Abbreviations used in this paper: ey, eyeless gene.

Cubozoan jellyfish: an Evo/Devo model for eyes and other sensory systems
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ABSTRACT Cnidaria are the most basal phylum containing a well-developed visual system located on specialized sensory structures (rhopalia) with eyes and statocytes. We have been exploring the cubozoan jellyfish, Tripedalia cystophora. In addition to containing simple photoreceptive ocelli, each rhopalium in Tridedalia has a large and small complex, camera-type eye with a cellular lens containing three distinct families of crystallins which apparently serve non-lenticular functions. Thus, Tripedalia recruited crystallins by a gene sharing strategy as have mollusks and vertebrates. Tripedalia has a single Pax gene, PaxB, which encodes a structural and functional Pax 2/5/8-like paired domain as well as an octapeptide and Pax6-like homeodomain. PaxB binds to and activates Tripedalia crystallin promoters (especially J3-crystallin) and the Drosophila rhodopsin rh6 gene in transfection tests and induces ectopic eyes in Drosophila. In situ hybridization showed that PaxB and crystallin genes are expressed in the lens, retina and statocytes. We suggest from these results that an ancestral PaxB gene was a primordial gene in eye evolution and that eyes and ears (mechanoreceptors) may have had a common evolutionary origin. Thus, the numerous structural and molecular features of Tripedalia rhopalia indicate that ancient cubozoan jellyfish are fascinating models for evo/devo insights into eyes and other sensory systems.

KEY WORDS: Cnidaria, rhopalia, eyes/ocelli, mechanoreceptors/ears, PaxB, evolution

Introduction

Eyes come in many forms and shapes in the animal kingdom (Land and Nilsson, 2002, Tomarev and Piatigorsky, 1996). These include the organelle eyes of some unicellular organisms, the simple eye spots of flatworms, the pin-hole eyes of some invertebrates, the varied compound eyes of insects, the mirror eyes of scallops and the complex, camera-type (lens-containing) eyes sprinkled throughout the animal kingdom. Even complex eyes can be varied and show anatomical differences. For example, the lens-containing eyes of vertebrates have inverted retinas in which the ciliated photoreceptors lie behind the ganglion cells with respect to the light path, while those of cephalopods (squids and octopus) have (generally) rhombodermic (microvillar) photoreceptors that are placed in front of the ganglion cells and are the first retinal cells to receive light (Arendt and Wittbrodt, 2001). The development and morphology of the lens and cornea also show differences between the complex eyes of vertebrates and cephalopods (Packard, 1972, Tomarev et al., 1997, West et al., 1995, West et al., 1994). Despite these variations, recent studies have indicated that all eyes may share a similar developmental cascade of transcription factors, suggesting that eyes have had a common evolutionary ancestor (Gehring and Ikeo, 1999). The initial molecular finding for the hypothesis of monophyletic eye evolution was that the gene for the eyeless (ey) mutation in Drosophila is Pax6 (Quiring et al., 1994), the very gene responsible for the Aniridia mutation in humans (Ton et al., 1991) and the Small eye mutation in mice (Hill et al., 1991). Subsequently, it was shown that misexpression of ey or of mouse Pax6 in leg, wing or antennae imaginal disc of Drosophila induces supernumerary ectopic eyes in the corresponding adult structures (Halder et al., 1995). These and other experiments showing that Pax6 from many species [i.e., squid (Tomarev et al., 1997), ascidian (Glardon et al., 1997), cephalochordate (Glardon et al., 1998), planarian (Callaerts et al., 1999) and ribbon worm (Loosli et al., 1996)] induce ectopic eyes in the fly have led to the idea that this transcription factor is encoded by a ‘master control’ gene for eye development (Gehring, 2002, Gehring and Ikeo, 1999). While the Pax6 master control gene for eye development has much to offer and has generated a flurry of

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Cubozoan eyes with special reference to Tripedalia

Cnidaria are the most basal animal phylum (Fig. 1) containing a well-developed visual system. In general among Cnidarians, it is the Cubozoa (known as ‘box jellyfish’ due to their square shape) that have lens-containing eyes (Coates, 2003, Piatigorsky, 2003b), although a few Hydrozoa do as well (for example, Cladonema radiatum) (Weber, 1981) (Fig. 2). Photoreceptive organs in Cnidaria have diverse structures, not only between species but within the same species. The cubozoan that we have been investigating, Tripedalia cystophora, has four equally spaced sensory structures (called rhopalia) dangling from a stalk and situated within open cavities surrounding the bell (Conant 1897, Laska and Hundgen, 1982, Pearse and Pearse, 1978, Piatigorsky et al., 1989, Yamasu and Yoshida, 1976) (Fig. 3). Each rhopalium has six separate eyes. There are two complex, lens-containing eyes, one larger than the other, situated at right angles to each other and two pairs (one pit-shaped, one slit-shaped) of simple ocelli comprising photoreceptors on either side of the complex eyes (Coates, 2003). This eye diversity within Tripedalia may provide new insights into the mechanisms used in evolution for achieving greater anatomical complexity of eyes. In addition to the eyes, each rhopalium of Tripedalia has a prominent statocyst described below. The function of the diverse eyes of Tripedalia is still elusive. Recent studies indicate that the camera-type eye has the ability to form a low resolution image on the photoreceptors despite the proximity of the lens to the retina (Laska and Hundgen, 1982, Pearse and Pearse, 1978, Piatigorsky et al., 1989). The behavioral role of the jellyfish eyes is under investigation (Coates, 2003). It seems likely that investigations of eye development in Cnidaria are directly relevant to eye development in other animal groups. The complex eyes of jellyfish show striking similarities in overall structure with the camera-type eye of vertebrates even though they differ in numerous anatomical details. In addition to a cellular lens and cornea, adult cubomedusan jellyfish eyes have ciliated photoreceptors, as do vertebrate eyes, rather than rhabdomeric (microvillar) photoreceptors generally populating invertebrates (Eakin, 1962, Yamasu and Yoshida, 1976). Eakin believed from electron microscopic evidence that there was “a common ancestry of the taxa bearing light-sensitive cilia” (Eakin, 1979); however, close examination of diverse groups shows that the presence of ciliary or rhabdomeric photoreceptors is not neatly divided among species (Arendt and Wittbrodt, 2001). That Cnidarians have many genes believed previously to have arisen with the vertebrates (Ball et al., 2002) provides additional support for the possibility that eye development in jellyfish shares many common features with that of more recently evolved triploblastic metazoans. Indeed, studies have revealed considerable conservation of regulatory genes between the diploblastic cnidarians and chordates (Galliot and Schmid, 2002, Hayward et al., 2002), increasing the likelihood that there is overlap in the mechanisms of eye development between jellyfish and vertebrates. In addition, an investigation of the early embryogenesis of the marine hydrozoan jellyfish, Podocoryne carnea, suggested that the nervous system developed from anterior to posterior in serially repeated patterns, characteristic of bilaterally symmetrical metazoans (Groger and Schmid, 2001). Since jellyfish differ sufficiently from vertebrates, we anticipate that detailed studies of their eye development should provide new insights into whether eyes are monophyletic, convergent, or a combination of both as well as provide new information on eye development in general. There is one caveat concerning the evolutionary aspects of jellyfish eyes that seems appropriate to keep in mind. Although Cnidaria are ancient and predate the Cambrian explosion, the time at which jellyfish evolved eyes is not known. It has been predicted that eyes may develop relatively rapidly during evolution (Nilsson and Pelger, 1994) and it remains possible that jellyfish eyes are relatively recent acquisitions.

Obtaining and culturing Tripedalia for studies on eye development

One of the difficulties of using Tripedalia for experiments is obtaining the adult medusae, rearing the embryos through meta-
morphosis and culturing the immature medusae to adulthood. We have captured adult *Tripedalia* medusae during the summer months swimming along the surface in the mangroves of La Parguera, Puerto Rico, where there is a Marine Station (University of PR). Sunbeams penetrate the foliage and water surface and reflect from the tentacles of the jellyfish. The live animals are caught with a dipnet from a small boat, placed in a bucket containing sea water and taken to the laboratory.

*Tripedalia*, as other jellyfish, undergo an alternation of generations between sessile, non-sexual polyps and swimming, sexually dimorphic medusae. Their life cycle and successful cultivation have been described (Kostrouch *et al.*, 1998, Werner, 1971).

Fertilization is internal and the planulae larvae develop in the gastric pocket of the females; the swimming larvae are released within 2 days into the sea water, settle on the bottom and become pyriform shaped polyps. By the time they form 5 or 6 tentacles near the mouth the primary polyps bud secondary polyps that are eventually released. The primary or secondary polyps grow to approximately 1 mm in size by 8-10 weeks and contain a whorl of 7-9 tentacles. Interestingly, the cubozoan polyp of *Tripedalia* differs from scypho- and hydrozoan polyps in having a nerve ring before the medusae stage (Werner, 1976). Full-grown polyps metamorphose into swimming ephydrae (immature medusae) within 4 or 5 days. Early studies indicated that the *Tripedalia* polyps are fully transformed into the tissues of the medusae (Werner, 1971). This includes the four rhopalia developing at the base of the resorbing polyp tentacles, which group first into a tetraradiate pattern. A new, primary tentacle forms between the rhopalia in the young medusa and within a day two new tentacles form next to each primary tentacle (Fig. 4). It would be of great interest to examine this transformation of polyp tissue into rhopalia with ocelli at the molecular level, another fascinating aspect of the jellyfish model of eye development.

**Jellyfish crystallins and gene sharing**

The abundant, water-soluble crystallins, responsible for the optical properties of the transparent lens, have been considered historically among the most characteristic and specialized proteins of the complex eye. In striking contrast to the conservation of opsins in the retina as the visual pigments, the lens crystallins are diverse, multifunctional proteins that are often taxon-specific: the crystallins are heterogeneous and many are used selectively in different species (de Jong *et al.*, 1989, Piatigorsky and Wistow, 1989, Wistow and Piatigorsky, 1988). Thus, while there is phylo-
...netic inheritance of crystallins, such as for example the δ-crystallins in birds and reptiles (Piatigorsky, 1984), the precise crystallin composition within the lens is not diagnostic for evolutionary relationships. Although the protein(s) used as lens crystallins often differ among species, many if not all are related or identical to common, ubiquitously expressed metabolic enzymes or physiological stress proteins. Of the crystallins present in all vertebrate lenses, the α-crystallins are small heat shock proteins (de Jong et al., 1993, Ingolia and Craig, 1982) and the β/γ-crystallins are related to microbial stress proteins (D’Alessio, 2002, Wistow, 1990). In addition to its optical role as a crystallin, αB-crystallin remains as a stress-inducible, widely expressed small heat shock protein (Klemenz et al., 1991); both the sibling, lens-specialized α A-crystallins and the small heat shock protein/αB-crystallin are effective chaperones that protect partially denatured proteins from aggregation in the lens (Horwitz, 1992). This is an important function that retards cataract formation during aging. Most of the taxon-specific crystallins are derived from or are active metabolic enzymes (Piatigorsky, 1992, Tomarev and Piatigorsky, 1996, Wistow and Piatigorsky, 1988). An exception to the rule of taxon-specific crystallins being enzyme-crystallins is γ-crystallin in the gecko, which is cellular retinol-binding protein type 1 (Werten et al., 2000). Although not an enzyme, γ-crystallin has been co-opted from another function for its optical role in the lens. This crystallin hallmark of being recruited from proteins with ubiquitous metabolic functions has led to the idea that crystallins are unified less by their protein phenotype than by their mode of high, lens-preferred gene expression (Carosa et al., 2002, Piatigorsky, 1993, Piatigorsky and Wistow, 1991). We have called the dual use of a single protein encoded in one gene, ‘gene sharing’ (Piatigorsky et al., 1988, Piatigorsky and Wistow, 1989). An important implication of gene sharing illustrated by the lens crystallins is that a protein can evolve a new role, without losing its original function, by a change in gene expression in the absence of gene duplication (Piatigorsky, 2003a, Piatigorsky and Wistow, 1991). As in other species, cubomedusan jellyfish crystallins are also taxon-specific, borrowed proteins that appear to have non-optical functions. There are three distinct crystallins (J1-, J2- and J3-crystal-
et al., 1992, Jordan et al., 1992, Ton et al., 1991) and ocular structures are virtually absent in Pax6 homozygote mice (Hill et al., 1991). The observation that mutations in the Drosophila homologue of Pax6 result in the eyeless (ey) phenotype (Quiring et al., 1994), along with the facts that misexpression of ey (Halder et al., 1995), toy (the second Pax6 gene in Drosophila; Czerny et al., 1999) or Pax6 from many other species induces ectopic eyes has led to the proposal of Pax6 being a universal master control gene for eye morphogenesis (Gehring, 2002, Gehring and Ikeo, 1999). Despite the importance of Pax6 for eye morphogenesis (Gehring, 2002, Gehring and Ikeo, 1999), thyroid gland dysgenesis (Mansouri et al., 1998) and ocular development of ommatidial cone and pigment cells (sparkling [Fu and Noll, 1997]) as well as mechanosensory bristles (shaven) (Fu et al., 1998, Kavaler et al., 1999). In mammals, Pax5 is essential for B-lymphopoiesis (Nutt et al., 1999, Rolink et al., 1999). Loss of Pax5 in mice results in severe kidney, eye and inner ear defects (Torres et al., 1996). In addition, Pax2 collaborates with Pax5 in midbrain and cerebellum development (Schwarz et al., 1997, Urbanek et al., 1997). Pax6 deficient mice display thyroid gland dysgenesis (Mansouri et al., 1998). Pax2 and Pax8 are required for the specification of the nephric lineage, as mouse embryos lacking both Pax2 and Pax8 are unable to form the pronephros (Bouchard et al., 2002). Thus, Pax2, which is closely related to PaxBo of Tripedalia, plays developmental roles in many tissues as well as in the eye in higher metazoans.

Much less is known about the function of cnidian PaxB genes. The expression of PaxB has been studied in the hydrozoan Podocoryne carneae (Groger et al., 2000), which does not have eyes. The Podocoryne PaxB gene is expressed in the eggs, the ectodermal layer of larva and endoderm of the developing and adult medusa. Based on the in vitro transdifferentiation assays, it has been argued that PaxB is involved in the development of adult medusa (Groger et al., 2000).

The Tripedalia PaxB gene is expressed in swimming larvae as well as in the rhopalia of adult jellyfish (Kozmik et al., 2003). Within the rhopalia, PaxB expression occurs both in the eye (lens and retina) and in the statocyst (Fig. 5A). Interestingly, PaxB expression shows similarities to that of vertebrate Pax6 in the eye and Pax2/5/8 family members in the eye and inner ear. We have performed a detailed structure-function study of PaxB protein and have shown that this ancient transcriptional activator represents a functional hybrid of Pax2/5/8 and Pax6 subfamilies (Kozmik et al., 2003). PaxB has a number of Pax2/5/8 features. In addition to having the paired domain DNA-binding specificity of the Pax2/5/8 subfamily, PaxB also has a functional transactivation and inhibitory domain characteristic of the Pax2/5/8 class. It has been shown that the N-terminus of all Pax proteins studied so far carries the transactivation function (Czerny and Busslinger, 1995, Dorfler and Busslinger, 1996, Lechner and Dressler, 1996, Nornes et al., 1996, Tang et al., 1998). Pax6 contains a relatively long transactivation domain composed of shorter regions that act synergistically with each other (Tang et al., 1998). In contrast, Pax2/5/8 proteins have an inhibitory domain in close proximity to a strong, short transactivation domain (Dorfler and Busslinger, 1996, Kreslova et al., 2002). The most prominent Pax6-like feature of PaxB is a functional homeodomain as a second DNA-binding domain. It was shown previously that Drosophila Pax6 (ey) directly activates expression of rhodopsin genes through homeodomain binding sites in the proximal region of the promoters (Papatsenko et al., 2001, Sheng et al., 1997). We have shown that PaxB is able to activate the Drosophila rhodopsin rh6 gene through synergistic interactions between the two domains (Balcerek et al., 1997). The Pax2/5/8 subfamily consists of a single Drosophila member, D-Pax2 (Czerny et al., 1997, Fu and Noll, 1997) and three mammalian genes, Pax2, Pax5 and Pax8, which arose by gene duplication at the onset of vertebrate lineage (Pfeffer et al., 1998) and cnidian PaxB genes (Groger et al., 2000, Kozmik et al., 2003, Sun et al., 2001, Sun et al., 1997). D-Pax2 is required for development of ommatidial cone and pigment cells (sparkling [Fu and Noll 1997]) as well as mechanosensory bristles (shaven) (Fu et al., 1998, Kavaler et al., 1999). In mammals, Pax5 is essential for B-lymphopoiesis (Nutt et al., 1999, Rolink et al., 1999). Loss of Pax5 in mice results in severe kidney, eye and inner ear defects (Torres et al., 1996). In addition, Pax2 collaborates with Pax5 in midbrain and cerebellum development (Schwarz et al., 1997, Urbanek et al., 1997). Pax6 deficient mice display thyroid gland dysgenesis (Mansouri et al., 1998). Pax2 and Pax8 are required for the specification of the nephric lineage, as mouse embryos lacking both Pax2 and Pax8 are unable to form the pronephros (Bouchard et al., 2002). Thus, Pax2, which is closely related to PaxBo of Tripedalia, plays developmental roles in many tissues as well as in the eye in higher metazoans.
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promoter via its homeodomain in transient transfection assays (Kozmik et al., 2003). Finally, in vivo data using Drosophila as the test system indicated that PaxB is a multifunctional hybrid protein. It rescues the spa mutation (D-Pax2 deficiency) and, when over expressed, induces ectopic eyes on Drosophila legs (as does Pax6), although with lower efficiency than Pax6 (Kozmik et al., 2003).

A PaxB-like gene was an ancestral regulator of lens crystallin genes

Pax6 is involved in the regulation of lens crystallin genes in mouse, chicken and guinea pig (Cvekl and Piatigorsky, 1996, Duncan et al., 2004; Duncan et al., 1998, Gopal-Srivastava et al., 1996, Kamachi et al., 2001, Kralova et al., 2002) and, possibly, scallop (Carosa et al., 2002). We have previously identified three major lens crystallins, J1-, J2- and J3-crystallin, in the cellular lenses of Tripedalia (Piatigorsky et al., 1989, Piatigorsky et al., 1993, Piatigorsky et al., 2001). The J3-crystallin gene, expressed primarily in the jellyfish lens and statocyst, appears to be a natural target gene of PaxB (Kozmik et al., 2003). Two paired domain binding sites that fit well with the Pax2/5/8 paired domain consensus (Czerny et al., 1993) were identified within the –66/-30 region of the TATA box containing promoter of J3-crystallin gene. PaxB activated expression of the J3-reporter gene construct but not the reporter gene construct in which PaxB binding sites were mutated. Remarkably, only PaxB (or Pax2) but not vertebrate Pax6, Pax1 or Pax3 activated the J3-reporter gene in transfection tests (Kozmik et al., 2003). J3-crystallin gene activation in jellyfish thus seems to be restricted to PaxB/Pax2/5/8 class of transcription factors.

Mutagenesis tests reinforced the requirement for the Pax2/5/8-like paired domain for activation of jellyfish crystallin promoters. Three amino acids (at positions 42, 44 and 47) within the paired domain are responsible for the difference in the DNA-binding specificity between Pax2/5/8 and Pax6. The amino acids IQN at these positions specify the Pax6 class of transcription factors whereas amino acids QRH determine Pax2/5/8 specificity (Czerny and Busslinger, 1995). It is also known that Pax2/5/8 DNA-binding specificity can be generated by converting residues IQN into QRH in positions 42, 44 and 47 of the Pax6 paired domain (Czerny and Busslinger, 1995). In accordance with this data, transfection tests using a PaxB(IQN) cDNA encoding a PaxB with a Pax6-like DNA-binding specificity did not activate the J3-crystallin promoter.

Fig. 6. Hypothesis suggesting that a PaxB-like gene, rather than a modern-like Pax6 gene, was the primordial Pax gene involved in eye formation. The model suggests that a PaxB-like protein was an initial regulator of eye development, of lens crystallin gene expression and of rhodopsin gene expression. In triploblasts, the PaxB gene duplicated, giving rise to early Pax2/5/8 and Pax6 genes. At least in some animal species, Pax6 has been recruited for the regulation of crystallin and rhodopsin genes.
Cnidarians suggest that PaxB, not Pax6, was the primordial gene during evolution of complex eyes

A gene with a typical Pax6-like structure has not been detected in the cubozoan, Tripedalia cystophora (Kozmik et al., 2003) or in the hydrozoans, Podocoryne carnea (Groger et al., 2000) and Cladonema californicum (Sun et al., 2001), despite that Tripedalia and Cladonema have complex eyes. Even if Pax6 does exist in Tripedalia but has escaped our efforts to detect its gene or cDNA, our co-transfection tests suggest that the jellyfish Pax6 protein would neither bind nor activate the J3-crystallin promoter, as does PaxB (Kozmik et al., 2003). Four Pax genes (PaxA, PaxB, PaxC and PaxD) have been found in corals (also cnidarians) (Miller et al., 2000). However, none represents a true Pax6 ancestor and none has the three characteristic amino acids (IQN) at positions 42, 44 and 47 of the Pax6 paired domain (Kozmik et al., 2003). Analyses of transgenic flies carrying chimeric Pax transgenes derived from the coral, Acropora millepora, are not consistent with the presence of a classical Pax6 gene in cnidarians (Plaza et al., 2003). Thus, although negative results do not establish the absence of Pax6, the data suggest that the Pax6 gene originated after the separation of Cnidaria from Bilateria. This implies that development of the jellyfish eye may have preceded brain evolution (Gehring, 2001). The eyespot of this unicellular alga orchestrates a positive phototaxis in low intensity light and a negative phototaxis in high intensity light by directly affecting the beating pattern of the two attached flagellae. Surprisingly, the Chlamydomonas ey2-1 mutant revealed that formation of the eyespot requires a member of the thioredoxin protein family and that this developmental role does not depend on the catalytic redox capability of the thioredoxin protein. The sponge larva, Reniera sp., provides another example of coordinated phototaxis in a multicellular organism that lacks nerve cells altogether (Leys and Degnan, 2001). A posterior ring of columnar epithelial cells containing a cilium and pigmented-filled protrusions respond directly to light, leading to negative phototaxis and directed swimming behavior. Increased light intensity makes the cilia rigid and subsequently bend, shielding the pigmented vesicles; decreased light intensity reverses the process. The resulting negative phototaxis is similar to the shadow response of tunicates and the unicellular Euglena. Spectral sensitivity tests suggest that the photoreceptive pigment in the sponge larva may be a flavin or carotenoid (Leys et al., 2002). This would make the sponge larva the first metazoan to use a rhodopsin-like protein as the primary photoreceptive pigment. It is not known yet whether expression of PaxB, which is present in sponges (Hoshiyama et al., 1998), is associated with the photoreceptive cilia in the sponge larva. Clearly, detailed studies on ancestral eyes and photoreponses are a rich source of new and unexpected insights.

Planula larvae of Tripedalia also have a photoreceptive system that appears to be directly connected to the cilium for steering towards particular light conditions. A series of single-cell, pigment cup ocelli, lacking neural connections, surround the posterior half of the larval ectoderm (Nordstrom et al., 2003) (see also Gehring in this issue). The positions of these ocelli vary in different species of cubozoan larvae. These light sensors apparently have photosensitive microvilli and a motor-cilium. The cilium responds directly to light and may act as a rudder to steer the larva. Thus, while ciliated and rhabdomeric photoreceptors are occasionally found in the same species, this is the first instance of the latter being reported in cnidarians.

Early developmental studies on embryonic induction have delineated the complex developmental relationships between the presumptive ear, nose and lens fields (Jacobson, 1963a, Jacobson, 1963b, Jacobson, 1963c). The primary role of Pax6 in eye development (see above) links the visual system with other sensory systems, since Pax6 and other transcription factors that shape the eye are widely expressed in the nervous system (Simpson and Price, 2002, van Heyningen and Williamson, 2002). Interestingly, Pax6 is also expressed in non-neural cells of the visual (i.e. lens, cornea) and olfactory (i.e. sustentacular cells, basal cells and Bowman’s glands) systems (Davis and Reed, 1996, Tomarev et al., 1997), linking these distinct sensory modalities.

Our studies on Tripedalia have revealed an intriguing, putative relationship between evolution of the eye and the inner “ear” (Kozmik et al., 2003, Piatigorsky, 2003b). Like eyes, “ears” (i.e. mechanoreceptors) of invertebrates and vertebrates are believed to be evolu-

The Jellyfish eye and other sensory systems and the putative eye/“ear” connection

Study of the jellyfish eye has implications for the evolution of other sensory systems. Gehring has reviewed the possibility that the jellyfish eye may have preceded brain evolution (Gehring, 2002). He supports this view by noting that unicalcellular algae (i.e. Chlamydomonas) or dinoflagellates (i.e. Erythropsid) have eye organelles and no brain. Jellyfish do, however, have a number of specialized ganglia associated with the rhopalia as well as an interconnected nerve ring which may, arguably, be a type of brain for a radially symmetrical animal (Coates, 2003). An attractive feature of eye before brain is that it places sense reception before information processing. Examples of ancestral photoreception preceding a central nervous system are fascinating. One is Chlamydomonas rehnhardii (Roberts et al., 2001). The eyespot of this unicellular alga orchestrates a positive phototaxis in low intensity light and a negative phototaxis in high intensity light by directly affecting the beating pattern of the two attached flagellae. Surprisingly, the Chlamydomonas ey2-1mutant revealed that formation of the eyespot requires a member of the thioredoxin protein family and that this developmental role does not depend on the catalytic redox capability of the thioredoxin protein. The sponge larva, Reniera sp., provides another example of coordinated phototaxis in a multicellular organism that lacks nerve cells altogether (Leys and Degnan, 2001). A posterior ring of columnar epithelial cells containing a cilium and pigmented-filled protrusions respond directly to light, leading to negative phototaxis and directed swimming behavior. Increased light intensity makes the cilia rigid and subsequently bend, shielding the pigmented vesicles; decreased light intensity reverses the process. The resulting negative phototaxis is similar to the shadow response of tunicates and the unicellular Euglena. Spectral sensitivity tests suggest that the photoreceptive pigment in the sponge larva may be a flavin or carotenoid (Leys et al., 2002). This would make the sponge larva the first metazoan to use a rhodopsin-like protein as the primary photoreceptive pigment. It is not known yet whether expression of PaxB, which is present in sponges (Hoshiyama et al., 1998), is associated with the photoreceptive cilia in the sponge larva. Clearly, detailed studies on ancestral eyes and photoreponses are a rich source of new and unexpected insights.

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Two sensory organs in jellyfish are anatomically diverse eyes and a statocyst, which play a role in the “righting response”. The relevance of this evolutionary relationship is that various sensory organs, including eyes and mechanoreceptors, evolved from an atonal-dependent protosensory organ.

The linkage between eyes and ears has clinical overtones. A functionally important protein overlapping vision and hearing/balance is class III myosin (Dose et al., 2003, Walsh et al., 2002). In Drosophila, the homologous protein, called NINAC, interacts with multiple components to organize the phototransduction machinery into a signaling complex (Wes et al., 1999); NINAC is also responsible for a recessive retinal degeneration in the fly. The human homolog of NINAC, myosin IIIA, is expressed most highly in the retina and ear (cochlea) and recessive loss-of-function mutations of myosin IIIA are associated with hearing loss. A scaffolding protein, hormarin, connected with Usher’s syndrome, affecting both vision and hearing, is another clinically intriguing association between these two sensory modalities (Montell, 2000, Verpy et al., 2000). The choroideremia gene also bridges eye and ear diseases (Starr et al., 2004). An example is the human choroideremia gene, which is responsible for a slow degeneration of rod photoreceptors and retinal pigment cells. Choroideremia encodes Rab escort protein 1 which is essential for prenylation of Rab5. Zebrafish carrying ru848, the recessive homologue of Choroideremia, are unresponsive to acoustic stimuli and lack balance. The recent finding that Norrie disease and familial exudative vitreoretinopathy both implicate a Norrin-Frizzled-4 (a presumptive Wnt receptor) signaling system provide another link between eye and ear pathology in humans (Xu et al., 2004).

Taken together, the jellyfish rhopalia, with their integrated ocelli and statocysts, have provided tantalizing new hints of information relating vision with other sensory modalities that warrant further study. We believe that the sophisticated sensory rhopalia containing anatomically diverse eyes and a statocyst make cubozoan jellyfish, such as Tripedalia, an advantageous model for eye and sensory research.

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