anatomically sophisticated visual system of the cubozoan jellyfish *Carybdea marsupialis* is described. Individual cubomedusae have eight complex eyes, each with a cornea, lens, and retina of ciliated photoreceptor cells, eight slit ocelli, and eight dimple ocelli. The photoreceptor cells of the complex eyes are bipolar and resemble vertebrate rod cells. Each photoreceptor has an outer cylindrical light-receptive segment that projects into a vitreous space that separates the lens and the retina, an inner segment rich in pigment granules, and a basal region housing the nucleus. The outer segment is a modified cilium with a 9 + 2 arrangement of microtubules plus stacks of membrane. These stacks of membrane form numerous discs that are oriented transversely to the long axis of the cell. The outer segment is connected to the inner segment by a slender stalk. The basal end of each photoreceptor forms an axon that projects into an underlying layer of interneurons. Each ocellus is composed of ciliated photoreceptor cells containing pigment granules. Rhodopsin-like and opsin-like proteins are found in the membrane stacks of the outer segments of the photoreceptors of the complex eyes. An ultraviolet-sensing opsin-like protein is present in the inner segments and basal regions of some of the photoreceptors of the complex eyes. Rhodopsin-like proteins are also detected in the photoreceptors of the slit ocelli. The cellular lens, composed of crystallin proteins, shows a paucity of organelles and a high concentration of homogeneous cytoplasm. Neurons expressing RFamide (Arg-Phe-amide) comprise a subset of interneurons found beneath the retinas of the complex eyes. RFamide-positive fibers extend from these neurons into the stalks of the rhopalia, eventually entering into the subumbrellar nerve ring. Vision may play a role in the navigation, feeding, and reproduction of the cubomedusae.

Introduction

Cnidarians have multicellular light detecting organs called ocelli (eyes). These photoreceptive organs include simple eyespots, pigment cups, complex pigment cups with lenses, and camera-type eyes with a cornea, lens, and retina (for a review, see Martin, 2002). Ocelli contain sensory photoreceptor cells interspersed among nonsensory pigment cells. Photoreceptor cells are bipolar, the apical end forming a light receptor process and the basal end forming an axon. A cilium with a 9 + 2 pattern of microtubules projects from the receptor-cell process. The membrane covering the cilium may show several variations, including evaginating microvilli. Pigment cells are rich in pigment granules, and in some animals the distal regions of these cells form tubular processes that project into the cavity of the ocellus. Microvilli may extend laterally from these tubular processes and interdigitate with the microvilli from the ciliary membranes of photoreceptor cells.

As cnidarians exhibit a diversity of eye designs, the evolution of these ocelli is fascinating (Singla, 1974; Eakin, 1982; Westfall, 1982). Photosensitive cells underwent morphological changes to produce

eye cups, eye cups with lenses, and complex eyes with a cornea, lens, and retina. These changes in eye pattern illustrate improvements of design wherein there is an increase in detectable spatial information (Gregory, 1967; Nilsson, 1989, 1990; Osorio, 1994). The ocelli of cubomedusae represent the most highly evolved eyes in the Cnidaria. In these ocelli, the opening of the eye cup constricted and a spherical, graded-index lens formed in the center of curvature of the retina, constructing a camera-type eye. Similar camera-type eyes, as seen in fish and cephalopods, achieve virtually aberration-free imaging over a full 180° visual field (Land & Fernald, 1992). The intricate visual system of a cubomedusa is reviewed in this paper, documenting that these animals do contain the optical-eye tools (opsins, crystallin proteins) required for photoreception and imaging.

Materials and methods

Jellyfish of *Carybdea marsupialis* Linnaeus were collected in the early afternoon in October 1998–1999 from shallow waters around Santa Barbara, California. Specimens were placed in one gallon ziplock plastic bags filled with seawater and transported to the lab. Medusae were placed in large finger bowls of seawater and observed and photographed using a Wild dissecting microscope. The stalks of the four eye-bearing structures, called rhopalia, were severed with a razor blade, thus freeing the rhopalia from the jellyfish bell. Larger pieces of the cubomedusae that included the rhopalia, their stalks, and surrounding bell tissue were dissected using fine scissors.

For immunocytochemistry, whole rhopalia and rhopalia with their stalks and pieces of surrounding bell tissue were fixed overnight at 4 °C in 4% paraformaldehyde in 0.2 M Millonig's buffer, pH 7.2, then washed 3× for 20 min each in 0.2 M Millonig's buffer, followed by 1 h in 0.4 M glycine, pH 7.2. Samples were again washed 3×, 20 min each, in 0.25% Triton X-100 in 0.1 M Millonig's buffer, and incubated overnight at 4 °C in a primary antibody diluted in 0.1 M Millonig's buffer containing 0.25% Triton X-100 plus 0.25% human serum albumin. Primary antibodies included affinity purified rabbit antisera raised against either blue-, green-, red-, or ultraviolet-sensing opsin proteins of zebrafish, rho-c (a rabbit polyclonal to the carboxyl terminus of zebrafish rhodopsin), rhodopsin 1 and rhodopsin 4 (rabbit polyclonals to *Drosophila* rhodopsins), Mab24B10 (a mouse monoclonal that recognizes the protein chaoptin in the membranes of the photoreceptors [larval and adult] of *Drosophila*), and RFamide antiserum 146 III (a rabbit polyclonal). Samples were washed 3× for 20 min each in 0.1 M Millonig's buffer containing 0.25% Triton X-100, followed by an incubation (1 h) at room temperature in either anti-rabbit FITC-IgG diluted 1:20 or anti-mouse FITC-Igs diluted 1:60 in 0.1 M Millonig's buffer containing 0.25% Triton X-100 and 0.25% human serum albumin. Specimens were rinsed 3× for 20 min each in 0.1 M Millonig's buffer and mounted on microscope slides using 50% glycerin in 0.1 M Millonig's buffer containing 5% n-propyl gallate. Whole mounts were examined and serially reconstructed with a BioRad MRC 1024 laser scanning confocal microscope.

For controls, rhopalia were also processed as described above except that the primary antibodies were eliminated. No staining was observed in these samples.

Results

General observations

Swarming cubomedusae of *Carybdea marsupialis*, a box jellyfish, appear seasonally during late summer and fall into January along the California coastline near Santa Barbara. This jellyfish has a clear bell, except for some small brownish flecks, giving it a slightly peppered appearance. Bell size ranges from 20 to 40 mm in height, with extended tentacles close to 1500 mm long. *Carybdea* is found in the shallow waters in the near shore part of the kelp beds known as sand channels. They are very patchy, however; when found, they are usually in densities of 1–2 over a 9 m^2. Densities of 30–50 m^{-3} have been reported in recent years. The jellyfish seem to stay in the sunny areas between the kelp beds over the clean sand. When they venture into the shadows of the kelp they reverse course and head back to the sunny areas. Shading them with a hand also results in their changing direction. They are positively phototactic and are