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Evolution of eyes and photoreceptor cell types

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ABSTRACT The evolution of the eye is a matter of debate ever since Darwin's *Origin of Species*. While morphological comparisons of eye anatomy and photoreceptor cell types led to the view that animal eyes evolved multiple times independently, the molecular conservation of the *pax6* eye-specifying cascade has indicated the contrary - that animal eyes evolved from a common, simple precursor, the proto-eye. Morphological and molecular comparative approaches are combined here in a novel Evo-Devo approach, the molecular comparison of cell types ("comparative molecular cell biology"). In the eye, the various types of photoreceptor cells, as well as pigment and lens cells, each require distinct combinations of specifying transcription factors that control their particular differentiation programmes, such as opsin expression in photoreceptors, specific neurotransmitter metabolism, or axonal outgrowth. Comparing the molecular combinatorial codes of cell types of animal extant eyes, their evolutionary histories can be reconstructed. This is exemplified here on the evolution of ciliary and rhabdomeric photoreceptor cells in bilaterian eyes and on the evolution of cell type diversity in the vertebrate retina. I propose that the retinal ganglion, amacrine and horizontal cells are evolutionary sister cell types that evolved from a common rhabdomeric photoreceptor cell precursor.

KEY WORDS: *eye, evolution, opsin, photoreceptor, retinal ganglion cell*

The quest for the proto-eye

The evolution of eyes remains a tantalising topic for the same reason that had already enthused Darwin, who found it hard to explain "that natural selection could produce … an organ so wonderful as the eye"(Darwin, 1859). What was the most ancient precursor of eyes – the 'proto-eye' (Pichaud and Desplan, 2002) – and when did it emerge on the animal evolutionary tree? What was its initial structure and function? Gehring and Ikeo have suggested a two-celled proto-eye made up of one photoreceptor cell and one pigment cell (Gehring and Ikeo, 1999), resembling the two-celled eyes that exist in today's primary ciliary larvae such as the polychaete trochophore (Fig. 1A) (Arendt *et al.*, 2002). Such very simple eye could have accomplished some primitive form of vision by detecting the direction of light for phototaxis. Also, it could have entrained a primitive circadian clock (Gehring and Rosbash, 2003). If such proto-eye existed – how did it evolve to the enormous complexity seen for example in the vertebrate camera eye?

The proto-eye can be reconstructed by the structural and molecular comparison of extant eyes such as the insect compound eye, the vertebrate camera eye, and the simple pigment-cup eyes found in many invertebrate groups (with photoreceptors embedded in a cup-shaped layer of shaded pigment; Fig. 1C). (For recent overviews of eye types see Fernald, 2000; Arendt and Wittbrodt, 2001). What characteristics do the diverse types of eyes share so specifically that this is most plausibly explained by common ancestry? Structures that trace back to a common precursor structure in the last common ancestor of the compared groups are referred to as homologous.

The classical units of morphological comparison in homology research are entire organs or bits of organs (such as skulls or their precisely defined bones). For animal eyes, this means that anatomical units have been compared such as lenses, retinae and irises in vertebrates, and ommatidia in insects, and in light of the vast anatomical differences it was concluded that these parts, as well as the eyes they constitute, should be non-homologous (Nilsson, 1996; Fernald, 1997). This view, however, has been challenged by the astounding, and apparently conserved capacity of *pax6* to act as a 'master control gene' of eye development (Quiring *et al.*, 1994; Halder *et al.*, 1995), and, meanwhile, by a wealth of additional molecular similarities (reviewed in e.g., Gehring and Ikeo, 1999; Pineda *et al.*, 2000; Wawersik and Maas, 2000; Fernald, 2000; Arendt and Wittbrodt, 2001; Kumar and Moses, 2001; Pichaud and Desplan, 2002).

This review aims to illustrate how the morphological and molecular approach can be combined, and reconciled, by focusing on the cell type as the main unit of reference in eye homology research. A *cell type* is *a homogenous population of cells express-*

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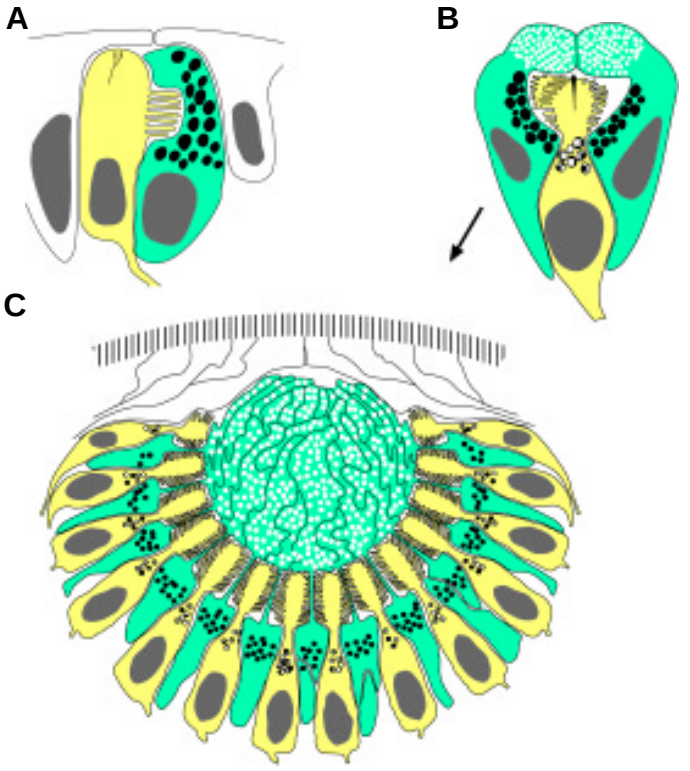

Fig. 1. Two-celled larval eye and prototype pigment-cup eye with rhabdomeric photoreceptors in *Platynereis dumerilii* (Polychaeta, Annelida, Lophotrochozoa). *Ultrastructure of* **(A)** *larval (24 h),* **(B)** *adult (72 h) and* **(C)** *fully grown eyes. Redrawn from EM micrographs (A,B; data not shown) and (C) after (Fischer and Brökelmann, 1966). Yellow, rhabdomeric photoreceptor cells; green, pigment cells.*

ing the same set of orthologous genes for specification and differentiation, to implement a defined cellular phenotype. Examples are the rods and cones and the various other cell types of the vertebrate retina; or R1-8 photoreceptors and other cell types that make up the insect ommatidum. So far, cell types of animal eyes were merely compared on morphological grounds (Salvini-Plawen and Mayr, 1977). We can now compare the cell types that make up the eye, most important the photoreceptor cells proper, on the molecular level, and by this gain insight into their evolutionary history. This approach is referred to as **comparative molecular cell biology**.

A molecular combinatorial code for cell type specification

It is now well established that in the entire nervous system cell-type specific differentiation depends on the expression of specific combinations of transcription factors, largely of the bHLH and homeodomain superfamilies, in the insect CNS (Bossing *et al.*, 1996; Schmidt *et al.*, 1997) and brain (Urbach and Technau, 2003), as well as in the vertebrate neural tube (Jessell, 2000; Andrews *et al.*, 2003) and brain (Wilson and Rubenstein, 2000). This combinatorial code of transcription factors also defines cell types of the insect ommatidium (Kumar and Moses, 1997; Frankfort and Mardon, 2002; Hsiung and Moses, 2002; Cook *et al.*, 2003) and the cell types of the vertebrate retina (Jean *et al.*, 1998; Cepko, 1999; Livesey and Cepko, 2001; Marquardt *et al.*, 2001; De Melo *et al.*, 2003). In the differentiating cell, the specific combination of transcription factors regulate cell-type specific differentiation programmes controlling cellular morphology, axonal outgrowth, the expression of effector molecules such as opsins or other receptors, neurotransmitter-synthesising enzymes, secreted hormones, ion channels, etc.

Homologous cell types and sister cell types

Molecular comparative cell biology explores the combinatorial code of gene expression to compare the cell types of a given species, (1) among themselves, and (2) to those of other closely, or even distantly related species. Comparing within a given species, some cell types will differ in their molecular characteristics only slightly, and will exhibit similar (but not identical) cellular phenotypes. Such similarities are indicative of a common evolutionary history, meaning that some ancestor of that species had a single precursor cell type that subsequently diversified into these cell types. I refer to the descendant cell types as 'sister cell types', defined as follows: *Sister cell types evolve from one common precursor by cell type diversification*. The rods and cones in the retina of a given vertebrate species are a good example for sister cell types because it is generally assumed that they have evolved from a common ciliary photoreceptor precursor that diversified in some vertebrate ancestor.

The molecular characteristics of cell types also reveal the genealogical interrelationships of cell types *between* species, and even between remote groups. Comparing across the evolutionary tree, *homologous cell types* are those that *have evolved from the same precursor cell type in the last common ancestor of the compared groups.* For example, the rods of the fish retina, and the rods of the mammalian retina, are considered homologous cell types (e.g., Meyer-Rochow and Stewart, 1996). (Notably, the rods of the fish retina, and the cones of the mammalian retina, are non-homologous, because they do not trace back to the very same precursor cell type in the last common ancestor of fishes and mammals).

The criteria to identify sister cell types in a given species, and homologous cell types between species, are necessarily the same: (1) deployment of similar combinations of orthologous transcription factors for cell type specification, (2) deployment of orthologous effector genes at differentiation stages, and (3) similar cellular morphologies reflecting the molecular resemblances. It must be stressed, however, that neither sister cell types, nor homologous cell types, should be completely identical – but for different reasons: As to sister cell types, molecular and morphological differences should reflect the cell type divergence that brought them into existence. In the case of homologous cell types, differences between them should reflect the evolutionary divergence of the species compared (making homologous cell types distinct even if the common precursor cell type did not diversify in any of the descending lines).

Pioneer studies compared the molecular combinatorial code of motor neurons between vertebrates and *Drosophila* and revealed a number of apparently homologous cell types (Thor *et al.*, 1999; Shirasaki and Pfaff, 2002), and a similar comparative cellular approach can be conducted now on the various cell types that make up bilaterian eyes. This review will focus on the emergence of ciliary and rhabdomeric photoreceptor sister cell types, on the

possible homology of invertebrate rhabdomeric photoreceptor cells and the vertebrate retinal ganglion cells, and on sister cell type relationships within the vertebrate retina.

Homology of animal photoreceptor cells

The prevalence of pigment-cup eyes in Bilateria, and their stereotype, simple design, tells us that eyes started off with merely two cell types, photoreceptor cells that associated with pigment cells to detect the direction of light (Arendt and Wittbrodt, 2001). Additional cell types were added during subsequent eye evolution, such as lens cells, various kinds of support cells, muscle cells etc. that also formed part of the eyes. Cell type diversity reached its maximum in the vertebrate and cephalopod camera eye, as well as in the arthropod compound eye. Thus, comparing bilaterian eyes at the cell-type level, the first central issue is whether their photoreceptor cells are homologous according to the above criteria for cell type homology.

On the molecular level it is long known that all eye photoreceptor cells so far described use a vitamin-A-based light-sensitive photopigment, comprising a chromophore and an apoprotein, opsin. Phylogenetic analysis approves that all opsins trace back to one opsin precursor molecule that predated bilaterians. Phototransduction always requires the binding of photoactivated opsin to the alpha subunit of a G-protein. Subsequent quenching of phototransduction cascades also employs similar molecular mechanisms, involving rhodopsin kinase that phosphorylates the photoactivated opsin and arrestin that competes with the alpha subunit of the G-protein for binding to opsin (e.g. Krupnick *et al.*, 1997).

At the level of the specifying transcription factors, developing animal eyes in a wide range of groups share an at least early involvement of *pax6*, and this can best be understood as the reflection of a very ancient *pax6* requirement for the specification of a pre-bilaterian photoreceptor cell precursor (Gehring and Ikeo, 1999; Pichaud and Desplan, 2002). In the vertebrates, *pax6* is required for the formation of virtually all retinal cell types (Marquardt *et al.*, 2001) (that might have collectively evolved from photoreceptive precursors, as will be outlined in more detail below, see Fig. 4). In *Drosophila*, the *pax6* orthologs *eyeless* and *eye gone* are required for the formation of the entire eye disc (Jang *et al.*, 2003), which gives rise to all ommatidial cell types including the photoreceptor cells. *Eyeless* and another *pax6* ortholog*, twin of eyeless* are also expressed in precursor cells of the photoreceptive Bolwig organ and ocelli (Czerny *et al.*, 1999), and in the late Bolwig organ (Sheng *et al.*, 1997). In line with a general affiliation with photoreceptor cell specification, *pax6* expression also covers the early eye anlagen in cephalopods (Tomarev *et al.*, 1997; Hartmann *et al.*, 2003), planarians (Callaerts *et al.*, 1999) (Salo *et al.*, 2002), nemertines (Loosli *et al.*, 1996), and polychaetes (Arendt *et al.*, 2002). However, and even if *pax6* started off as an early photoreceptor specification gene in pre-bilaterians, in none of the species investigated is *pax6* photoreceptor cell-specific, or even eye-specific (Simpson and Price, 2002). This means that the ancestral function of *pax6* in cell type specification or differentiation (whatever it was) is not exclusively required in photoreceptor cells. It is also clear that in few cases photoreceptor cells can form in the complete absence of *pax6*, such as the Hesse eyecups in *Branchiostoma* (Glardon *et al.*, 1998).

The *orthodenticle/otx* genes play an equally conserved role in eye and photoreceptor cell development. In the vertebrates, *otx* orthologs are required for the formation of many, if not all, retinal cell types: *crx, otx2* for rod and cone photoreceptors and bipolar cells (Chen *et al.*, 1997; Furukawa *et al.*, 1997a; Furukawa *et al.*, 1997b; Viczian *et al.*, 2003) and *otx1* for retinal ganglion and amacrine cells (Martinez Morales *et al.*, 2000). They are also expressed in the photoreceptors of the fly ommatidia, Bolwig organ, and ocelli(Vandendries *et al.*, 1996), and of the planarian pigment-cup eyes (Umesono *et al.*, 1999). However, as with *pax6*, the role of *otx* is considerably broader than photoreceptor cell specification. - Other transcription factors with an apparently very old role in photoreceptor cell type development belong to the *six* family of homeodomain proteins. In *Drosophila* (Serikaku and O'Tousa, 1994), in planarians (Pineda *et al.*, 2000), and in the polychaete *Platynereis* (Arendt *et al.*, 2002; Tessmar-Raible and Arendt, 2003), *six1/2* ortholgs, beside an early role in eye specification, remain expressed in photoreceptor and pigment cells at differentiation stages. In the vertebrates, *six2* is apparently not involved in early eye development, but shows expression in photosensitive cells of the retina at late differentiation stages (Kawakami *et al.*, 1996; Ghanbari *et al.*, 2001), hinting at an ancestral role of this gene in photoreceptor differentiation. The vertebrate *Six3* gene plays a pivotal role in eye formation in the vertebrates (Carl *et al.*, 2002; Tessmar *et al.*, 2002; Zhu *et al.*, 2002; Lagutin *et al.*, 2003; Lopez-Rios *et al.*, 2003), and in insects where it acts independently of *pax6* (Seimiya and Gehring, 2000). While not expressed in the developing planarian eye (Salo *et al.,* 2002) it is also involved in polychaete eye formation (D. A., J. Wittbrodt, unpublished data). Again, however, the roles of *six* family members are broader than eye development. *Six1/2* genes are also involved in myogenic specification (Heanue *et al.*, 1999), and the specifying role of *six3* extends to a whole region of the most anterior brain that includes the developing eye (Loosli *et al.*, 1998; Seimiya and Gehring, 2000). Nevertheless, even if none of these factors alone can account for eye specificity, the combinatorial expression of *pax6, otx,* and *six1/2,* and *six3* apparently forms part of an ancestral code for general photoreceptor cell fate in development and evolution.

In summary, the molecular comparison indicates that a common precursor of today's photoreceptor cell types expressing an Ur-opsin for light perception evolved on the pre-bilaterian branch of animal evolution ("animal photoreceptor cell precursor" in Fig. 2); that it employed arrestin and rhodopsin kinase for quenching the light signal, and that *pax6*, *otx*, and *six1/2,* and *six3* transcription factors were involved in its specification and differentiation. If so, then animal photoreceptor cells are homologous on large scale. During subsequent evolution, the prebilaterian photoreceptor precursor would then have diversified into sister cell types, as discussed in the subsequent chapters.

Rhabdomeric and ciliary photoreceptor sister cell types

It is long known from electron-optic studies that animal photoreceptor cells can be of two distinct morphologies. All photoreceptor cells enlarge the membraneous surface for the storage of photopigment, but the rhabdomeric photoreceptor cells do so by folding the apical cell surface, while the ciliary photoreceptors cells fold the ciliary membrane (Eakin, 1968; Eakin, 1982). Rhabdomeric

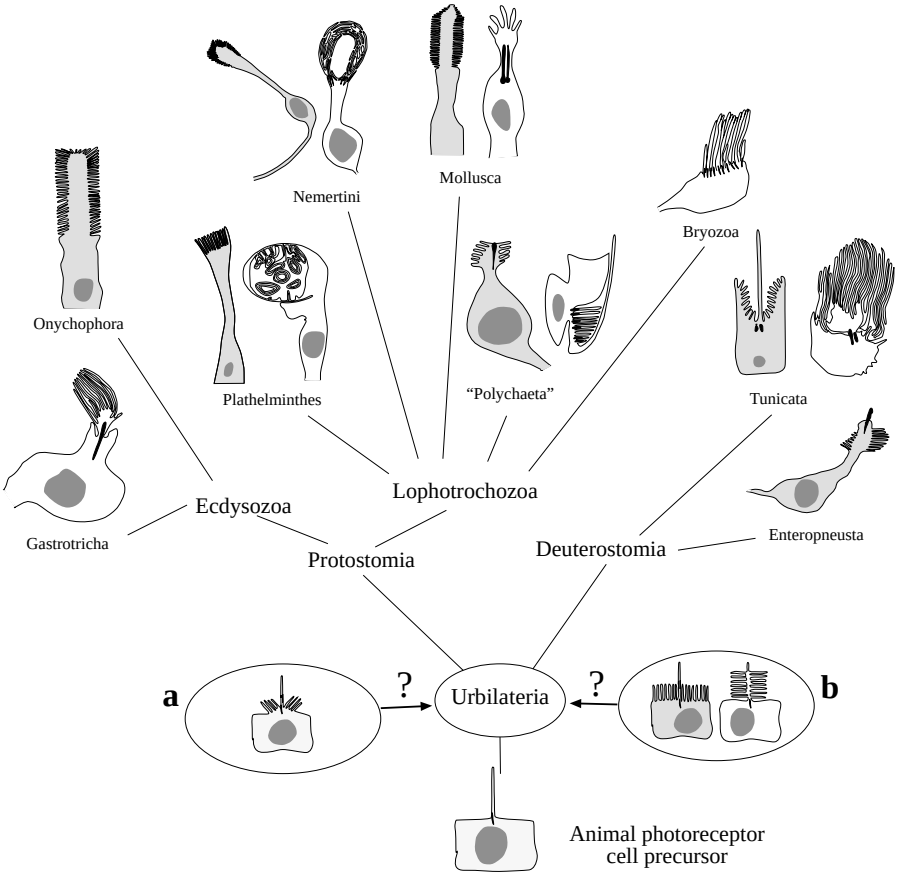

Fig. 2. Conflicting scenarios of photoreceptor cell type evolution. *Dark grey, rhabdomeric photoreceptor cell; white, ciliary photoreceptor cell. Photoreceptor cell types in Urbilateria could have been (a) a bimodal ciliary/rhabdomeric precursor cell or (b) ciliary and rhabdomeric precursor cells. Redrawn from various sources, modified after (Arendt and Wittbrodt, 2001).*

and ciliary photoreceptor cells co-exist in many bilaterian groups (Fig. 2). However, the significance of the rhabdomeric versus ciliary surface extension is yet unclear. While some authors think that rhabdomeric and ciliary photoreceptor sister cell types have evolved multiple times independently from an intermediate photoreceptor cell type inherited from Urbilateria (Fig. 2; alternative 'a'), others view a more fundamental difference between the two distinct photoreceptor sister cell types that had emerged and co-existed already in Urbilateria (Fig. 2; alternative 'b') (reviewed in (Arendt and Wittbrodt, 2001).

The molecular comparative approach now indicates that the latter view is most likely correct: that indeed two distinct *opsin*-employing photoreceptor cell types had already diversified in Urbilateria (Arendt and Wittbrodt, 2001). Constructing phylogenetic trees for the conserved molecules involved in phototransduction and its quenching (opsin, G-alpha, arrestin, rhodopsin kinase), the surprising result for each of them has been that at least two distinct paralogs exist in Bilateria, and that the invertebrate rhabdomeric and vertebrate ciliary photoreceptor cells deploy distinct paralogs (Fig. 3). This is best explained if one assumes that the initially single pre-bilaterian photoreceptor cell type precursor had diversified into two distinct types at the base of the Bilateria (Fig. 2; alternative 'b'), and that this diversification was paralleled by gene duplication (and subsequent sub- and neofunctionalisation) of many of the cell-type specific genes. After their splitting apart, both of the new sister cell types specialised on different intracellular messenger pathways (phosphodiesterase versus phospholipase C) (Fig. 3) (Lott *et al.*, 1999; Mayeenuddin and Mitchell, 2001; Mayeenuddin and Mitchell, 2003) that probably co-existed in their common precursor. (Notably, this scenario implies that invertebrate 'rhabdomeric photoreceptors' and vertebrate 'ciliary photoreceptors' are non-homologous cell types – because they trace back to distinct sister cell types in Urbilateria. Note also that this does not conflict with the previous statement that 'photoreceptor cells' as such should be homologous in all animals).

This scenario could explain some 'discrepancies' in the combinatorial code of the transcription factors specifying the rhabdomeric versus ciliary photoreceptor cell type. For example, the *rx* (*retina homeobox*) transcription factor plays a crucial role in vertebrate early eye development (Mathers *et al.*, 1997; Loosli *et al.*, 2001), and continues to be expressed in the rod and cone ciliary photoreceptor cells at differentiation stages (Chuang *et al.*, 1999; Deschet *et al.*, 1999). However, *rx* is not required for (Davis *et al.*, 2003), and completely absent from (Eggert *et al.*, 1998), the developing *Drosophila* rhabdomeric eyes, neither is it detected in the planarian rhabdomeric photoreceptors (Salo *et al.*, 2002). If this indeed reflected an early divergence of the rhabdomeric and ciliary sister cell types, then we would expect ciliary photoreceptors outside vertebrates to express *rx* (and *c-opsin*, the opsin ortholog specific for cilliary photoreceptors, see Fig. 3C), a hypothesis currently tested for the polychaetes (D.A., K. Tessmar-Raible, J. Wittbrodt, unpublished data).

The proto-eye employed the rhabdomeric photoreceptor cell type

If two types of photoreceptor cells existed in the Urbilateria: which of the two formed part of the proto-eye? The predominance of the rhabdomeric type in the cerebral pigment-cup eyes of Protostomia, as well as its occurence in larval eyes in lower Deuterostomia (Arendt and Wittbrodt, 2001) allows a clear statement that it should have been the rhabdomeric type. In most cases ciliary photoreceptors do not form part of the eyes, with few exceptions such as the single ciliary photoreceptor cell interspersed between rhabdomeric photoreceptor cells in the left larval eye of the polyclad planarian *Pseudoceros* (Eakin and Brandenburger, 1980) or the ciliary photoreceptor cells of the scallop mantle edge eye (Barber *et al.*, 1967; Barber and Wright, 1969). In light of this overwhelming rhabdomeric majority, it is a mystery why the ciliary photoreceptor cell type made the race in the evolution of chordate vision. And relating to this question, what was the fate of the rhabdomeric photoreceptors in the vertebrates – one of the few bilaterian groups where the rhabdomeric type has never been described?

Homology of retinal ganglion cells with the rhabdomeric photoreceptor cell type?

The comparison of molecules involved in the specification and differentiation of rhabdomeric photoreceptor cells with those of the different cell types of the vertebrate retina has surprisingly revealed many resemblances not with the rod and cone ciliary photoreceptor cells, but with the retinal ganglion cells (RGCs) (Arendt *et al.*, 2002; Frankfort and Mardon, 2002; Hsiung and Moses, 2002). This has prompted us to propose that RGCs and rhabdomeric photoreceptor cells are homologous cell types that trace back to the same precursor cell type in Urbilateria (Arendt *et al.*, 2002).

First *Pax6,* apart from its earlier role in generating most retinal cell types, remains expressed in the retinal ganglion cells of the differentiating retina, and this later expression is shared with a subset of differentiating rhabdomeric photoreceptors, as present for example in the dipteran late Bolwig organ (Sheng *et al.*, 1997), in the polychaete larval eye (Arendt *et al.*, 2002), or in intact and regenerating planarian pigment cup eyes (Callaerts *et al.*, 1999). Second, both cell types have in common to specifically express *atonal* orthologs, bHLH family members. In the *Drosophila* compound eye (Frankfort and Mardon, 2002; Hsiung and Moses, 2002) and larval Bolwig's organ (Daniel *et al.*, 1999), expression of *atonal* precedes differentiation, and drives eye precursor cells into the rhabdomeric photoreceptor cell fate. In the polychaete *Platynereis*, rhabdomeric photoreceptor cells also arise from within *atonal*-positive cell clusters (Arendt *et al.*, 2002). In mouse, the onset of RGC differentiation coincides with the expression of *math5*, an *atonal* ortholog, that is likewise required for RGC differentiation (Wang *et al.*, 2001), and the same is true for zebrafish (Kay *et al.*, 2001). Interestingly, *ath5* activates the POU family member *brn3*(Hutcheson and Vetter, 2001; Liu *et al.*, 2001),

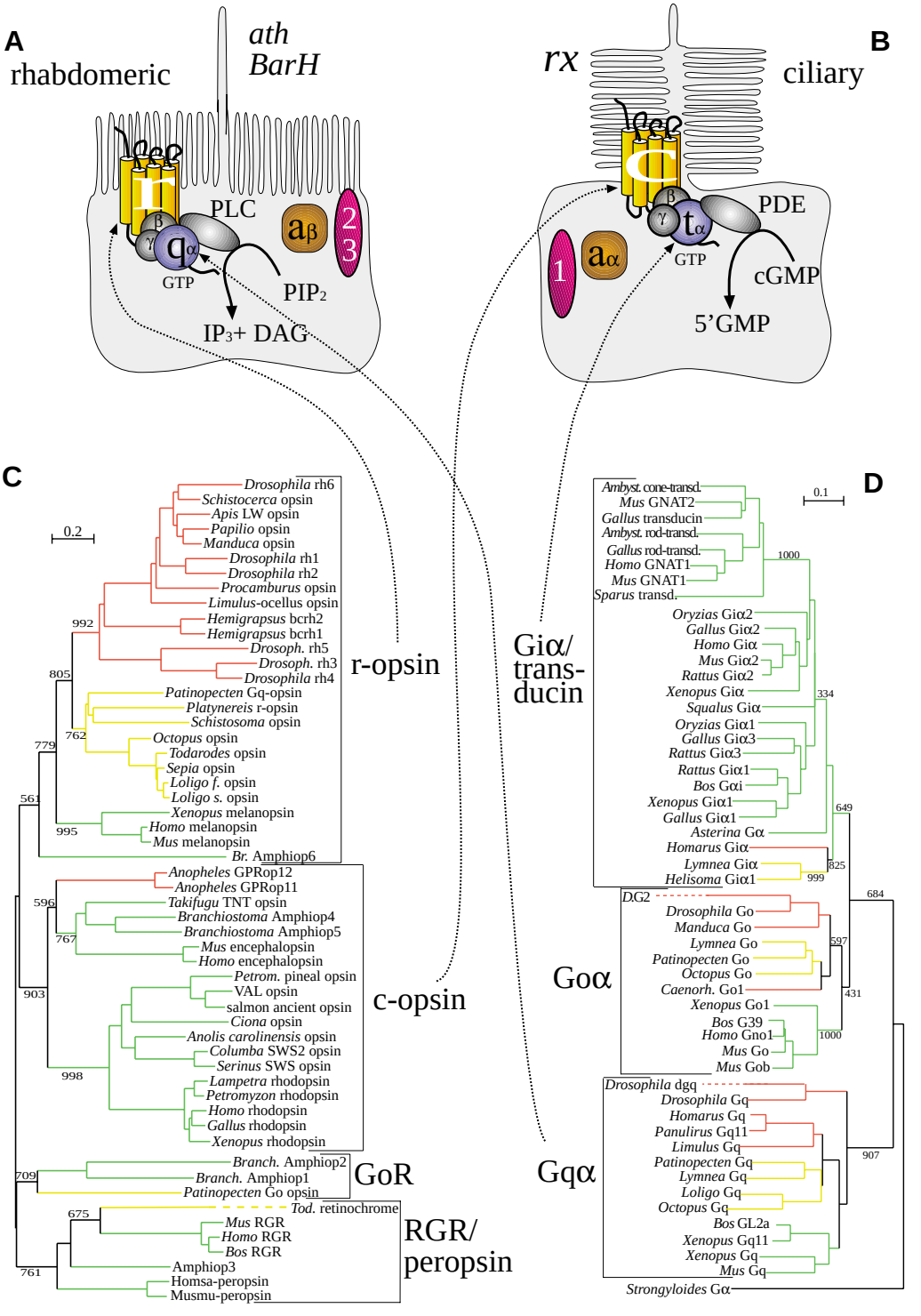

Fig. 3. Paralogy of effector genes of rhabdomeric and ciliary photoreceptor cells. *Schematic reprensentation of rhabdomeric* **(A)** *and ciliary* **(B)** *photoreceptor cells with relevant components of their respective pototransduction cascades. Rhabdomeric (r, orange) and ciliary (c, orange) opsins, G-α subunits (blue), arrestin α and β (brown) and rhosopsin kinases (purple). Abbreviations: cGMP, cyclic guanosylmonophosphate; DAG, diacylglycerol; GTP, Guanosytrisphos–phate; IP3, inositol-1,3,5-trisphosphate; PDE, Phosphodiesterase; PIP2, Phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C. (C,D) The trees were calculated using ClustalX on opsin protein sequences* **(C)** *and on G-α DNA sequences* **(D)**. *Brackets enclose orthologous genes that can be traced back to the same precursor gene in Urbilateria. The colour code in the trees uses green for Deuterostomia, yellow for Lophotrochozoa and red for Ecdysozoa. Relevant bootstrap values are given.*

which in the vertebrates is specific for differentiating RGCs and promotes their differentiation (Liu *et al.*, 2000). Similarly, we have detected expression of a *brn3* ortholog in the rhabdomeric photoreceptor cells of the polychaete larval eye (K. Tessmar, D. A., unpublished results). Note that in the vertebrate retina *Brn3* is required for the outgrowth of axons from the RGCs towards the optic chiasm (Erkman *et al.*, 2000; Wang *et al.*, 2000; Wang *et al.*, 2002); and the axonal projection to the brain is another similarity that RGCs share with the rhabdomeric photoreceptors (Arendt *et al.*, 2002; Frankfort and Mardon, 2002). A third specification factor specifically shared between RGCs and rhabdomeric photoreceptors is encoded by the *BarH* homeobox gene, another putative downstream gene of *atonal* (Bermingham *et al.*, 2001). In the *Drosophila* eye disc, *BarH1* specifies outer photoreceptor cell fates (and primary pigment cells; Hayashi *et al.*, 1998; Higashijima *et al.*, 1992). In the vertebrate retina, *BarH* orthologs are expressed in the differentiating RGCs, in *Xenopus* (Patterson *et al.*, 2000), fish (Poggi *et al.*, 2002), and rat (Saito *et al.*, 1998).

The molecular similarities between RGCs and rhabdomeric photoreceptor cells extend to the effector gene level. Strikingly, RGCs express *melanopsin* (Hattar *et al.*, 2002; Provencio *et al.*, 2002), the vertebrate ortholog of invertebrate *rhabdomeric opsins (r-opsins)* (Figs. 2,3), and they have recently been identified as additional photosensitive cells in the vertebrate retina (Hattar *et al.*, 2002); unfortunately, however, the phototransduction cascade of the ganglion cells is unknown (Rollag *et al.*, 2003).

Diversification of cell types in the vertebrate retina

Having identified RGCs and invertebrate rhabdomeric photoreceptors as possible homologous cell types – what about other cell types of the retina such as amacrine, horizontal, or bipolar cells? Molecular comparative cell biology is a means to identify cell type relationships in the vertebrate retina (Fig. 4). Considering both the specifying transcription factors and the cell-type specific effector genes, it appears that RGCs, amacrine cells, and horizontal cells are sister cell types (that would all have evolved from an ancient, formerly rhabdomeric precursor cell type). Note that the concept of sister cell types necessarily implies that the molecular identities of the three cell types should be non-identical, reflecting the func-

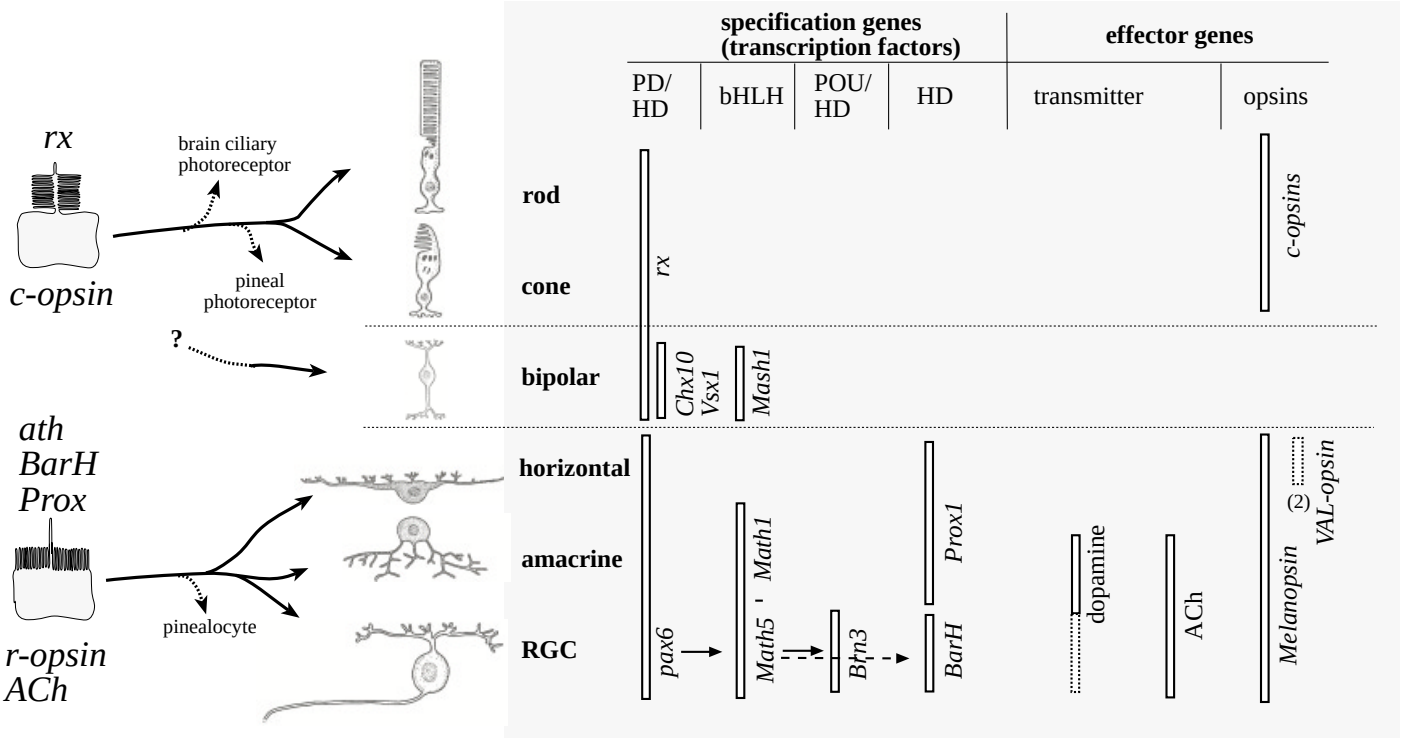

Fig. 4. Diversification of cell types in the vertebrate retina. *Molecular comparative cell biology indicates that rods and cones have evolved from a common ciliary photoreceptor precursor, while retinal ganglion, amacrine and horizontals have evolved from a rhabdomeric photoreceptor precursor. Black arrows represent cell type evolution. The evolutionary origin of bipolars is unclear. For the regulation of transcription factors, see text. Most expression patterns deduced from mouse data.* Pax6 *in retinal ganglion cells, amacrine and horizontal cells (Belecky-Adams* et al.*, 1997; Perron* et al.*, 1998; Marquardt* et al.*, 2001; De Melo* et al.*, 2003).* Math5 *in ganglion cells (Brown* et al.*, 2001; Liu* et al.*, 2001; Wang* et al.*, 2001).* Math1 *in amacrine cells (pers. comm.).* Mash-*1 in bipolars (Hatakeyama* et al.*, 2001);* rx *in rods, cones + INL (Mathers* et al.*, 1997).* Rx *expression was extrapolated from zebrafish where* Zrx1*/2 are expressed in cones (Chuang* et al.*, 1999) and* Zrx3 *in bipolars (Chuang* et al.*, 1999);* Chx10 *in bipolars (Belecky-Adams* et al.*, 1997; De Melo* et al.*, 2003).* Vsx1 *in bipolars (Chow* et al.*, 2001);* Brn3 *in ganglion cells (Liu* et al.*, 2000). Expression of* BarH *orthologs in the differentiating RGCs has been extrapolated from* Xenopus *(Patterson* et al.*, 2000) and fish(Poggi* et al.*, 2002).* Prox1 *in amacrine and horizontals (Dyer* et al.*, 2003). Note that* Prox1 *is also weakly expressed in bipolars. Cholinergic ganglion and amacrine cells (Yasuhara* et al.*, 2003). ChAT in* Xenopus *(Lopez* et al.*, 2002). Dopaminergic amacrines (Marquardt* et al.*, 2001; De Melo* et al.*, 2003). The VAL opsin specific immunoreactivity (1) was detected in a subset of non-GABAergic horizontal cells in the zebrafish retina (Kojima* et al.*, 2000).* Melanopsin *in amacrine and ganglion cells (Provencio* et al.*, 2000).* Melanopsin *expression in horizontals refers to zebrafish (Bellingham* et al.*, 2002; Drivenes* et al.*, 2003).*

tional divergence implied in the sister cell concept (for example, entrainment of circadian rhythmicity and optic nerve formation for RGCs, versus lateral information flow for amacrine and horizontal cells).

In specific, all three cell types share the late expression of *pax6* at differentiation stages (Belecky-Adams *et al.*, 1997; De Melo *et al.*, 2003; Marquardt *et al.*, 2001; Perron *et al.*, 1998). Amacrine and horizontal cells share expression of *Prox1*, a homeobox transcription factor. Interestingly, vertebrate *Prox* genes are orthologous to *Drosophila prospero*, which controls the distinction between colour photoreceptor cell fates in the ommatidia of the eye disc (Cook *et al.*, 2003). Attributing *prospero/prox* function to an ancestral rhabdomeric photoreceptor, this function would have been lost in the RGCs, but persisted in the amacrine and horizontal cells. (The functional significance of this sharing of *prospero* expression is so far obscure; but it should be stressed that amacrine and horizontal cells should also be photosensitive as judged from their *melanopsin* expression, see below).

On the effector gene level, dopamine and acetylcholine (ACh) are restricted to amacrine cells and RGCs (Marquardt *et al.*, 2001; Lopez *et al.*, 2002; Yasuhara *et al.*, 2003; De Melo *et al.*, 2003). Again, the deployment of ACh as transmitter is another similarity shared with the invertebrate rhabdomeric photoreceptors (Yasuyama *et al.*, 1995; Yasuyama and Meinertzhagen, 1999). Most important, in fish the three sister cell types share expression of *melanopsin* paralogs (Bellingham *et al.*, 2002; Drivenes *et al.*, 2003) – while in mouse horizontal cells would have lost *melanopsin* expression.

It must be stressed that this comparative survey is far from complete – not only because the list of genes active in specification and differentiation of retinal cell types has to be extended considerably (compare, e.g., with the extensive listings in (Jean *et al.*, 1998; Marquardt *et al.*, 2001)), but also because retinal cell types are more diverse than depicted in Fig. 4. There is molecular evidence that there are subtypes not only of rod and cone photoreceptor cells (see, e.g., (Witkovsky, 2000), but also of bipolar cells (De Melo, *et al.,* 2003, and see above), and of retinal ganglion cells (as judged from differential *melanopsin* expression but also distinct axonal projections (Belenky *et al.*, 2003), and last but not least the Müller glia cells have so far been omitted from the scheme. It is intended here only to exemplify a novel approach in comparative eye research, and to give a starting point and guideline for future cell type comparions.

Outlook

The evolution of photoreceptor cell types can now be traced by the comparative analysis of their molecular combinatorial codes. More efficiently than previous anatomical comparisons, this novel evo-devo approach will help to elucidate the evolution of animal photoreceptor cells and thus of animal eyes. Conducted here on the vertebrate retina, this approach can equally be applied on the *Drosophila* photosensitive system with its three types of eyes (compound eyes, Bolwig's organ/eyelet, ocelli) that also harbours a wealth of different cell types, and on the less complex but more ancestral invertebrate photosensitive systems of high comparative interest, as found for example in the lower chordate *Branchiostoma*, or in the polychaete *Platynereis*, to finally assemble a more general picture of cell type evolution in animal photosensitive systems.

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References

ANDREWS, G.L., YUN, K., RUBENSTEIN, J.L. and MASTICK, G.S. (2003). Dlx transcription factors regulate differentiation of dopaminergic neurons of the ventral thalamus. *Mol. Cell. Neurosci.* 23: 107-20.

ARENDT, D., TESSMAR, K., DE CAMPOS-BAPTISTA, M.I., DORRESTEIJN, A. and WITTBRODT, J. (2002). Development of pigment-cup eyes in the polychaete Platynereis dumerilii and evolutionary conservation of larval eyes in Bilateria. *Development* 129: 1143-54.

ARENDT, D. and WITTBRODT, J. (2001). Reconstructing the eyes of Urbilateria. *Phil. Trans. Roy. Soc. Lond. B* 356: 1545-1563.

BARBER, V.C., EVANS, E.M. and LAND, M.F. (1967). The fine structure of the eye of the mollusc *Pecten maximus*. *Z. Zellforsch.* 76: 295-312.

BARBER, V.C. and WRIGHT, D.E. (1969). The fine structure of the eye and optic tentacle of the mollusc *Cardium edule*. *J. Ultrastruct. Res.* 26: 515-528.

BELECKY-ADAMS, T., TOMAREV, S., LI, H.S., PLODER, L., MCINNES, R.R., SUNDIN, O. and ADLER, R. (1997). Pax-6, Prox 1 and Chx10 homeobox gene expression correlates with phenotypic fate of retinal precursor cells. *Investigative Ophthalm. Vis. Sci.* 38: 1293-1303.

BELENKY, M.A., SMERASKI, C.A., PROVENCIO, I., SOLLARS, P.J. and PICKARD, G.E. (2003). Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *J. Comp. Neurol.* 460: 380-93.

BELLINGHAM, J., WHITMORE, D., PHILP, A.R., WELLS, D.J. and FOSTER, R.G. (2002). Zebrafish melanopsin: isolation, tissue localisation and phylogenetic position. *Brain Res. Mol. Brain Res.* 107: 128-36.

BERMINGHAM, N.A., HASSAN, B.A., WANG, V.Y., FERNANDEZ, M., BANFI, S., BELLEN, H.J., FRITZSCH, B. and ZOGHBI, H.Y. (2001). Proprioceptor pathway development is dependent on Math1. *Neuron* 30: 411-22.

BOSSING, T., UDOLPH, G., DOE, C.Q. and TECHNAU, G.M. (1996). The embryonic central nervous system lineages of Drosophila melanogaster. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. *Dev. Biol.* 179: 41-64.

BROWN, N.L., PATEL, S., BRZEZINSKI, J. and GLASER, T. (2001). *Math5* is required for retinal ganglion cell and optic nerve formation. *Development* 128: 2497-2508.

CALLAERTS, P., MUNOZ-MARMOL, A.M., GLARDON, S., CASTILLO, E., SUN, H., Li, W.H., GEHRING, W.J. and SALO, E. (1999). Isolation and expression of a Pax-6 gene in the regenerating and intact Planarian Dugesia(G)tigrina. *Proc. Nat. Acad. Sci. USA* 96: 558-563.

CARL, M., LOOSLI, F. and WITTBRODT, J. (2002). Six3 inactivation reveals its essential role for the formation and patterning of the vertebrate eye. *Development* 129: 4057-63.

CEPKO, C.L. (1999). The roles of intrinsic and extrinsic cues and bHLH genes in the determination of retinal cell fates. *Curr. Opin. Neurobiol.* 9: 37-46.

CHEN, S., WANG, Q.L., NIE, Z., SUN, H., LENNON, G., COPELAND, N.G., GILBERT, D.J., JENKINS, N.A. and ZACK, D.J. (1997). Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron* 19: 1017-30.

CHOW, R.L., SNOW, B., NOVAK, J., LOOSER, J., FREUND, C., VIDGEN, D., PLODER, L. and McInnes, R.R. (2001). Vsx1, a rapidly evolving paired-like homeobox gene expressed in cone bipolar cells. *Mech. Dev.* 109: 315-22.

CHUANG, J.C., MATHERS, P.H. and RAYMOND, P.A. (1999). Expression of three *Rx* homeobox genes in embryonic and adult zebrafish. *Mech. Dev.* 84: 195-198.

COOK, T., PICHAUD, F., SONNEVILLE, R., PAPATSENKO, D. and DESPLAN, C. (2003). Distinction between Color Photoreceptor Cell Fates Is Controlled by Prospero in Drosophila. *Dev. Cell* 4: 853-64.

CZERNY, T., HALDER, G., KLOTER, U., SOUABNI, A., GEHRING, W.J. and BUSSLINGER, M. (1999). twin of eyeless, a second Pax-6 gene of Drosophila, acts upstream of eyeless in the control of eye development. *Mol. Cell* 3: 297-307.

DANIEL, A., DUMSTREI, K., LENGYEL, J.A. and HARTENSTEIN, V. (1999). The control of cell fate in the embryonic visual system by atonal, tailless and EGFR signaling. *Development* 126: 2945-54.

DARWIN, C. (1859). "On the Origin of Species by Means of Natural Selection: or the Preservation of Favoured Races in the Struggle for Life." John Murray, London.

DAVIS, R.J., TAVSANLI, B.C., DITTRICH, C., WALLDORF, U. and MARDON, G. (2003). Drosophila retinal homeobox (drx) is not required for establishment of the visual system, but is required for brain and clypeus development. *Dev. Biol.* 259: 272-87.

DE MELO, J., QIU, X., DU, G., CRISTANTE, L. and EISENSTAT, D.D. (2003). Dlx1, Dlx2, Pax6, Brn3b and Chx10 homeobox gene expression defines the retinal ganglion and inner nuclear layers of the developing and adult mouse retina. *J. Comp. Neurol.* 461: 187-204.

DESCHET, K., BOURRAT, F., RISTORATORE, F., CHOURROUT, D. and JOLY, J.-S. (1999). Expression of the medaka (*Oryzias latipes*) *Ol-Rx3 paired*-like gene in two diencephalic derivatives, the eye and the hypothalamus. *Mech. Dev.* 83: 179-182.

DRIVENES, O., SOVIKNES, A.M., EBBESSON, L.O., FJOSE, A., SEO, H.C. and HELVIK, J.V. (2003). Isolation and characterization of two teleost melanopsin genes and their differential expression within the inner retina and brain. *J. Comp. Neurol.* 456: 84-93.

DYER, M.A., LIVESEY, F.J., CEPKO, C.L. and OLIVER, G. (2003). Prox1 function controls progenitor cell proliferation and horizontal cell genesis in the mammalian retina. *Nat. Genet.* 34: 53-8.

EAKIN, R. (1968). "Evolution of photoreceptors." Appleton-Century-Crofts, New York.

EAKIN, R.M. (1982). Continuity and diversity in photoreceptors. *In* "Visual cells in Evolution" (J. A. Westfall, Ed.), pp. 91-105. Raven Press, New York.

EAKIN, R.M. and BRANDENBURGER, J.L. (1980). Unique eye of probable evolutionary significance. *Science* 211: 1189-1190.

EGGERT, T., HAUCK, B., HILDEBRANDT, N., GEHRING, W.J. and WALLDORF, U. (1998). Isolation of a Drosophila homolog of the vertebrate homeobox gene Rx and its possible role in brain and eye development. *Proc. Nat. Acad. Sci. USA* 95: 2343-2348.

ERKMAN, L., YATES, P.A., MCLAUGHLIN, T., MCEVILLY, R.J., WHISENHUNT, T., O'CONNELL, S.M., KRONES, A.I., KIRBY, M.A., RAPAPORT, D.H., BERMINGHAM, J.R., O'LEARY, D.D. and ROSENFELD, M.G. (2000). A POU domain transcription factor-dependent program regulates axon pathfinding in the vertebrate visual system. *Neuron* 28: 779-92.

FERNALD, R.D. (1997). The evolution of eyes. *Brain Behav. Evol.* 50: 253-9.

FERNALD, R.D. (2000). Evolution of eyes. *Curr. Opin. Neurobiol.* 10: 444-50.

FISCHER, A. and BRÖKELMANN, J. (1966). Das Auge von *Platynereis dumerilii (Polychaeta).* Sein Feinbau im ontogenetischen und adaptiven Wandel. *Z. Zellforsch.* 71: 217-244.

FRANKFORT, B.J. and MARDON, G. (2002). R8 development in the *Drosophila* eye: a paradigm for neural selection and differentiation. *Development* 129: 1295-1306.

FURUKAWA, T., MORROW, E.M. and CEPKO, C.L. (1997a). Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* 91: 531-41.

FURUKAWA, T., MORROW, E.M. and CEPKO, C.L. (1997b). Crx, a Novel Otx-Like Homeobox Gene, Shows Photoreceptor-Specific Expression and Regulates Photoreceptor Differentiation. *Cell* 91: 531-541.

GEHRING, W. and ROSBASH, M. (2003). The co-evolution of blue-light photoreception and circadian rhythms. *Proc. Natl. Acad. Sci.* in press

GEHRING, W.J. and IKEO, K. (1999). Pax 6: mastering eye morphogenesis and eye evolution. *Trends Genet.* 15: 371-7.

GHANBARI, H., SEO, H. C., FJOSE, A. and BRANDLI, A.W. (2001). Molecular cloning and embryonic expression of Xenopus Six homeobox genes. *Mech. Dev.* 101: 271-7.

GLARDON, S., HOLLAND, L.Z., GEHRING, W.J. and HOLLAND, N.D. (1998). Isolation and developmental expression of the amphioxus Pax-6 gene (AmphiPax-6): insights into eye and photoreceptor evolution. *Development* 125: 2701-2710.

HALDER, G., CALLAERTS, P. and GEHRING, W.J. (1995). Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. *Science* 267: 1788-1792.

HARTMANN, B., LEE, P.N., KANG, Y.Y., TOMAREV, S., DE COUET, H.G. and CALLAERTS, P. (2003). Pax6 in the sepiolid squid Euprymna scolopes: evidence for a role in eye, sensory organ and brain development. *Mech. Dev.* 120: 177-83.

HATAKEYAMA, J., TOMITA, K., INOUE, T. and KAGEYAMA, R. (2001). Roles of homeobox and bHLH genes in specification of a retinal cell type. *Development - Suppl.* 128: 1313-22.

HATTAR, S., LIAO, H. W., TAKAO, M., BERSON, D.M. and YAU, K.W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections and intrinsic photosensitivity. *Science* 295: 1065-70.

HAYASHI, T., KOJIMA, T. and SAIGO, K. (1998). Specification of primary pigment cell and outer photoreceptor fates by BarH1 homeobox gene in the developing Drosophila eye. *Dev. Biol.* 200: 131-45.

HEANUE, T.A., RESHEF, R., DAVIS, R.J., MARDON, G., Oliver, G., TOMAREV, S., LASSAR, A.B. and TABIN, C.J. (1999). Synergistic regulation of vertebrate muscle development by Dach2, Eya2 and Six1, homologs of genes required for Drosophila eye formation. *Genes Dev.* 13: 3231-43.

HIGASHIJIMA, S., KOJIMA, T., MICHIUE, T., ISHIMARU, S., EMORI, Y. and SAIGO, K. (1992). Dual Bar homeo box genes of Drosophila required in two photoreceptor cells, R1 and R6 and primary pigment cells for normal eye development. *Genes Dev.* 6: 50-60.

HSIUNG, F. and MOSES, K. (2002). Retinal development in Drosophila: specifying the first neuron. *Hum. Mol. Genet.* 11: 1207-14.

HUTCHESON, D.A. and VETTER, M.L. (2001). The bHLH factors Xath5 and XNeuroD can upregulate the expression of XBrn3d, a POU-homeodomain transcription factor. *Dev. Biol.* 232: 327-38.

JANG, C.C., CHAO, J.L., JONES, N., YAO, L.C., BESSARAB, D.A., KUO, Y.M., JUN, S., DESPLAN, C., BECKENDORF, S.K. and SUN, Y.H. (2003). Two Pax genes, eye gone and eyeless, act cooperatively in promoting *Drosophila* eye development. *Development* 130: 2939-2951.

JEAN, D., EWAN, K. and GRUSS, P. (1998). Molecular Regulators Involved in Vertebrate Eye Development. *Mech. Dev.* 76: 3-18.

JESSELL, T.M. (2000). Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat. Rev. Genet.* 1: 20-9.

KAWAKAMI, K., OHTO, H., TAKIZAWA, T. and SAITO, T. (1996). Identification and expression of six family genes in mouse retina. *FEBS Letters* 393: 259-263.

KAY, J.N., FINGER-BAIER, K.C., ROESER, T., STAUB, W. and BAIER, H. (2001). Retinal Ganglion Cell Genesis Requires lakritz, a Zebrafish atonal Homolog. *Neuron* 30: 725-36.

KOJIMA, D., MANO, H. and FUKADA, Y. (2000). Vertebrate ancient-long opsin: a green-sensitive photoreceptive molecule present in zebrafish deep brain and retinal horizontal cells. *J. Neurosci.* 20: 2845-51.

KRUPNICK, J.G., GUREVICH, V.V. and BENOVIC, J.L. (1997). Mechanism of quenching of phototransduction. Binding competition between arrestin and transducin for phosphorhodopsin. *J. Biol. Chem.* 272: 18125-31.

KUMAR, J. and MOSES, K. (1997). Transcription factors in eye development: a gorgeous mosaic? *Genes and Development* 11: 2023-2028.

KUMAR, J. P. and MOSES, K. (2001). Eye specification in Drosophila: perspectives and implications. *Semin. Cell Dev. Biol.* 12: 469-74.

LAGUTIN, O.V., ZHU, C.C., KOBAYASHI, D., TOPCZEWSKI, J., SHIMAMURA, K., PUELLES, L., RUSSELL, H.R., Mckinnon, P.J., SOLNICA-KREZEL, L. and OLIVER, G. (2003). Six3 repression of Wnt signaling in the anterior neuroectoderm is essential for vertebrate forebrain development. *Genes Dev.* 17: 368-79.

LIU, W., KHARE, S.L., LIANG, X., PETERS, M.A., LIU, X., CEPKO, C.L. and XIANG, M. (2000). All Brn3 genes can promote retinal ganglion cell differentiation in the chick. *Development - Suppl.* 127: 3237-47.

LIU, W., MO, Z. and XIANG, M. (2001). The Ath5 proneural genes function upstream of Brn3 POU domain transcription factor genes to promote retinal ganglion cell development. *Proc. Natl. Acad. Sci. USA* 98: 1649-54.

LIVESEY, F.J. and CEPKO, C.L. (2001). Vertebrate neural cell-fate determination: lessons from the retina. *Nat. Rev. Neurosci.* 2: 109-18.

LOOSLI, F., KMITA-CUNISSE, M. and GEHRING, W.J. (1996). Isolation of a Pax-6 homolog from the ribbonworm Lineus sanguineus. *Proc. Nat. Acad. Sci. USA* 93: 2658-63.

LOOSLI, F., KOSTER, R.W., CARL, M., KRONE, A. and WITTBRODT, J. (1998). Six3, a medaka homologue of the Drosophila homeobox gene sine oculis is expressed in the anterior embryonic shield and the developing eye. *Mech. Dev.* 74: 159-64.

LOOSLI, F., WINKLER, S., BURGTORF, C., WURMBACH, E., ANSORGE, W., HENRICH, T., GRABHER, C., ARENDT, D., CARL, M., KRONE, A., GRZEBISZ, E. and WITTBRODT, J. (2001). Medaka eyeless is the key factor linking retinal determination and eye growth. *Development* 128: 4035-44.

LOPEZ, J.M., MORENO, N. and GONZALEZ, A. (2002). Localization of choline acetyltransferase in the developing and adult retina of Xenopus laevis. *Neurosci. Lett.* 330: 61-4.

LOPEZ-RIOS, J., TESSMAR, K., LOOSLI, F., WITTBRODT, J. and BOVOLENTA, P. (2003). Six3 and Six6 activity is modulated by members of the groucho family. *Development* 130: 185-95.

LOTT, J.S., WILDE, J.I., CARNE, A., EVANS, N. and FINDLAY, J.B. (1999). The ordered visual transduction complex of the squid photoreceptor membrane. *Mol. Neurobiol.* 20: 61-80.

MARQUARDT, T., ASHERY-PADAN, R. andREJEWSKI, N., SCARDIGLI, R., GUILLEMOT, F. and GRUSS, P. (2001). Pax6 is required for the multipotent state of retinal progenitor cells. *Cell* 105: 43-55.

MARTINEZ MORALES, J., SIGNORE, M., ACAMPORA, D., SIMEONE, A. and BOVOLENTA, P. (2000). Otx genes are required for tissue specification in the developing eye *Development* 128(11): 2019-2030.

MATHERS, P.H., GRINBERG, A., MAHON, K.A. and JAMRICH, M. (1997). The Rx homeobox gene is essential for vertebrate eye development. *Nature* 387: 603-607.

MAYEENUDDIN, L.H. and MITCHELL, J. (2001). cDNA cloning and characterization of a novel squid rhodopsin kinase encoding multiple modular domains. *Vis. Neurosci.* 18: 907-15.

MAYEENUDDIN, L.H. and MITCHELL, J. (2003). Squid visual arrestin: cDNA cloning and calcium-dependent phosphorylation by rhodopsin kinase (SQRK). *J. Neurochem.* 85: 592-600.

MEYER-ROCHOW, V.B. and STEWART, D. (1996). Review of larval and postlarval eye ultrastructure in the lamprey (Cyclostomata) with special emphasis on Geotria australis (Gray). *Microsc. Res. Tech.* 35: 431-44.

NILSSON, D.-E. (1996). Eye ancestry: old genes for new eyes. *Curr. Biol.* 6: 39-42.

PATTERSON, K.D., CLEAVER, O., GERBER, W.V., WHITE, F.G. and KRIEG, P.A. (2000). Distinct expression patterns for two Xenopus Bar homeobox genes. *Dev. Gen. Evol.* 210: 140-4.

PERRON, M., KANEKAR, S., VETTER, M.L. and HARRIS, W.A. (1998). The genetic sequence of retinal development in the ciliary margin of the Xenopus eye. *Dev. Biol.* 199: 185-200.

PICHAUD, F. and DESPLAN, C. (2002). Pax genes and eye organogenesis. *Curr. Opin. Genet. Dev.* 12: 430-4.

PINEDA, D., GONZALEZ, J., CALLAERTS, P., IKEO, K., GEHRING, W.J. and SALO, E. (2000). Searching for the prototypic eye genetic network: Sine oculis is essential for eye regeneration in planarians. *Proc. Nat. Acad. Sci. USA* 97: 4525-4529.

POGGI, L., CARL, M., VIGNALI, R., BARSACCHI, G. and WITTBRODT, J. (2002). Expression of a medaka (Oryzias latipes) Bar homologue in the differentiating central nervous system and retina. *Mech. Dev.* 114: 193-6.

PROVENCIO, I., RODRIGUEZ, I.R., JIANG, G., HAYES, W.P., MOREIRA, E.F. and ROLLAG, M.D. (2000). A novel human opsin in the inner retina. *J. Neurosci.* 20: 600-5.

PROVENCIO, I., ROLLAG, M.D. and CASTRUCCI, A.M. (2002). Photoreceptive net in the mammalian retina. *Nature* 415: 493.

QUIRING, R., WALLDORF, U., KLOTER, U. and GEHRING, W.J. (1994). Homology of the eyeless gene of drosophila to the small eye gene in mice and aniridia in humans. *Science* 265: 785-789.

ROLLAG, M.D., BERSON, D.M. and PROVENCIO, I. (2003). Melanopsin, ganglion-cell photoreceptors and mammalian photoentrainment. *J. Biol. Rhythms* 18: 227-34.

SAITO, T., SAWAMOTO, K., OKANO, H. andERSON, D.J. and MIKOSHIBA, K. (1998). Mammalian BarH homologue is a potential regulator of neural bHLH genes. *Dev. Biol.* 199: 216-25.

SALO, E., PINEDA, D., MARSAL, M., GONZALEZ, J., GREMIGNI, V. and BATISTONI, R. (2002). Genetic network of the eye in Platyhelminthes: expression and functional analysis of some players during planarian regeneration. *Gene* 287: 67-74.

SALVINI-PLAWEN, L.V. and MAYR, E. (1977). "On the evolution of photoreceptors and eyes." Plenum, New York.

SCHMIDT, H., RICKERT, C., BOSSING, T., VEF, O., URBAN, J., TECHNAU, G.M., UDOLPH, G. and DOE, C.Q. (1997). The embryonic central nervous system lineages of Drosophila melanogaster. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. *Dev. Biol.* 189: 186-204.

SEIMIYA, M. and GEHRING, W.J. (2000). The Drosophila homeobox gene optix is capable of inducing ectopic eyes by an eyeless-independent mechanism. *Development* 127: 1879-1886.

SERIKAKU, M.A. and O'TOUSA, J.E. (1994). sine oculis is a homeobox gene required for Drosophila visual system development. *Genetics* 138: 1137-50.

SHENG, G., THOUVENOT, E., SCHMUCKER, D., WILSON, D.S. and DESPLAN, C. (1997). Direct regulation of rhodopsin 1 by Pax-6/eyeless in Drosophila: evidence for a conserved function in photoreceptors. *Genes Dev.* 11: 1122-31.

SHIRASAKI, R. and PFAFF, S.L. (2002). Transcriptional codes and the control of neuronal identity. *Annu. Rev. Neurosci.* 25: 251-81.

SIMPSON, T.I. and PRICE, D.J. (2002). Pax6; a pleiotropic player in development. *Bioessays* 24: 1041-51.

TESSMAR, K., LOOSLI, F. and WITTBRODT, J. (2002). A screen for co-factors of Six3. *Mech. Dev.* 117: 103-13.

TESSMAR-RAIBLE, K. and ARENDT, D. (2003). Emerging systems: between vertebrates and arthropods, the Lophotrochozoa. *Curr. Opin. Genet. Dev.* 13(4): 331-340

THOR, S. andERSSON, S.G., TOMLINSON, A. and THOMAS, J.B. (1999). A LIM-homeodomain combinatorial code for motor-neuron pathway selection. *Nature* 397: 76-80.

TOMAREV, S.I., CALLAERTS, P., KOS, L., ZINOVIEVA, R., HALDER, G., GEHRING, W. and Piatigorsky, J. (1997). Squid Pax-6 and eye development. *Proc. Natl. Acad. Sci. USA* 94: 2421-6.

UMESONO, Y., WATANABE, K. and AGATA, K. (1999). Distinct structural domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. *Dev. Genes. Evol.* 209: 31-9.

URBACH, R. and TECHNAU, G.M. (2003). Molecular markers for identified neuroblasts in the developing brain of Drosophila. *Development* 130: 3621-37.

VANDENDRIES, E.R., JOHNSON, D. and REINKE, R. (1996). orthodenticle is required for photoreceptor cell development in the Drosophila eye. *Dev. Biol.* 173: 243-55.

VICZIAN, A. S., VIGNALI, R., ZUBER, M. E., BARSACCHI, G. and HARRIS, W.A. (2003). XOtx5b and XOtx2 regulate photoreceptor and bipolar fates in the Xenopus retina. *Development* 130: 1281-94.

WANG, S.W., GAN, L., MARTIN, S.E. and KLEIN, W.H. (2000). Abnormal polarization and axon outgrowth in retinal ganglion cells lacking the POU-domain transcription factor Brn-3b. *Mol. Cell. Neurosci.* 16: 141-56.

WANG, S.W., KIM, B.S., DING, K., WANG, H., SUN, D., JOHNSON, R.L., KLEIN, W.H. and GAN, L. (2001). Requirement for math5 in the development of retinal ganglion cells. *Genes Dev.* 15: 24-9.

WANG, S.W., MU, X., BOWERS, W.J., KIM, D.S., PLAS, D.J., CRAIR, M.C., FEDEROFF, H.J., GAN, L. and KLEIN, W.H. (2002). Brn3b/Brn3c double knockout mice reveal an unsuspected role for Brn3c in retinal ganglion cell axon outgrowth. *Development* 129: 467-77.

WAWERSIK, S. and MAAS, R.L. (2000). Vertebrate eye development as modeled in Drosophila. *Hum. Mol. Genet.* 9: 917-925.

WILSON, S.W. and RUBENSTEIN, J.L. (2000). Induction and dorsoventral patterning of the telencephalon. *Neuron* 28: 641-51.

WITKOVSKY, P. (2000). Photoreceptor classes and transmission at the photoreceptor synapse in the retina of the clawed frog, Xenopus laevis. *Microsc. Res. Tech.* 50: 338-46.

YASUHARA, O., TOOYAMA, I., AIMI, Y., BELLIER, J.P., HISANO, T., MATSUO, A., PARK, M. and KIMURA, H. (2003). Demonstration of cholinergic ganglion cells in rat retina: expression of an alternative splice variant of choline acetyltransferase. *J. Neurosci* 23: 2872-81.

YASUYAMA, K., KITAMOTO, T. and SALVATERRA, P.M. (1995). Localization of choline acetyltransferase-expressing neurons in the larval visual system of Drosophila melanogaster. *Cell Tissue Res.* 282: 193-202.

YASUYAMA, K. and MEINERTZHAGEN, I.A. (1999). Extraretinal photoreceptors at the compound eye's posterior margin in Drosophila melanogaster. *J. Comp. Neurol* 412: 193-202.

ZHU, C.C., DYER, M.A., UCHIKAWA, M., KONDOH, H., LAGUTIN, O.V. and OLIVER, G. (2002). Six3-mediated auto repression and eye development requires its interaction with members of the Groucho-related family of co-repressors. *Development* 129: 2835-49.