ON THE EVOLUTION OF CNIDARIAN EYES

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Dekan

“ la plus libre, la plus souple, la plus voluptueuse des danses possibles, m’apparut sur un écran où l’on montrait de grandes méduses: ce n’étaient point de femmes et elles ne dansaient pas.

Point des femmes, mais des êtres d’une substance incomparable, translucides et sensibles, chairs de verre follement irritables, dômes de soie flottante, couronnes hyalines, longues lanières vives toutes courues d’ondes rapides, franges et fronces qu’elles plissent, déplissent; cependant qu’elles se retournent, se déforment, s’envolent, aussi fluides que le fluide massif qui les presse, les épouse, les soutient de toutes parts, leur fait place à la moindre inflexion et les remplace dans leur forme. Là, dans la plénitude incompressible de l’eau qui semble ne leur opposer aucune résistance, ces créatures disposent de l’idéal de la mobilité, y détendent, y ramassent leur rayonnante symétrie. Point de sol, point de solide pour ces danseuses absolues; point de planches, mais un milieu où l’on s’appuie par tous les points qui cèdent vers où l’on veut. Point de solides, non plus, dans leur corps de cristal élastique, point d’os, point d’articulations, de liaisons invariables, de segments que l’on puisse compter…”

Paul Valéry
Degas, danse, dessin, 1936, in Oevres II, Pièces sur l’art

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SUMMARY

Cnidarians and their medusa stage are generally considered to be diploblasts and therefore ancestral to Bilaterians. They represent the most primitive phylum where striated muscle tissue, a complex system of nerve rings and different sense organs of high complexity, including eyes have evolved in the jellyfish stage.

We demonstrated that jellyfish and the triploblast Bilateria use homologous gene cascades and developmental pathways to build these muscle systems. The expression of *JellyD*, a derived jellyfish homolog of the master regulator of muscle tissue *MyoD*, is correlated with that of bilaterian muscle determination factors.

Furthermore, the eye determination genes of the *Pax* and *Six* families of cnidarians have bilaterian-like expression patterns. Although no *bona fide Pax6* homolog could be found, it can be shown that among the four *Pax* genes characterized, cnidarians do have a *Pax* gene (*PaxA-Cr*) that is exclusively expressed in the maintenance and regeneration of eye tissue. Additionally the hypothesis of a loss of *Pax* genes within the cnidarians can be rebut as well as the claim that cubozoans would possess only one *Pax* gene. *Cladonema* jellyfish have three cognate members of the *sine oculis/Six* class family of which *Six1/2-Cr* and *Six3/6-Cr* are upregulated during eye regeneration. Analysis of gene expression patterns during eye regeneration shows that the cnidarian *Pax* gene is upregulated before the *Six* genes, indicating a possible upstream position in the gene regulatory network.

The results are in agreement with monophyly of eye evolution and indicate that the common ancestor between Cnidaria and Bilateria had a more complex anatomy than commonly anticipated.

INTRODUCTION

Are Cnidaria diploblasts?

Evolution is an excruciatingly slow process and cannot be understood without understanding the evolution of development, and how the process of development itself constrains evolution. Mutation and selection are the basis to portray evolution and provide a steady supply for new genes. Developmental mechanisms control body shape, pattern, and therefore establish the field wherein mutations act. In other words, evolution roots in changes found in developmental mechanisms and evolution is primarily the evolution of genomes (Davidson, 2001).

The comparative study of the spatio-temporal expression patterns of developmental genes, mainly transcription factors, has been one principal focus in evolutionary developmental biology over the last years. Studies on the evolution of development yielded in the astonishing finding that shared regulatory genes have conserved roles in development across phyla and morphological diversity is often based on changes in the developmental roles of transcription factors and not necessarily in the appearance of completely new genes. Additionally it is true that the number of genes, or the size of the genome, is not correlated with an animal's complexity (Davidson, 2001). The discovery of the homeobox (McGinnis et al., 1984; Scott and Weiner, 1984) and its widespread phylogenetic conservation was one of the most important key events linking molecular data to body plan architecture and so helped much to explain the relationship within phyla.

Therefore the expression pattern of developmental genes of a species is useful to define the evolutionary position of its phylum. Today about 35 different animal phyla, each with visibly distinct body plans, are distinguished. Since fossil records demonstrated that most of them suddenly appeared during the Cambrian explosion (Carroll, 2001) it was suggested that this

great evolutionary diversification occurred before the onset of the Cambrian period (Chen et al., 2000). At the time a deep reorganization of the metazoan phylogenetic tree is taking place as a result of the availability and input of DNA sequence analysis (Adoutte et al., 2000) and the genesis of bilaterian complexity has to be reinterpreted.

The new rRNA-based phylogeny leaves diploblasts as the only sister group to Bilateria. According to the textbooks diploblasts are animals with a two-layered body structure. Ctenophores and Cnidaria, and according to some, Sponges and Placozoa constitute the diploblasts. A bilateral symmetry along an anterior-posterior axis, the presence of three germ layers, a coelom, a through gut, a central nervous system, and the principle of colinearity of Hox cluster gene expression are characteristics of bilateral animals. Cnidaria display also many bilaterian-like traits. In *Podocoryne carnea* (Cnidaria, Hydrozoa) the expression pattern of the homeobox gene Gsx-Pc (Yanze et al., 2001), the formation of a subset of nerve cells (Gröger and Schmid, 2001) and the expression of atonal in endodermal cells (Seipel et al., 2004) indicate the existence of an anterior-posterior polarity in axis formation in the development of the planula larva. During medusa development, the entocodon, a third ECM bordered (Bölsterli, 1977) cell layer is formed from the early bud ectoderm (Bouillon, 1994; Hyman, 1940; Kühn, 1910). Then the entocodon cavitates and the outer layer will differentiate striated and smooth muscle of the bell, the inner layer the smooth muscle of the manubrium. This cavity could represent a coelom-like structure which gives rise to the subumbrellar cavitiy of the bell, in which later (the adult medusa) the gametes are shed. Many molecular markers and regulatory proteins typical for the mesoderm and myogenic lineage in Bilateria were isolated from jellyfish (see chapter 3 and 4 of this thesis) (Schuchert et al., 1993; Gröger et al., 1999; Müller et al., 1999, 2003; Spring et al., 2000; 2002). Their expression patterns in the entocodon or its derived tissues strengthen the idea that the entocodon is a mesoderm-like structure. Even a jellyfish homolog of the master regulator of

muscle development MyoD (Davis et al., 1987) was characterized from *Podocoryne* (Müller et al., 2003). The high sequence conservation of cnidarian genes with insect and vertebrate homologues confirms again the hypothesis, that the striated muscle of jellyfish is related to the striated muscle of bilaterians. The argument that the lack of a through gut and a central anteriorized nervous system (brain) clearly separates Cnidaria from Bilateria has to be interpreted cautiousely. Another big phylum with radial symmetry, the echinoderms which are believed to be true bilaterians, have reduced the through gut in the Ophiuroidea and some asteroid species and have given up an anteriorized nervous system.

Evolution of eyes

A discussion about eye evolution leads to date ultimately into a discussion of a possible monophyletic, polyphyletic or perhaps biphyletic origin. The ubiquity and perplexing Pax6 gene expression in developing visual organs throughout the Bilateria and mutant results in mice and fruit flies provide a compelling case for a key position of this gene throughout phyla in the development of animal eyes and as a conclusion, seem to justify the claim of a monophyletic origin of eye evolution. Those overwhelming molecular data stay obviously in strong contrast to the classical morphological view of extreme polyphyly of eyes. Eyes and photoreceptive cells would have originated in at least 40 if not 65 or more different lines (Salvini-Plawen and Mayr, 1977). Salvini-Plawen and Mayr argue also, that earliest invertebrates (sessile carnivores like cnidarians) did not bear eyes. Eyes would have evolved late because early Bilateria would have lived interstitially or tunneled in the substrate.

From their anatomy the different eye types in the animal kingdom are fundamentally different. Eyes can be discriminated by their ontogenetic origin, structure of photoreceptor cells, position of receptor axons and the organisation of phototransduction.

Cnidaria are in a key position to unravel the enigma of the eye evolution representing the most basal phylum with a nervous system and eyes. Recent work showed that this basal phylum already contains a surprising diversity of transcription factors and metabolic enzymes previously assumed to be restricted to vertebrates (Kortschak et al., 2003). Genes formerly thought to be vertebrate inventions must have been present in the common metazoan ancestor. The results of this thesis confirm this hypothesis as is demonstrated by the identification of the Six class homeobox family genes (chapter 1) and members of the paired box family (chapter 2) display. Expression analysis and regeneration experiments demonstrate that *Cladonema* employs the same highly conserved eye-specification network as proposed for Bilateria. But the question remains: Does the mere ownership of a highly conserved gene-cascade (master control gene network) and its corresponding function legitimate the claim of a monophyletic eye evolution?

To answer this question we first have to define and focus on the prerequisite of vision. All animals (including the eyes of cnidarians, see below) use rhodopsin as a photoreceptor molecule. Opsins of all animals are probably homologous and when activated they all couple to a trimeric, GTP binding G-protein. A G-protein coupled receptor with the characteristic seven transmembrane helices is used as a light sensitive receptor even in bacteria. Bacteriorhodopsin is perhaps among the simplest known ion pumps which functions by converting light energy into an electrochemical gradient pumping protons out of the cytoplasm. The fact that already bacteria possess light sensitive receptors shows the importance of light as a selective force, probably the most profound selective force during evolution (Fernald, 2000). Light influences movement, photosynthesis, navigation, timing, vision and behavior. Hence we may assume that a unicellular ancestor of all Metazoa possessed some kind of a simple light perception unit consisting of receptor protein, protein cascade and corresponding nuclear reaction to that incoming stimulus. Although this

unicellular ancestor was in a way certainly influenced by light it did not bear eyes and we are far from organogenesis. Sooner or later evolution must have passed the status of a myosensory cell, a cell equipped with cilia or with a flagellum. This myosensory cell would potentially represent the common ancestor of muscle and nerve cells. Light information from receptor proteins can be used to change the direction of flagellar beat, as was demonstrated in *Euglena* (Lebert and Hader, 1997).

It appears plausible that the evolution of the zootype did not pass diploblasty but assembled for functional reasons from the beginning the three different germ layers. Genes, gene networks, or gene cascades that were established within the myosensory cell could have been co-opted in forging the basic Bauplan. In this view it is not astonishing that myogenesis and eye development are controlled by a similar synergistic genetic network (Heanue et al., 1999). It would also explain why Otx is used in muscle cells of medusa whereas it occupies nerve specific roles in Bilateria (Müller et al., 1999). Locomotion and feeding have certainly been of high selective value in evolution and therefore been tightly coupled to the development of muscle and nerve cells. With the evolution of the anatomy for fast locomotion sensory adaptations became necessary leading to diverse eye structures. In essence it does not matter how those sensory structures are realized, the already evolved genetic cascade from the myoepithelial-neuro-sensory ancestor was implemented and the specific members of these gene families selected for specific functions. A discussion about the homology of highly evolved eye structures is therefore redundant if it can be demonstrated that the simplest animals with eye-like organs, jellyfish, used the same genetic inventory to develop eyes.

Are all eyes homologous?

What precisely does the term homology signify when there are so many differences among the descendant structures and between them and the ancestral eye form? If in the view of classical phylogeny, a character shared between two species was present in the common ancestor (convergent) of the two species, it is termed homologous, if not it is an analogous character. Homologies are further divided into ancestral and derived homologies. If a character is homologous it must have the same fundamental structure and the same relationship to surrounding characters and development in the two species. A gene performs a homologous function in two animals if at least some of its upstream linkages or both remain the same in the two genomes, and the function it performs is descendant from their common ancestor (Davidson, 2001). Wilkins (2002) tries to answer the question of homologous eye structures as he demands some changes in the way the term homology is used. Morphological traits and genes need not to share tight, invariant relationship. There exists a continuity of genetic information and therefore the basic concept (of homology) of shared possession remains intact. In this context the eyes of insects and vertebrates are homologous even though they look different from each other, develop differently and may have arisen independently in separate lineages from ancestors lacking eyes. What they share is the inherited regulatory machinery and the ancestral function of that machinery for light sensing or some rudimentary form of vision.

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Cnidarians – the model organism of choice

The research of cnidarians has a long history. In the 1740s the Swiss scientist Abraham Trembley (1710-1784) started to work with the freshwater polyps, today known as Hydra. He discovered that hydra could regenerate heads and feet, and if cut into small pieces, all of them would regenerate to form new individuals. He was able to split the head of the polyp longitudinally and allow two heads to regenerate. By repeatedly splitting the new heads, he was able to generate a multiheaded animal that he named Hydra in reference to the mythological creature. Trembley was one of the first scientists to demonstrate that animals could reproduce asexually.

Cnidaria are well positioned to study questions related to evolution. They represent an old phylum with tissue level anatomy but already have differentiated striated and smooth muscle tissues, complex nerve rings and sense organs. Furthermore their big potential for regeneration and transdifferentiation can be additionally exploited for the evolution of development and gene interacting networks. Therefore, this phylum occupies a unique position with respect to information from basal genomic characteristics like gene structure, the ancestral function of genes and the gene complement of the common ancestor (Miller and Ball 2000).

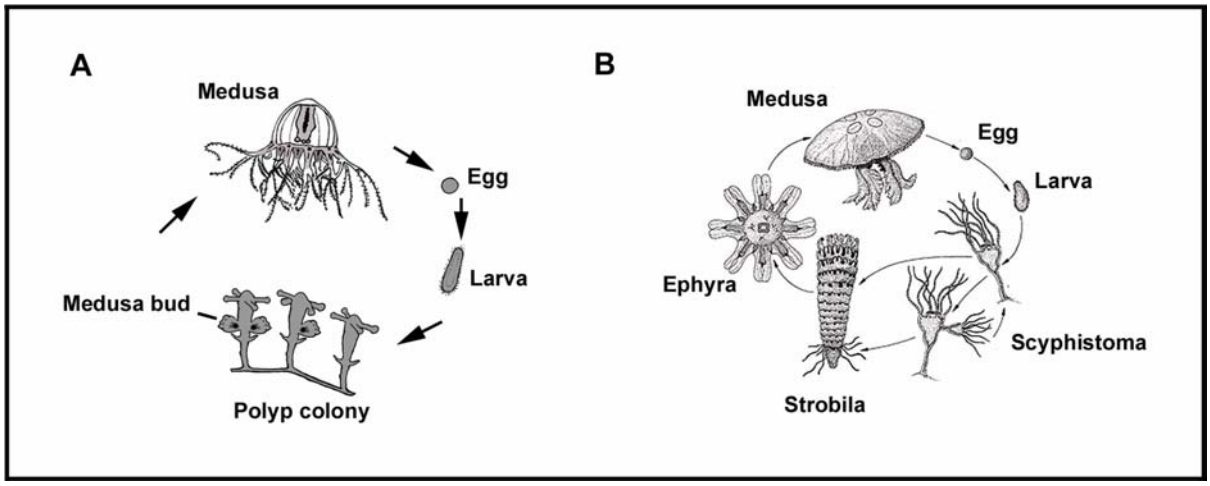

Figure1 The life cycle of one representative of the Hydrozoa, *Cladonema radiatum* (A), and a representative of the Scyphozoa, *Aurelia aurita* (B).

The phylum cnidaria comprises four different classes: Anthozoa, Hydrozoa, Scyphozoa and Cubozoa. Their relationships are still controversial. These mostly marine carnivorous animals show a primary radial symmetry and hence it is considered to place the cnidarians separately into the Radiata (Ruppert and Barnes, 1994). Fossil records are poor but those fossils found date back to the Precambrian. Most of the animal phyla that are represented in the fossil record first appear "fully formed" some 550 million years ago in the Cambrian (Ruppert and Barnes, 1994). Therefore the origin and early diversification of the various animal phyla must have occurred in the Precambrian between 600 and 1000 million years ago.

Approximately 9500 living species are combined in the cnidarian phylum, making it the seventh largest (Miller, 2000). Most cnidarians exhibit a metamorphic life cycle including a planula larva, a stationary benthic polyp stage and a free-swimming medusa, the sexually reproducing form. The variation of life cycle forms is outstanding with the highest degree of diversity within the Hydrozoa.

The polyp shape (Fig. 2) is in essence that of a tube with one end carrying the mouth and the other a basal disc that attaches the animal to the substratum. There is only one body

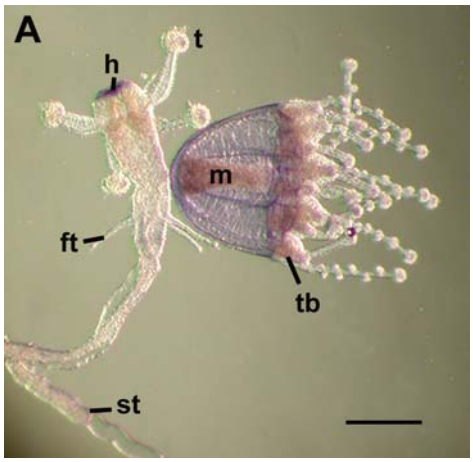

Figure 2 A polyp of *Cladonema radiatum* bearing a medusa bud, stained with an antisense RNA probe for *Six1/2-Cr*. The message localizes to exumbrella, striated muscle and hypostome (mouth region). h, hypostome; ft, filiformous tentacle; m, manubrium; st, stolon; t, tentacle; tb, tentacle bulb. Bar is 100 μm.

opening serving for food intake and ejection of indigestible material. The body wall consists of two tissue layers separated by an ECM (mesogloea). The mesogloea is composed of collagen (type IV), fibronectin, heparan sulfate proteoglycan, and laminin (reviewed in Schmid and Reber-Müller, 1995). Epitheliomuscular cells, interstitial cells, cnidocytes, gland cells and sensory nerve cells are the main cell types found interspersed in the two layers. The cnidocytes contain the stinging structures, particulary nematocysts, and are unique among the metazoans. But nematocyst-like structures are not an exclusively cnidarian feature in that similar organelles are present in a number of protists (Myxozoa, Microspora and Dinophyta). Nerve cells are associated in an irregular nerve net or plexus in the polyp. The medusa is the more elaborate life stage and its tissue architecture is completely different to that of the polyp. The mesogloea of the medusa bell is increased and striated muscle tissue and sense organs differentiate only in the medusa. In general the nervous system of the medusa is more highly specialized than that of the polyp. At the margin of the bell nerve cells are organized in a nerve ring. Some medusae contain even two nerve rings. It has been shown that these nerve rings can contain large motor neurons that connect to the swimming muscles. The

nerve ring should contain also the peacemakers, the center for rhythmic pulsation (Mackie and Meech, 1995). In *Polyorchis* it has been demonstrated that central neurons of the inner nerve ring respond to light (Anderson and Mackie, 1977). The nervous system of cnidarians is remarkable in the multifunctionality of the nerve cells: all neurons are sensory-motor-interneurons with neurosecretory granules (Koizumi, 2002). True sense organs of the medusa are the light sensitive ocelli and statocysts.

In Hydra germ cells arise from interstitial cells (Tardent, 1969). Cnidarian sperm with one exception lack an acrosome (Carré, 1984), but many contain several mitochondria. Fertilization is predominantly external in the water, but internal fertilization is known and in Cubozoa even an example of copulation is described (Brusca and Brusca, 1990). Fertilization takes place when oocyte meiosis is completed. Several different cleavage patterns are known and gastrulation is very diverse in cnidarians. By the end of gastrulation a bilayered ciliated planula larva is formed. Planulae swim for several hours to several days. They are planktonic and serve for dispersal. Planulae can be planktotrophic (many Anthozoa) or lecithotrophic (many Hydrozoa). It is believed that the planulae receive an external stimulus that signals the entry to metamorphosis. The body plan of the cnidaria is regarded to be as successful neither did it give rise to any other phlya nor any other known animal group derive from Cnidaria. It was suggested that the ancestry of the cnidarians must lie within the protista.

Anthozoa

Anthozoa are generally believed to be the most ancient cnidarians as the structure of mitochondrial DNA, mitochondrial 16S ribosomal DNA sequences as well as 18S ribosomal DNA sequence data suggest (Bridge et al., 1995). Only anthozoans have circular mitochondrial genomes. Those of the other cnidarian classes are linear and often fragmented. The medusoid stage is completely absent in this largest cnidarian class. The Anthozoa can

form large solitary or colonial polyps. The most familiar cnidarians like sea anemones or corals belong to the Anthozoa.

Hydrozoa

In contrast to the Anthozoa most people are unaware of the existence of the Hydrozoa (see Fig. 3). Hydrozoa are of small size and they often grow attached to rocks where they are usually dismissed as seaweed. A few freshwater species belong to this class with the most popular Hydrozoan *Hydra*. *Hydra* is an untypical member of this class. It not only lost the medusa stage and its polyps are solitary, it also owns an infrequent and unpredictable sexual reproduction. Without any doubt the freshwater *Hydra* can not serve as a representative of this overwhelmingly marine phylum. The uniting characters of the Hydrozoa are the lack of cells in the mesogloea, the gastrodermis contains no cnidocytes and the gonads are mostly of epidermal origin (Ruppert and Barnes, 1994). The hydromedusae are typically small ranging from half of a centimeter up to six centimeter in diameter. The medusa bears a velum. The most primitive hydrozoans are probably species in which the pelagic actinula develops directly into an adult medusa (Ruppert and Barnes, 1994). The polypoid state is missing. This life cycle is realised in the order of the trachymedusa.

Scyphozoa

Scyphozoa-medusae are similar to the hydromedusae but differ in the following: the manubrium is tentaculate, the medusa lacks a velum, the mesogloea can contain amoebocytes and gonads are gastrodermal. It is reported that nerve rings in Scyphozoan medusa are rare (Ruppert and Barnes, 1994). Pulsation control is centered on the marginal concentrations of neurons in structures called rhopalia (Hyman, 1940). Rhopalia carry also statocysts and

sometimes ocelli. The polyp forms a scyphistoma that buds by fission ephyra larvae. Ephyra larvae form juvenile medusa.

Cubozoa

The representative of the cubozoan medusa investigated is *Carybdea marsupialis* (Fig. 3). Cubomedusa are fast swimmers and known to possess a vicious sting. Cubozoa are commonly called box jellies because they have a cubical shape. There are about 20 known species found in tropical and semitropical waters. The medusa is the dominant phase in the life cycle. The very small polyp produces a single medusa by complete metamorphosis. Cubozoa have evolved the most elaborate ocelli of this phylum and the only medusa whose behaviour can be studied (Nielson, pers. communication).

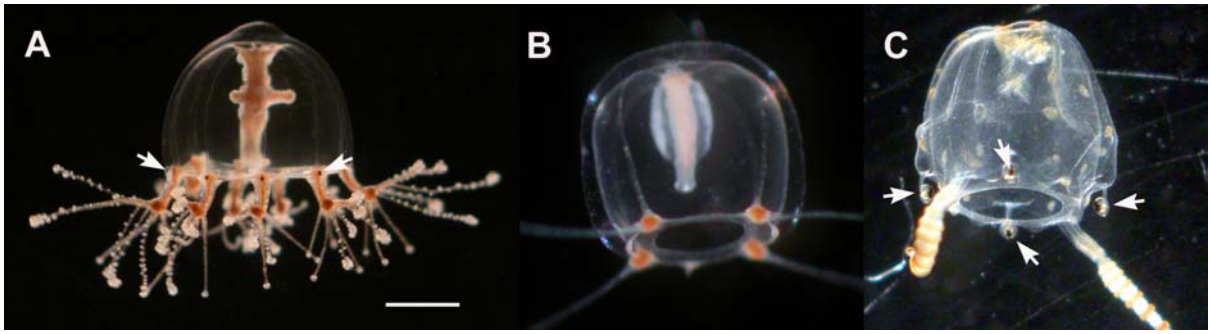

Figure 3 The species used for this thesis: (A) *Cladonema radiatum*, (B) *Podocoryne carnea*, (C) *Carybdea marsupialis*. *Cladonema* and *Podocoryne* are members of the Hydrozoa whereas *Carybdea* is a representative of the Cubozoa. The lens eyes are located at the margin of the bell (arrows in A, C). Note that the eyes in *Carybdea* are stalked and arranged together with additional ocelli. *Podocoryne* does not bear any eyes. Bar is (in μm) 350 in (A), 240 in (B), 330 in (C).

The eye – a classical model system for evolutionary studies

The vertebrate eye is one of the classical models used to demonstrate many important principles, including the concepts of inductive tissue interactions first investigated in the early 1900s. Developing imaginal discs from *Drosophila* have been described already in 1864, but the real study of the development of the compound eye started in the mid-seventieths of the 20th century (Moses, 2002). During the past 30 years there has been an explosion in the study of the fly eye. One of the most astonishing discoveries in the last years has been the molecular homology between invertebrates and vertebrates, especially the specification of the eye via the Pax6/Eyeless (Quiring et. al., 1994).

Photoreception must be phylogenetically very old. A consequence of the phylogenetic antiquity of photoreception is its near ubiquitousness in the animal kingdom. The slight variability of visual systems within a species is a sign of high selection pressure. Invertebrates have evolved a greater variety of evolutionary adaptations of their light sensitive organs than vertebrates. It is not only the huge number of species which suggests this multiformity of eye structures but also the remarkably different biotopes they occupy. Each phylum has pecularities of its own, in morphology as well as in physiology and behavior. A sedentary habit can lead to a complete absence of eyes, although some molluscs like pecten have highly developed eyes. Invertebrates have evolved adaptations that are never or only rarely found among vertebrates for example to detect the polarization of light and to orient by the pattern of polarization. As animals evolved new activity patterns, changing from the diurnal habit to a preferance for twilight and finally to life in the dark, a large number of adaptations developed. Frequently larval animals have well-developed eyes that later degenerate to a considerable extent (f.e. Ascidians). Many endoparasites completely lack eyes.

Many aspects of eye development can be investigated with the fly eye. *Drosophila* as a model system has been invaluable to elucidate eye development. A discussion of eye development without concerning results from this system seems to be impossible. However this genus lacks some developmental aspects. Arthropods precede the evolutionary appearance of neural crest, an embryonic structure from which much of the anterior of the vertebrate eye is formed. In *Drosophila* it has been estimated that more than 2500 genes may be required to construct the visual system (Gehring and Ikeo, 1999).

Note there are also a number of important eye structures that have barely been investigated, work on cornea and anterior chamber development has lagged behind lens and retina and almost nothing is known on the molecular level regarding development of the glands associated with the anterior of the vertebrate eye (see Moses, 2002).

Definition of an eye

The gradation from light-sensitive single cells through localized groups of such cells that serve as photoreceptive organs, to more complex organs with focusing devices is so continuous that it is difficult to define what an eye is. The presence of pigmentation is not a prerequisite for photoreceptors. Gehring and Ikeo (1999) propose the definition of a prototype eye: a prototype eye consists of at least two cells, a pigment cell and a photoreceptor cell carrying the visual pigment.

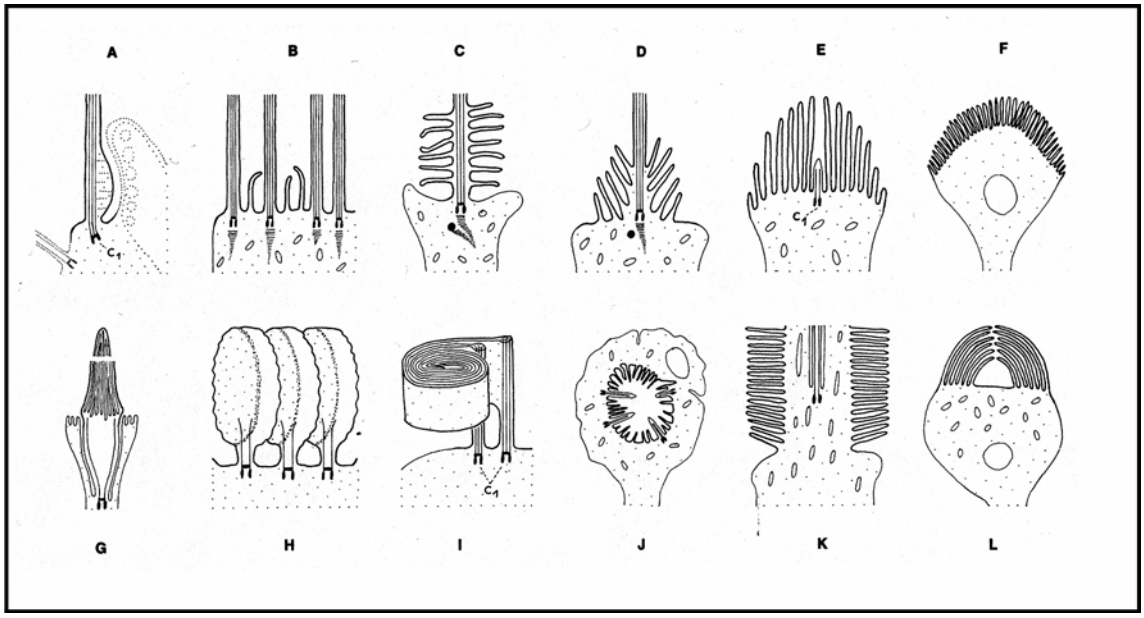

Figure 4 Different photoreceptor types from (A) Protozoa, Phytoflagellata, (B) Bryozoa, (C) Cnidaria, Hydrozoa, (D) Echinodermata, Astereoidea, (E) Mollusca, Gastropoda, (F) Cephalochordata, Branchiostoma, (G) Chaetognatha, (H) Polychaeta, (I) Placophora, (J) Annelida, Clitellata, (K) Onychophora, (L) Rotatoria. Figure modified after Salvini-Plawen and Mayr (1977).

The most basic function of a photoreceptor (Fig. 4) is to measure changes in light intensities. Two basic forms underlie photoreceptor structure: either cilia are present and the visual cell is modified in various ways, or the membrane of the visual cell is greatly enlarged by microvilli or lamellae. The visual cells of vertebrates are quite uniform. All of them contain ciliumlike structures with the typical 9+2 arrangement. In contrast, invertebrates form many different structures. These falls into two categories: (1) the ciliary photoreceptors and (2) the rhabdomeric photoreceptors. The membrane of the cilium can be enlarged in various ways: tunicates show disc-like processes of the cilium, the ocelli of sea stars are of ciliary type but from their cilia arise irregularly twisted microvilli, lamellar processes are found in the polyplacophoran mollusc *Onithochiton neglectus* while *Euglena* shows paraflagellar bodies. Micorvilli can also be arranged in a remarkable variety of ways. Ciliary and rhabdomeric photoreceptors occur side by side in *Pecten* and *Lima*.

However there exist photoreceptors that do neither contain microvilli nor cilliary structures. A great number of freshwater and marine decapod crustaceans have a paired photosensitive neuron in the sixth abdominal ganglion that lacks both optical structures. Neural photoreception is known from numerous invertebrates like the giant ganglion cells of *Aplysia* or in the metasoma of scorpions (Salvin-Plawen and Mayr, 1977). Pigment cup cells in *Branchiostoma* (Hesse's cells) are secondarily modified ganglia cells which never bear cilia. Light sensory cells of some Nematoda appear to be modified bipolar neurons.

One aspect in which the photoreceptors of vertebrates and invertebrates differ markedly is the nature and function of their receptor membrane. Vertebrate photoreceptor cells respond to light with hyperpolarization whereas invertebrate photoreceptors depolarize to light. The optical characteristics of the eye are primarily determined by whether it is used in air or water, or whether it is used under diurnal or nocturnal conditions. Lenticular structures show nearly as much diversity as receptor cells (Salvini-Plawen and Mayr, 1977).

Different eye types in the animal kingdom

Photoreceptors are present in most animal groups (see Fig. 5). Some dinoflagellates (*Erythropis*, *Liarnovia*, *Glenodinium*) are described to possess highly differentiated photosensitive structures. A number of phytoflagellates, i.e. *Euglena* (including the spermatozoids of Phaeophyceae) bear eyespots (Salvini-Plawen and Mayr, 1977). Minchin (1896) described some unicellular photoreceptors in the larvae of *Leucosolenia* (Porifera) situated in the central portion of the organism. Many endoparasitic species may have lost secondarily eye-like structures.

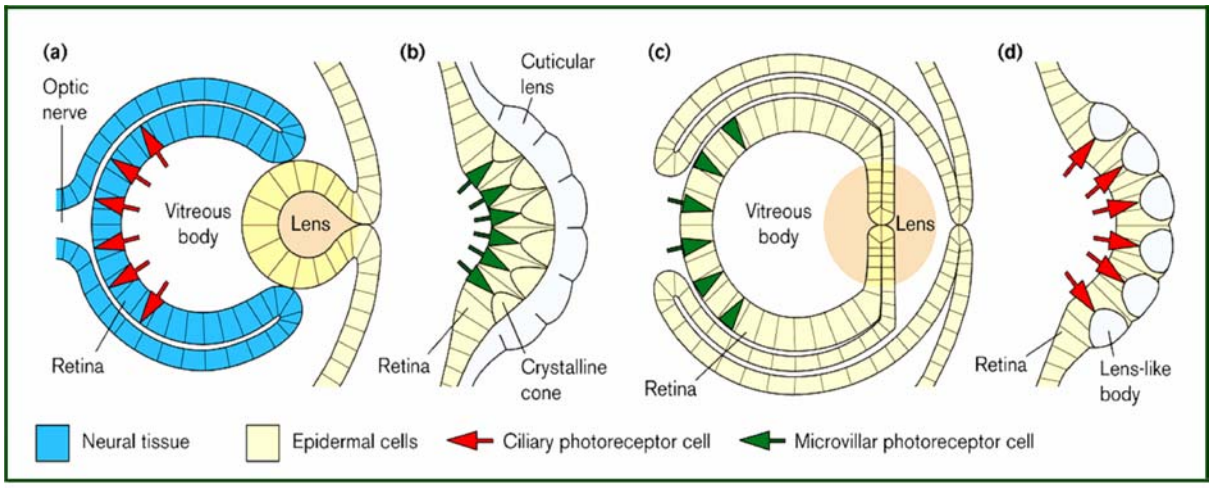

Figure 5 Building plans of four types of eye. (a) A vertebrate eye. (b) An arthropod compound eye. (c) A cephalopod lens eye. (d) A compound eye in polychaete tube-worms and arcoid clams. Note that the construction of eyes varies considerably. For example, in chordates, photoreceptor cells differentiate from the central nervous system, whereas cephalopod and arthropod eyes differentiate from the epidermis. In addition, the retina is inverse (e.g. photoreceptors are at the back of the eye) in vertebrates and everse (e.g. photoreceptors are at the front of the eye) in cephalopods. Figure taken from Fernald, 2000.

The fine structure of vertebrate eyes had been studied since the beginning of histology. Therefore the development of the vertebrate camera eye is well understood. Molluscs display the greatest diversity in the differentiation of eyes among all groups of animals. The best understood eye of invertebrates is the compound eye of *Drosophila*.

The arthropod compound eye

The compound eye of *Drosophila* contains approximately 800 individual light-sensing units called ommatidia. An ommatidium consists of 19 cells including 8 photoreceptors, 4 cone cells, 6 pigment cells and a mechanosensory bristle. The position of each cell within an ommatidium is precisely stereotyped so that each unit is an exact replica of its neighbors. Compound eyes have a single lens for each ommatidium. There are about 16000 viable cells in the adult eye. Approximately 2000 of the cells generated during eye development are

eliminated by apoptosis (Wolff and Ready, 1993) so finally there have been almost 18000 cells created during eye development. Cell death occurs in a tight band just ahead and following of the advancing morphogenetic furrow (see Fig. 6) (Wolff and Ready, 1991). The adult eye develops from the so called eye imaginal disc (monolayer epithelia), a structure which derives from about 20 cells set aside during embryonic development. This means that there is an almost 1000 fold increase in the number of cells during eye development (Neufeld and Hariharan, 2002).

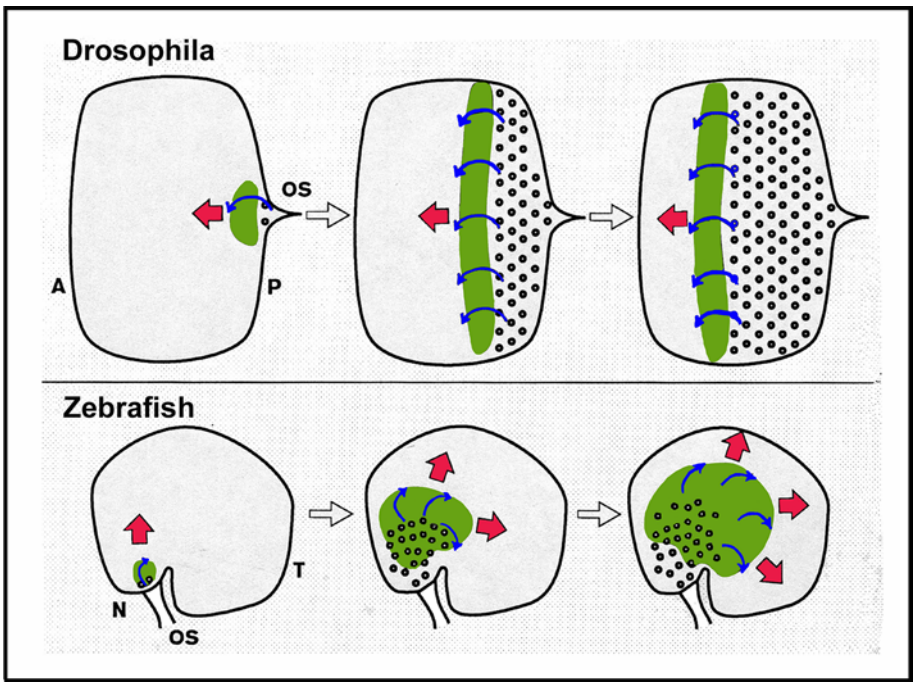

Figure 6. A wave of differentiation called the morphogenetic furrow. The way the genes are deployed in insects and vertebrates is remarkably similar. Neurogenesis in the *Drosophila* eye imaginal disc and the zebrafish inner optic cup is schematically displayed (after Jarman, 2000). Expression of atonal/ath5 (in green) precedes the appearance of initial neurons (R8 in *Drosophila*, retinal ganglion cells in zebrafish). Short-range hedgehog signaling (blue arrows), produced by newly formed neurons, appears to drive the wave of neurogenesis. Red arrows mark the direction of the wave. It remains speculative if sonic hedgehog acts via ath5 activation. A, anterior; p, posterior; N, nasal; T, temporal.

The early development of the eye imaginal disc is marked by the expression of a set of nuclear factors, the eye specification genes that will be discussed later. Eye specification does

not occur during embryonic development as previously thought, but in the second larval stage (Kumar and Moses, 2001). It is only during the second larval stage that all seven eye specification factors have overlapping expression patterns in the eye imaginal disc. The eye disc of *Drosophila* has a progressive pattern of differentiation. During embryogenesis and the first two larval instars, cells within the eye imaginal disc are unpatterened and undifferentiated. Differentiation of photoreceptors starts at the posterior margin of the eye disc and proceeds anteriorly. Prior to their differentiation cells constrict and show an apical-basal contraction that leads to an indentation which is called the morphogenetic furrow (MF; Ready et al., 1976). Anterior to this dorso-ventral groove the cells are unpatterned and divide actively. In the MF cells are arrested in the G1 phase while posterior to the MF cells undergo one round more of division, the second mitotic wave. Photoreceptor differentiation takes about 2 days. R8 cells are the first to differentiate in each ommatidium and are required for recruitment, mostly mediated via Epidermal growth factor receptor (EGFR), of other cells. R8 specification is dependent on atonal, a basic Helix-Loop-Helix (bHLH) transcription factor. Atonal null mutants lack nearly all the eye. It is expressed in a period of less than three hours within the corresponding specifying ommatidial column and controls the levels of EGFR signaling (Baker et al., 1996). EGFR is a tyrosine kinase membrane receptor and its activity leads to the RAS/MAPK signaling cascade. R8 cells require EGFR signaling only for their maintenance after the proneural gene atonal is downregulated, a function that is separable from roles in specification (Kumar et al., 1998). It has been shown that EGFR signaling is also needed to suppress programmed cell death in the eye (Bergmann et al., 1998). The receptor protein Notch plays several roles patterning the atonal expression and activated Notch expression can abolish atonal expression. On the other hand a reduced function of Notch leads to the differentiation of more than one R8 cell at the same place (Baker and Zitron 1995). EGFR and Notch signaling pathways control therefore the initiation

of the MF. It has been shown that those pathways have homeotic functions that are genetically upstream of the eye specification genes (Kumar and Moses, 2001). The complete homeotic transformation of the eye into an antenna can be induced by a hyperactivation of EGFR or a downregulation of Notch signaling (Kumar and Moses, 2001). After founding of the R8 cells development of the ommatidia seem to be self-organizing. Photoreceptor cells R2, R3, R4, R5 and R8 differentiate but those cells do not divide any more. After passing of the MF all other cells enter a synchronous round of cell division (Wolff and Ready, 1991) and differentiate into the remaining photoreceptor cells (R1, R6, R7), cone cells, pigment cells and cells of the interommatidial bristle. BarH1 is critical for the differentiation of the pigment cells (Hayashi et al., 1998). Six of the photoreceptor cells, R1-R6, extend the full depth of the retina whereas the remaining two, R7 and R8, are restricted to the upper and lower halves of each ommatidium. Each photoreceptor cells contains a specialized microvillar structure that is the site for light reception and phototransduction. The rhabdomeres are functionally equivalent to the outer segments of human rod and cones. Both R1-6 cells and rods are very sensitive to light, express a single visual pigment, and make up the majority of photoreceptor cells. By contrast, R7-8 cells and cones are less sensitive to light, express multiple visual pigments and comprise a high-acuity system. R7 and R8 cells occupy the central region of the ommatidia and are smaller in cross-sectional area. The outer photoreceptors R1-6 are mainly responsible for image formation and contain a visible light sensitive opsin, Rh1 (Papatsenko et al., 1997).

Cnidarian eye types

In general it is the medusa stage that carries eye-like structures, although polyps from all cnidarian classes are described to be light-sensitive (Tardent, 1969). One identified polyp (Stylocoronella riedli, Scyphozoa) seems to have multicellular light-detecting organs. 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 (Blumer et al., 1995). However its life cycle classifies this polyp as a member of the stauromedusae (sessile medusa). Recently a Cubozoan larva was reported to possess one-celled ocelli that even lack nerve cells (Nordström, 2003). The ocelli of the larva disappear as they settle to form polyps. Cnidarian photoreceptors range from simple eyespots and eyecups to complex eyes with a lens (Fig. 7). The number of such ocelli varies from a few to sometimes several hundreds (*Spirocodon saltatrix*). Extraocular photosensitivity is widespread throughout the cnidarians, with neurons, epithelial cells, and muscle cells mediating light detection.

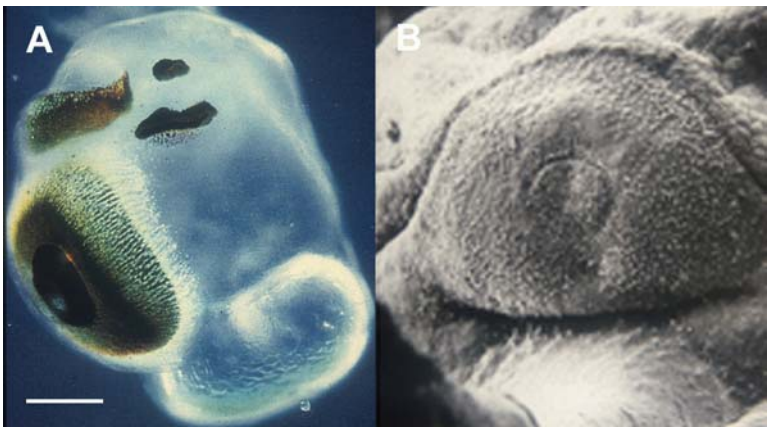

Figure 7 Cnidarian lens eyes. Stalked rhopalium of a Cubomedusa (A) and an electron microscopic picture of a hydrozoan eye. Bar is (in µm) 50 in (A), 15 in (B).

Cubomedusae have four rhopalia, each with a statocyst, two slit eyes, two pit eyes and two lens-eyes (Pearse and Pearse, 1978). The rhopalium can twist and swing back and forth.

Salvini-Plawen and Mayr (1977) argue based on morphology that cnidarian photoreceptors evolved independently in four or five different lines. All photoreceptors of jellyfish are of the ciliary type (Eakin and Westfall, 1962; Eakin, 1963) hyperpolarizing in response to light like vertebrate photoreceptors do. It has therefore been suggested that the photoreceptors of Cnidaria belong to the same evolutionary line as those of vertebrates (Eakin 1963, 1968, 1979). Photoreceptor cells of Hydrozoans are coupled to each other through gap junctions

(Singla and Weber, 1982). Such electrical coupling allows amplification of low-intensity light. Nerves and synapses operate in much the same way as those of higher animals (Mackie and Meech, 2000). Eyes seem to be directly coupled to the muscles. Photoreceptor cells have axonal contacts onto second-order neurons that group together to form an ocular nerve. Those ocular nerves enter into the main net of the animal, the nerve ring (see Fig. 9A, B). These nerve rings could be understood as the animal's central nervous system (Macki and Meech, 1995). The nerve ring neurons are large to facilitate fast transmission around the bell margin (Mackie and Meech, 1995). The precursor of the photoreceptor cells in cnidarians was probably a photosensitive ciliated ectodermal cell (Martin, 2002).

Physiological studies demonstrate that the photoreceptor cells of cnidarians respond to light intensity with graded potentials which are directly proportional to the range of changes in the light level. It has been shown that some cnidarian ocelli are most sensitive to blue-green and green light with spectral curves ranging from 363 to 675 nm (Weber, 1982). Furthermore it is believed that Cubomedusae are able to distinguish light spectra ranging from ultra-violet to deep red light (pers. communication D. Nilsson), a capacity requiring several rhodopsin types. The spectral range covered from the cubozoan eyes would be greater than from any other known animal. Behavioural experiments suggest that changes in illumination influence the movements of the animal (Hyman, 1940). From the evolutionary point of view it remains unclear why so anatomically simple structured, but certainly not primitive animals evolved sophisticated lens eyes. It remains enigmatic as the question about their quality of vision.

Cladonema radiatum is a benthic hydrozoan jellyfish that undergoes the full life cycle consisting of asexually reproducing polyp colonies liberating gonochoristic medusae. The medusa stage carries eight to twelve genuine anatomical lens eyes in the tentacle bulbs (at the margin of the bell) that derive exclusively from the ectoderm (Fig. 8).

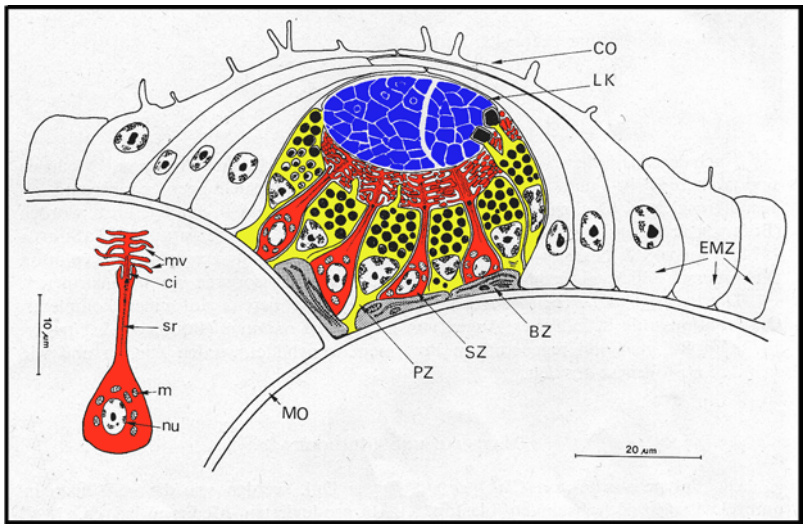

Figure 8. Schematic cross-section of a *Cladonema* lens eye. Ciliary-type photoreceptor cells are in red, melanin containing pigment cells are in yellow and the tripartite biconvex lens is in blue (modified after Weber, 1978). ci, cilium; CO, cornea; EMZ, epithelial muscle cell; LK, lens; m, mitochondria; MO, mesogloea; mv, microvilli; Nu, nucleus; PZ, pigment cell; sr, striated root; SZ, photoreceptor cell.

Structure, development and regeneration of those ocelli have been studied in detail (see Weber, 1981a, b). An ocellus has a diameter of 45-55 µm and contains a tripartite, biconvex lens. The lens body originates from the apical portion of the pigment cells whose pigmentation has been identified as melanin (Weber, 1981a, b). It is generally believed that all metazoans share the same visual pigment rhodopsin although it has never been shown to be the case for cnidarians, nor is the sequence of any cnidarian opsin available.

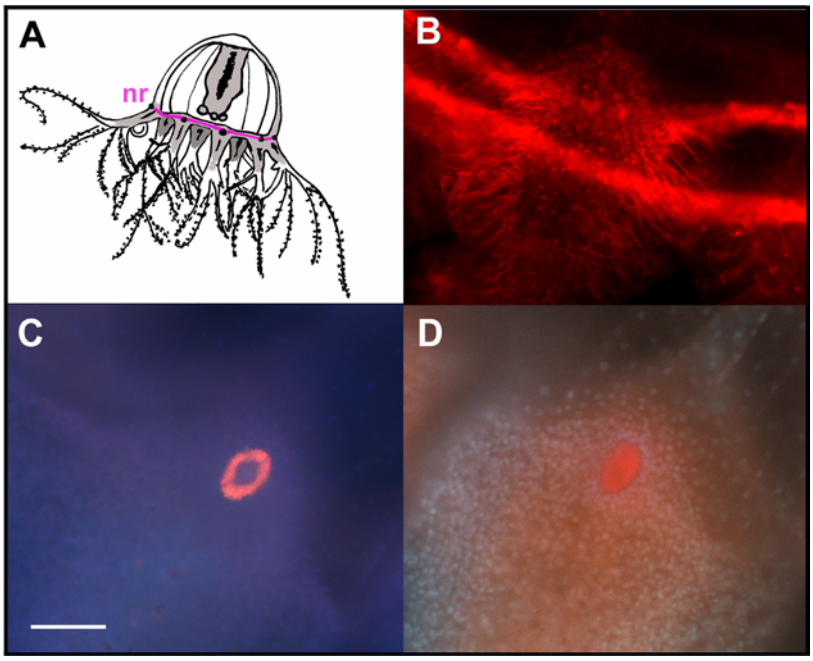

Figure 9 (A) A scheme of a *Cladonema* medusa displaying the localization of the nerve ring (in pink) along the ring canal. Tyrosine-tubulin positive nerve fibers (B) connecting the innervation of the eye to the nerve ring. (C) Cross-reaction of a monoclonal rhodopsin antibody developed against Drosophila rhodopsin 1. Note that the lens area is free of the antibody labeling (C) whereas a polyclonal squid anti-opsin antiserum stains the whole eye area (D). Nuclei are stained with DAPI. Bar is (in µm) 450 in (A), 180 in (B), 50 in (C), and 60 in (D). Immunohistology is described in chapter 1.

A *Drosophila* monoclonal antibody directed against *Drosophila* rhodopsin 1 stains specifically photoreceptor cells of *Cladonema* (Fig. 9C). Its circular staining pattern can be explained by the central position of the lens and therefore fits perfectly with the electron microscopic analysis of the eye (see Weber, 1981a). The staining pattern is specific and any artefact staining can be excluded. The specificity permits the use of this antibody as a differentiation marker. A polyclonal squid anti-opsin antiserum, originally developed against a *Loligo* eye extract, stains also specifically *Cladonema* eyes (Fig. 9D). Its crossreaction is not as strong and precise as with the insect monoclonal antibody, staining probably also pigment cells or a different opsin type. The epitope of both antibodies used is not known.

The eye specification genes

All of the master control genes are expressed anterior to the morphogenetic furrow and before the initiation of the neural differentiation in *Drosophila*. With the exception of sine oculis (so) any of those eye specification genes are sufficient to initiate the entire programme of retinal development when they are ectopically expressed. Synergistic induction of ectopic eye formation can be observed by most combinations of ectopic gene expressions. Physical interactions of the encoded proteins of these eye specification genes have been observed. Each gene of the eye specification network (Fig. 10) is absolutely required for ectopic eye induction.

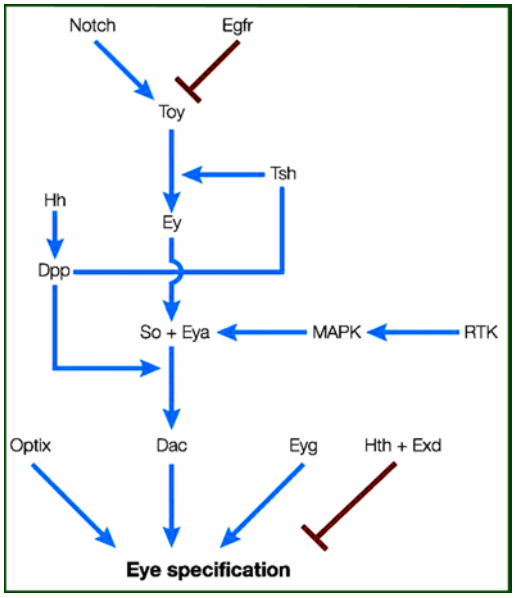

Figure 10

Genetic control of eye specification in Drosophila. A set of nuclear proteins, patterning pathways and signal-transduction cascades form a complicated regulatory network and are together required to specify the compound eye in Drosophila. The arrows indicate the direction of the genetic, molecular and biochemical relationships.

(taken from Kumar, 2001)

Removal of any of the eye specification genes results in drastic reduction or deletion of the adult compound eye and loss-of function of one gene can result in a loss of expression of another (summarized in Fig. 13). There are several feedback loops to ensure normal eye development. The genes do not function in a linear pattern but rather in a complex network of interactions that constantly cross-regulate. Transcriptional and post-translational regulation of

those eye specification genes is achieved through interactions within the network and with extracellular signaling pathways, including EGFR/RAS/MAPK, TGF-β/DPP, Wingless, Hedgehog, and Notch.

eyeless/Pax6

Pax genes are transcription factors characterized by a DNA binding motif called the paired domain. The paired domain (PD) is a stretch of 128 amino acids named after the prototypical Drosophila segment polarity gene paired in which it was first identified (Bopp et al.,1986). The PD is organized into distinct N- and C-terminal subdomains, termed PAI and RED respectively. Each subdomain consists of three alpha-helices arranged in a helix-turn-helix motif (Xu et al., 1995) and encodes a sequence-specific DNA binding activity. Both the N- and C-terminal subdomains make contact with the DNA (Xu et al., 1999). Some Pax genes contain in addition other conserved domains such as a complete or partial paired type homeodomain (HD), or an octapeptide. The octapeptide is located between the PD and the HD. The paired type homeodomain found in Pax genes is characterized by the presence of a crucial residue found at position 50, a serine (S_{50}), whereas most homeoproteins including all Hox proteins bear a glutamine at this position. In human nine different Pax genes have been identified that can be grouped into four different classes: (1) Pax1 and Pax9, (2) Pax3 and Pax7, (3) Pax4 and Pax6, (4) Pax2, Pax5 and Pax8.

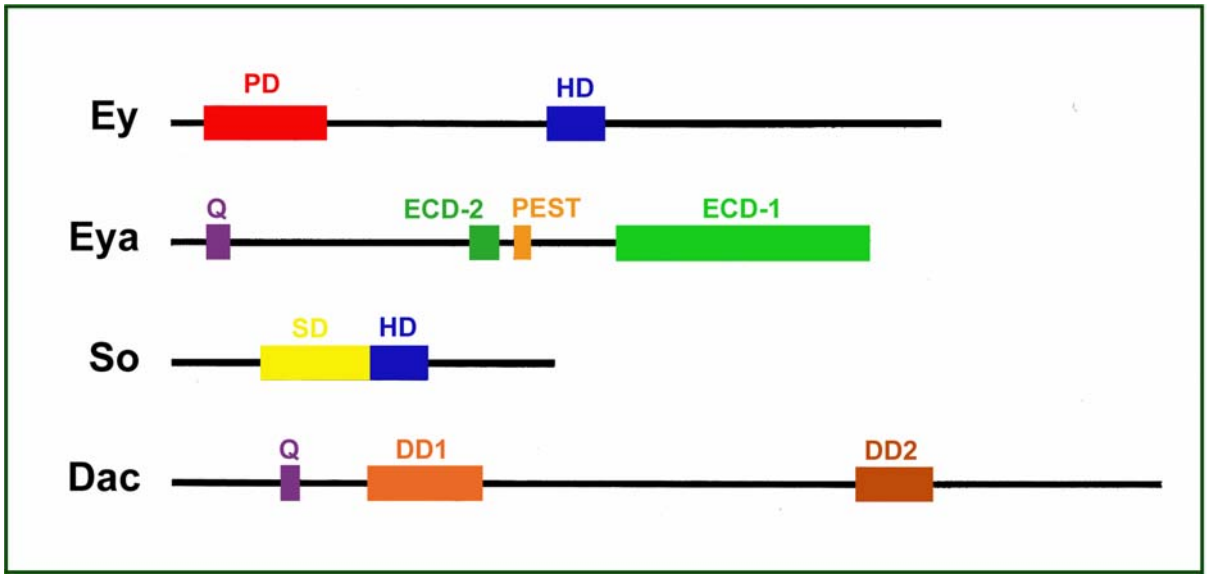

Figure 11 Predicted structures of the proteins encoded by the core eye specification genes in Drosophila. Figure modified after Kumar, 2001.

Pax6 is essential for the development of tissues including the eyes, central nervous system and endocrine glands of vertebrates and invertebrates. Pax6 takes also part in the early formation of the neural tube and the olfactory epithelium. It regulates the expression of a broad range of molecules, including transcription factors, cell adhesion and short-range cell-cell signaling molecules, hormones and structural proteins (Simpson and Price, 2002). Pax6 is involved in many biological processes like cell proliferation, migration, adhesion, signaling and oncogenesis. A number of human allelic variants of the Pax6 gene have been identified like aniridia, Peter's anomaly, keratitis, foveal hypoplasia and ectopia pupillae (Simpson and Price, 2002). But the proposed reciprocal inductive signals from presumptive lens ectoderm to presumptive retina are not dependant on Pax6 (Treisman and Lang, 2002). Initially Pax6 was identified in mouse (Walther and Gruss, 1991) but to date homologues have been isolated in a broad range of species. It is known from human, rat, chick, *xenopus*, zebrafish, ascidians, sea urchins, cephalopods, *C.elegans*, *Drosophila*, planarians. Injections of Pax6 RNA into Xenopus embryos induce optic lenses, the majority without associated neural tissue

(Chow et al., 1999). The activation of Notch signaling in Xenopus embryos causes eye duplications and proximal eye defects which are also induced by over-expression of eyeless (ey) and twin of eyeless (toy) (Onuma et al., 2002). In mice, a naturally occurring mutation in the Pax6 gene have characteristic small eye and experiments using those mutants indicate a requirement for Pax6 only in the surface ectoderm and not in the optic vesicle for lens induction (Fujiwara et al., 1994). Vertebrate Pax6 mRNA comprises 15 exons, the first four of which are noncoding (Glaser et al., 1992). Several of these exons are alternatively spliced, some of them without a PD and at least 5 different Pax6 products have been characterized in quail (Carrière et al. 1995). Pax6 may be regulated by a diverse array of factors, including retinoic acid (Hyatt et al. 1996). A series of transcriptional control elements are characterized in the Pax6 gene. Both the ectoderm enhancer (a conserved region 531 bp located 3.5 kb upstream of the first promoter) and the SIMO element (135 kb 3' to Pax6 in the last intron of the adjacent gene) mediate *Pax6* expression during the placodal phase.

The Drosophila homologues of the vertebrate Pax6 gene are toy and ey. Both are located near to each other on the fourth chromosome and share splice sites that are not found in Pax6 genes from other species (Czerny et al. 1999). It seems likely that toy and ey arose as a result of a gene duplication event during arthropod evolution (Czerny et al. 1999). Toy has to date only been described from arthropods. The early expression of toy precedes expression of ey in the embryo (Quiring et al, 1994). It has been shown that the ability of the *Drosophila* ey to induce ectopic eyes is heavily dependant upon Dpp signaling (Chen et al. 1999).

The paired domain of Ey is sufficient for its function in eye development. The role of the homeodomain is less clear. Punzo et al. (2001) showed that the eyeless homeodomain is dispensible for eye development in *Drosophila*. When ey is ectopically expressed it is the homeodomain but not the paired domain that is required to repress the antennal and leg determinant Distal-less (Dll). In human patients with aniridia or other anterior segment

defects, multiple mutations in the Pax6 paired domain have been found while only one homeodomain missense mutation is known to cause a mild eye phenotype (Hanson et al., 1999).

371 genes, mainly transcription factors involved in photoreceptor specification, signal transducers, cell adhesion molecules and proteins involved in cell division are expressed in the eye imaginal discs and up-regulated when an eye morphogenetic field is ectopically induced in the leg discs (Michaut et al., 2003). Only 40% of the genes ectopically induced by ey in the leg discs were also found to be transcribed in the eye discs.

To date no *bona fide* Pax6 homolog has been found in cnidarians (see chapter 2). A Pax6-like fragment of a paired-like HD could be isolated from *Cladonema* (see Appendix), but all attempts to elongate this fragment failed. However, several Pax genes have been characterized from Cnidaria and PaxB and/or PaxC have been suggested to play the ancestral role of a Pax6 in this phylum (Miller et al., 2000; Kozmik et al., 2003).

Sine oculis / Six genes

Six proteins are characterized by a Six domain and the Six-type homeodomain (see chapter1). Six genes have been characterized from various different phyla. *Drosphila sine oculis (so)* is required for the development of the entire visual system with its main function in the establishment of the MF (Cheyette et al., 1994). It was shown to be a direct target for both ey and toy (Punzo et al., 2002). The *Drosophila* orthologous gene for mouse Six3 is *optix*, which is involved in eye morphogenesis by an ey-independent mechanism (Seimiya, 2000). The vertebrate six gene responsible for eye development is *Six3/6*. Six genes are involved in several genetic diseases in humans like holoprosencephaly, anophthalmia and myotonic dystrophy. Recently it has been reported that Six1 has an essential role in determining the

metastatic fate of rhabdomyosarcoma, the most common soft-tissue sarcoma in children (Yu et al., 2004).

Three different Six genes could have been identified from *Cladonema* and two different six genes from *Podocoryne*. These are the only known Six genes from cnidarians.

Eyes absent

Eya family members are defined by a conserved 275 aa motif referred to as the Eya domain. The Drosophila *eya* gene, which is also termed *clift*, is required for the survival of eye progenitor cells at a critical stage in morphogenesis (Bonini, 1993). Ectopic expression of eya together with ey is more effective in eye formation and additionally can occur on genitalia, a condition which has never been observed when either gene is ectopically expressed alone (Bonini, 1997). Clift has been identified as a regulator of Drosophila gonadogenesis, it determinates somatic gonadal precursor cells. Later it turned out that clift and eya are identical. To date target genes of *eya/clift* are not discovered and therefore it remains open, if this gene unifies a role in eye and mesoderm.

Ectopic eyeless expression in the context of eyes absent or sine oculis mutations results in apoptosis (Clark et al., 2002). Mutations in EYA1 are responsible for cataracts and anterior segments defects, branchiootic syndrome and branchio-oto-renal syndrome (Abdelhak et al., 1997). There is an amino acid sequence similarity between the Eya domains and enzymes of the halocid dehalogenase (HAD) superfamily. This family includes a class of dephosphorylating enzymes (phosphatases) of which some remove phosphate groups specifically from serine amino acids in target proteins.

Eya protein is a tyrosine phosphatase (Tootle et al., 2003). Eya3 can dephosphorylate RNA polymerase II and has also an autocatalytical dephosporylation activity (Li et al., 2003). It is speculated that Eya3's phosphatase activity is required to switch Dach1 from a repressor to

an activator. Eya has probably phosphatase-dependent and –independent biological activities (Tootle et al., 2003). It is the first example of a transcription factor with intrinsic phosphatase activity and it is suggested to represent a method for fine-tuning transcriptional regulation (Tootle et al., 2003).

To date no eya homolog has been reported from Cnidaria. The hunt for eya in *Cladonema* was not successful although an antibody cross-reaction with a monoclonal *Drosophila* anti-eya antibody could have been observed (Fig. 12B). Labeling is restricted to cells in the manubrium, the feeding and sex organ. The staining protocol was performed according to the one described in chapter 1. It remains unclear if *Cladonema* does posses an Eya homolog and if the obtained labeling pattern of the cross-reaction is specific or not.

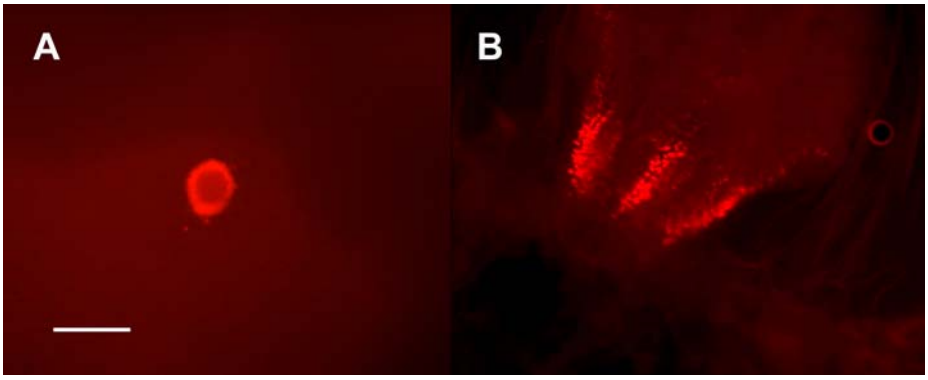

Figure 12 Immunohistology displaying the cross-reaction of a monoclonal antibody developed against *Drosophila* Dac (A) and of a monoclonal antibody developed against *Drosophila* Eya (B) with *Cladonema radiatum*. The labeling obtained with the anti-dac antibody seems to be restricted to the eye whereas anti-eya labels cells located close to the manubrium lips. Bar is (in µm) 65 in (A) and 100 in (B).

Dachshund

The fly mutant phenotype of dachshund has extremely short legs in relation to its body length and therefore inspired for the name. Dachshund was originally identified as a dominant suppressor of a mutation of the epidermal growth factor receptor *Ellipse*. *Drosophila*

dachshund is necessary and sufficient for comopound eye development and is required for normal leg and brain development (Davis et al., 2001). Dac function is required for the normal movement of the MF. In dachshund mutants cells fail to adopt a neural fate and remain in an undifferentiated state and die eventually (Mardon, 1994). Dac may be a direct target of ey. Remarkably, the external morphology of the adult ocelli in Drosophila appear normal in all dachshund mutants (Mardon, 1994). It is differentially expressed in the male and female genital discs, and plays sex-specific roles in the development of the genitalia. (Keisman and Baker, 2001). Three zebrafish dac homologues have been characterized (Hammond et al., 2002). All three are expressed in sensory organs, the central nervous system and pectoral fin buds. Its expression overlaps extensively with those of zebrafish pax, eya and six family members. A mouse homologue is expressed in the developing retina and limbs, suggesting functional conservation. Homozygous mouse mutants survive to birth but exhibit postnatal lethality associated with a failure to suckle, cyanosis and respiratory distress (Davis et al., 2001). Histological examination of the eyes reveals no abnormalities in these mice. The DACH protein exhibits two domains (DD1 and DD2) highly conserved from *Drosophila* to human, although the function of these two domains is unknown. The amino-terminal domain DD1 has approximately 35% amino acid identity to the Ski/Sno family of oncoproteins (Ayres et al., 2001). No targets of Dach1 transcriptional activity have been identified in vertebrates and the regulation of Dach1 expression by growth factors has not yet been characterized. The mouse DACH1 protein was detected in several organs in which epithelial/mesenchymal interactions are known to be important in patterning and cell fate determination, including the developing kidneys, eyes, limb buds (Ayres et al., 2001). DACH1 protein and message was detected in cells of the optic cup and in some of the mesenchymal cells surrounding the eye (Ayres et al., 2001). The expression of mouse Dach2 suggests a partially redundant role of the dach genes (Davis et al., 2001). The expression of

Dach2 in the forebrain of Pax6 mutants and in dermamyotome mutants of Pax3 is not detectably altered (Davis et al., 2001).

Ayres et al. (2001) found that DACH1 is expressed in association with other retina determination genes in the developing mammalian eyes, inner ears, limbs, and kidneys. No mutations in human DACH contributing to human disorders have been identified (Ayres et al., 2001). Human DACH maps to a chromosome region that has been associated with digital abnormalies. DACH represents an attractive candidate gene for limb malformations because it is expressed in the distal limb bud during digital patterning (Ayres et al., 2001). Dach1 in mice acts as a corepressor and as a co-activator of Six1 and Eya proteins (Li et al., 2003).

There is no cnidarian Dach homolog identified yet but preliminary data indicate, that Cnidaria seem to posses a dachshund gene (not shown). A monoclonal *Drosophila* anti-dac antibody cross-reacted specifically with the *Cladonema* eye (Fig. 12A).

Teashirt

Teashirt encodes a transcription factor with zinc finger motifs and it was originally identified for the specification of the trunk segments in *Drosophila.* The targeted expression of teashirt in imaginal discs is sufficient to induce ectopic eye formation in non-eye tissues and teashirt and ey induce the expression of each other (Pan et Rubin, 1998). It is suggested that teashirt acts upstream of eya, so and dac in ectopic eye development (Pan et Rubin, 1998). Nothing is known about a teashirt homolog from Cnidaria.

Eyegone

Eyegone, originally called Lune, is a Pax protein but contains only a partial PD, with an incomplete PAI subdomain and a complete RED subdomain. The linker region between PAI

and RED is most closely related to Pax2, Pax5, Pax8 but its helix-turn-helix region is most related to Pax6. It contains a paired-class HD with a characteristic serine at position 50. Eyg promotes eye development primarily by repressing wingless. Both eya and eyg are required for the activation of dpp in the retinal tissue. Eyg seems to be involved in growth and specification of the fly eye independently of ey (Dominguez, 2004). It regulates probably different target genes than those regulated by ey but it was speculated that eyg and ey could form heterodimers via their HD. In vertebrates no homolog of eyg has yet been identified. It has been speculated that eyg probably plays a role equivalent to the vertebrate Pax6-5 isoform (Jang et al., 2003). No eyg homolog has been identified yet from Cnidaria.

Gene	Loss of function	Gain of function
twin of eyeless (*toy*)	Not reported	Strong ectopic eye induction
eyeless (*ey*)	Reduced or no eyes	Strong ectopic eye induction
eyes absent (*eya*)	Reduced or no eyes	Weak ectopic eye induction
sine oculis (*so*)	Reduced or no eyes	No phenotype
dachshund (*dac*)	Reduced or no eyes	Weak ectopic eye induction
teashirt (*tea*)	No phenotype in the eye	Weak ectopic eye induction
optix (*optx*)	Not reported	Weak ectopic eye induction
eyegone (*eyg*)	Reduced or no eyes	Ectopic PRs in the eye disk
homothorax (*hth*)	Ectopic PRs in eye disk clones	Loss of PRs
extradenticle (*exd*)	Ectopic PRs in eye disk clones	Loss of PRs

Figure 13 *Drosophila* eye specification genes and results of their loss of function and gain of function experiments. Figure taken from Kumar and Moses, 2001.

Secreted factors required for eye development in *Drosophila*

The secreted factors encoded by hedgehog (hh), dpp and wingless (wg) are required for normal development of the Drosophila eye but these genes do not specify cell fate directly. The ectopic expression of those genes does not change the imaginal disc fate but their misexpression causes pattern duplication.

Hedgehog

Members of the Hedgehog family are key mediators of many fundamental processes in embryonic development and their activities are central to the growth, patterning and morphogenesis of many different regions within the body plans of vertebrates and insects. In *Drosophila* hh is a central patterning signal in the wing and eye discs as well as regulating several other processes like germ cell migration, development of the optic lamina and gonad, abdomen and tracheal system (reviewed in Ingham and McMahon, 2001). *Drosophila* has only one hh gene, there are several related genes in vertebrate species. One notable exception is the nematode *C. elegans*, which has no hh ortholog but does possess several genes encoding proteins homologous to the hh receptor Patched (Kuwabara et al., 2000). The vertebrate Sonic hedgehog (Shh) is involved in the separation of the eye fields and the formation of the optic stalk (Chiang et al., 1996; Perron et al., 2003). A wave of sonic hedgehog (Shh) patterns the zebrafish retina, as in the fly eye.

The intensive hunt for an hh-homolog from *Cladonema* was not successful. No cnidarian hedgehog homolog is known although previously believed differently.

Decapentaplegic

In the fly decapentaplegic (dpp) is responsible for the dorsal/ventral polarity, for the definition of boundaries between segmental compartments, between appendage compartments assuring correct anterior/posterior polarity and functions analogous in the development of the eye. In the eye it is primarily responsible for the progression of the MF. Bmp4 has been identified as a potential lens inducer (Furuta and Hogan, 1998) by regulating the expression of Sox2, a transcription factor that has been implicated in the regulation of crystallin genes. Pax6 and Sox2 form a complex that can regulate δ-crystallin gene

expression in the chick (Kamachi et al., 2001). Bmp4 regulates early differentiation in the lens lineage and Bmp7 null mice have eye defects ranging from microophthalmia to anophthalmia. Bmp and fibroblast growth factor (Fgf) signaling pathways cooperate in some way. Fgf receptor and Bmp7 signaling probably combine upstream of the placodal phase of Pax6 expression in a genetic pathway defining lens induction.

Dpp homologs and different members of the BMP/TGF-β family have been identified from Cnidaria. Although a Dpp as well as a BMP 5-8 homolog could have been identified in *Podocoryne*, the *Cladonema* representatives are still unknown.

Wingless

Like several other pathways wingless (wg) signaling has several functions in eye development. In Drosophila wg signaling establishes the border between the retina and adjacent head structures by inhibiting the expression of the eye specification genes eya, so, dac (Baonza and Freeman, 2002). Ectopic wg signaling leads to a repression of these genes and the loss of eyes (Baonza and Freeman, 2002). Wnt proteins function by binding to seven-pass transmembrane receptors belonging to the frizzled family. Wnts acitvate frizzled receptors by binding to the cysteine-rich extracellular domain of the receptor. The frizzled signaling is both necessary and sufficient to regulate eye development in Xenopus (Rasmussen et al., 2001). These findings demonstrate a requirement for wnt/frizzled signaling in regulating vertebrate eye development. Stump et al. (2003) report a role of Wnt signaling in lens epithelial differentiaion.

Wnt signaling has been reported from hydra (Hobmayer et al., 2000) and identification of Wnt from *Cladonema* has been successful (not shown).

Crystallins

Crystallins are soluble proteins in eye lenses, which play an important role in the maintenance of lens transparency, optical clarity and refractive index. They are essentially defined by their abundance, collectively 80-90% of the water-soluble proteins in the lens (Piatigorsky et al., 2001). In terrestrial vertebrates about one third of refraction is done by the lens whereas it accounts for all the refraction in aquatic vertebrates (Tomarev and Piatigorsky, 1996). Lens crystallins represent a surprisingly diverse group of multifunctional proteins and some display taxon-specificity. In general, vertebrate crystallins have been recruited from stress-protective proteins, like heat-shock proteins, and a number of metabolic enzymes (Tomarev and Piatigorsky, 1996). The crystallins (α and βγ crystallins) that show sequence similarity to small heat-shock proteins of *Drosophila*, are ubiquitously used in vertebrates, and must therefore have occurred in a common ancestor and be quite ancient (Janssens and Gehring, 1999). As different visual systems became more elaborate the more recent taxon specific crystallins must have arisen. All invertebrate crystallins examined so far are different and novel proteins. A stress protective metabolic enzyme, glutathione S-transferase (GST) seems to provide the major cephalopod crystallins. GSTs share sequence motifs with the γ subunit of the eukaryotic elongation factor 1 (EF1γ). Cephalopods have at least two taxon-specific crystallins related to aldehyde dehydrogenase and related to a superfamily of lipid-binding proteins.

In the acellular corneal lens of *Drosophila* three calcium binding taxon-specific crystallins have been found, while antigen 3G6 is a highly conserved protein present in the ommatidial crystallin cone and central nervous system of numerous arthropods (Tomarev and Piatigorsky, 1996). Drosocrystallin is a secreted protein that shows sequence similarities to some insect cuticular proteins. It is expressed in the brain as well as in the ommatidia and it is therefore likely that it serves for an additional function. There are suggestions that crystallins

of compound eyes of arthropods are expressed outside of the lens. Aldehyde dehydrogenase is an example of an enzyme-crystallin that is used by both the vertebrates and invertebrates (Piatigorsky et al., 2000). The gene sharing strategy to use multifunctional proteins for refraction may have occurred in invertebrates as it did in invertebrates (Tomarev and Piatigorsky, 1996).

A rabbit antiserum from a soluble protein of the bovine lens was able to produce immunofluorescence specifically in the lens of *Cladonema radiatum* (Weber, 1981). It is possible that the immunofluorescence was due to one or more common epitopes between jellyfish and vertebrate crystallins. A lens crystallin of the cubomedusa *Tripedalia cystophora* has been identified and shows sequence similarity to vertebrate saposins (Piatigorsky et al., 2001). Its message was detected in embryonic and larval stages as well as in the rhopalia. A GST has been characterized from *Cladonema* and its expression analysed to verify a role as a crystallin in jellyfish. However, GST from *Cladonema* is expressed in the manubrium but not in the lens (Fig. 14). The existence of a βγ-crystallin-type gene from the sponge Geodia cydonium has also been reported (Di Maro et al., 2002).

In situ hybridization with an anti-sense GST Dig RNA probe. The staining is restricted to the upper part of the manubrium, mainly the gonads (arrow). The presence of message in the eyes is too weak as it could play a role of a possible lens crystallin in jellyfish. Bar is 450 μm.

Figure 14

Phototransduction

Phototransduction is one of the fastest known G protein-coupled signaling cascades. In *Drosophila* exposure of the photoreceptor cells to light leads to activation of the light-induced cation influx channels, transient receptor potential and transient receptor potential-like within 20ms. In less than 100ms after cessation of the light stimulus the deactivation of the cascade is completed (Ranganathan et al., 1991). Recent studies have provided evidence that many of the components functioning in *Drosophila* phototransduction form a supramolecular signaling complex, consisting of a minimum of seven signaling proteins bound to scaffold protein referred to as INAD. These include rhodopsin, phospholipase C-β, protein kinase C, calmodulin, the myosin III NINAC, and two light-sensitive ion channels, TRP and TRPL. More than 40 genes function in phototransduction. *Drosophila* and vertebrate phototransduction have some notable similarities and differences. Both *Drosophila* and vertebrate visual transduction are initiated by the light-induced isomerization of the photopigment rhodopsin and subsequent interaction with the heterotrimeric G protein. The effector for the *Drosophila* G protein is a PLC, which catalyzes PIP2 to IP3 and DAG and the activation of the PLC leads to a Na and Ca influx as a result of the opening of the cation influx channels. In contrast, the effector for the G protein in vertebrates is a phosphodiesterase, which hydrolyzes cGMP to GMP and as a consequence closes the cGMP-gated ion channels and hyperpolarizes photoreceptor cells by termination of the Na and Ca influx (Montell, 1999).

Rhodopsin consists of two components: a protein containing seven transmembrane segments (opsin) and a chromophore, typically 11-cis-retinal, which is covalently attached to a lysine residue in the seventh transmembrane domain (Montell, 1999). Retinal is the molecule transducing light energy into electrical signals and opsin is the covalently bound protein carrier. Exposure to light results in conversion from the cis to the trans configuration in the

chromophore, the only step in phototransduction that is directly regulated by light. This cis-trans isomerization causes a conformational change in the opsin moiety. A major difference in the rhodopsin cycle between fly and vertebrates is the regeneration of rhodopsin.

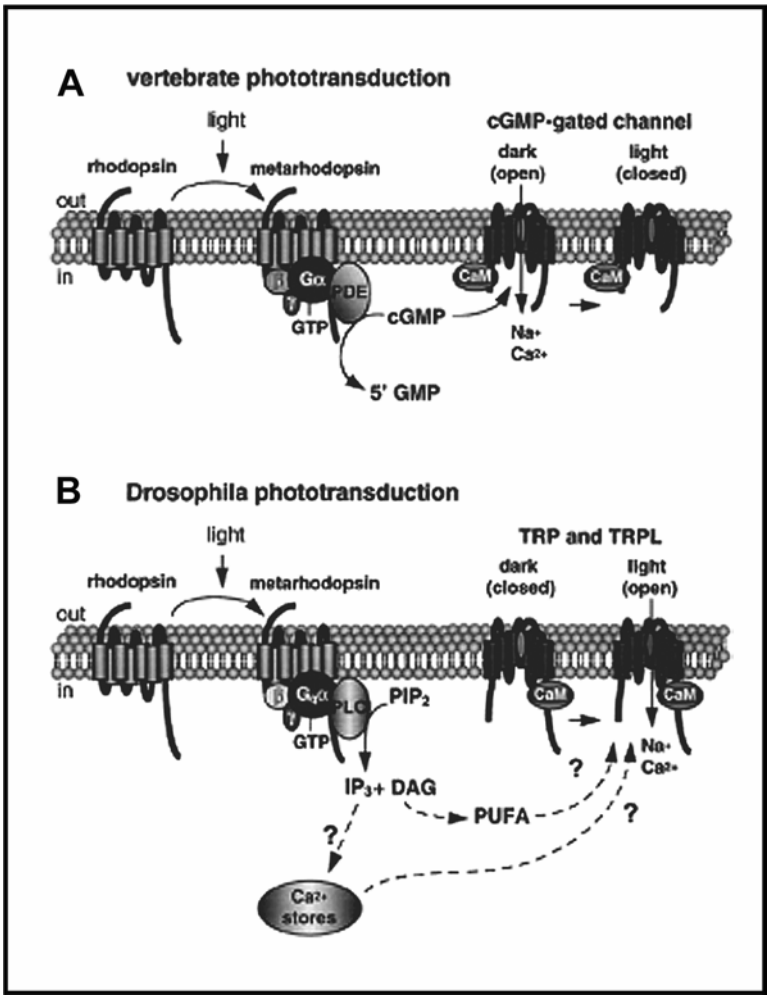

Figure 15. A scheme comparing vertebrate and *Drosophila* phototransduction (taken from Montell, 1999).

Following exposure to light, the chromophore in *Drosophila* photoreceptor cells does not dissociate from the opsin whereas vertebrate opsin and 11-cis retinal dissociate after arrestin binding. Vertebrate opsins fall into five fundamental subfamilies. It is speculated that the phototransduction proteins seem to have co-evolved as a system (Hisatomi and Tokunaga, 2002). Many vertebrates have duplicate photoreceptor type cells, rods and cones, responsible for twilight and daylight vision respectively (Hisatomi and Tokunaga, 2002). Phototransductory molecules such as opsins and arrestins are directly regulated by Otx- and Pax6 transcription factors (Kimura et al., 2000).

Drosophila and vertebrate phototransduction results in opposite effects on the ion channels, opening versus closing (Fig. 15). But both cascades share several features like sensitivity over a vast range of light intensities, high speed and temporal resolution, and enormous signal amplification.

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