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Retinal development in the lobster *Homarus americanus*
Comparison with compound eyes of insects and other crustaceans

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**Abstract** Pattern formation and ommatidial differentiation were examined in the developing retina of the lobster *Homarus americanus* using light and electron microscopy. In the lobster the retina differentiates from the surface ectoderm that covers the optic primordia. Initially a single band of proliferation moves across this surface ectoderm. Immediately following this wave of proliferation, rows of ommatidial cell clusters appear. The earliest cell clusters are often seen adjacent to dividing cells of the proliferation band. The changing organization of the first seven rows of ommatidial clusters, visible at the surface of the retina, reveals events in early ommatidial differentiation. A rosette-like cluster of 18 cells forms the first row. Each stage following the rosette clusters occurs in a separate staggered row. Developing ommatidia have a central cluster of retinula cells, whose organization changes at each stage. Four cone cells enclose the retinula cells in each cluster and extend to the surface. In the seventh row, rhabdome formation begins and the retinula cells recede, leaving only cone cells visible at the retinal surface. This change initiates the two-tiered organization of the adult ommatidium. In 70% embryos, asymmetries in the position of the R8 axon around R7 create an equatorial line separating the dorsal and ventral halves of the retina. Possible mechanisms for the formation of these asymmetries are discussed. Postembryonic growth of the retina continues in stage VI juvenile animals along the ventral edge of the retina.

**Keywords** Retina · Development · Equator · Ommatidium · Lobster, *Homarus americanus* (Crustacea)

**Introduction**

One of the insights resulting from gene expression studies in a variety of developing systems is that certain developmental mechanisms are conserved in animals from a wide range of different phyla. One example, with relevance to the eye and nervous system, is the expression of the *Pax 6* gene (Callearts et al. 1997). This gene is expressed in the eyes and developing nervous system of animals ranging from Cephalopods to Arthropods and mice to man (Callearts et al. 1997). In some of these animals, such as *Drosophila melanogaster* (Insecta, Diptera, Brachycera), interpretation of gene expression data in the eye anlage has been enhanced by an understanding of the basic process of pattern formation and differentiation in the retina. In order to focus the present broad comparisons of gene expression, we must expand the numbers of animals within a particular group or phylum in which these types of studies are done. Such investigations will help to identify parallelisms and convergently similar features within morphological regions such as the eye and brain (Wray 2000). It has also been pointed out that the selection of representative organisms should be either for similarities in their structure and/or differences in their developmental mechanism (Hughes and Kaufman 2000).

This study of retinal development in the lobster *Homarus americanus* (Malacostraca, Decapoda, Homarida), along with our previous work on retinal development in the crayfish *Procambarus clarkii* (Malacostraca, Decapoda, Astacida; Hafner and Tokarski 1998), establishes a basic understanding of the process of retinal development and ommatidial differentiation in two species of decapod crustaceans. These two representatives were selected because of their similar retinal morphologies and different developmental histories. The stalked eyes of *H. americanus* and *P. clarkii* are nearly identical in appearance and organization of the ommatidia that make up their retinas. In *H. americanus*, like *P. clarkii*, a single ommatidium is composed of five different cell types and a total of at least 16 cells (Parker 1890; Hafner and
Tolarks (1998). These cells include two corneagenous cells, four cone cells, two distal screening pigment cells, eight retinula cells, and an undetermined number of proximal accessory pigment cells per ommatidium.

In contrast to their many similarities, the two species have significant differences in their mode of development. The development of *H. americanus* differs from *P. clarkii* in that it is longer and contains several free-swimming larval stages before a final metamorphic molt gives rise to the bottom-dwelling adult form (Helluy and Beltz 1991). In general, crayfish embryonic development is half as long as that of *H. americanus* and more direct. The three postembryonic stages resemble the adult body form but contain a significant yolk deposit that is rapidly consumed (Helluy et al. 1993). After the third postembryonic molt, individuals independent of the mother are present (Sandeman and Sandeman 1991). In both cases, percent staging systems which describe embryonic development as a percentage of the total embryonic period have been proposed (Helluy and Beltz 1991; Sandeman and Sandeman 1991).

In *H. americanus*, optic primordia appear between 5% and 8% development. The first eye pigmentation (E13%) appears at approx. day 21 as a crescent line at the posterior border of the optic lobe (Helluy and Beltz 1991). Pigmentation of the retina progresses in an anterior-medial direction and becomes oval in shape at 40% development (Helluy and Beltz 1991).

Optic primordia in the crayfish *Cherax destructor* (Malacostraca, Decapoda, Astacidae) first appear at E35%. Eye pigmentation first appears at approx. day 29 (E65–70%) at the lateral border of the optic primordia. As development proceeds, pigmentation increases within the eye, and at hatching a band covering approximately one-quarter of the retina is present (Sandeman and Sandeman 1991; Helluy et al. 1993). Hafner et al. (1982) have made similar observations in *P. clarkii*.

Retinal development in *H. americanus* was first described by Parker (1890). He found that the optic primordia appeared as two oval disks on the ventral surface of the embryo at its anterior end. Within these regions both the retina and neuropils of the eyestalk develop. The retina forms from the surface ectoderm at the lateral edge of the disk. As the retina and associated neuropils grow and differentiate, the eye disks shift from their ventral facing orientation to a dorsal-lateral position so that the lateral edge of the disk is now posterior and the medial side of the disk is more anterior. Also, with the growth of the neuropils, the optic disks elongate, taking on the cylindrical shape of the adult eyestalk. Each eyestalk extends anteriorly and medially toward the rostral end of the embryo and connects with the brain via the protocerebral tract. The retina forms at the distal or posterior end of the stalk.

Many of Parker’s observations are similar to those reported for *C. destructor*, by Sandeman and Sandeman (1991), and for eye development in *P. clarkii*, by Hafner et al. (1982) and Hafner and Tokarski (1998). This study focuses on the process of retinal formation and ommatidial differentiation in the lobster *H. americanus*. We compare our findings with previous work on the freshwater crayfish *P. clarkii*, the fruitfly *D. melanogaster*, and other Arthropods where details of these processes have been reported.

**Methods**

Embryos of the North American lobster, *H. americanus*, were collected and fixed in the laboratory of Dr. B. Beltz, Department of Biology, Wellesley College, Wellesley, Mass. A variety of embryonic stages were examined, beginning at 46% development and extending through 95%. No larval stages were examined, but stage VI juveniles were studied for their adult characteristics. The staging of all embryos is based on the percent development criteria of Helluy and Beltz (1991) for *H. americanus*. Embryos were fixed for light and electron microscopy using the protocol of King (1976). The fixative consisted of 2.5% glutaraldehyde and 1.0% paraformaldehyde in 0.2 M Millonig’s phosphate buffer, pH 7.4, plus 0.14 M NaCl. Dissected embryos were placed in fixative at room temperature for 1–2 h with agitation. After the initial fixation, the tissue was rinsed for 10 min in 0.2 M phosphate buffer containing 0.3 M NaCl. Animals were postfixed on ice for 1 h in 1% OsO4 in 0.1 M phosphate buffer, pH 7.0, plus 0.38 M NaCl. Osmicated blocks were stained in aqueous 2% uranyl acetate, dehydrated in a cold acetone series to propylene oxide, and embedded in Epon 812. Thin sections were stained with lead citrate and uranyl acetate and examined on a Siemens 101 electron microscope.

**Results**

**Light microscopy**

In the earliest embryonic stage examined (46%), the eyes were elongated and extended from the medial-anterior end of the embryo dorsally and laterally. At this stage, the ommatidia in the retina exhibit a nearly complete range of differentiation, from the earliest ommatidial clusters to small but mature-looking ommatidia. The more mature ommatidia are found in the posterior-lateral region of the retina and the degree of differentiation decreased in an anterior-medial direction. Thus, in almost any embryonic stage, a complete or nearly complete spectrum of ommatidial development can be found.

Many of the light-microscopic observations of Parker (1890) were confirmed by this study. Figure 1 is a longitudinal section of the eye from a 46% embryo. Here, the earliest changes in retinal development can be followed along the ventral ectodermal surface. The earliest recognizable event is an initial wave of mitotic activity, indicated here by a single mitotic figure (small arrow in Fig. 1). Initially, in the ectoderm layer, cells elongate and, during the mitotic cycle, round up at the surface and divide. In serial thick sections, the mitotic band within the surface ectoderm is semicircular, extending dorsally to ventrally over the developing retina. This single band of mitotic activity was first identified in *H. americanus*, by Harzsch et al. (1999), using bromodeoxyuridine (BrdU) staining of dividing cells.