REVIEW / SYNTHÈSE

Photoreceptors of cnidarians[1]

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Abstract: Cnidarians are the most primitive present-day invertebrates to have multicellular light-detecting organs, called ocelli (eyes). These photodetectors include simple eyespots, pigment cups, complex pigment cups with lenses, and camera-type eyes with a cornea, lens, and retina. Ocelli are composed of sensory photoreceptor cells interspersed among nonsensory pigment cells. The photoreceptor cells are bipolar, the apical end forming a light-receptor process and the basal end forming an axon. These axons synapse with second-order neurons that may form ocular nerves. A cilium with a 9 + 2 arrangement of microtubules projects from the receptor-cell process. Depending on the species, the membrane covering the cilium shows several variations, including evaginating microvilli. In the cubomedusae stacks of membranes fill the apical regions of the photoreceptor cells. 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. Photoreceptor cells respond to changes in light intensity with graded potentials that are directly proportional to the range of the changes in light intensity. In the Hydrozoa these cells may be electrically coupled to each other through gap junctions. Light affects the behavioral activities of cnidarians, including diel vertical migration, responses to rapid changes in light intensity, and reproduction. Medusae with the most highly modified photoreceptors demonstrate the most complex photic behaviors. The sophisticated visual system of the cubomedusan jellyfish *Carybdea marsupialis* is described. Extraocular photosensitivity is widespread throughout the cnidarians, with neurons, epithelial cells, and muscle cells mediating light detection. Rhodopsin-like and opsin-like proteins are present in the photoreceptor cells of the complex eyes of some cubomedusae and in some neurons of hydras. Neurons expressing glutamate, serotonin, γ-aminobutyric acid, and RFamide (Arg-Phe-amide) are found in close proximity to the complex eyes of cubomedusae; these neurotransmitters may function in the photic system of the jellyfish. *Pax* genes are expressed in cnidarians; these genes may control many developmental pathways, including eye development. The photobiology of cnidarians is similar in many ways to that of higher multicellular animals.

Résumé : Les cnidaires sont les invertébrés les plus primitifs à posséder des organes pluricellulaires à fonction photoréceptrice appelés ocelles (yeux). Ces photorécepteurs sont de simples taches oculaires ou des coupes de pigments, ou encore des coupes complexes de pigments avec un cristallin, ou alors ce sont des yeux semblables à des appareils-photo avec cornée, cristallin et rétine. Les ocelles sont composés de cellules sensorielles photoréceptrices dispersées parmi des cellules pigmentaires non sensorielles. Les cellules photoréceptrices sont bipolaires; leur extrémité apicale émet un processus photorécepteur et leur extrémité basale forme un axone. Les axones font des synapses avec des neurones de second ordre, formant parfois des nerfs oculaires. Un cil avec des microtubules disposés selon un arrangement 9 + 2 prend son origine sur le processus des cellules réceptrices. La membrane qui recouvre le cil est très variable selon l'espèce et porte parfois des microvillosités évaginées. Chez les cuboméduses, des empilements de membranes remplissent les régions apicales des cellules photoréceptrices. Les cellules pigmentaires sont riches en granules de pigments et, chez certaines espèces, la région distale de ces cellules émet des processus tubulaires dans la cavité de l'ocelle. Il arrive que des microvillosités issues de ces processus tubulaires s'étendent latéralement et s'entrecroisent avec les microvillosités issues des membranes ciliaires des cellules photoréceptrices. Les cellules photoréceptrices réagissent aux changements d'intensité lumineuse par des potentiels gradués directement proportionnels à l'étendue de ces changements. Chez les hydrozoaires, ces cellules peuvent s'unir pour former des couples électriques au niveau des jonctions intercellulaires de type adherens (gap junctions). La lumière a des effets sur les activités comportementales des cnidaires, notamment les migrations verticales, les réactions aux changements brusques de l'intensité lumineuse et la reproduction. Les méduses qui ont les photorécepteurs les plus modifiés ont aussi les comportements photiques les plus complexes. Le système visuel sophistiqué de la cuboméduse *Carybdea marsupialis* est décrit. La photosensibilité

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[1]This review is one of a series dealing with aspects of the biology of the phylum Cnidaria. This series is one of several virtual symposia on the biology of neglected groups that will be published in the Journal from time to time.

extraoculaire est une propriété répandue chez les cnidaires et ce sont des neurones, des cellules épithéliales et des cellules musculaires qui assurent la détection. On trouve des protéines de types rhodopsine et opsine dans les cellules photoréceptrices des yeux complexes de certaines carboméduses et dans certains neurones des hydres. On trouve aussi des neurones contenant du glutamate, de la sérotonine, le GABA et le RFamide (Arg-Phe-amide) dans le voisinage immédiat des yeux complexes des cuboméduses; ces neurotransmetteurs peuvent être fonctionnels dans le système photique des méduses. Des gènes *Pax* sont exprimés chez les cnidaires; on croit qu'ils peuvent être responsables du contrôle de plusieurs voies de développement, dont celle de l'oeil. La photobiologie des cnidaires se rapproche de celle des animaux pluricellulaires supérieurs par plusieurs aspects.

[Traduit par la Rédaction]

Introduction

Sensitivity to light is widespread in living organisms, as light influences movement, photosynthesis, vision, and behavior. Organisms have evolved photosensory and effector mechanisms for detecting light that range from simple to complex. In unicellular organisms such as *Euglena* sp., the photoreceptor–effector system is part of a single cell, whereas in multicellular organisms, specialized cells contain the photosensory components (Land 1990; Wolken 1995). These photosensitive cells may be arranged to form distinct, complex structures called ocelli, or eyes, or they may be contained in extraocular structures. Many invertebrates and some vertebrates have both ocular and extraocular photoreceptors (Land 1990; Wolken 1995).

Members of the phylum Cnidaria include the familiar hydras, jellyfish, sea anemones, and corals, as well as the less familiar hydroids, sea fans, siphonophores, and zoanthids. Some jellyfish and one identified polyp have multicellular light-detecting organs, the ocelli. These animals are the most primitive present-day invertebrates to have ocelli. These photodetectors range from simple eyespots and eyecups to complex eyes with a lens. The photoreceptor cells of cnidarians resemble those of vertebrates in that they are of the ciliary type, unlike those of most other invertebrates, which are rhabdomeric (Eakin and Westfall 1962; Eakin 1982). Thus, cnidarian photoreceptor cells may represent a very early stage of evolution leading to the vertebrate eye (Eakin and Westfall 1962; Eakin 1979). Cnidarians also show extraocular photosensitivity (Yoshida 1979; Wolken 1995; Musio 1997; Taddei-Ferretti and Musio 2000). Hence, the cnidarians are a good group in which to examine the first photoreceptors and photosensory mechanisms.

In this paper I examine several parameters of photobiology in the Cnidaria, focusing on (*i*) the structure and function of the different types of ocelli, (*ii*) photic behaviors, (*iii*) extraocular photosensitivity, (*iv*) photopigments, (*v*) neurotransmitters that are possibly involved in photoreception, (*vi*) the *Pax* genes, developmental control genes that play a role in invertebrate/ vertebrate eye formation, and (*vii*) how the photobiology of cnidarians compares with that of higher metazoans.

Structure and function of ocelli

The photoreceptive organs of cnidarians (ocelli) include simple eyespots, pigment cups, complex pigment cups with a lens, and camera-type eyes with a cornea, lens, and retina (for Hydrozoa see Eakin and Westfall 1962; Yamasu and Yoshida 1973; Bouillon and Nielsen 1974; Singla 1974; Yamamoto and Yoshida 1980; Weber 1981*a*, 1981*b*; Singla

and Weber 1982*a*, 1982*b*; Thomas and Edwards 1991; for Scyphozoa and Cubozoa see Berger 1898, 1900; Bouillon and Nielsen 1974; Yamasu and Yoshida 1976; Pearse and Pearse 1978; Piatigorsky et al. 1989; Lesh-Laurie and Suchy 1991; Hamner 1994; Matsumoto 1995; Mackie 1999; Martin and Givens 1999; Martin 2000) (Fig. 1). A simple eyespot is an epithelial patch of sensory photoreceptor cells interspersed with nonsensory pigment cells (Fig. 1B), whereas in a pigment-cup ocellus, the pigment cells produce a cup into which the photoreceptor cells project (Figs. 1C, 1D, and 2). In some cases the cup is closed by a layer of epidermal cells. If the photoreceptor cells extend between the pigment cells into the lumen of the cup, the ocellus is termed everted (Figs. 1C and 1D). If the pigment cup is found below the epidermis and the photoreceptor cells project into the opening of the cup but not between the pigment cells, the ocellus is termed inverted (Fig. 2). In an inverted ocellus the receptive portions of the photoreceptor cells reach the pigment layer via the cup opening and their axons emerge via the cup opening.

In hydromedusae the ocelli appear as red, brown, or black spots, usually one on each tentacular bulb (the swollen base of a tentacle where the tentacle joins the bell) (Fig. 3). Some hydromedusae also have ocelli on their bell margins, and the number of ocelli may approach several hundred. Various grades of ocellular organization are found in hydromedusae. Simple eyespot ocelli, flush with the animal surface, are demonstrated in *Turris* species and *Leuckartiara octona* (Hyman 1940; Singla 1974) (Fig. 1B). Pigment-cup ocelli are found in *Polyorchis penicillatus*, *Spirocodon saltatrix*, and *Bougainvillea principis* (Figs. 1C, 1D, and 3) (Eakin and Westfall 1962; Singla 1974; Toh et al. 1979; Lin et al. 2001) and pigment-cup ocelli with a lens are observed in *Cladonema radiatum* and *Sarsia tubulosa* (Hyman 1940; Weber 1981*a*, 1981*b*; Singla and Weber 1982*a*). The most complex hydromedusan ocellus is an inverted type found in *Tiaropsis multicirrata* (Hyman 1940; Singla 1974). This ocellus has a gastrodermal pigment cup separated from the photoreceptor cells by a layer of mesoglea. The tips of the photoreceptors project into the lumen of the cup, while their basal ends pass out of the opening of the cup (Fig. 2).

Scyphomedusae and cubomedusae have club-shaped sensory structures called rhopalia located around the bell margin, on pedalia, or on the sides of the bell (cubomedusae) (Hyman 1940). The rhopalia of some scyphomedusae and cubomedusae bear ocelli that may be pigment spots (eyespots) (*Aurelia aurita*), pigment cups (*A. aurita* (inverted), *Cassiopeia* species), or complex eyes with a lens (*Carybdea marsupialis*, *Carybdea rastonni*, *Carybdea sivickisi*, *Tamoya bursaria*, *Tripedalia cystophora*) (Fig. 1E) (Berger 1898, 1900; Hyman

Fig. 1. Evolution of photosensitive ocelli in the Cnidaria, ranging from simple ciliated photoreceptors to complex eyes with ciliated photosensitive cells forming a retina. The bottom row of diagrams shows the basic designs of the various types of ocelli and the top row is a visualization of the structures of the photoreceptor cells and pigment cells in the ocelli. A–D are modified from Singla (1974). (A) Hypothetical indistinct ocellus composed of simple ciliated sensory cells and supporting cells. (B) Primitive eyespot ocellus as found in *Leuckartiara octona*. Ciliated photoreceptor cells (*r*) and pigment cells (*p*) intermingle to constitute the simple eyespot. Microvilli (*m*) emerge from the surfaces of both types of cells (*c*, cilium). (C) Cup-shaped ocellus as found in *Polyorchis penicillatus*. The photosensitive epithelium of photoreceptor cells (*r*) and pigment cells (*p*) has invaginated to form a cup-shaped ocellus. Microvilli (*m*) emerge from the membranes of the cilia (*c*) of the photoreceptor cells and from projections of the pigment cells. The villous processes of these cells interdigitate to fill the lumen of the ocellus. (D) Cup-shaped ocellus as found in *Bougainvillia principis*. The microvillous processes (*m*) of the photoreceptor cells (*r*) and the pigment cells (*p*) have spatially differentiated from each other so that they no longer interdigitate (*c*, cilium). (E) Complex ocellus with a cornea, lens, and retina as found in cubomedusae such as *Carybdea marsupialis*. Ciliated photoreceptor cells (*r*) compose the retina of the eye. The apical ends of these cells are rich in vesicles and stacks of membranes, while the basal ends form axons that feed into a plexus of neurons (*n*).
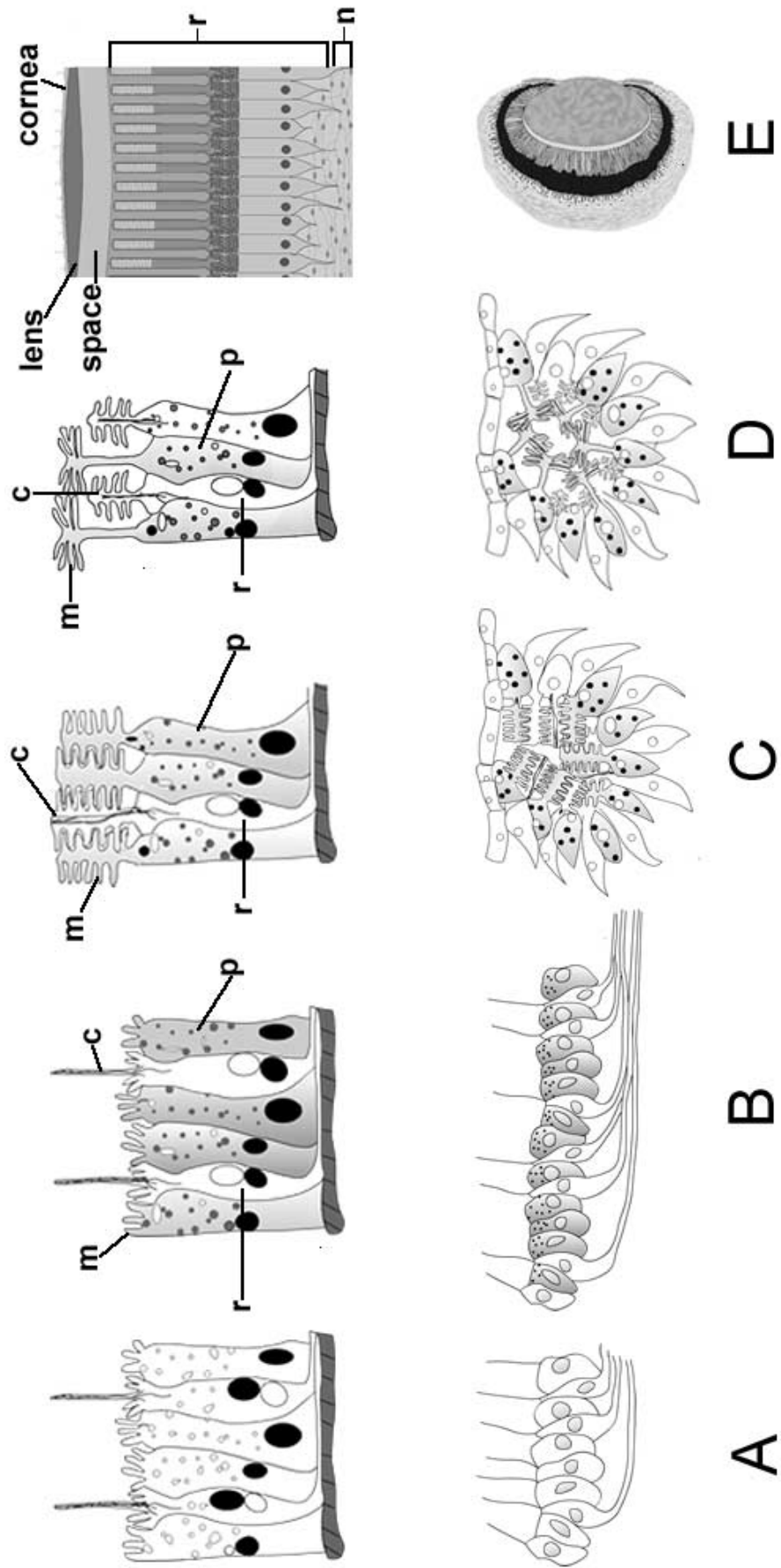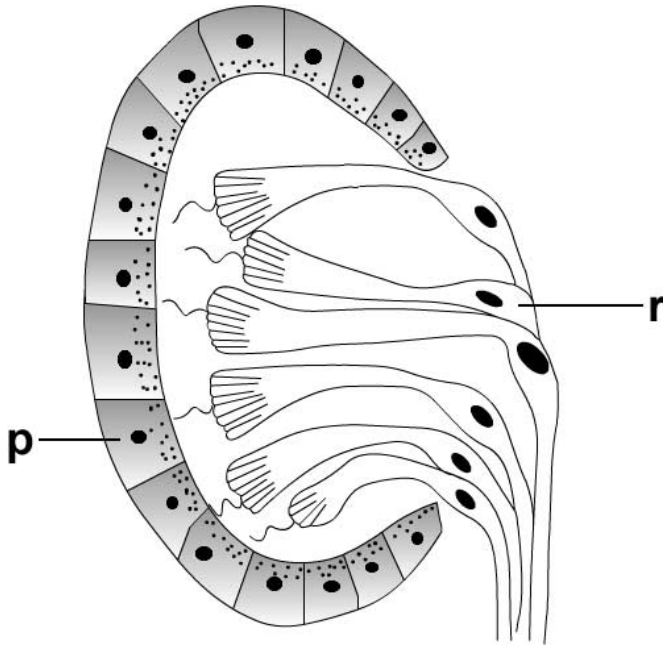
Fig. 2. Inverted pigment-cup ocellus as found in *Tiaropsis multicirrata* and *Aurelia* species. Pigment cells (*p*) form a pigment cup into which project the ciliated tips of the photoreceptor cells (*r*). The basal ends of these photoreceptor cells pass out of the opening of the cup.

Fig. 3. Overview of the hydromedusa *Polyorchis penicillatus*, showing the ocelli. Red dots, which are cup-shaped ocelli, rim the margin of the bell where the tentacles emerge. These ocelli contain photosensory cells and pigment cells. Photograph courtesy of Yi-Chan James Lin.
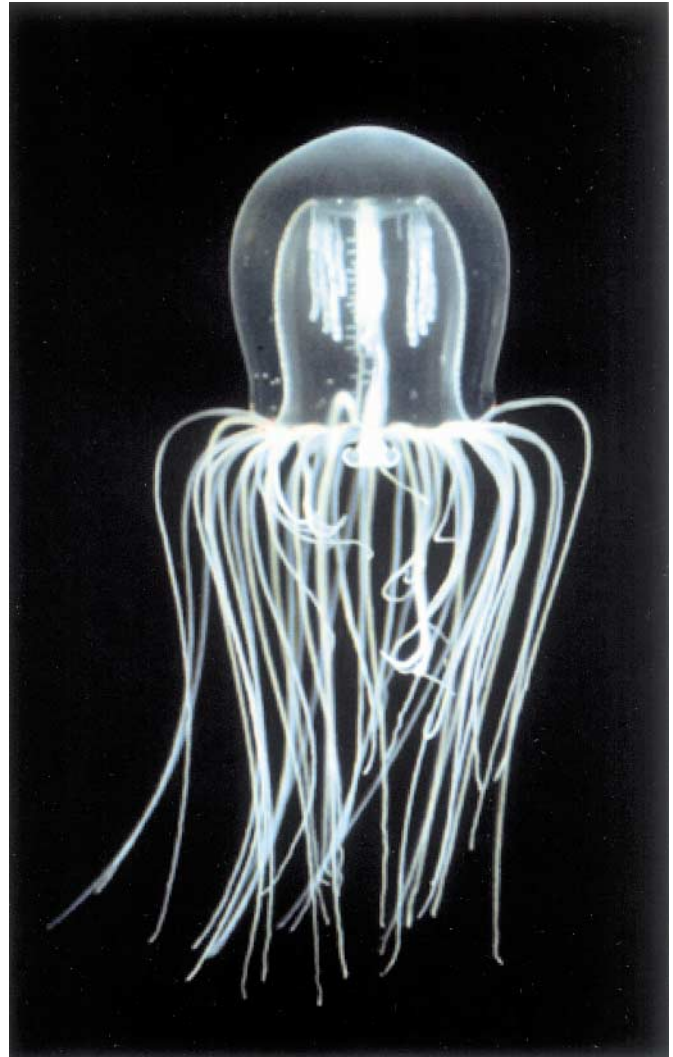

1940; Bouillon and Nielsen 1974; Yamasu and Yoshida 1976; Hamner 1994; Matsumoto 1995; Stewart 1996; Martin and Givens 1999). The structure of the amazing complex eye of the cubomedusa *C. marsupialis* is described in detail below.

Within the Cnidaria, the polyps of hydrozoans, scyphozoans, cubozoans, and anthozoans react to light stimuli and show light-sensitive behaviors; however, the receptors responsible for these responses have not been identified (Mori 1960; Tardent and Frei 1969; Frei 1973; Imafuku 1973; Mori and Tanase 1973; Gladfelter 1975; Tardent and Weber 1976; Tardent et al. 1976; Dickinson 1978; Lasker 1979; Shick 1991; Sawyer et al. 1994; Taddei-Ferretti and Musio 2000; Gorbunov and Falkowski 2002). To date, the only polyp stage known to possess distinct ocelli is the scyphozoan *Stylocoronella riedli* (Blumer et al. 1995). This interstitial-living polyp has up to 24 pigment-spot ocelli, located at the base of the tentacles, composed of monociliated sensory cells and pigment cells. The cilia associated with the sensory cells show a strange axonemal pattern, with a third central microtubule in one region and disorganized microtubules in the distal regions.

Cnidarian ocelli are composed of sensory photoreceptor cells interspersed among nonsensory pigment cells. The photoreceptor cell is a bipolar cell with its apical end forming a light-receptor process and the basal end forming an axon (Figs. 1, 4, and 6). These cells vary in shape and size depending on the species. For example, *P. penicillatus* has spindle-shaped receptor cells approximately 15 μm long (Fig. 4), *B. principis* has variably shaped cells 8–10 μm long, and *C. marsupialis* has slender cells 50–60 μm long. A cilium with a 9 + 2 arrangement of microtubules projects from the receptor-cell process (Figs. 4C and 6B). These cilia often

have long striated rootlets that may reach the level of the nucleus. It is doubtful that the cilia are motile (Eakin and Westfall 1962). Depending on the species, the membrane covering the cilium may show several variations (Fig. 1). Such adaptations of the ciliary membrane form the putative photoreceptive organelle specialized for photon detection. In the simple eyespots of *L. octona* the receptor cilia do not exhibit membrane evaginations (Fig. 1B), whereas in the ocelli of *S. tubulosa*, *S. saltatrix*, *P. penicillatus* (Fig. 1C), and *B. principis* (Fig. 1D) the cilia form several microvillous processes that extend into and fill the ocellar cup. In the most organized type of ocellus, the eye of cubomedusae, photoreceptor cells displaying ciliary sheaths with balloonlike swellings and emerging microvilli have been observed (*T. bursaria*; Yamasu and Yoshida 1976). At some locations these microvilli show a packed, parallel alignment. Also, in *T. bursaria* the ciliary microtubules are arranged in a 9 + 2 pattern in the proximal part of the ciliary sheath but are dis-

Fig. 4. Ocelli of *P. penicillatus* stained with the monoclonal antibody 5C6. Scale bars are in micrometres. (From Lin et al. 2001, reproduced with the permission of Invertebrate Neuroscience, Vol. 4, © 2001 Springer-Verlag.) (*a*) Whole mount of the base of a tentacle showing an ocellus (OC) and paired ocellar nerves (arrows) extending back into the outer nerve ring (ONR). (*b*) Immunoreactive photoreceptor cells of the ocellus. These cells are supported by pigment cells, which do not label. (*c*) Labeled photorereceptor cell showing a central cilium (solid arrow) and a surrounding circlet of microvilli (open arrow).
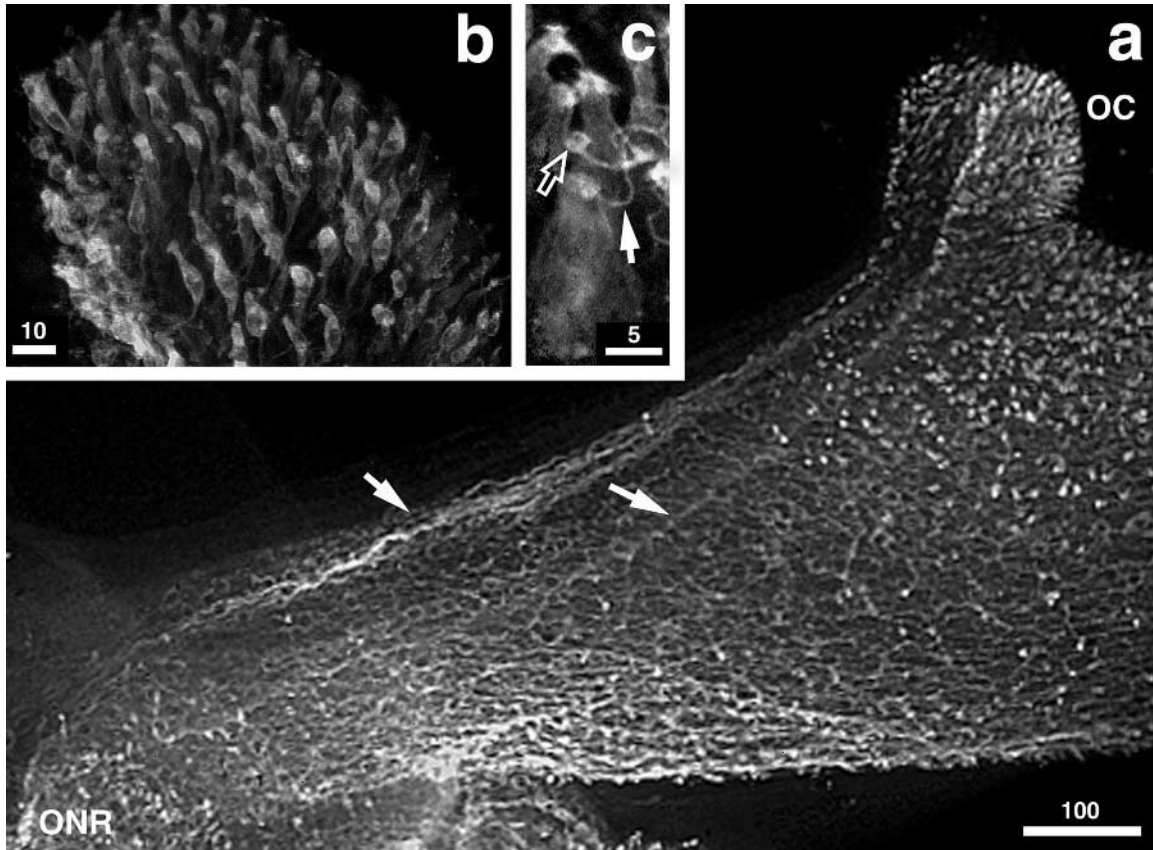

organized in the distal part of the sheath. In the cubomedusa *C. marsupialis* the apical portion of each photoreceptor cell is filled with stacked membranes (Figs. 1E and 6D). The cytoplasm of photoreceptor cells is rich in mitochondria, microtubules, and vesicles. The nuclei of these cells are mid to basally located. Pigment cells may be round, cuboid, or columnar in shape and vary in size depending on the species (Singla 1974; Singla and Weber 1982*a*, 1982*b*). Their cytoplasm is rich in dark-staining pigment granules. The pigment in pigment cells of *C. radiatum* has been identified as melanin (Weber 1981*a*). In addition to pigment bodies, the cytoplasm contains mitochondria, Golgi bodies, and vesicles. In some animals the distal regions of the pigment cells form tubular processes that project into the cavity of the ocellus (Figs. 1C and 1D). Microvilli extend laterally from these tubular processes and interdigitate with the microvilli from the ciliary membranes of photoreceptor cells. Further, in some ocelli the tubular processes from pigment cells pass through the sensory region to form a mass of tubules that fills the distal region of the ocellus (Fig. 1D). In this type of ocellus there is spatial differentiation of the villous processes of the receptor and pigment cells. In the ocelli of the hydromedusa *C. radiatum*, apical extensions of the pigment cells pinch off to form a biconvex lens in the center of the pigment cup (Weber 1981*a*).

Photoreceptor cells have axonal contacts onto second-order neurons (nerve cells located just outside the ocellus) (Toh et al. 1979; Yamamoto and Yoshida 1980; Singla and Weber 1982*a*, 1982*b*). Synaptic vesicles 60–80 nm in diameter have been observed on the receptor cell side only (centripetal synapse), on the second order neuron side only (centrifugal synapse), or on both the receptor-cell and the second order neuron side (two-way synapse) (Yamasu and Yoshida 1973, 1976; Toh et al. 1979; Yamamoto and Yoshida 1980; Singla and Weber 1982*a*, 1982*b*; Westfall 1982). These second-order neurons may synapse with other second-order neurons. The axons of these second-order neurons group together to form an ocular nerve (Fig. 4). In some cases each ocellus forms a pair of ocular nerves, each of which enters independently into the main nerve net of the animal, usually into a nerve ring (Singla and Weber 1982*a*, 1982*b*; Martin 2000; Lin et al. 2001). In some ocelli synapses are found between receptor cell bodies and between receptor cell body and adjacent axons (Singla and Weber 1982*b*).

Because cnidarians display a diversity of eye designs, the evolution of these ocelli is intriguing (Singla 1974; Eakin 1982; Salvini-Plawen 1982; Vanfleteren 1982) (Fig. 1). Through gradual modifications of a patch of photosensitive cells, morphological changes occurred, producing eye cups, eye cups with a lens, and complex eyes with a cornea, lens, and retina. Such changes in eye design represent improvements of design wherein there is an increase in the amount of detectable

Fig. 5. Scanning electron micrograph of the rhopalium of the cubomedusa *C. marsupialis*. The club-shaped rhopalium hangs from the bell by a stalk (S). Each rhopalium has six eyes: a small complex eye (SE) pointed up toward the apex of the bell, a large complex eye (LE) directed in toward the center of the bell, two simple slit ocelli (single arrow), and two simple dimple ocelli (double arrow). Scale bar = 100 μm.
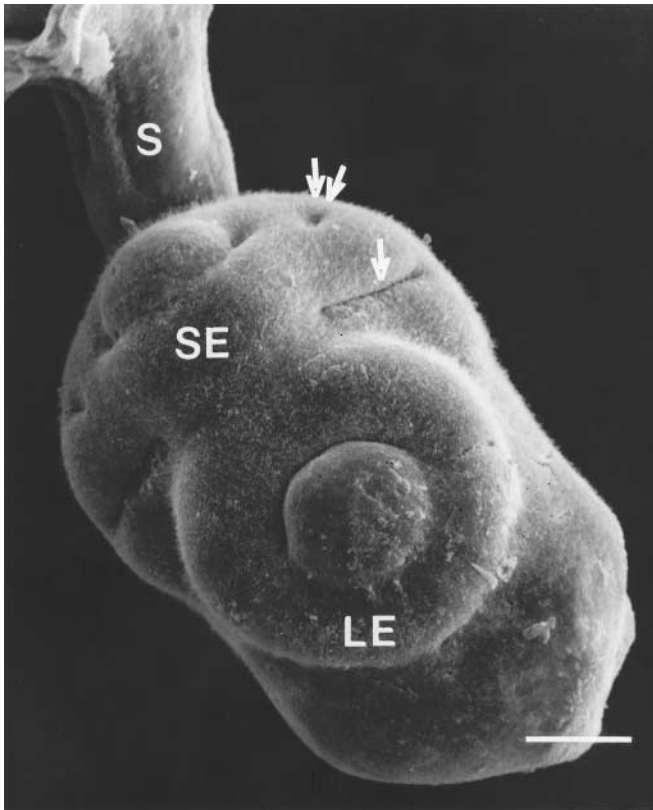
spatial information (Gregory 1967; Nilsson 1989, 1990; Nilsson and Pelger 1994; Osorio 1994). Salvini-Plawen (1982) proposed that a photosensitive monociliated ectodermal cell served as a stem cell from which other photoreceptors are derived. Many cnidarians are sensitive to light, yet they bear no distinct ocelli. Lentz and Barnett (1965) observed ciliated sensory cells in the outer epithelium of hydra and suggested that these cells are photosensitive. The precursor of the photoreceptor cells in cnidarians was probably a photosensitive ciliated ectodermal cell, similar to those described in hydra (Fig. 1A). Such ciliated photosensory cells possess greater information capacity if they are grouped with nonciliated pigment cells to form a primitive distinct ocellus. The ocelli of *L. octona* illustrate this design, as they are composed of a simple patch of ciliated photosensory cells intermingled with pigment cells (Fig. 1B). The photosensory cells expanded their apical, light-receptive surfaces with microvilli, and their basal ends were drawn out to form axons. Such simple eyespots would be useful for informing an animal about the distribution of light and dark in the surroundings. Over time the light-sensitive patch invaginated to form a cup-shaped structure, and the plasma membrane covering the cilium of the photoreceptor cell evaginated to form villous processes, thus increasing the surface area for photon detection. The pigment cells also formed microvillous processes that interdigitated

with the villous processes of the sensory cells, both processes filling the ocellar cup. This design is seen in *P. penicillatus* (Fig. 1C). Through the formation of a pigmented cup, spatial resolution was introduced, as the angle through which the individual photoreceptor cells received light was reduced. Spatial differentiation of the villous processes of the pigment cells and photoreceptor cells occurred, as is seen in *B. principis* (Fig. 1D). In some animals, such as *C. radiatum*, primitive lenses derived from villous extensions of pigment cells formed in the ocellar cups. Finally, the ocelli of cubomedusae represent the most highly evolved eyes in the Cnidaria. In these ocelli, the opening to the eye cup constricted and a spherical, graded-index lens formed in the center of curvature of the retina, producing a camera-type eye (Fig. 1E). Such camera-type eyes in other aquatic animals (fish and cephalopods) can achieve virtually aberration-free imaging over a full 180° visual field (Land and Fernald 1992). The imaging capacity of the cubozoan eye is not known. Recent behavioral studies suggest that these jellyfish do "see" images, and recent molecular analyses indicate that they do have the optical-eye tools (crystallin proteins, opsins) required for imaging (Piatigorsky et al. 1989; Hamner 1994; Hamner et al. 1995; Martin and Givens 1999). What is questionable is whether these animals have the advanced neural processing required for image formation, however crude the images might be (see the section Structure of the complex eye of a cubozoan, *C. marsupialis*).

Physiological studies demonstrate that the photoreceptors of cnidarians respond to changes in light intensity with graded potentials which are directly proportional to the range of changes in light level. Such graded responses provide very precise information about small changes in light intensity. Electrical recordings from ocelli of *S. tubulosa*, *P. penicillatus*, and *S. saltatrix* show graded positive potential changes in response to the onset of varying light intensities (Ohtsu and Yoshida 1973; Weber 1982*b*; Ohtsu 1983*a*, 1983*b*). Arkett and Spencer (1986*a*) proposed that an electrically coupled "O" system (an identifiable neuronal system in the outer nerve ring) is the primary photoreceptor system in *P. penicillatus*. Portions of this system extend as far as each ocellus. Intracellular electrode recordings from this O system and from the ocelli show a graded response to increases and decreases in light level (Arkett and Spencer 1986*b*). The photoreceptors respond to increases in light intensity with graded depolarizations and to decreases in light intensity with graded hyperpolarizations. In other invertebrates the photoreceptors depolarize in response to illumination, while vertebrate photoreceptors hyperpolarize (Wolken 1995).

Morphological and physiological studies show that hydrozoan photoreceptors are electrically coupled to each other through gap junctions (Singla and Weber 1982*b*; Spencer and Arkett 1984). Such electrical coupling of photoreceptors allows for amplification and detection of low-intensity light (Laughlin 1981). Gap junctions between receptor cell bodies have been seen in the ocelli of *S. tubulosa* (Singla and Weber 1982*b*). Further, the O system in *P. penicillatus*, which appears to connect to the ocelli, is electrically coupled (Spencer and Arkett 1984). The electrical coupling properties of this O system and the radially arranged ocelli may be important in the integration of photic information (Arkett and Spencer 1986*b*).

Structure of the complex eye of a cubozoan, *C. marsupialis*

The cubomedusa *C. marsupialis* has a sophisticated visual system (Figs. 1E and 5). This cubozoan has four sensory structures called rhopalia, one on each quadrant of the bell. Each rhopalium is a club-shaped structure suspended by a stalk from the bell. The rhopalia lie in small indentations of the bell tissue; each indentation is partially covered by a flap of tissue, similar to an eyelid. Each rhopalium has six eyes: two complex eyes and four simple eyes. The complex eyes, one small and one large, have a cornea, a lens, and a retina of ciliated photoreceptors (Fig. 1E). The large eye is directed laterally in toward the center of the transparent bell (away from the eyelid), and the small complex eye is directed upward, toward the apex of the bell. The simple eyes (ocelli) are located between the large and small eyes and also face inward. Two slit ocelli are found just above and on either side of the large complex eye, and two invaginated, dimple ocelli are found on either side of the small complex eye. Pigmented photoreceptor cells form these simple ocelli (Martin and Givens 1999). As the stalks of the rhopalia can swing, twist, and turn, the animal has an unobstructed view of its surroundings, with possibly some limitation directly forward along the body axis. The transparency of the bell allows each eye multidirectional vision, while the opening of the indentation may provide a clear field of photoreception in certain directions.

The large eye is 350–400 μm in diameter, with a lens measuring 150 μm in diameter, whereas the small eye is 250–300 μm in diameter, with a lens diameter of 150 μm. The structure of the large complex eye will now be described (Figs. 1E and 6). The cornea covers the lens and is composed of a layer of flattened, squamous epithelial cells bearing numerous microvilli and an occasional short cilium (Figs. 1E and 5). Below the corneal epithelium is a heterogeneous biconvex transparent cellular lens composed of crystallin proteins (Piatigorsky et al. 1989; Martin 2000). The lens cells are round at the center of the lens and flattened at its periphery. Cells located deeper in the lens stain darker than the cells at the periphery, indicating a greater concentration of proteins in the center cells. Electron micrographs show that the cytoplasm of the lens cell is a homogeneous matrix with sparse organelles, so it is similar in morphology to the vertebrate lens cell (Fawcett 1994). A small vitreous space separates the lens and retina (Martin and Givens 1999). A layer of long, slender, closely packed photoreceptor cells, each 50–60 μm in length, composes the retina of the eye. Each photoreceptor cell has an outer cylindrical segment that projects toward the vitreous space. The outer segment is a modified cilium with a 9 + 2 arrangement of microtubules plus stacks of parallel lamellae (Fig. 6). These stacks of membranes form numerous discs oriented transversely to the long axis of the cell, similar to the stacks of membranes found in vertebrate rod cells (Fawcett 1994). These membranes are rich in rhodopsin-like and opsin-like photopigments, visual pigments involved in phototransduction (see the section Photopigments) (Figs. 7 and 8). The outer segment of the photoreceptor cell is connected to an inner segment by a slender stalk. The distal portion of the inner segment is rich in pigment granules, which are elliptical or round (Fig. 6A).

The alignment of these pigment granules in the inner segments of the photoreceptors of the retina produces a dark, dense band that extends across the entire retina, giving the eye a dark color when viewed from the exterior (Fig. 1E). The nuclei of the photoreceptor cells are located in the basal region of the cell. The base of each photoreceptor cell tapers to form an axon that projects into a neural plexus located directly below the retina. The plexus contains bipolar neurons, many of which contact neurons in the stalk of the rhopalium (Fig. 12). Some of these stalk neurons send extensions into the main nerve ring of the bell of the jellyfish (Fig. 13). At least two types of photoreceptor cells are found in the eye: an occasional dark-staining cell intermingled with the more numerous light-staining cells (Fig. 6A). The two types of cells display similar morphologies and both contain pigment granules.

Carybdea marsupialis has advanced camera-type eyes that are optically competent. The lens is a graded-index lens, meaning that the refractive index gradually changes across the lens, forcing light to bend continuously toward the area of higher refractive index (Piatigorsky et al. 1989). The refractive index is very high in the center of the lens and gradually decreases toward the periphery. With a graded-index lens, spherical aberration can be eliminated completely, and the lens has a shorter focal length than a homogeneous lens (Nilsson 1990). Behind the lens is a vitreous space into which the photoreceptors of the retina project their expansive receptive surfaces. These receptive surfaces contain numerous membranes derived from cilia, which are arranged in disc-like structures. The discs are rich in rhodopsin-like and opsin-like proteins. These photoreceptors house pigment granules that move under changing light conditions; such movements may allow the eye to adjust its sensitivity according to changes in ambient light. The photoreceptor cells in the apposition eyes of flies, butterflies, bees, and wasps also contain pigment granules that migrate in response to changes in light intensity (Stavenga and Hardle 1989). These pigment granules screen out light and affect the angular sensitivity and spectral filtering of the eye.

Camera-type eyes are refracting eyes in which a focused image is produced on a retina by refraction of light rays through a lens. In *C. marsupialis* a small vitreous space was observed between the lens and the retina, but nothing comparable to the space found in the camera-type eyes of vertebrates and molluscs. Nilsson (1989) states that many small eyes with a retina behind a lens are enigmatic and do not form images, as the retina is too close to the lens to be in focus. Whether or not the retina of *C. marsupialis* is too close to the lens to form an image is not known, as the refracting power of the lens has not yet been determined. Further, there are animals, such as the "four-eyed fish", *Anableps anableps*, that focus images onto a retina in eyes which have very little space between the lens and the retina (Sivak 1976). If the cubomedusan lens does not resolve an image onto the retina, perhaps it is functioning as a light-collecting instrument regulating the light-sensitivity threshold of the photoreceptors.

Where does visual processing occur in cnidarians? These animals lack brains; their nervous systems consists of nerve nets and nerve rings (Hyman 1940). The nervous system of cubomedusae is complex by cnidarian standards and is composed of a diffuse synaptic nerve net throughout the bell
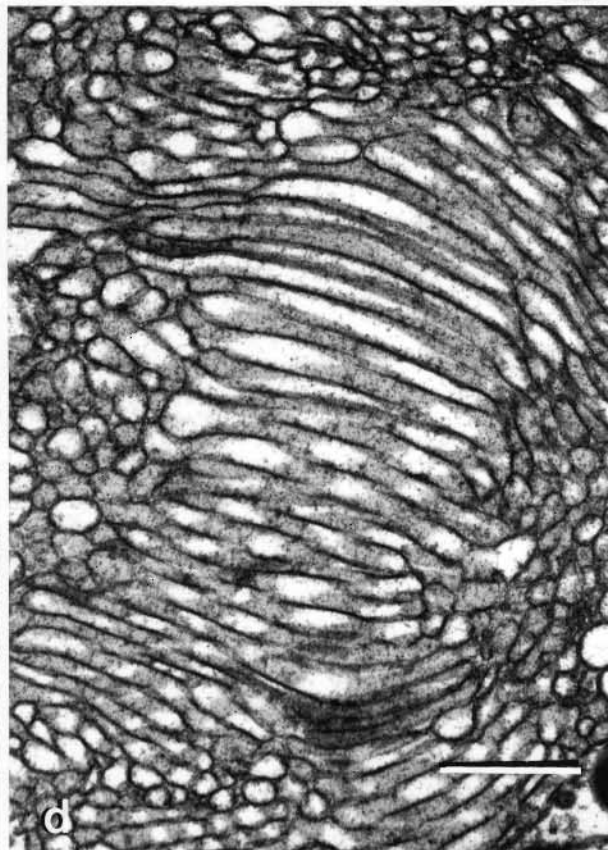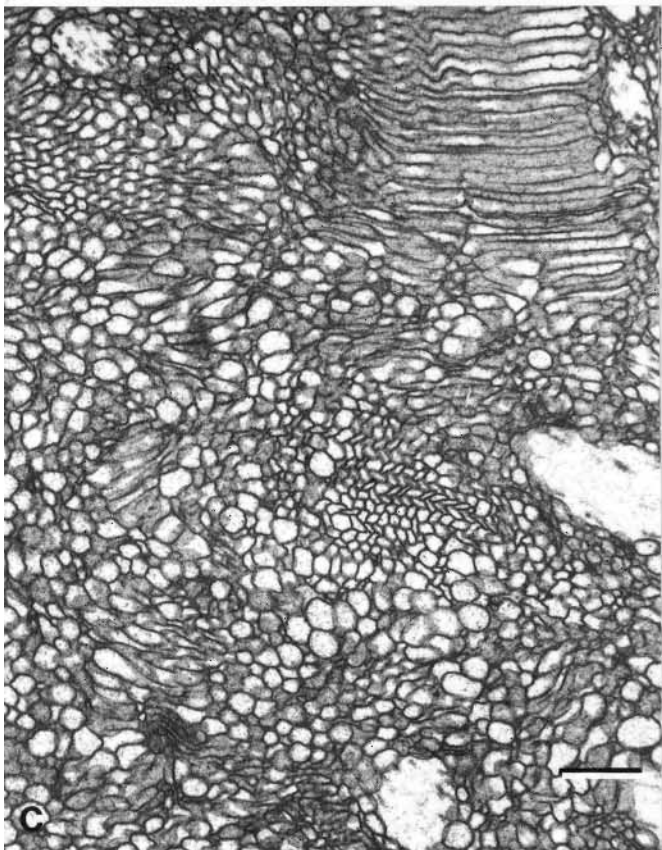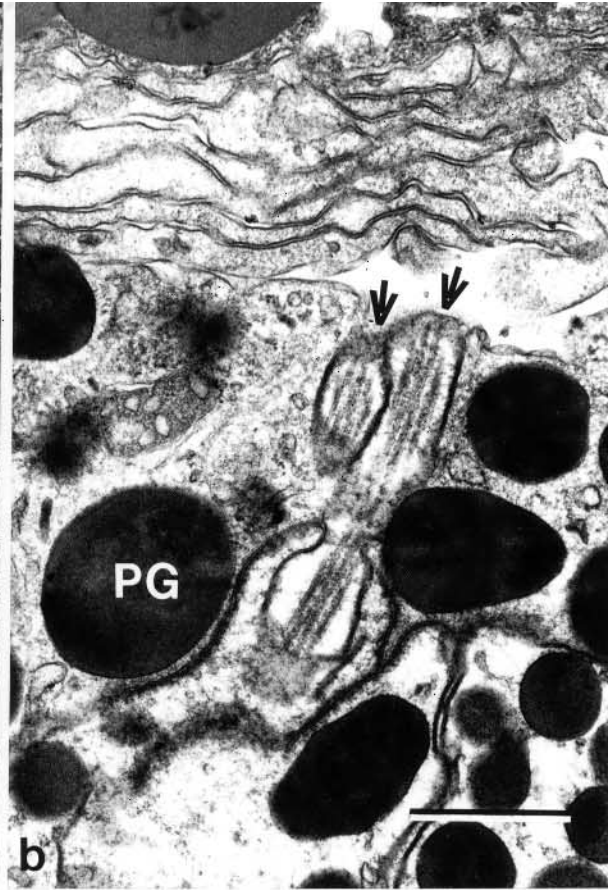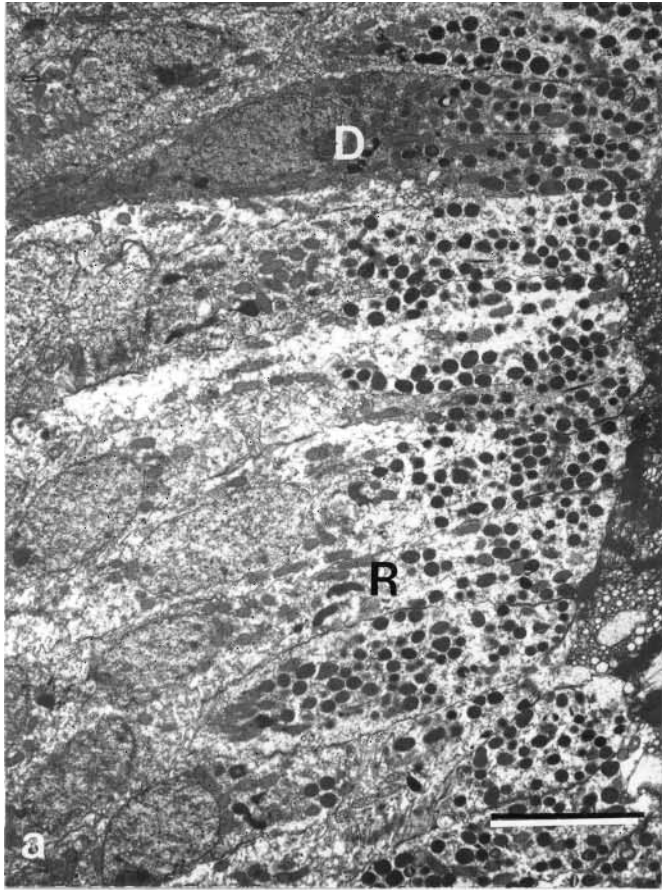
Fig. 6. Transmission electron micrographs of the photoreceptor cells of the large eye of *C. marsupialis*. (*a*) Inner segments of the photoreceptor cells, showing electron-dense pigment granules and basally located nuclei. Two types of photoreceptor cells are observed: light-staining cells (R) and dark-staining cells (D). The two types of cells have similar morphologies. Scale bar = 5 μm. (*b*) Cilia (arrows) of the outer segments of the photoreceptor cells projecting into a vitreous space. Note the pigment granules (PG) in the cytoplasm of the cells. Scale bar = 1 μm. (*c*) Outer segments of the photoreceptor cells, showing stacks of lamellae. Scale bar = 1 μm. (*d*) Parallel discs of membranes in the outer segment of a photoreceptor cell. Scale bar = 1 μm.

Fig. 7. Whole mount of the large complex eye of *C. marsupialis* labeled with the antibody RhoC directed against zebrafish rhodopsin. (*a*) Small dots of label in the outer segments of the photoreceptor cells. These regions are rich in vesicles and lamellae of membrane. Scale bar = 50 μm. (*b*) Rhodopsin-like proteins in the outer segments of the photoreceptor cells. Scale bar = 50 μm.
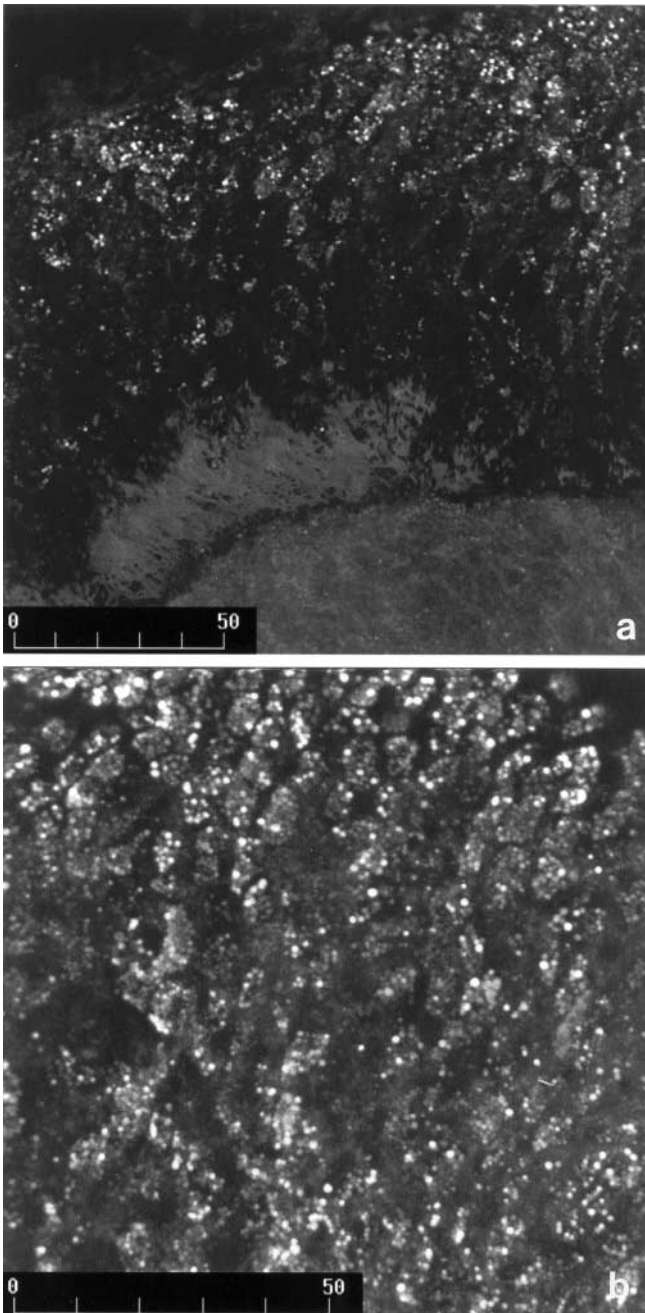
connected to a subumbrellar nerve ring (Satterlie 1979). The nerve net contains neurons of all sizes, including giant neurons with cell bodies 40 μm in diameter and neurites 5 μm in diameter. The subumbrellar ring consists of hundreds of neurons, some of which are giant neurons, that run circularly in the ring. Nerve bundles pass from the subumbrellar ring into the subumbrellar nerve net. Interneuronal synapses occur throughout the animal. Subumbrellar neurons also synapse onto muscle cells, as swimming is controlled by the subumbrellar nerve net (Satterlie 1979). The rhopalia are interconnected by the subumbrellar nerve ring, as processes from the ring extend into the stalks of the rhopalia. The complex eyes of the rhopalia have neuronal processes that synapse onto second-order neurons. These second-order neurons contact other neurons by means of processes that extend up the rhopalial stalk, eventually feeding into the subumbrellar nerve ring. Processing of visual information probably occurs in this subumbrellar nerve ring.

Photic behaviors

Ocellus-bearing animals

Light affects many behavioral activities in cnidarians, including diel vertical migration, responses to rapid changes in light intensity, and reproduction. Curiously, these behaviors are seen in animals both with and without distinct ocelli (see the section Extraocular photosensitivity). In general, medusae with the most highly modified photoreceptors demonstrate the most complex photic behaviors (Arkett 1989; Martin 2000). Many medusae undertake diel vertical migrations (Arkett 1989). Such migrations usually entail an upward movement of the animal in the water column at dusk and a downward movement at dawn. The hydromedusae *B. principis* and *P. penicillatus* display this pattern of migration (Arkett 1989). Many scyphomedusae display distinct phototaxis, coming to the surface of the water during cloudy weather and at twilight and moving down in the water column during bright sunlight and at night (Ruppert and Barnes 1994). In *Aurelia* species the pulsation rhythm is affected by light; however, it is not clear if the behavior is mediated through the ocelli (Horstmann 1934).

Rapid changes in light intensity may trigger a behavior called the shadow response (also referred to as the off response or shadow reflex) in certain hydromedusae (Kikuchi 1947; Hisada 1956; Tamasige and Yamaguchi 1967; Singla 1974). Such responses occur within a few seconds after the change in light intensity and can be triggered by either an increase or a decrease in illumination. For example, the hydromedusae *B. principis* and *P. penicillatus* respond to a decrease in light level with a burst of swimming contractions and tentacle contractions. Presumably this behavior functions as an escape response. Removal of the ocelli from

Fig. 8. Outer segments of photoreceptor cells of the large complex eye of *C. marsupialis* labeled with antibodies directed against zebrafish opsin proteins. (*a*) Outer segments of photoreceptor cells labeled with antisera that detect the blue-sensing opsin protein of zebrafish. Staining is confined to the outer segments of some of the photorecptor cells, as the inner segments do not label. Scale bar = 50 μm. (*b*) Outer segments of photoreceptor cells labeled with antisera that detect the green-sensing opsin protein of zebrafish. Labeling is found exclusively in the outer segments of some the photoreceptor cells. Scale bar = 50 μm. (*c*) Outer segments of photoreceptor cells labeled with antisera that detect the red-sensing opsin protein of zebrafish. Note the absence of stain. Scale bar = 50 μm. (*d*) Outer segments of photoreceptor cells labeled with antisera that detect the ultraviolet-sensing opsin protein of zebrafish. Note the absence of label from the cells in this region. Scale bar = 50 μm.
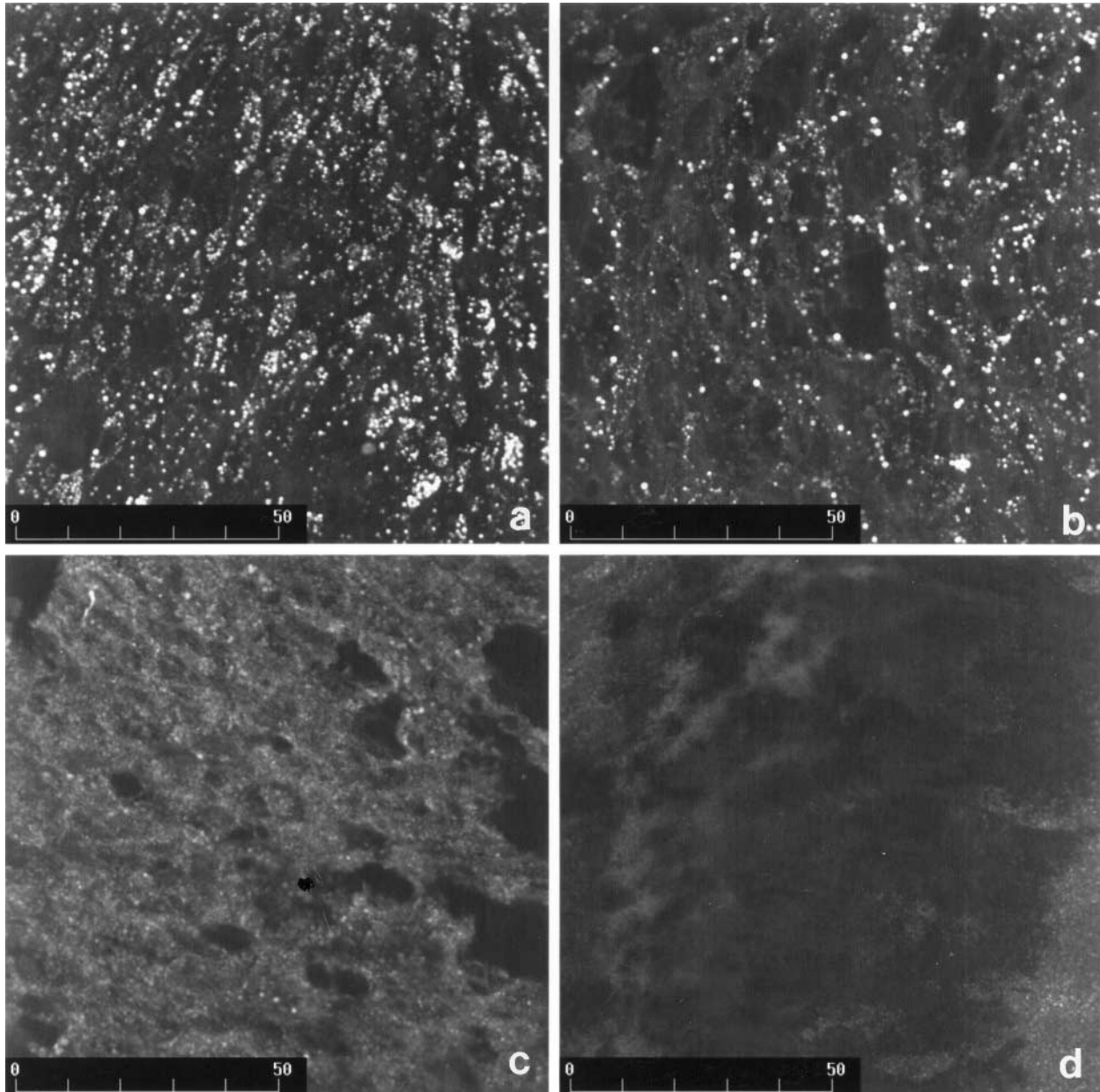
P. penicillatus alters the shadow response, indicating that these structures and their connection to the outer nerve net are important for the initiation and execution of the shadow reflex (Arkett and Spencer 1986*a*).

Light or dark stimulates many reproductive activities such as oocyte maturation, gonad rupture, gonophore swelling, and gonophore detachment and pulsation (Campbell 1974). Numerous species respond to changes in intensity of illumination, either lunar or solar. Some individuals spawn shortly after dark, after a period of darkness, or after exposure

to dark followed by light (Ballard 1942; Fadlallah 1983). *Polyorchis penicillatus* begins spawning within an hour after dark, whereas *B. principis* releases gametes at dawn and dusk (Mills 1983; Arkett 1989). It is not clear how light triggers these reproductive behaviors; however, Yoshida et al. (1980) proposed that a conducting system of gap junctions spreads light information around gonadal tissue. In many species gonadal tissue is pigmented, and the pigment may function in photosensitivity (Roosen-Runge 1962).

Cubomedusae exhibit more complex behaviors than any

other cnidarian. Because these medusae possess sophisticated visual systems, the role of vision and image formation in these behaviors has often been discussed and (or) proposed (Conant 1898; Agassiz and Mayer 1902; Larson 1976; Pearse and Pearse 1978; Hartwick 1991a, 1991b; Hamner 1994; Hamner et al. 1995; Matsumoto 1995; Stewart 1996; Martin 2000). Many cubomedusae are active swimmers, traveling at speeds of 3–6 m/min (Larson 1976). They are active during the day and night, positively phototactic, and easily captured using night lights. The jellyfish *C. marsupialis* has been observed in densities of 30–50/m³ along the California coastline near Santa Barbara during late summer and fall into January. These jellies stay in the sunny areas between kelp beds over 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 causes them to change direction. *Carybdea rastonii* and *T. cystophora* move away from dark objects such as divers and the oars of a boat (Matsumoto 1995). Further, *Chironex fleckeri* navigates accurately between pier pilings and takes evasive action when people venture too close (Hamner 1994). In the laboratory, *Chiropsalamus quadrigatus* and *C. fleckeri* have been seen to orient to the light of a match up to 5 ft (1.52 m) away, even if the match is extinguished before the turning movement is completed (Barnes 1961). *Carybdea sivickisi* grown in an aquarium are attracted to narrow strands of opaque plastic twine placed in the tank (Hartwick 1991b). In the laboratory, *C. fleckerii* will avoid dark objects positioned in an aquarium but will readily move toward and knock over white standpipes in the tank (Hamner et al. 1994). Further, when black panels are placed at one end of a tank and white panels at the opposite end, these cubomedusae swim away from the black end and toward the white end. If black panels are placed at both ends of a tank, the cubomedusae move to the center of the tank (Hamner et al. 1994).

Vision may play a role in the feeding and reproduction of cubomedusae. Many of these jellyfish chase, catch, and eat fish (Larson 1976). Also, some cubomedusae engage in copulatory behavior during mating (Studebaker 1972; Werner 1973; Stewart 1996). Male medusae chase female medusae. After a female is caught, the tentacles of the male medusa are extended to grasp the tentacles of the female, which are then positioned below the male's bell. Once the medusae pair, the male injects spermatophores onto the tentacles of the female. The medusae then part and usually the female ingests the attached spermatophores into her gastric pouch using her tentacles, resulting in internal fertilization.

Extraocular photosensitivity

Ocelli are not the only means of photoreception in the Cnidaria, as many of these animals lack distinct ocular structures, yet they exhibit specific photic behaviors. In these animals photosensitivity is mediated through an extraocular surface. Such sensitivity, called extraocular photosensitivity, is widespread throughout the animal kingdom, in both invertebrates and vertebrates (Yoshida 1979; Wolken 1995; Musio 1997; Taddei-Ferretti and Musio 2000). In extraocular photosensitivity the photosensitive cells are not organized into a complex organ like an ocellus. Instead, these cells are solitary or grouped and are scattered or localized throughout the

animal. Identification of the cells involved in extraocular photodetection has often proved difficult; in some animals, however, neurons, epithelial cells, and muscle cells have been shown to be photosensitive (Yoshida 1979; Arkett and Spencer 1986a; Sawyer et al. 1994; Wolken 1995; Musio et al. 2001). These extraocular photoreceptors function as light detectors, informing the animal of the presence of light, measuring light intensity, and activating rhythmic behaviors.

Examples of extraocular photosensitivity are abundant in the Cnidaria (for reviews see Yoshida 1979; Arkett 1989; Musio 1997; Taddei-Ferretti and Musio 2000). Many hydromedusae lacking ocelli undergo diel vertical migrations (*Gonionemus vertens*, *Proboscidactyla ornata*, *Stomotoca atra*, *Phialidium gregarium*, *Mitrocoma cellularia*, *Euphysa* species, *Aequoria victoria*, *Obelia* species, *Aegina citrea*, *Solmissus albescens*, *Aglantha digitale*) and exhibit a shadow response (*Rathkea octopunctata*, *G. vertens*, *S. atra*). Further, many eyeless hydromedusae are triggered to spawn with the changing light conditions at dawn or dusk (*Mitrocomella* species, *P. gregarium*, *Phialidium hemisphaericum*, *Proboscidactyla flavicirrata*, *Tesserogastria musculosa*, *A. victoria*, *A. digitale*, *Euphysa* species, *M. cellularia*, *Obelia* species, *R. octopunctata*, *G. vertens*, *S. atra*) (Arkett 1989). Cilium-based lamellar bodies have been found in the distal regions of sensory cells in the outer nerve ring of *P. hemisphaericum*; these sensory cells may be involved in the extraocular photoresponse (Bouillon and Nielsen 1974). Similar cells have been described in ctenophores, molluscs, insects, and *P. penicillatus* (an ocellus-bearing hydromedusa), where extraocular photodetection has been demonstrated (Horridge 1964; Wiederhold et al. 1973; Henkart 1975; Aronova 1979; Arikawa et al. 1980; Satterlie 1985). Polyps of the freshwater hydra respond to light by body contractions and tactic movements (Wilson 1891; Feldman and Lenhoff 1960; Passano and McCullough 1962; Tardent and Frei 1969; Taddei-Ferretti and Cordella 1975; Tardent et al. 1976). Recently, a rhodopsin-like protein was localized in sensory nerve cells of the ectodermal layer of *Hydra vulgaris* (Musio et al. 2001). This rhodopsin-like visual pigment may be functioning in the photoresponse of *H. vulgaris*, as opsin proteins have been shown to be the universal visual pigments involved in invertebrate and vertebrate photoreception, both ocular and extraocular (Gartner and Towner 1995; Kojima and Fukada 1999; Yokoyama 2000).

Within the Anthozoa, many sea anemones, corals, and sea pens show distinct behavioral responses to light, including phototaxis, expansion and contraction, and spawning (Fleure and Walton 1907; Parker 1918; Batham and Pantin 1950, 1954; North 1957; North and Pantin 1958; Mori 1960; Mori and Tanase 1973; Pearse 1974; Gladfelter 1975; Lasker 1979; Fadlallah 1983; Shick 1991; Sawyer et al. 1994; Gorbunov and Falkowski 2002). Many tropical sea anemones and corallimorpharians show negative phototaxis to full sunlight, preferring partially shaded microhabitats (Zahl and McLaughlin 1959; Sebens and DeRiemer 1977; Shick 1991). In the zooxanthellate sea anemone *Anthopleura elegantissima*, Pearse (1974) demonstrated that specimens expand within 5–10 min after exposure to light. Conversely, the azooxanthellate sea anemone *Urticina felina* contracts within 5 min after exposure to bright light (Fleure and Walton 1907). The majority of reef corals are contracted during the day and fully expanded at night; however, such expansion activity cycles

vary according to the species (Lasker 1979). In many species of symbiotic corals, spawning occurs synchronously several nights after the full moon. This process is correlated with the level of lunar illumination (Bull 1986; Wyers et al. 1991; Gorbunov and Falkowski 2002). Recently, Gorbovunov and Falkowski (2002) demonstrated that coral polyps show very sensitive photoreception in the blue region of the spectrum, hence they are capable of detecting the blue portion of lunar irradiance. The sea pen *Cavernularia obesa* shows a daily rhythm of contraction/expansion of the tentacles that appears to be a circadian rhythm entrained by light (Mori 1960; Mori and Tanase 1973). Although many anthozoans respond to light, no specific photoreceptor cell has been identified in these animals. Sensory neurons, muscle cells, and even zooxanthellate symbionts have been proposed to be involved in anthozoan photosensitivity (North 1957; Marks 1976; Shick 1991; Sawyer et al. 1994). Finally, some neurons of the sea pansy *Renilla kollikeri* produce melatonin (a daily photoperiodic and seasonal messenger in vertebrates), which is suggested to function as a seasonal marker for reproduction (Mechawar and Anctil 1997).

Photopigments

Opsin proteins are the functional visual pigments in vertebrate and most invertebrate photoreceptors (Tsuda 1987; Lythgoe and Partridge 1989; Goldsmith 1990; Land and Fernald 1992; Gould 1994; Gartner and Towner 1995; Wolken 1995). In fact, the visual pigment rhodopsin and its chromatophore retinal have been found in the retinas of all animal eyes (Wolken 1995). Such rhodopsins extracted from vertebrate and invertebrate eyes show a range of absorption from the near ultraviolet through the visible into the infrared (340 nm to beyond 700 nm). Several studies on hydromedusae and cubomedusae indicate that rhodopsin-like and opsin-like proteins are present in their photoreceptors. A retinol-like substance was detected in the ocelli of the hydromedusa *S. saltatrix* (Yoshida 1972). Furthermore, the photoreceptors comprising these ocelli contained intramembranous particles 8–9 nm in diameter with a density of 5000–6000/μm^2 associated with their microvillar and ciliary membranes (Takasu and Yoshida 1984). Similar particles have been identified in the outer membranes of the rods and cones of *Xenopus* eyes and are believed to contain rhodopsin (Jan and Revel 1974). A rhodopsin-like protein has been identified in cells of the ectodermal layer of *H. vulgaris* that may function in its photoresponse (Musio et al. 2001). Three of the four visual organs of the cubozoan jellyfish *C. marsupialis* exhibit rhodopsin-like and opsin-like photopigments (Figs. 7–10) (Martin and Givens 1999; Martin 2000). In both the large and small complex eyes, rhodopsin-like proteins were detected in the retina in the outer segments of some of the photoreceptor cells. Specifically, the photopigment was associated with the stacks of parallel membranes forming the discs in the outer segments (Fig. 7). In the slit ocelli, rhodopsin-like proteins were visualized in the tips and bases of some of the photoreceptors (Martin 2000). When antisera that detected blue-, green-, red-, or ultraviolet-sensing opsin proteins in zebrafish were applied to cubozoan visual organs, positive staining was seen for blue-, green-, and ultraviolet-sensing opsin in the large and small complex eyes (Figs. 8–10).

Fig. 9. Complex eyes of the cubomedusa *C. marsupialis* labeled with antisera that detect the ultraviolet-sensing opsin protein of zebrafish. (*a*) Large complex eye, showing labeling of the inner segments (arrow) and basal regions of some of the photoreceptor cells. Note the absence of label in the outer segments of the photoreceptors (the region closest to the lens (L)). Scale bar = 50 μm. (*b*) Small (single arrow) and large (double arrow) complex eyes, showing label in the inner segments and basal regions of some of the photoreceptor cells. The outer segments of the photoreceptors lack label. L, lens of the small eye. Scale bar = 50 μm.
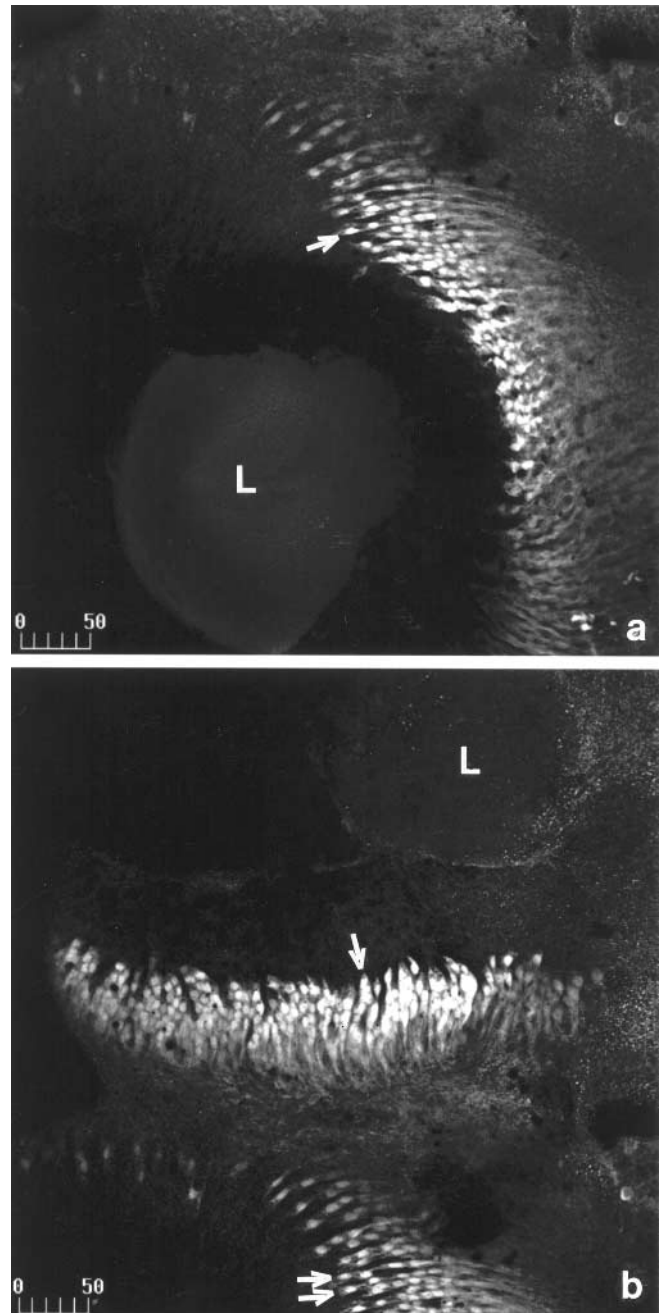
Antisera for red-sensing opsin proteins showed no binding to any of the visual organs. The blue- and green-sensing opsin protein staining was found in the outer segments of some of the photoreceptors of the retina. Small dots of stain were

observed in the stacks of disc membranes. No staining was seen in the inner segments or basal areas of the photoreceptors. Staining for ultraviolet-sensing opsin proteins was detected in some of the photoreceptors of the retina. Unlike the staining for the blue- and green-sensing opsins that was found in the outer segments of the photoreceptors, the staining for ultraviolet-sensing opsin was detected in the inner segments and basal regions of some of the photoreceptors (Figs. 8–10). The photopigment-staining patterns of the cubozoan complex eyes suggest that the jellyfish have a blue-wavelength receptor, a green-wavelength receptor, and an ultraviolet-wavelength receptor and imply that jellyfish, like may other invertebrates, can distinguish different wavelengths of light (Goldsmith 1990).

Physiological studies on several hydromedusae suggest that rhodopsin-like proteins are functioning in their photoreceptors. *Sarsia tubulosa* exhibits a maximum electroretinogram response at 540 nm, while *P. penicillatus* has a maximum response at 530 nm (Weber 1982*b*, 1982*b*; Arkett 1985). *Polyorchis penicillatus* shows a shadow response at 550 nm, while *S. saltatrix* responds between 480 and 500 nm (Yoshida 1969; Arkett 1985). As rhodopsin absorbs maximally around 500 nm, the responses of these hydromedusae are in the range which suggests that a rhodopsin-like protein is functioning in the photoreceptors.

Neurotransmitters

Anatomical and physiological studies indicate that in most invertebrate and vertebrate visual systems, synaptic transmission from photoreceptors to second-order neurons is chemically mediated. Transmitters that function in the vertebrate retina and invertebrate photoreceptors include glutamate, acetylcholine, γ-aminobutyric acid (GABA), serotonin, and dopamine (Lam et al. 1982; Quay 1986; Daw et al. 1989). In several hydromedusae (*P. penicillatus*, *S. tubulosa, Nemopsis dofleini*), electron-lucent and electron-dense synaptic vesicles 60–110 nm in diameter have been found in the photoreceptor cells (Toh et al. 1979; Yamamoto and Yoshida 1980; Singla and Weber 1982*a*, 1982*b*). However, the transmitters contained in these vesicle have not been identified. In the cubozoan jellyfish *C. marsupialis*, neurons expressing glutamate, serotonin, GABA, and RFamide (Arg-Phe-amide) have been identified in close proximity to the complex eyes (Figs. 11 and 12). Such neurons may well function in the photic system of jellyfish. Further, tracks of neurons expressing the neuropeptide RFamide project away from the eyes into the stalk of the rhopalium, pass up the stalk, and eventually feed into the main nerve ring of the jellyfish (Fig. 13). These neurons may constitute part of the ocular nerves. Ocular nerves extending from the ocelli to the outer nerve ring have been identified in the hydromedusa *P. penicillatus* (Lin et al. 2001). Further, some of the neurites of these ocular nerves, as well as some cell bodies in the ocelli, express RFamide (Grimmelikhuijzen and Spencer 1984; Spencer 1991).

Pax genes: developmental-control genes

Pax genes are developmental-control genes that play a role in the development of the central nervous system and certain organs such as the eye (Quiring et al. 1994; Tremblay and Gruss 1994; Callaerts et al. 1997). *Pax-6* and *Pax-6*

Fig. 10. Photoreceptor cells of the complex eyes of *C. marsupialis* labeled with antisera that detect the ultraviolet-sensing opsin protein of zebrafish. (*a*) Large complex eye. The inner segments of some of the photoreceptors are stained with the antibody. These labeled photoreceptor cells exhibit a very different morphology from that of the labeled photoreceptors of the small eye (shown in *b*). Scale bar = 50 μm. (*b*) Small complex eye. The inner segments of some of the photoreceptor cells are labeled with the antibody. Scale bar = 50 μm.
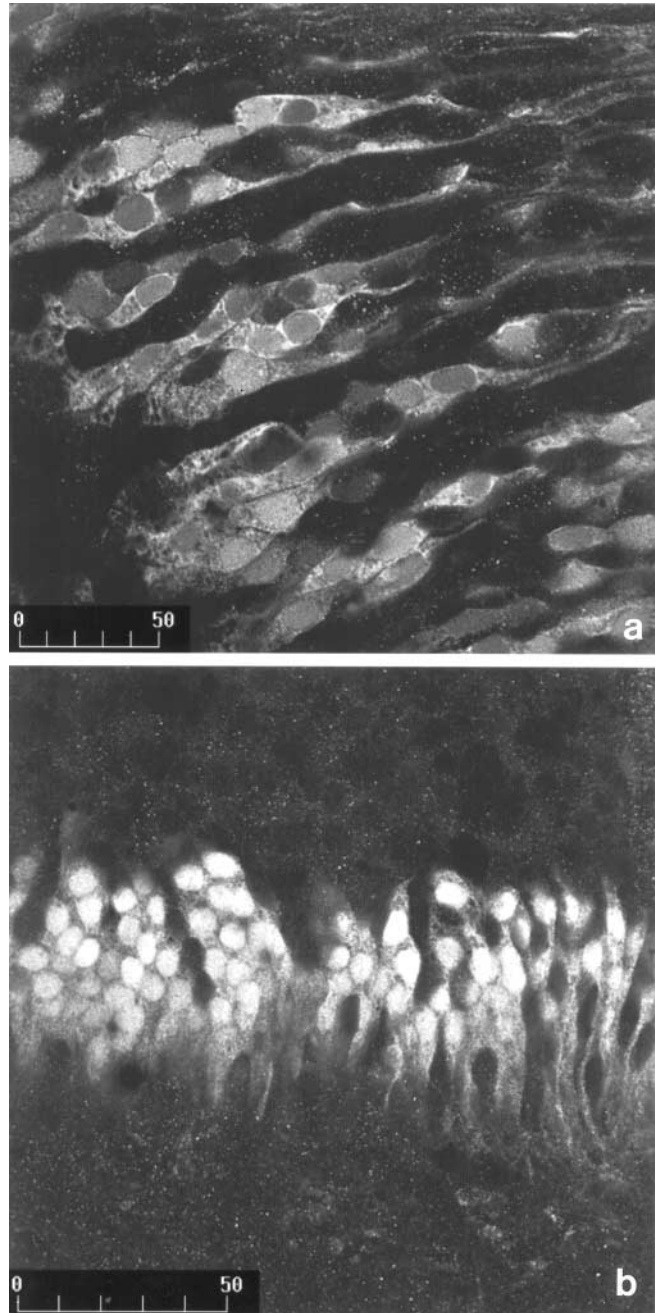

homologs are key control genes involved in eye development in both vertebrates and invertebrates (Hill et al. 1991; Jordan et al. 1992; Quiring et al. 1994; Tremblay and Gruss 1994; Callaerts et al. 1997; Bromham 2002). As cnidarians are the most primitive present-day metazoans to possess eyes, the idea has been raised that a *Pax-6* homolog is also

Fig. 11. Rhopalial neurons in close proximity to the complex eyes of *C. marsupialis*. (*a*) Neurons labeled with antibodies directed against serotonin. Scale bar = 50 µm. (*b*) Neurons labeled with antibodies directed against glutamate. Scale bar = 50 µm. (*c*) Neurons labeled with antibodies directed against GABA. Scale bar = 50 µm.

used to control eye development in this group. In recent years several *Pax* genes have been discovered in cnidarians (Sun et al. 1997; Catmull et al. 1998; Groger et al. 2000; Miller et al. 2000; Nordstrom et al. 2001; Sun et al. 2001). Four *Pax* genes have been identified in the coral *Acropora millepora*: *Pax-A*, *Pax-B*, *Pax-C*, and *Pax-D* (Miller et al. 2000). *Pax-C* and *Pax-D* are unique to *A. millepora*; however, *Pax-A* and *Pax-B* have been identified in other cnidarians such as the sea nettle *Chrysaora quinquecirrha*, the hydroid *Hydra littoralis*, and the hydromedusa *Cladonema californicum* (Sun et al. 1997, 2001). Further, *Pax-A* has been isolated from the scyphomedusa *A. aurita* and *Pax-B* has been isolated from the hydromedusa *P. penicillatus* and the cubomedusa *C. quadrigatus* (Nordstrom et al. 2001). *Pax-A* may be orthologous with *Drosophila Pax neuro*, *Pax-B* belongs to the vertebrate *Pax-2/5/8* class, *Pax-C* most closely resembles the vertebrate *Pax-6*, and *Pax-D* belongs to the vertebrate *Pax-3/7* class (Miller et al. 2000). Using phylogenetic analysis and gene sequencing, Sun et al. (1997) showed that *Pax-A* and especially *Pax-B* were more closely related to *Pax-2/5/8* and *Pax-4/6* than to *Pax-1/9* or *Pax-3/7*. Further, they classified the *Pax* genes into two supergroups: *Pax-A/Pax-B/Pax-2/5/8/4/6* and *Pax-1/9/3/7* and proposed that modern *Pax-4/6* and *Pax-2/5/8* genes evolved from an ancestral gene similar to cnidarian *Pax-B*. Thus far, a definitive *Pax-6* gene has not been found in cnidarians; however, only two gene studies on ocellus-bearing cnidarians (*C. californicum* and *C. quinquecirrha*) exist in the literature (Sun et al. 1997, 2001). It will be interesting to examine cubomedusae with complex eyes for the presence of a definitive *Pax-6* gene and determine how *Pax-B* functions in these jellyfish. With further investigations, the *Pax* genes of cnidarians may prove to be multifunctional, controlling many developmental pathways, including eye development.

How cnidarian photobiology compares with that of higher metazoans

The photobiology of cnidarians is similar in many ways to that of higher multicellular animals. The photoreceptors of many animals demonstrate some basic properties: a graded response to changes in light intensity, electrical coupling between cells, and connections to higher order neurons (Fain et al. 1976; Fain 1981; Goldsmith 1990). The photoreceptors of cnidarians react to changes in light intensity with graded potentials that are proportional to the degree of these changes (Weber 1982*a*, 1982*b*; Ohtsu 1983*a*, 1983*b*; Arkett and Spencer 1986*b*). Such responses relay precise information about minute changes in light intensity to higher order neurons. Hydrozoan photoreceptors are electrically coupled through gap junctions (Singla and Weber 1982*b*; Mackie et al. 1984). Such coupling probably functions in photic integration by increasing the chances of detecting light of low intensity (Fain et al.
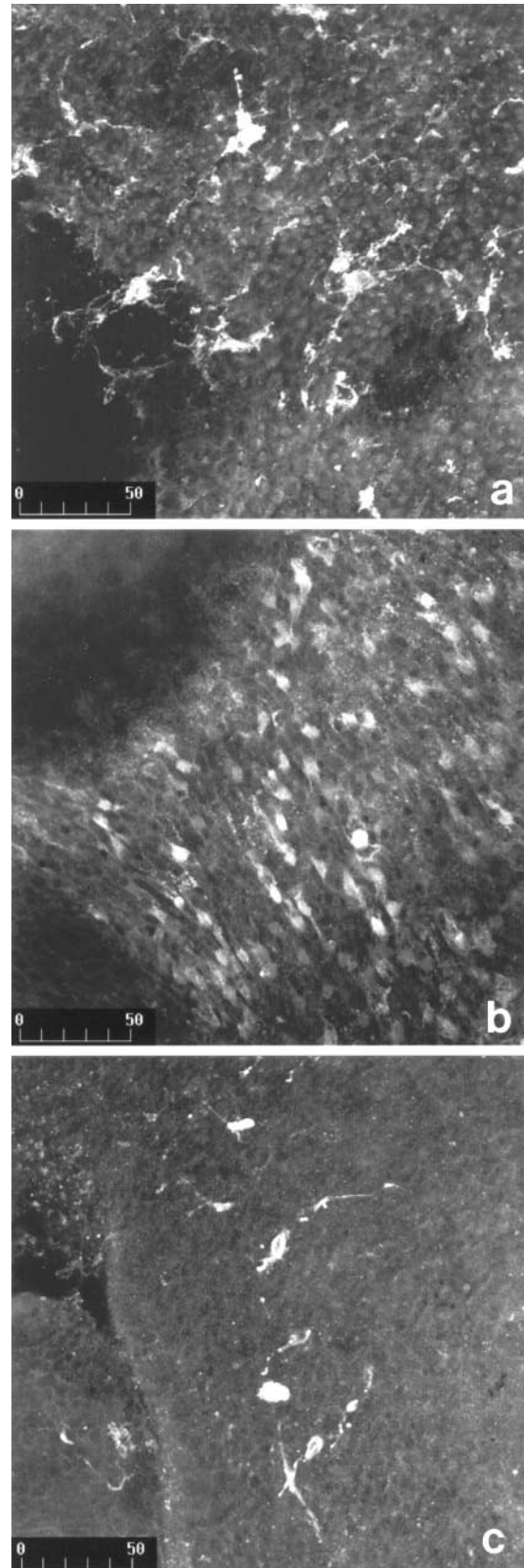
1976; Schwartz 1976; Laughlin 1981). In cnidarian ocelli the photoreceptor cells synapse with second-order neurons (Toh et al. 1979; Yamamoto and Yoshida 1980; Singla and

Fig. 12. Rhopalium of *C. marsupialis* labeled with antibodies directed against the neuropeptide RFamide. (*a*) RFamide-positive neurons (single arrows) are found in the region of the rhopalium that connects to the stalk (S), beneath the retina of the complex eyes (shown in *b*), and in the stalk (see Fig. 13). RFamide immunoreactivity is also detected in some of the cells of the simple ocelli (double arrow). L, lens. Scale bar = 100 μm. (*b*) Large complex eye, showing RFamide-positive neurons (arrows) directly beneath the retina. L, lens. Scale bar = 100 μm.
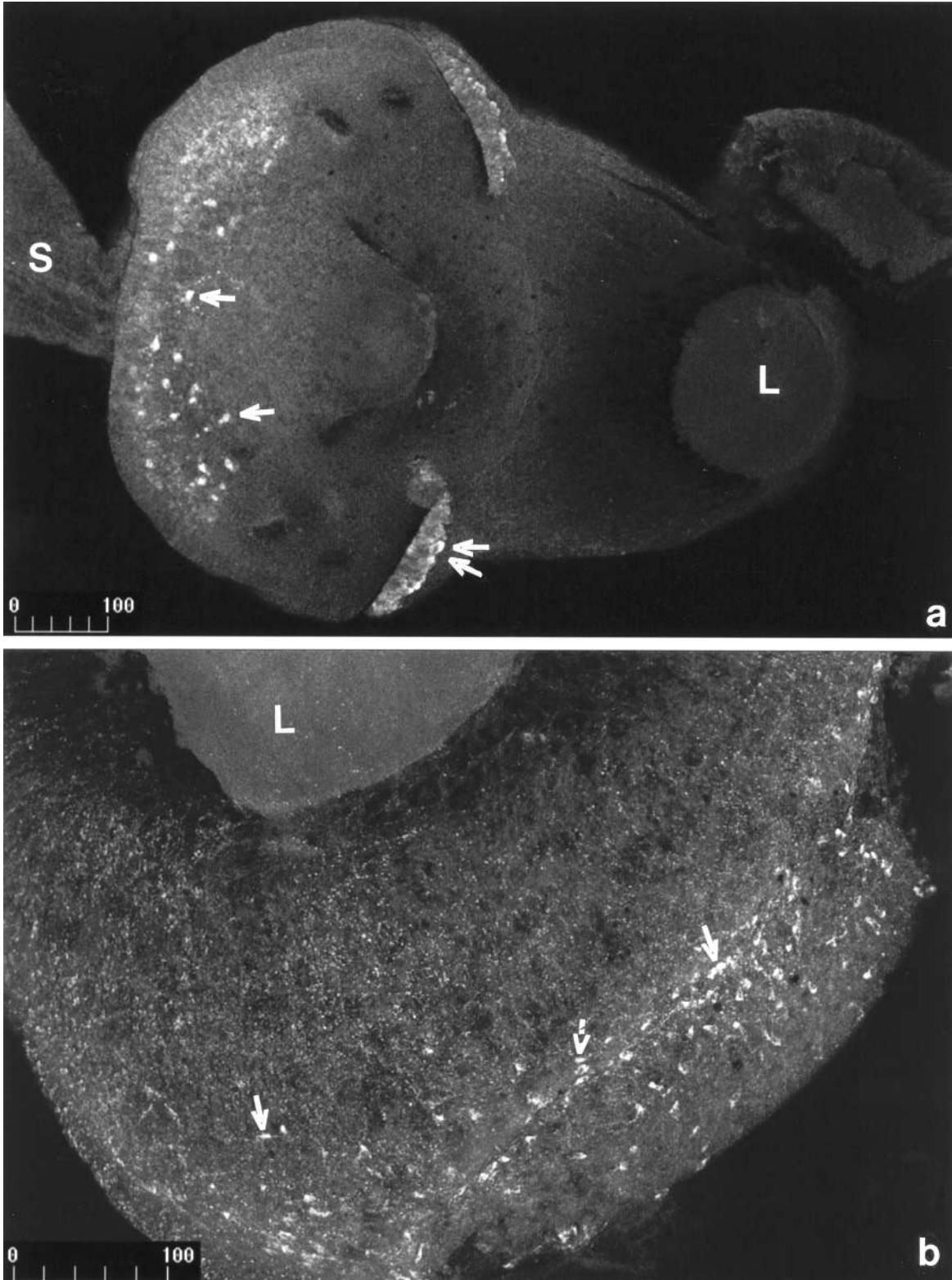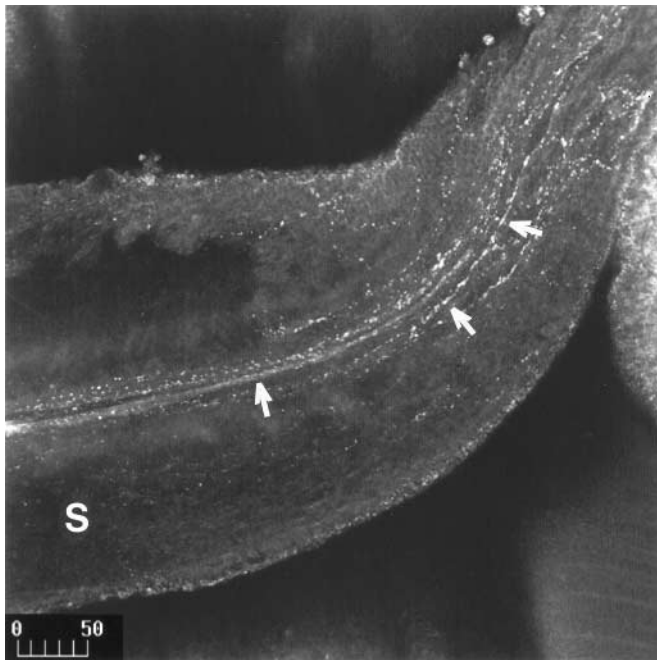
Fig. 13. Stalk (S) of the rhopalium of *C. marsupialis*, showing RFamide-positive neurons and their processes (arrows). These processes project back into a main nerve ring of the jellyfish. Scale bar = 50 μm.

Weber 1982*a*). Synapses have also been seen between receptor-cell bodies, between the receptor-cell body and adjacent axons, and among axons forming the ocular nerves (Yamasu and Yoshida 1973, 1976; Yoshida et al. 1978; Singla and Weber 1982*a*, 1982*b*). Further, neurons containing the neurotransmitters glutamate, serotonin, and GABA are found in close proximity (just below the retina) to the complex eyes of cubomedusae. The same neurotransmitter molecules play a role in photoreception in higher metazoans and most likely function in photoreception in cnidarians. Where and how neural processing of visual information takes place in cnidarians remain to be discovered; however, the processing centers probably reside in the nerve rings, as ocular nerves feed into these rings (Singla and Weber 1982*a*, 1982*b*; Martin 2000; Lin et al. 2001).

Cnidarian photoreceptor cells possess some of the same basic features of vertebrate photoreceptors: a cilium and associated structures, stacks of photosensitive membranes that increase the surface area, and a cytoplasm rich in mitochondria and microtubules (Eakin 1963, 1982; Salvini-Plawen and Mayer 1977; Land 1981; Salvini-Plawen 1982; Vanfleteren 1982; Burr 1984; Cronin 1986). The cellular lenses of cubomedusan jellyfish are morphologically similar to vertebrate lenses in that the lens cells show a paucity of organelles and a high concentration of homogeneous cytoplasm (Kuwabara 1968; Piatigorsky et al. 1972; Laska and Hundgen 1982). Further, these lenses are composed of crystallin proteins, as are the lenses of all vertebrate eyes (Bioemendal 1982; Wistow and Piatigorsky 1988; Piatigorsky et al. 1989). The jellyfish crystallins and the vertebrate crystallins are, however, different proteins.

Several key genes and (or) gene products instrumental in eye development and eye function in higher metazoans have recently been identified in cnidarians. A phospholipase C cDNA has been discovered in hydra, indicating the presence of genes involved in the inositol phospholipid signaling pathway (Koyanagi et al. 1998). Phospholipase C is a key player in the visual cascade mediated by inositol 1,4,5-triphosphate and calcium in invertebrates (O'Day et al. 1997). Whether or not this pathway plays a role in photosensitivity in cnidarians remains to be seen. Opsin-like and rhodopsin-like proteins are found in the photoreceptor cells of cubomedusae and in some of the sensory nerves of hydroid polyps (Martin and Givens 1999; Martin 2000; Musio et al. 2001). In the cubomedusae these proteins were visualized using antisera directed against vertebrate zebrafish opsins, and staining was restricted to photoreceptor cells (Martin and Givens 1999; Martin 2000). The opsins found in the eyes of molluscs, arthropods, and vertebrates show sequence and structure conservation, indicating a common origin of the photoreceptor molecule (Tsuda 1987; Gould 1994; Gartner and Towner 1995; Wolken 1995). The demonstration of the association of opsin-like proteins with distinct photoreceptors of the complex eyes of cubomedusae, using antibodies directed against vertebrate opsins, suggests some structure/sequence commonalities of these jellyfish proteins with those of vertebrates. Hence, the opsins may date back to the cnidarians, the most primitive present-day animals displaying eyes. *Pax-6*, a transcription factor found in most phyla, is involved in the development of the central nervous system and has been shown to play a central role in the development of the eyes of arthropods and vertebrates (Glaser et al. 1994; Quiring et al. 1994; Halder et al. 1995). The structure of the *Pax-6* gene is extremely conserved across groups. *Pax* genes have been identified in cnidarians; in fact, a coral expresses a *Pax-C* gene that closely resembles *Pax-6* (Miller et al. 2000). The expression pattern of *Pax-C* in neurons is consistent with an ancestral role of the *Pax* family in neural differentiation and patterning. Further, several cnidarians express *Pax-B*, a gene that is proposed to be similar to the ancestral gene that gave rise to modern *Pax-2/5/8* and *Pax-6* (Sun et al. 1997). The detection of *Pax*-gene expression in cubomedusae with sophisticated eyes and morphologically complex nervous systems will be of importance, as the expression of specific *Pax* genes in their eyes would strengthen the idea of a common origin of vision in all animals.

Acknowledgements

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