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Reconstructing the eyes of Urbilateria

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The shared roles of *Pax6* and *Six* homologues in the eye development of various bilaterians suggest that Urbilateria, the common ancestors of all Bilateria, already possessed some simple form of eyes. Here, we re-address the homology of bilaterian cerebral eyes at the level of eye anatomy, of eye-constituting cell types and of phototransductory molecules. The most widespread eye type found in Bilateria are the larval pigment-cup eyes located to the left and right of the apical organ in primary, ciliary larvae of Protostomia and Deuterostomia. They can be as simple as comprising a single pigment cell and a single photoreceptor cell in inverse orientation. Another more elaborate type of cerebral pigment-cup eyes with an everse arrangement of photoreceptor cells is found in adult Protostomia. Both inverse larval and everse adult eyes employ rhabdomeric photoreceptor cells and thus differ from the chordate cerebral eyes with ciliary photoreceptors. This is highly significant because on the molecular level we find that for phototransduction rhabdomeric versus ciliary photoreceptor cells employ divergent rhodopsins and non-orthologous G-proteins, rhodopsin kinases and arrestins. Our comparison supports homology of cerebral eyes in Protostomia; it challenges, however, homology of chordate and non-chordate cerebral eyes that employ photoreceptor cells with non-orthologous phototransductory cascades.

Keywords: eye; evolution; photoreceptor; Urbilateria; *Pax6*; *sine oculis*

1. INTRODUCTION

Pax6 homologues are essential for eye formation in vertebrates (Hill *et al.* 1991; Walther & Gruss 1991; Chow *et al.* 1999) and *Drosophila* (Quiring *et al.* 1994; Halder *et al.* 1995*a*). This led to the idea that *Pax6* has an evolutionary conserved function in triggering initial steps of eye development (master control gene hypothesis) (Quiring *et al.* 1994; Halder *et al.* 1995*a*; Gehring & Ikeo 1999). *Pax6* homologues have meanwhile been cloned from various other Bilateria. Whenever eyes are present in the Bilateria investigated, they express the respective *Pax6* homologue (Appendix A), with the exception of the Hesse organs in *Branchiostoma* (Glardon *et al.* 1998). On these grounds, it has recently been proposed that the various eye types found in Metazoa are derived from a common *Pax6*-dependent precursor resembling a two-celled 'prototype eye' with a single photoreceptor cell (Halder *et al.* 1995*b*; Callaerts *et al.* 1997; Gehring & Ikeo 1999; Pichaud *et al.* 2001). Genes belonging to the *Six*/*sine oculis* family equally share common roles in eye development of insects (Cheyette *et al.* 1994; Seimiya & Gehring 2000), vertebrates (Oliver *et al.* 1995; Loosli *et al.* 1998, 1999) and planarians (Pineda *et al.* 2000). Homology of eyes across the Bilateria—and even across the Protostomia and Deuterostomia split, a notion strongly rejected from the morphological viewpoint (Salvini-Plawen & Mayr 1977; Nilsson 1996)—is again open for discussion.

The congruities in insect and vertebrate eye development add to a series of recent comparative studies that have revealed unexpected similarities between these phylogenetically remote groups. These studies have revived the notion that ventral in insects corresponds to dorsal in vertebrates (Arendt & Nübler-Jung 1994; Holley *et al.* 1995), and allow a reconstruction—via the detection of putative interphyletic homologies—of the body plan of Urbilateria, the stem species of all Bilateria (De Robertis & Sasai 1996). Urbilateria should have formed a rather elaborate centralized nervous system with an apical brain (Thor 1995; Arendt & Nübler-Jung 1996; Reichert & Simeone 1999), and longitudinal trunk cords along the ventral body side (Arendt & Nübler-Jung 1999).

It is intended here to collect and review morphological and molecular data available that relate to the question: what type(s) of eyes existed in Urbilateria (if any at all)? Nilsson (1996) has stated that 'for an assessment of the potential homology between eyes of animals from different phyla, useful indicators are the eye's ontogenetic origin, the way photoreceptor cells are constructed, and the molecular machinery responsible for light detection'. In brief, this outlines the three major sections of this paper. We also add a section about comparative anatomy of bilaterian eyes, to explore what urbilaterian eyes might have looked like.

(a) *What is an eye?*

The minimum setting for an eye involves a photoreceptor in the vicinity of shading pigment, which allows the detection of the direction of light. A simple eye consists of one photoreceptor and one pigment cell. Such prototype two-celled eyes are found, for example, in trochophora larvae (see below) and in planarians (Gehring & Ikeo 1999). Our definition also includes single photosensitive cells containing both photo- and shading pigment (e.g. unicellular algae, turbellarian 'epidermal eyes', see Appendix B), but excludes photoreceptor cells not combined with shading pigment (e.g. deep brain photoreceptor cells).

The starting point for the evolution of photoreceptor cells is an epidermal ciliated cell with photopigment (opsin + retinal = rhodopsin) molecules embedded in its membrane. Retinal is the molecule transducing light energy into electrical signals, and opsin is the covalently bound protein carrier. To enhance light sensitivity, the membranous surface enlarges locally by in- or outfolding, to form a light-sensitive organelle (= photoreceptor). In ciliary photoreceptors the ciliary membrane folds into internal discs or tubules, or into outer microvilli or lamellae. In rhabdomeric photoreceptors the apical cell membrane folds into microvilli or lamellae, while the cilium remains unchanged (but nevertheless is present—though often rudimentary). (For examples, see figure 8.)

Pigment cells contain non-photosensitive, light-absorbing pigment such as melanin or pterins. They often acquire the capacity to secrete lens-forming material (Eakin & Westfall 1964, 1965; Fischer & Brökelmann 1966).

Classification of eyes is based on levels of complexity. 'Ocelli' are simple, multicellular eyes comprising photoreceptor cells, pigment cells, and, optionally, additional support cells—the two-celled eye being the simplest variant. Structurally, ocelli often resemble 'pigment cup eyes' where photoreceptors are embedded in a cup-shaped layer of pigment cells. Because light can enter only through the cup opening, the pigment-cup eye already detects direction of light with some accuracy. Optionally, light-harvesting lenses (or lens-resembling 'Füllmasse' (filling mass) of unknown function) are present.

Bilaterian eyes can be of inverse or everse design. In inverse eyes, the receptive organelles of photoreceptor cells project towards the pigment cup, while in everse eyes they project away from it, towards the light. This formal distinction was first introduced for *Polychaeta* (Hesse 1899) and extended to the whole Bilateria (see figure 4 for prototypic inverse eyes and figure 5*a*,*b* for everse eyes). However, there are transitions between the two, both ontogenetically and phylogenetically (see below).

Compound eyes are composed of a (species specific) number of distinct units called ommatidia, described for Polychaeta, Bivalvia and Arthropoda. Structurally, an individual ommatidium on its own resembles a one pigment-cup ocellus.

Complex eyes are found in Cephalopoda and Vertebrata. They consist of cornea, iris, lens and retina.

(b) *Homology of eyes in Bilateria?*

Homologous features of two given animal groups are those 'that stem phylogenetically from the same feature . . . in the immediate common ancestor of these organisms' (Ax 1989) so that their 'non-incidental resemblances are based on shared information' (Schmitt 1995).

In discussing the homology of bilaterian eyes, it is essential first to clarify the level of complexity implied in the homology proposal. 'Homology of cephalopod and vertebrate *eyes*' (Halder *et al.* 1995*b*; Gehring & Ikeo 1999) implies not more (and not less) than that Urbilateria formed a prototype two-celled eye, as suggested by the authors. Beyond that, 'homology of cephalopod and vertebrate *complex eyes*' would imply that Urbilateria already formed complex eyes with cornea, iris, lens and retina, and has inherited all this to extant vertebrates and cephalopods. This is rather unlikely. Instead, *complex eyes* (and also *compound eyes*) can 'serve as textbook examples of functional convergence or parallelism' (Nilsson 1996).

The homology criteria formulated by Remane (1952) have eased the identification of homologous structures. These criteria were applicable at the classical morphological level but nowadays can also be applied at the molecular level. First, the most basic test for homology is to check whether the compared structures form at similar positions in a shared system of spatial reference, such as conserved body plans, early axonal scaffolds, or expression patterns of conserved early regionalization genes (see below). This 'criterion of position' already excludes eyes forming at 'aberrant' positions from any long-range homology proposal. For instance, pygidial eyes at the posterior end of sabellid polychaetes, or the cushion-like eyespots on the arms of starfish will not find interphyletic counterparts. (The possibility of organ displacement remains to be considered, however, but this is unlikely in the above cases.) Second, the 'criterion of specific quality' asks for similarities of the compared structures in specific characteristics. Again, this can apply at the structural or at the molecular level. Structurally, we consider the inverse versus everse design of bilaterian eyes and the eye-constituting cell types, such as ciliary versus rhabdomeric photoreceptor cells. Molecularly, one can compare the specific expression of developmental control genes such as *Pax6* (Appendix A) or of downstream genes involved in the phototransductory cascade (see below). Third, the 'criterion of continuity' asks for interconnecting forms that can be identified in the ontogeny and/or phylogeny of living species, or of extinct species. We will discuss the phylogenetic 'continuity' of eyes for the major bilaterian branches.

2. ANCESTRALITY OF CEREBRAL EYES?

In Bilateria there is a plethora of eyes, at various locations and of remarkable structural diversity, portrayed extensively for Polychaeta (e.g. Hesse 1899; Verger-Bocquet 1984; Rhode 1991) and Molluscsa (Barber *et al.* 1967; Barber & Wright 1969; Hughes 1970; Land 1984*b*). In an extensive survey, Salvini-Plawen & Mayr (1977) have listed various eye types, many of which they consider to be new evolutionary acquisitions (see also Salvini-Plawen 1982). What is intended here is to distil out recurrent eye types in Bilateria—which may, in the end, reflect interphyletic homology. Most promising candidates for evolutionary conservation across phyletic boundaries are the paired cerebral eyes that form in anterior body regions of various Bilateria, innervated by the cerebral ganglia. We prefer the term 'cerebral eyes' to 'cephalic eyes' because the occurrence of a brain (cerebrum) appears to be a more constant and reliable character than that of a head (cephalon). (For example, in Bivalvia, there is no head due to the rather derived morphology while a brain still forms.) The widespread larval eyespots are 'cerebral' in nature because they form in close vicinity to the developing brain. Notably, in all Bilateria so far studied, the *Pax6*-expressing eyes are cerebral eyes, while the non-cerebral Hesse eyecups of the lancelet do not express *Pax6* (Appendix A).

Following the criterion of continuity, any homology of cerebral eyes across Bilateria would require their presence

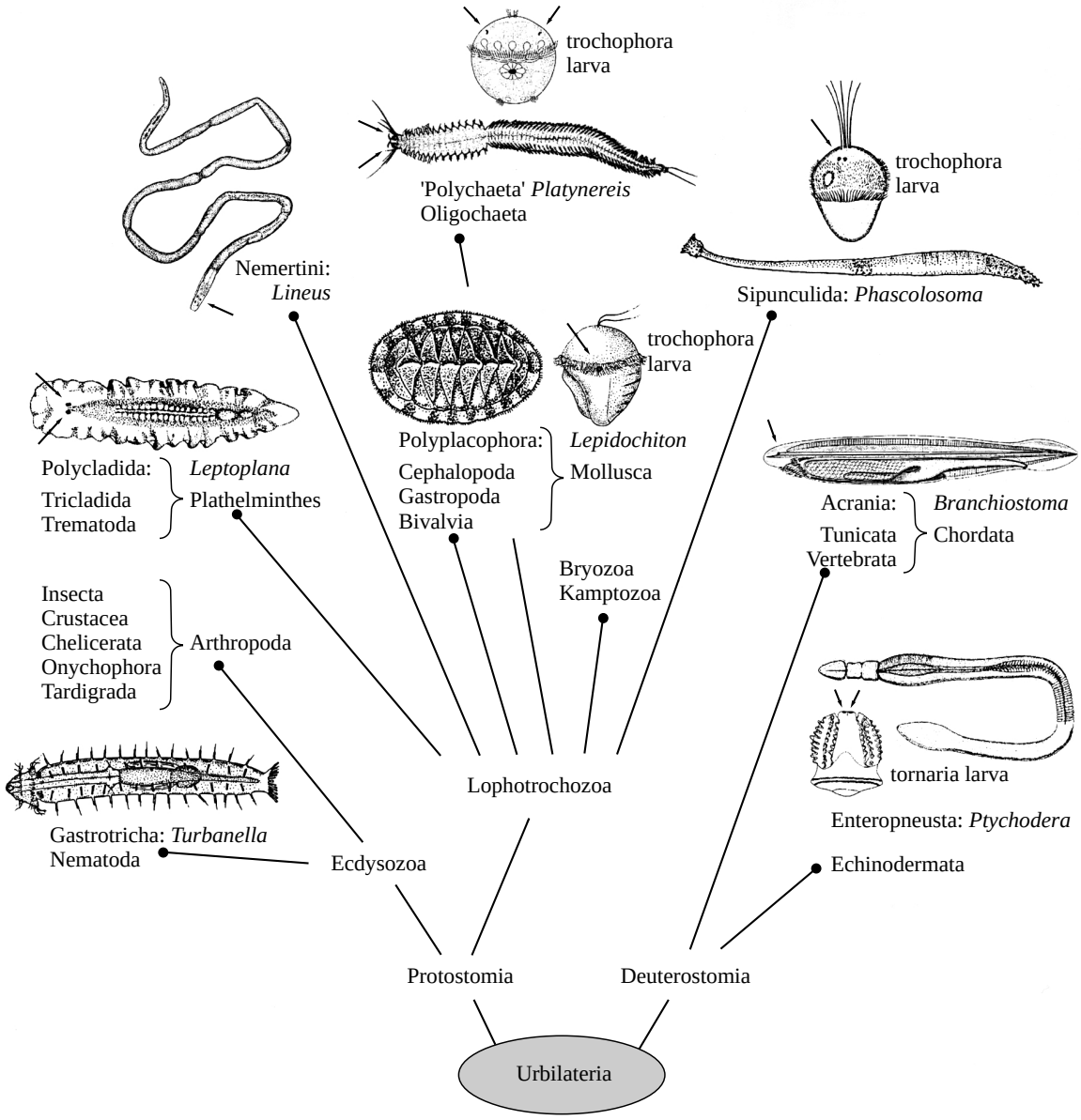

Figure 1. Phylogenetic tree of the Bilateria, with cerebral eyes marked by arrows in an exemplary manner. For clarity, some groups have been omitted. Current molecular phylogenies divide the Bilateria into three major branches, Deuterostomia, Lophotrochozoa and Ecdysozoa (Field *et al*. 1988; Halanych *et al*. 1995; Kim *et al*. 1996; Aguinaldo *et al*. 1997; and compare also Peterson *et al*. 2000). We hold to this basal subdivision here although uncertainties remain (Winnepenninckx *et al*. 1998). Drawings adapted from Dorresteijn *et al*. 1993 with kind permission of Springer-Verlag; Gerould 1906; Peterson *et al*. 1999 with kind permission of The Company of Biologists Ltd; Riedl 1983, with kind permission of Blackwell Wissenschafts-Verlag; Willey 1898).

at the base of each of the major branches (figure 1). However, the still limited resolution in the branching pattern of phylogenetic trees (Field *et al*. 1988; Halanych *et al*. 1995; Kim *et al*. 1996; Aguinaldo *et al*. 1997; Winnepenninckx *et al*. 1998) does not yet allow the reliable identification of basal groups in the Lophotrochozoa. Moreover, even if an identified (extant) basal group lacked eyes, we cannot infer that this was also true for an (extinct) stem group. Secondary loss of eyes is a probable process—even found in extant sister species—when living in an aphotic environment. 'Degeneration (of eyes) may . . . occur when a phyletic line of marine invertebrates with well-developed eyes invades a niche in which photoreceptors are no longer maintained by selection (aphotic zone, tunnelling in the substrate, etc.)' (Salvini-Plawen & Mayr 1977, p. 210). For example, this accounts for the absence of eyes in some mollusc groups (Caudofoveata, Scaphopoda, Monoplacophora) (Rosen *et al*. 1979), in Clitellata (Annelida), or in Phoronida (Tentaculata). However, other eyeless groups such as Solenogastres (Mollusca), or Pterobranchia do not live in especially aphotic habitats. Thus, should some kind of cerebral eyes be ancestral for Bilateria, secondary eye loss will have to be accounted for in these latter groups (Salvini-Plawen 1982). As a conclusion, ancestrality of cerebral eyes appears to be a tenable hypothesis in the light of the widespread occurrence of cerebral eyes in Protostomia (Ecdysozoa and Lophotrochozoa) and their presence in lower Deuterostomia (in enteropneust tornaria larvae).

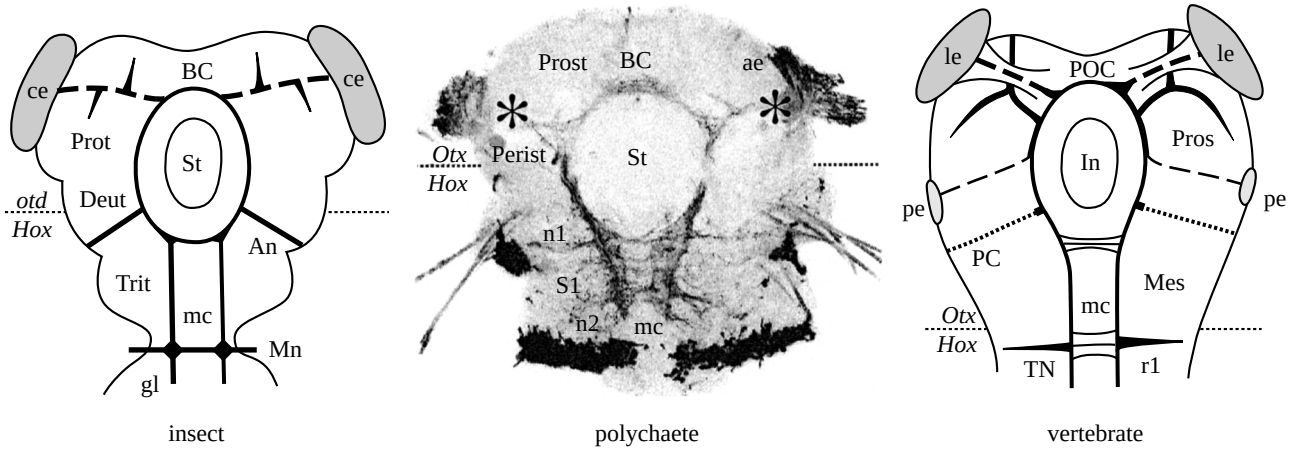

Figure 2. Innervation of eyes from axonal scaffold in Insecta, Polychaeta and Vertebrata, and formation within the *Otx*-region. Stars indicate position of polychaete adult eyes. Insect and vertebrate scaffolds modified after Arendt & Nübler-Jung (1996). Abbreviations: ae, adult eyes; An, antennal nerve; BC, brain commissure; ce, compound eyes; Deut, deutocerebrum; In, infundibulum; le, lateral eyes; mc, midline cells; Mes, mesencephalon; Mn, mandibular nerve; Perist, peristomium; PC, posterior commissure; pe, pineal eyes; POC, postoptic commissure; Pros, prosencephalon; Prost, prostomium; Prot, protocerebrum; St, stomodaeum; Trit, tritocerebrum.

(a) *The early axonal scaffold*

Considering next the criterion of position, cerebral eyes in Bilateria obviously share their anterior position. Beyond that, early axonal scaffolds can be utilized as a conserved system for spatial reference (figure 2). In representatives of all bilaterian superphyla, early axonal scaffolds consist of two longitudinal axon bundles on both sides of the neural midline that form from the *NK-2.2*-specified medial column of the nervous system anlage (Arendt & Nübler-Jung 1999). These run into an anterior loop, the most anterior part of which is the prominent brain commissure (Wilson *et al.* 1990; Boyan *et al.* 1995; Therianos *et al.* 1995; D. Arendt & J. Wittbrodt, unpublished data). On these grounds, homology of early axonal scaffolds has been proposed (Arendt & Nübler-Jung 1996), making them a suitable reference system for the developing visual system. In insects, annelids and vertebrates, the outgrowing axons of the developing cerebral eyes connect to the axonal scaffold at similar positions, namely at the level of the very anterior brain commissure (figure 2). However, since this similarity reflects rather obvious functional constraints for cerebral eyes it is a precondition rather than support for their homology.

(b) *Molecular framework for spatial reference*

The criterion of position can nowadays be tested at the molecular level, in that the structures compared should form in conserved body regions specified by homologous regionalization genes. One such region is the *Otx* territory located in the anterior body regions of all species examined (Bruce & Shankland 1998; Mitsunaga-Nakatsubo *et al.* 1998; Stornaiuolo *et al.* 1998; Acampora & Simeone 1999; Hirth & Reichert 1999; Reichert & Simeone 1999; Umesono *et al.* 1999; Wada & Saiga 1999; Kimura *et al.* 2000) (figure 2). Cerebral eyes originate from the *Otx*-region in vertebrates, insects, ascidians (Wada & Saiga 1999), planarians (Umesono *et al.* 1999) and polychaetes (Arendt *et al.* 2001), and cerebral eye formation requires functional *Otx*, at least in insects (Finkelstein *et al.* 1990; Vandendries *et al.* 1996) and in vertebrates (Acampora & Simeone 1999; Suda *et al.* 1999). Promoter studies indicate that phototransductory molecules, such as opsins and arrestins, are directly regulated by *Otx*- and *Pax6*-transcription factors (Kimura *et al.* 2000). This underscores the affiliation of cerebral eyes to the *Otx*-expression territory.

3. COMPARATIVE ANATOMY OF CEREBRAL EYES IN BILATERIA

(a) *Larval cerebral eyes*

Conspicuous eyespots can be observed in primary, ciliary larvae commonly found in Lophotrochozoa and in basal Deuterostomia (figure 1). Their basic design and widespread distribution in the Bilateria makes them a plausible starting point for a comparative survey of bilaterian cerebral eyes. In many cases they locate to similar positions, namely left and right of, but variable distances to the apical organ. The 'trochophora', a primary, ciliary larva with conspicuous apical eyespots, is considered ancestral at least for Lophotrochozoa (Ax 1995). And since larval body plans appear to be widely conserved during evolution (Arendt *et al.* 2001; Peterson *et al.* 2000) (and see below), ancestrality of primary, ciliary larvae with apical, cerebral eyespots could well extend to Deuterostomia.

In Lophotrochozoa, the paired larval eyespots of the polychaete trochophora larva match the bilaterian prototype two-celled eye. Their structure is exemplified in figure 3*a* for *Platynereis dumerilii* (Rhode 1992). They are referred to as inverse, because the photoreceptor, the rhabdome, is orientated towards the concavity of the pigment cell. Similar inverse larval eyes are found in sipunculan worms and in flatworms (figure 3*b*), two other lophotrochozoan groups. However, in molluscs, pigment and photoreceptor cells of larval eyes are arranged in an everse manner (figure 3*c*). Homology of mollusc and polychaete larval eyes would thus imply a transition from

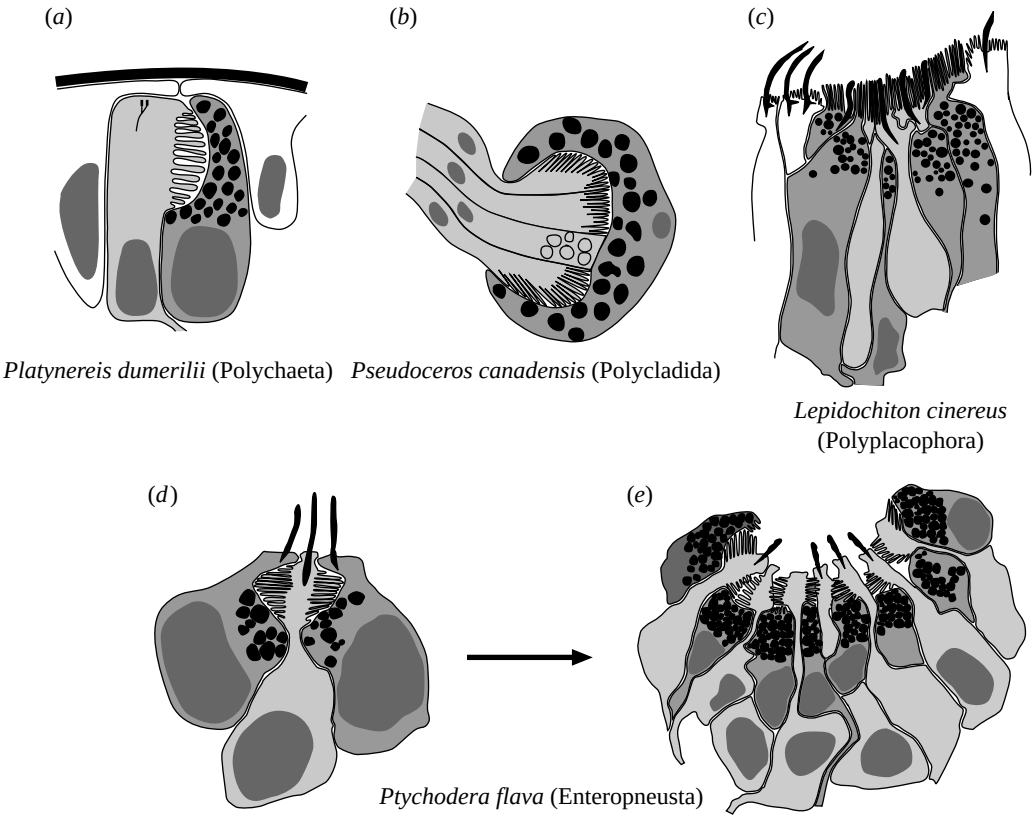

Figure 3. Two-celled and primitive pigment-cup eyes of primary ciliary larvae in Bilateria. Light grey, photoreceptor cell; dark grey, pigment cell. (*a*) Larval eye of polychaete trochophora (*Platynereis dumerilii*) after Rhode (1992). (*b*) Left larval eye of turbellarian Müller's larva (*Pseudoceros canadensis*, Polycladida) after Eakin & Brandenburger (1980, 1981) and Fournier (1984, p. 220, fig. 2c). Ciliary photoreceptor cell with transverse cilia indicated by open circles. (*c*) Larval eye of mollusc trochophora (*Lepidochiton*, Polyplacophora) after Bartolomaeus (1992*b*). (*d*) Early larval eye of six-day enteropneust tornaria larva (*Ptychodera flava*) after Brandenburger *et al*. (1973). (*e*) Late larval eye of tornaria (tentaculate stage) (*Ptychodera flava*) after Brandenburger *et al*. (1973).

inverse to everse design in the evolution of molluscs. A more detailed phylogenetic survey of larval eyes is given in Appendix B.

What about larval cerebral eyes outside Lophotrochozoa? In ecdysozoan Crustacea, the first free-living larval stage is the three-segmented nauplius larva. It shows a tripartite larval eye that may trace back to polychaete-like precursor forms (Appendix B). In lower Deuterostomia, on the other hand, larval eyespots are common in the tornaria larvae of Enteropneusta (e.g. Stiasny 1914). Notably, in early tornaria eyespots (figure 3*d*) the cellular arrangement very much resembles that of larval eyes in trochophora larvae in that the receptive organelles, the rhabdomens, are orientated towards the concavity of the pigment cells (compare figure 3*a* with 3*d*). Deviating from this, in the later tornaria the larval eyes acquire an everse design (figure 3*e*), a state analogous to the polyplacophoran trochophora (compare figures 3*c* and 3*e*).

In conclusion, larval cerebral eyespots in primary, ciliary larvae share an apical position and have in common a very simple structure, comprising one to a few photoreceptor cells and one to a few pigment cells, mostly arranged in a characteristic inverse manner. This is in line with the notion of evolutionary conservation. Homology is supported by the similar employment of rhabdomeric photoreceptor cells, as will be outlined in § 4. There is a general tendency towards increasing cell numbers of both photoreceptor and pigment cells. Evolutionary conservation of larval eyes in Bilateria would also involve a transition from inverse to everse in at least two independent lines (molluscs and enteropneusts). These are recurrent themes in the evolution of cerebral eyes in adult Bilateria.

(b) *Inverse cerebral eyecups: neoteny of larval eyes*

Outwardly directed eyecups of inverse design are not restricted to larvae, but also occur in adults. Examples of their structure are given in figure 4 and an evolutionary overview in given in Appendix C. In polychaetes, the structure of adult inverse eyes either closely matches the larval pattern (figure 4*a*), or shows a more elaborate design (figure 4*b*) revealing a tendency to increase pigment and photoreceptor cell numbers (Verger-Bocquet 1984, p. 291). As described for *Polygordius*, polychaete inverse cerebral eyes are persisting larval eyes (Brandenburger & Eakin 1981).

Inverse adult eyecups have also been described for other lophotrochozoan groups, for example Nemertini (Vernet 1970; Storch & Moritz 1971) (figure 4*c*) or flatworms (figure 4*d*,*e*). As in the polychaetes, adult flatworm inverse eyecups develop directly from the larval eyespots (Appendix C). Characteristic for flatworm inverse eyes,

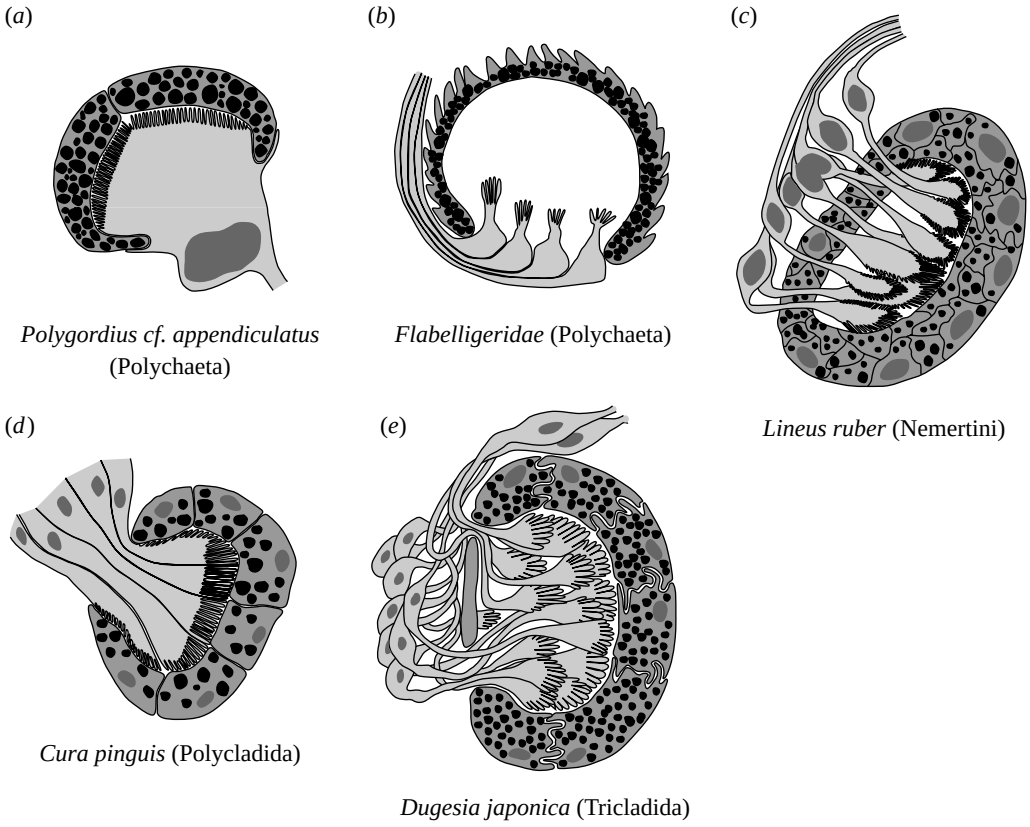

Figure 4. Inverse eyes in Bilateria. Light grey, photoreceptor cell; dark grey, pigment cell. (*a*) *Polygordius* cf. *appendiculatus*, Polychaeta, after Brandenburger & Eakin (1981, fig. 1F). (*b*) Flabelligeridae, Polychaeta after Spies (1975) and Verger-Bocquet (1984). (*c*) *Lineus ruber*, Nemertini, after Vernet (1970, fig. 15A). (*d*) *Cura pinguis*, Polycladida, after Durand & Gourbault (1977) and Fournier (1984). (*e*) *Dugesia japonica*, Tricladida, after Kishida (1967, fig. 32E).

there is a tendency of eye duplication, a tendency to generally increase cell number (Hesse 1897; Fournier 1984) and there is an evolutionary series from inverse to everse design, as noted by (Hesse 1902).

Due to their very similar structure, cerebral inverse eyes of polychaetes, flatworms and nemerteans are considered 'obviously homologous' by Salvini-Plawen & Mayr (1977), who also hold the view of extreme polyphyly of eyes. However, the similar structure of adult inverse eyes is apparently due to the fact that they represent larval inverse eyes neotenously taken over by the adults. This can easily have occurred several times convergently and thus would make adult inverse eyes a case of evolutionary parallelism (independent evolution from homologous sources) (Hodin 2000).

(c) *Everse pigment-cup adult eyes in Protostomia*

There is another recurrent type of cerebral eyes in adult Bilateria, the everse pigment-cup eyes. Examples of structure are given in figure 5, and an evolutionary overview in Appendix C. The everse eye type may represent a second, distinct type of eye conserved in Bilateria because, in contrast to the inverse adult eyes, it is not a derivative of the larval eyes but represents a separate formation, at least in the cases studied (polychaetes, sipunculans, arthropods: Appendix C). Everse eye development is exemplified for the polychaete *Platynereis* in figure 6. Characteristically, there is a transitory developmental state very reminiscent of inverse larval eye design (compare figure 3*a* and 3*d* to figure 6*c*). In this respect, *Platynereis* everse eye development resembles that of enteropneust larval eyes, where a similar inverse-to-everse transition occurs (figure 3*d*,*e*). This ontogenetic transition might recapitulate a phylogenetic inverse-to-everse transition as postulated above, e.g. for polyplacophoran larval eyes.

Among lophotrochozoans, everse adult eyes are found in carnivorous polychaetes (figure 5*a*), various molluscs (figure 5*b* and 5*c*), and sipunculans (figure 5*d*; Appendix C). They all have in common a very specific structure with photoreceptive cell processes traversing the pigment cell layer. Everse eyes have also been described for Ecdysozoa and repeatedly considered homologous to polychaete everse eyes on the basis of detailed structural comparisons (Eakin & Westfall 1965; Hermans & Eakin 1974; Salvini-Plawen & Mayr 1977). However, there is a strong caveat to this. Given that an evolutionary transition from inverse to very similar everse eyes took place in independent evolutionary lines, the everse eye design as such can only be a weak argument for evolutionary conservation. Clearly, a comparative analysis of everse-eye-specific molecular markers is a very promising tool to clarify this issue.

(d) *Cerebral adult eyes in chordates: inverse or everse?*

There are no examples of adult everse eyes in the lower Deuterostomia because in adult enteropneusts and pterobranchs eyes are entirely lacking (Dawydoff 1948). In

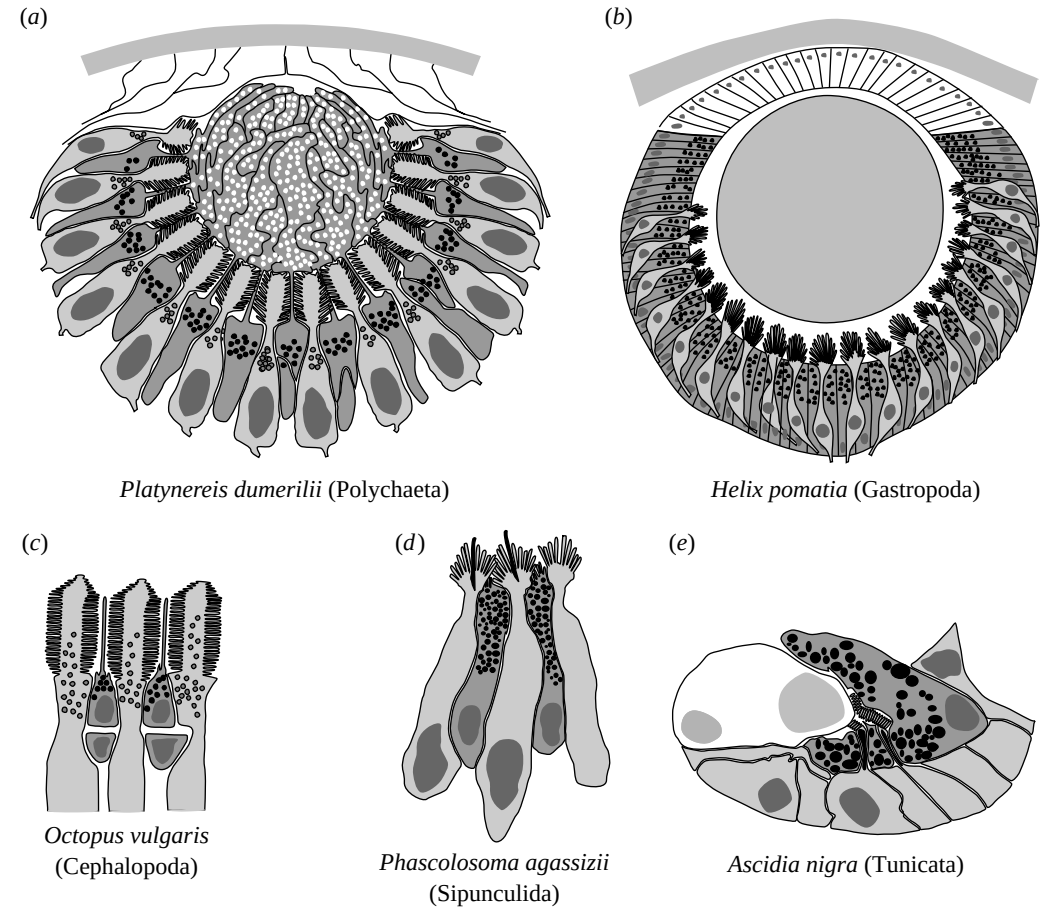

Figure 5. Everse cerebral eyes in Protostomia. Light grey, photoreceptor cell; dark grey, pigment cell; white circles, lens vesicles; black circles, photoreceptor cell pigment vesicles; spotted pattern represents lens. (*a*) Everse eye in *Platynereis dumerilii*, Polychaeta, after Fischer & Brökelmann (1966). (*b*) Everse eye in *Helix pomatia*, Gastropoda, after Hesse (1908) and Land (1984*b*). (*c*) Everse eye of *Octopus*, Cephalopoda, after Yamamoto *et al.* (1965). (*d*) Everse eye of *Phascolosoma agassizii*, Sipunculida, after Hermans & Eakin (1969). (*e*) Semi-inverse eye of *Ascidia nigra*, Tunicata, after Jefferies (1986, p. 107f).

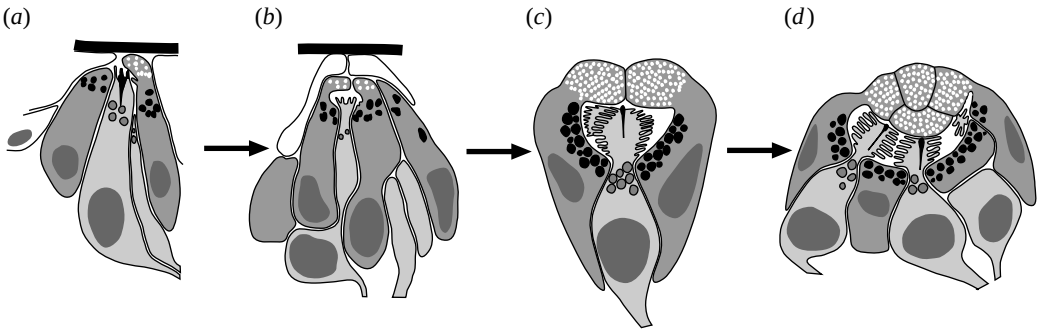

Figure 6. Developing adult eye in: (*a*) early; (*b*) late two-day-old larvae; (*c*) late three-day-old larvae; and (*d*) in three-week-old young worm of *Platynereis dumerilii* (Polychaeta). Light grey, photoreceptor cell; dark grey, pigment cell; white circles, lens vesicles; black circles, photoreceptor cell pigment vesicles. After Rhode (1992).

chordates, on the other hand, cerebral eyes are present. They are 'inverse' because photoreceptor cells in the ascidian ocelli (Dilly 1961; Eakin & Kuda 1971; Dilly & Wolken 1973), in the *Branchiostoma* frontal organ (Lacalli 1996) and lamellate organ (Ruiz & Anadon 1991*b*), as well as vertebrate lateral eyes and the medial pineal/parietal eyes, are all directed inwardly towards the pigment cells, away from the light. However, the inverse character of chordate eyes can essentially be traced back to an everse situation inverted by neurulation (figure 7). Since neurulation is a derived feature of the chordates, chordate cerebral eyes thus become conceptually equivalent to everse cerebral eyes in non-chordate groups. Obviously, this does not sustain homology but rather describes the way chordate and non-chordate eyes can be compared at all.

Depicted in figure 5*e* is the ocellus of *Ascidia nigra* (Dilly 1961; Jefferies 1986, p. 107f). It bears some resemblance to

non-chordate everse eyes in that the apical processes of photoreceptor cells project through the cup-shaped layer of pigment cells into the cavity of the cup (Eakin & Kuda 1971). However, ascidian ocelli differ in an important aspect: while in the vast majority of non-chordate everse eyecups rhabdomeric photoreceptor cells are employed, the photoreceptor of the ascidian ocelli is a modified cilium. This difference in the photoreceptor cell type is of high phylogenetic significance, as will be outlined in the next two sections.

4. RHABDOMERIC VERSUS CILIARY PHOTORECEPTORS: AN ENIGMATIC DICHOTOMY

There is a clear bias of photoreceptor cells to either enlarge the apical cell membrane or the ciliary membrane. They thus form part of the rhabdomeric versus ciliary types of photoreceptors (figure 8). This dichotomy in structure appears to be rather strict. Any 'intermediate type' (Salvini-Plawen & Mayr 1977, p. 245) that should exist, for example, in the starfish *Henricia* (Asteroidea) has been refuted. Starfish photoreceptor cells were reinvestigated and assigned to the rhabdomeric type (Eakin 1979). The coexistence of ciliary and rhabdomeric photoreceptor has never been established physiologically. The 'ciliary' versus 'rhabdomeric' duality of photoreceptor types was complemented repeatedly by the introduction of new photoreceptor types but these are of unclear or doubtful vindication (Appendix D). For a detailed overview of photoreceptor ultrastructure see Eakin 1963, 1966, 1968, 1979, 1982; Salvini-Plawen & Mayr 1977; Salvini-Plawen 1982; and Vanfleteren & Coomans 1976, 1982.

(a) *Phylogenetic distribution of ciliary and rhabdomeric photoreceptors*

In contrast to Eakin's initial proposition that ciliary photoreceptors should be 'characteristic' for Deuterostomia and rhabdomeric photoreceptors for Protostomia (= Lophotrochozoa + Ecdysozoa) (Eakin 1963, 1966, 1968, 1979, 1982), it turned out that both types coexist in Lophotrochozoa, Ecdysozoa and Deuterostomia (figure 8). Remarkably, however, the tissue distribution of rhabdomeric versus ciliary photoreceptors is not random. In Protostomia as well as in lower Deuterostomia, cerebral eyes have rhabdomeric photoreceptors (figures 3–5). This applies for Lophotrochozoa where, beside the numerous examples for rhabdomeric cerebral eyes, there are only few ciliary 'exceptions' (Ehlers & Ehlers 1977; Vanfleteren & Coomans 1982), such as the left larval eye of *Pseudoceros* (Polycladida) with one ciliary photoreceptor interspersed between rhabdomeric photoreceptor cells (Eakin & Brandenburger 1980) (figure 3*b*), or the larval eyes of Bryozoa composed entirely of ciliary photoreceptors (Woollacott & Zimmer 1972) and Kamptozoa (Woollacott & Eakin 1973). Most lophotrochozoan ciliary photoreceptors are found in non-cerebral eyes that form at highly divergent positions and are more likely to be phylogenetically young (Salvini-Plawen & Mayr 1977; Eakin 1982, p. 100; Burr 1984, p. 161). For example, in polychaetes ciliary photoreceptors have been detected in the branchial crown eyes (Eakin & Hermans 1988) and in molluscs ciliary photoreceptor cells form part of the mantle edge eyes and optic tentacles (Barber *et al.* 1967; Barber & Wright 1969; Hughes 1970). Ciliary brain photoreceptors that have been detected in the posterior brain of polychaetes (Dhainaut-Courtois 1965; Whittle & Golding 1974; Rhode 1991) and of Nemertini (Vernet 1974) are not associated with pigment cells and thus do not represent eyes.

In Ecdysozoa, the overwhelming majority of rhabdomeric photoreceptors in cerebral eyes is again complemented by a few 'exceptions' of a ciliary nature, in Nematoda (Burr & Burr 1975), Gastrotricha (Teuchert 1976) and presumably also in the Belloncini organ of Crustacea (Chaigneau 1984).

In Deuterostomia, photoreceptor cells in the apical eyespots of the tornaria larva are rhabdomeric (Brandenburger *et al.* 1973). Therefore, larval cerebral eyes with rhabdomeric photoreceptor cells might have existed at the very root of the Deuterostomia (see figure 8). Rhabdomeric photoreceptor cells have also been found in echinoderms, in the non-cerebral ocelli of the holothurian *Opheodesoma* (Yamamoto & Yoshida 1978) and in the cushion-like eyespots of three starfish genera (*Patiria*, *Leptasterias* and *Henricia*) (Eakin 1979). In the chordate line, rhabdomeric and ciliary photoreceptors coexist in the *Branchiostoma* cerebral vesicle where Joseph cells are rhabdomeric (Welsch 1968; Ruiz & Anadon 1991*a*) while lamellate cells are probably of ciliary design (Ruiz & Anadon 1991*b*; and compare with Meves 1973). These cells, however, are not associated with pigment cells and thus do not form part of an eye. Remarkably, in contrast to the vast majority of Bilateria, in chordates cerebral eyes have ciliary photoreceptors. This is true for the frontal organ in *Branchiostoma* (Lacalli *et al.* 1994) and for the cerebral pigment-cup eye in the ascidian tadpole (Barnes 1971). The vertebrates are in fact the only deuterostomes not possessing any rhabdomeric photoreceptors (Vanfleteren & Coomans 1982). On the other hand, the non-cerebral Hesse eyecups in *Branchiostoma* (Ruiz & Anadon 1991*a*), and siphon eye spots in *Ciona* (Dilly & Wolken 1973) are again rhabdomeric.

(b) *Bilaterian photoreceptors: single, dual or multiple origin?*

What is the genealogical relationship of the two widespread basic photoreceptor types—rhabdomeric and ciliary—in Bilateria? Or, to view it from a different angle, what kind of photoreceptor cells were present in Urbilateria—if at all?

Salvini-Plawen & Mayr (1977) have put forward the view of extreme polyphyly in photoreceptor evolution. They postulated that eyes and photoreceptive cells have originated several times independently in at least 40 if not 65 or more different lines. This would imply that Urbilateria did not possess any kind of photoreceptor cell (or eye) but merely some kind of indefinite sensory ciliary precursor cell (figure 8*a*).

Vanfleteren & Coomans (1976, 1982) have advanced the view that all photoreceptors can be traced back to a single type of photoreceptor precursor present in Urbilateria (figure 8*b*). Based on the observation that in both types of photoreceptors the photoreceptive organelle is induced by a ciliary formation—that, after initiating membrane expansion, may become more or less abortive (rhabdomeric type) or may develop further into a ciliary organelle (ciliary type)—they argue that the difference

between both types is more quantitative than qualitative. A characteristic of this view is that it allows transitions from one receptor type to the other.

Another possible view takes into account the widespread occurrence of both types of photoreceptor cells, ciliary and rhabdomeric, and infers that at least one type, and possibly both types, were present already in Urbilateria (figure 8*c*). They were then inherited to the diverging superphyla in a parallel manner (i.e. not in an either–or fashion).

Obviously, it is not possible to opt for or against any of these views on the basis of occurrence and the ultrastructure of photoreceptor cells only. Also, the views are not strictly exclusive because even if one or two types of photoreceptor cells already existed in Urbilateria, these were not necessarily the precursors for all photoreceptor cells in Bilateria. Convergent evolution of subsets of photoreceptor cells are a clear option. Salvini-Plawen & Mayr (1977) give an illustrative example, where the ciliary photoreceptor cells in the distal retina of the mantle edge eyes in Bivalvia are deduced from an epidermal, ciliary sense organ, previously unrelated to vision.

There is yet another level on which homology of photoreceptor cell types can be tested, namely, whether or not they employ orthologous molecules for light detection and phototransduction.

5. TWO NON-HOMOLOGOUS CASCADES FOR PHOTOTRANSDUCTION IN CILIARY VERSUS RHABDOMERIC PHOTORECEPTORS IN BILATERIA

As the first step in phototransduction, photoactivation of rhodopsin involves the isomerization of covalently bound retinoids. Photoactivated rhodopsin then activates a G-protein that in turn activates intracellular messengers to finally hyperpolarize or depolarize the photoreceptor cell. Subsequent quenching of phototransduction involves phosphorylation of photoactivated rhodopsin by the enzyme rhodopsin kinase followed by binding of the protein arrestin, which competes with the G-protein for binding to photoactivated rhodopsin (Krupnick *et al.* 1997). Factors related to these four molecules have been isolated for various Bilateria and found to be active in the light detection cascade (Van Veen *et al.* 1986; Hyde *et al.* 1990; Smith *et al.* 1990; Yamada *et al.* 1990; Cassill *et al.* 1991; Zuker 1994; Kikkawa *et al.* 1998). Accordingly it is postulated that rhodopsin, a G-protein, rhodopsin kinase and arrestin precursor molecules participated in light detection and phototransduction in putative photoreceptor cells in Urbilateria.

What is the significance of this for photoreceptor cell evolution? First, it seems unlikely that Urbilateria did not possess photoreceptor cells in some form (refuting figure 8*a*). Beyond this, we utilize the sequence information available for these molecules to test whether bilaterian ciliary and rhabdomeric photoreceptor cells derive from a common photoreceptor precursor cell (figure 8*b*) or from distinct precursors (figure 8*c*). In the former case, one would expect that rhodopsins, associated G-proteins, rhodopsin kinases and arrestins employed in ciliary and rhabdomeric receptor cells in the present should be homologous in a strict sense (orthologous), meaning that they should have emerged from one single precursor molecule of each type, present already in Urbilateria. In the latter case, the existence of distinct photoreceptor cell precursors at the very root of Bilateria might have involved a duplication and subsequent divergence of phototransductory molecules. In other words, distinct paralogues for rhodopsin, for the associated G-protein, for rhodopsin kinase and for arrestins would be expected and could have already existed in Urbilateria—one 'rhabdomeric', one 'ciliary' paralogue each. Strikingly, this is what the sequence comparisons of all four molecules indicates (figure 9).

(a) *Separate 'ciliary' versus 'rhabdomeric' opsins as common heritage of Bilateria*

The phylogenetic tree of rhodopsin molecules (figure 9*c*) shows that 'ciliary' and 'rhabdomeric' opsins are highly divergent. They probably trace back to distinct genes present in Urbilateria, meaning they are non-orthologous. Vertebrate 'ciliary' opsins—expressed in the ciliary photoreceptor cells of lateral eyes and pineal—are more closely related to retinochromes than to invertebrate 'rhabdomeric' opsins. In turn, the invertebrate 'rhabdomeric' opsins—expressed in the rhabdomeric photoreceptor cells in insects (O'Tousa *et al.* 1985; Zuker *et al.* 1985; Fryxell & Meyerowitz 1987; Salcedo *et al.* 1999) and molluscs (Kojima *et al.* 1997)—are more closely related to vertebrate melanopsins (Provencio *et al.* 1998, 2000) than to vertebrate 'ciliary' opsins. The evolutionary divergence of 'ciliary' and 'rhabdomeric' opsins makes it unlikely that present day vertebrate ciliary photoreceptor cells, and invertebrate rhabdomeric photoreceptor cells, stem from a common Urbilaterian precursor (figure 8*b*). On the other hand, photoreceptor cells expressing orthologous opsins are more likely to descend from a common photoreceptor cell precursor—although evolutionary co-option of the same molecule cannot be ruled out (see § 5b).

An interesting case is the occurrence of 'rhabdomeric' opsins in vertebrates given that they do not possess rhabdomeric photoreceptor cells. However, the occurrence of rhabdomeric photoreceptor cells in all other deuterostome groups (see § 4) makes it an attractive hypothesis that (a subset of) the vertebrate melanopsin-expressing cells traces back to rhabdomeric photoreceptor cells (with reduced rhabdomen). In the vertebrate eye, melanopsins are expressed in cells of the pigmented retinal epithelium and in retinal ganglion cells (Provencio *et al.* 1998, 2000). The apparent photosensitivity of these cells is consistent with the finding that naturally occurring and transgenic mice that lack ciliary photoreceptors are still capable of photoregulating circadian rhythms and pineal activity through their lateral eyes (Freedman *et al.* 1999; Lucas *et al.* 1999), while bilateral removal of the eyes abolishes such regulation (Nelson & Zucker 1981).

The scallop Go-rhodopsin is of unclear affinity (Kojima *et al.* 1997). Remarkably, it is expressed in the ciliary—not rhabdomeric—photoreceptors in the distal retina of the mantle edge eye, considered an evolutionary novelty (see above).

(b) *Non-orthologous phototransductory cascades*

In all bilaterian photoreceptors photoactivated rhodopsins activate heterotrimeric GTP-binding proteins

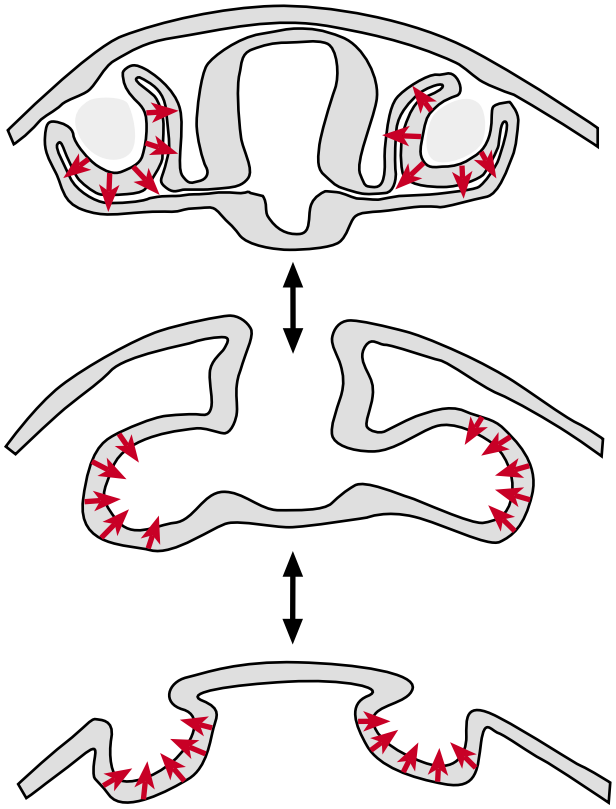

Figure 7. Possible transition from everse to inverse eyes in early chordates with the evolution of neurulation. Red arrows indicate basal–apical orientation of photoreceptors.

(G-proteins). These in turn convey the photoexcitement to internal messengers. Phylogenetic analysis has revealed that the α-subunit of G-proteins underwent extensive gene duplication in early animal evolution (Yokoyama & Starmer 1992; Suga *et al.* 1999). Strikingly, ciliary versus rhabdomeric photoreceptor cells employ non-orthologous molecules. Transducins are active in vertebrate ciliary photoreceptors. Direct invertebrate counterparts for transducins are lacking but phylogenetic analysis based on DNA sequences reveals that transducins have emerged from the Gi-α precursor, and not from the Go-α or Gq-α precursor (figure 9*d*) (Yokoyama & Starmer 1992; Suga *et al.* 1999). In line with this, vertebrate Gi-α is functionally analogous to transducin (Kanaho *et al.* 1984).

As to the rhabdomeric photoreceptors, the *Drosophila* Gq-α protein is expressed in the ocelli and in all eight photoreceptor cell rhabdomeres of the lateral eyes (Lee *et al.* 1994). A Gq protein also localizes to the rhabdomeric cerebral ocelli of *Perinereis* (Polychaeta) (Miyako-Shimazaki *et al.* 1999) and is involved in phototransduction in the rhabdomeric receptor cells of cephalopod cerebral eyes (Bhatia *et al.* 1996; Kikkawa *et al.* 1996), of crayfish lateral eyes (Terakita *et al.* 1993) and of *Limulus* lateral eyes (Munger *et al.* 1996). Notably, Gq-α homologues also exist in the vertebrates (figure 9*d*). They are widely expressed in neuroectodermally derived tissue—but not, however, in the ciliary receptor cells of the eye (Wilkie *et al.* 1991; Zhou *et al.* 1994).

The only invertebrate ciliary photoreceptor cells investigated so far for G-α expression are those of the scallop (Kojima *et al.* 1997). Remarkably, they specifically express a Go-α orthologue. Therefore, and remarkably, the scallop Go-α is non-orthologous both to 'ciliary' vertebrate transducins, and to 'rhabdomeric' invertebrate Gq-α. This underscores the notion that ciliary photoreceptors in the distal retina of the mantle edge eyes in Bivalvia are evolutionary novelties (i.e. not related to vertebrate ciliary photoreceptors, as also suggested by the morphological evidence, see above).

The second messenger system transducing the photoexcitement also differs between rhabdomeric and ciliary photoreceptor cells: as a rule, rhabdomeric photoreceptors employ the phospholipase C inositol 1,4,5-trisphosphate ($InsP_3$) system, whereas the ciliary photoreceptors use the cyclic guanosyl monophosphate (cGMP) system (Finn *et al.* 1997; Gomez & Nasi 1997).

Finally, also the quenching of phototransduction by rhodopsin kinase and arrestin employs related, but non-orthologous, molecules in ciliary versus rhabdomeric photoreceptor cells (figure 9*a*,*b*). Rhodopsin kinases belong to the family of G-protein-coupled receptor kinases (GRKs). This gene family is subdivided into two subfamilies, members of which have been isolated across the Bilateria (Premont *et al.* 1999). Urbilateria possessed at least two distinct members of this family, a *GRK-1/4/5/6* precursor and a *GRK-2/3* precursor. And again, while vertebrate rhodopsin kinase (GRK1)—active in ciliary photoreceptor cells—belongs to the former subfamily, the *Octopus* rhodopsin kinase—active in rhabdomeric photoreceptor cells (Kikkawa *et al.* 1998)—belongs to the latter. A similar distinction applies to bilaterian arrestin molecules, which can be subdivided into α- and β-arrestins (Nicolas-Leveque *et al.* 1999). While vertebrate ciliary photoreceptor cells make use of α-arrestins, *Drosophila* rhabdomeric arrestins belong to the β-arrestin subfamily (Hyde *et al.* 1990; Smith *et al.* 1990).

On these grounds, non-orthologous systems for light detection and phototransduction exist in Bilateria. The ciliary photoreceptor type employs a 'ciliary' rhodopsin, a G-protein of the Gi or Go superfamily, the cGMP second messenger system, a *GRK-1/4/5/6*-related rhodopsin kinase and α-arrestin. In contrast, in the rhabdomeric type a 'rhabdomeric' rhodopsin, a Gq protein, the phospholipase C $InsP_3$ second messenger system, a *GRK-2/3* related rhodopsin kinase and β-arrestin are active.

6. A PAIR OF LARVAL CEREBRAL EYES IN URBILATERIA?

The comparison of bilaterian cerebral eyes allows the following conclusions with regard to their possible homology.

1. Precondition for homology of bilaterian cerebral eyes is the formation in the *Otx*-territory and the connection to the early axonal scaffold at the level of the anterior brain commissure. This holds true for the developing adult eyes in insects, vertebrates and polychaetes (figure 2).
2. Larval, apical eyes are widespread in Lophotrochozoa, and also exist in lower Deuterostomia. They can be traced back to the two-celled prototype eye as present in the polychaete trochophora (figure 3), with few exceptions. Starting from an inverse condition, everse

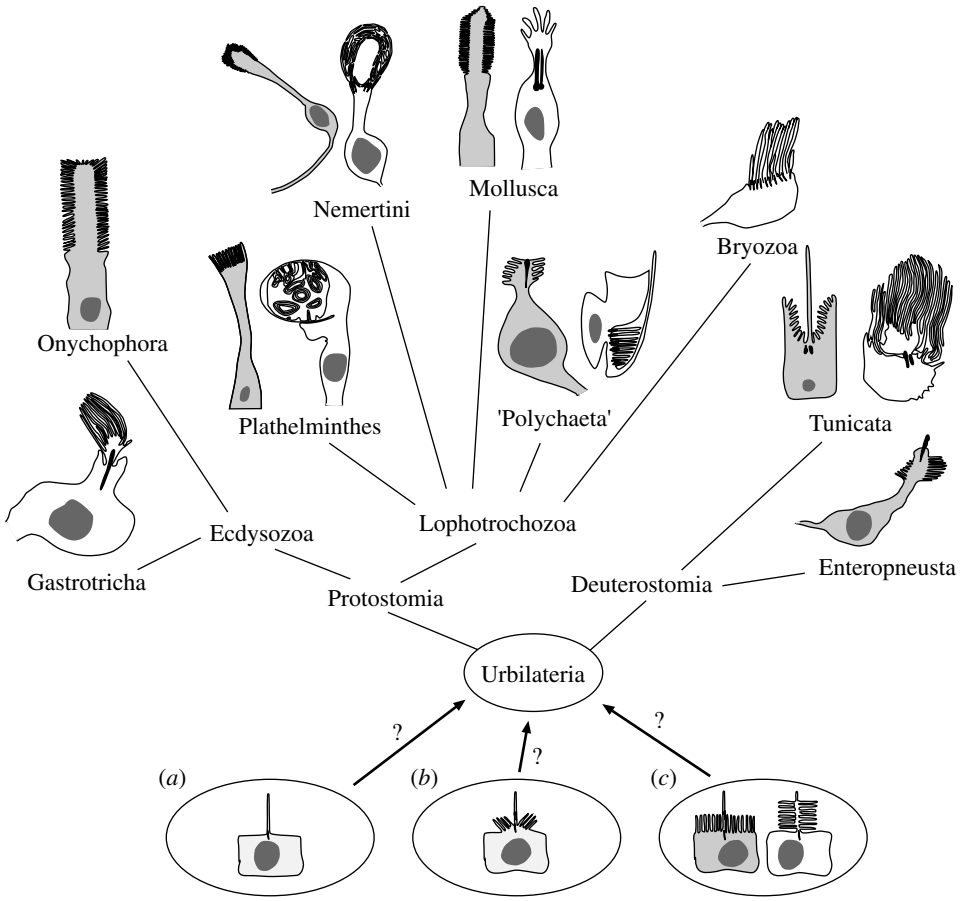

Figure 8. Phylogenetic distribution of ciliary and rhabdomeric photoreceptors in Bilateria and conflicting views of their evolution. Dark grey, rhabdomeric photoreceptor cell; white, ciliary photoreceptor cell. Precursors in Urbilateria could have been (*a*) a sensory ciliary precursor cell, (*b*) a bimodal ciliary/rhabdomeric precursor cell or (*c*) ciliary and rhabdomeric precursor cells. Redrawn from various sources.

eyes evolved independently in polyplacophoran and enteropneust larvae.

3. Inverse cerebral eyes present in adult Lophotrochozoa are neotenously taken over from the larvae (figure 4). Again, there is an inverse-to-everse transition traceable, e.g. in planarians.
4. There is a second, separate type of adult cerebral eyes of rather elaborate, everse design, which is not a larval-eye derivative. It is present in Polychaeta, Sipunculida, Mollusca, Onychophora and Arthropoda (figure 5), indicating possible conservation in Protostomia. Though an everse orientation of photoreceptor cells has apparently evolved several times convergently, homology of adult everse cerebral eyes is an option that should be further addressed.
5. Chordate cerebral 'inverse' eyes can be deduced from the everse eye type, if neurulation is conceptually reverted (figure 7).
6. Rhabdomeric and ciliary photoreceptor cells coexist in Lophotrochozoa, Ecdysozoa and Deuterostomia (figure 8). Their distribution is not random. In lophotrochozoan, ecdysozoan and enteropneust cerebral eyes rhabdomeric photoreceptor cells clearly predominate, while in chordate cerebral eyes exclusively ciliary photoreceptors are detected.
7. Non-homologous systems for light detection and phototransduction coexist in Bilateria (figure 9). For the molecules involved, one paralogue is active in rhabdomeric, the other in ciliary photoreceptors.

What is the significance of this on the reconstruction of eyes in Urbilateria and on the possible homology of bilaterian cerebral eyes? Clearly, our comparison strengthens the notion that the cerebral larval eyespots of, for example, Polychaeta, Mollusca, Plathelminthes, Crustacea and Enteropneusta are phylogenetically conserved, as they all employ the rhabdomeric photoreceptor type. We propose that bilaterian larval eyspots derive from an ancestral, two-celled pair of larval cerebral eyes with rhabdomeric photoreceptor cells present in Urbilateria. This 'prototype eye' then gave rise to a multitude of adult cerebral eyes in Lophotrochozoa, Ecdysozoa and, possibly, Deuterostomia.

7. OUTLOOK: EVOLUTION OF CHORDATE CEREBRAL EYES

It is also evident that chordate and non-chordate cerebral eyes employ non-orthologous—'ciliary' versus 'rhabdomeric'—molecules for light detection and phototransductory cascades. This seems to contradict the notion that chordate cerebral eyes are direct derivatives of a pair of cerebral eyes considered ancestral for Bilateria. However, the role of *Pax6* and *Six* homologues in the

control of eye development in Lophotrochozoa, Ecdysozoa and Deuterostomia (Appendix A) indicates that components of their eyes are homologous to some extent. Possibly, primary, ciliary larvae with 'rhabdomeric' eyespots (as found, for instance, in modern tornaria larvae) were present in ancestral chordates. Chordate descendants then lost the primary larvae but might have inherited the larval eyes. We propose that these were then complemented by a population of ciliary photoreceptor cells. In keeping with this, both rhabdomeric and ciliary receptor cells form part of the cerebral vesicle in extant *Branchiostoma* (Ruiz & Anadon 1991*a*,*b*). In the line of evolution leading to vertebrates, the ciliary photoreceptor cells then more and more replaced the rhabdomeric photoreceptor cells in the perception of light. What was the evolutionary fate of the rhabdomeric photoreceptor cells in the vertebrates? If they were not reduced, are there any 'legitimate' descendants? The expression of melanopsins in ganglion cells and pigment cells in the vertebrate retina may be taken as a first clue in that direction. However, clearly additional comparative molecular studies are needed to trace the transformation of ancestral cell types in the evolution of the chordate eye.

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APPENDIX A. *PAX6* IN BILATERIA

In Ecdysozoa, a *Pax6* homologue outside insects is known for *Caenorhabditis elegans* (Nematoda) (Chisholm & Horvitz 1995; Zhang & Emmons 1995) that is devoid of eyes. A possible involvement of *Pax6* in nematode eye development could be tested in other nematode species that form unique eye structures in close proximity to the olfactory amphid organs (Burr 1984, p. 137).

In Lophotrochozoa, *Pax6* homologues are known for nemerteans, planarians and cephalopods. In regenerating heads of the ribbonworm *Lineus sanguineus* (Nemertini), *Ls-Pax6* expression correlates well with the temporal appearance and position of the inverse eye anlage (Loosli *et al.* 1996). In regenerating heads of the planarian *Dugesia*/*Girardia tigrina* (Tricladida) *Dg-Pax6* is expressed in photoreceptor and pigment cells of the forming inverse eyes (Callaerts *et al.* 1999). In the developing squid *Loligo opalescens* (Cephalopoda, Mollusca), *Lo-Pax6* is expressed in the everse eye anlage (Tomarev *et al.* 1997). In contrast to planarians, however, *Lo-Pax6* is not expressed in the differentiating photoreceptor and pigment cells. We are currently investigating *Pax6* expression in the polychaete *Platynereis dumerilii* and find strong patches of expression of *Pd-Pax6* in the region of the inverse larval eyes (D. Arendt and J. Wittbrodt, unpublished data). As is characteristic for polychaetes, this species forms two distinct types of eyes (larval inverse and adult everse) that might be evolutionary ancient for Lophotrochozoa.

In Deuterostomia, *Pax6* expression data exist for echinoderms and chordates. However, they are lacking for the enteropneust tornaria larva, the larval eyespots of which may represent the starting point for the evolution of eyes in the deuterostome line of evolution (see below), and for starfish with optic cushions at the tip of each arm (Eakin & Westfall 1964). *Pax6* has been isolated for the sea urchin *Paracentrotus lividus* (Echinodermata), which lacks distinct eyes. Strong expression of *Pl-Pax6* was detected in adult tube feet, the peripheral organs characteristic of echinoids (Czerny & Busslinger 1995). Interestingly, in echinoids the entire body surface is assumed photosensitive, and tube feet are involved in the light-sensitive covering reaction, taking up objects from the substrata and carrying them to illuminated parts of the dermis (Yoshida & Takasu 1984). Also, tube feet perform phototactic movements towards or away from a source of light. Thus, possibly, *Pl-Pax6* is expressed in tube feet photoreceptor cells of unknown identity. In the lower chordate branch, *Pax6* has been isolated for the ascidian *Phallusia mammilata* (Tunicata). In the developing ascidian tadpole, *Pm-Pax6* is strongly expressed in the sensory vesicle, including the developing ocellus (Glardon *et al.* 1997). However, definite cellular resolution has not been obtained. Similarly, in the lancelet *Branchiostoma floridae* (Acrania) *AmphiPax6* expression covers the posterior brain vesicle where the photoreceptive lamellar organ is located (Glardon *et al.* 1998). Notably, *AmphiPax6* is not expressed in the developing organs of Hesse that later form conspicuous eyecups distributed along the length of the spinal chord.

APPENDIX B. LARVAL EYES IN BILATERIA

In Polychaeta, larval eyes are widespread and of common design (figure 3*a*). In the more than a dozen species investigated (Eakin & Westfall 1964; Holborow & Laverack 1972; Brandenburger & Eakin 1981; Verger-Bocquet 1984; Bartolomaeus 1987, 1992*a*; Marsden & Hsieh 1987) there are one to two photoreceptor cells and one to two pigment cells.

In Sipuncula, the inverse pigment-cup eyes of the trochophora larva (figure 1) show inverse design and thus have been homologized to polychaete larval eyes (Salvini-Plawen & Mayr 1977; Salvini-Plawen 1982). They have not been investigated ultrastructurally, however.

In 'Turbellaria', cerebral larval eyespots of inverse design are very frequent in the trochophora-type Müller/Götte larvae of Polycladida and in the free larvae of the parasitic Neodermata (e.g. Trematoda) (Fournier 1984). They consist of one cup-shaped pigment cell enclosing one to a few photoreceptor cells (Lanfranchi *et al.* 1981) (figure 3*b*). This 'regular' cerebral eye type is considered evolutionarily ancient by Salvini-Plawen & Mayr (1977) (and see Salvini-Plawen 1982). Another eye type, the single so-called 'epidermal eye', found in an anterolateral position in early Müller's larvae of *Pseudoceros* (Eakin & Brandenburger 1981) and *Thysanozoon brochii* (Lanfranchi *et al.* 1981), and Götte's larvae of *Stylochus mediterraneus* (Lanfranchi *et al.* 1981), appears structurally more derived. It consists of one pigment cell only, which is also considered photosensitive (Lanfranchi *et al.* 1981). However, at later larval stages this 'epidermal eye' moves inwards and gets complemented by two photoreceptor cells, to thus transform into a 'regular' larval inverse eye with separate pigment and photoreceptor cells (Lanfranchi *et al.* 1981).

In Mollusca, the larval ocelli of the polyplacophoran trochophora (figure 1) consist of several pigment cells and one photoreceptor cell in the black chiton *Katharina*

tunicata (Rosen *et al.* 1979) and of several pigment cells and several photoreceptor cells in *Lepidochiton cinereus* (figure 3*c*) (Fischer 1980; Bartolomaeus 1992*b*). Larval eyes are also present in the trochophora of Bivalvia (Rosen *et al.* 1978) and in the veliger of Gastropoda (Bartolomaeus (1992*b*) and references therein). Rosen *et al.* (1978, 1979) homologize larval eyespots among molluscs and between molluscs and polychaetes. Also, Bartolomaeus (Bartolomaeus 1987, 1992*a*,*b*; Ax 1995) is convinced that larval eyespots were present in the last common ancestor of polychaetes and molluscs. This has been refuted by Salvini-Plawen (1982), mainly because larval eyes of Polyplacophora are connected with the lateral nerve cord. However, those of Bivalvia and Gastropoda are connected with the developing cerebral ganglia and polyplacophoran lateral ganglia may proliferate from the cerebral ganglia (Nielsen 1995). In the light of molecular evidence pointing towards an annelid origin of molluscs (Ghiselen 1988), homology of polychaete and mollusc larval eyes appears a tenable option.

In Bryozoa = Ectoprocta, a potential, two-celled photoreceptor organ is present in the larva of *Bugula neritina* (Woollacott & Zimmer 1972). The mesodermal origin of the pigment cell, as well as the ciliary nature of the photoreceptor (see below), clearly indicate independent evolutionary origin (Salvini-Plawen & Mayr 1977).

In Kamptozoa = Entoprocta, 'aberrant' eyespots similar to bryzoan larval eyes are common in the larvae of Loxosomatidae (Nielsen 1971; Woollacott & Eakin 1973). Everse larval eyespots of unknown ultrastructure are also present in the lobate larva of Brachiopoda (Salvini-Plawen & Mayr 1977).

In Crustacea, the 'nauplius eyes' of copepod nauplius larvae consist of three inverse eyecups, each with eight to ten photoreceptor cells and the backing of one pigment cell (Fahrenbach 1964, 1965; Land 1984*a* and compare also Eloffson 1966). Though far more elaborate, nauplius eyes of entomostracan crustaceans have been likened to the larval eyespots of the polychaete trochophora based on their similar inverse design (Salvini-Plawen & Mayr 1977).

In Enteropneusta, larval eyes are present in tornaria larva (figure 1). Ultrastructural studies were carried out for *Ptychodera flava* (Brandenburger *et al.* 1973). Depicted in figure 3*d* are the eyespots of a six-day-old *Ptychodera* tornaria that very much resemble polychaete larval eyes.

APPENDIX C. ADULT CEREBRAL EYES IN BILATERIA

(a) *Inverse eyes*

In Polychaeta, inverse eyes are widespread, found for example in *Armandia* (Opheliidae) (Hermans & Cloney 1966), or in *Polygordius* (*Eunicidae*) (Brandenburger & Eakin 1981) (figure 4*a*). Most frequently, inverse adult eyes are found among limicolous and tubicolous polychaetes (e.g. *Terebellida*, *Serpulida*) (Salvini-Plawen & Mayr 1977; Verger-Bocquet 1984), groups with a tendency towards reduction of adult everse eyes (Rhode 1993) (see below), and in the so-called archiannelids (Eakin *et al.* 1977), a polyphyletic assemblage of neotenous polychaetes that retain various larval features.

In Platyhelminthes, inverse eyecups are observed in adult flatworms, namely turbellarians (Polycladida; figure 4*b*), planarians (Tricladida; figure 4*c*) and parasitic Neodermata (e.g. Trematoda) (for a review, see Vanfleteren & Coomans 1982; Fournier 1984, p. 108). As evidenced for *Stylochus uniporus* (Kato 1940), *Stylochus mediterraneus* and *Thysanozoon brochii* (Lanfranchi *et al.* 1981), flatworm inverse eyes develop from the larval eyespots: in the course of metamorphosis of the Götte's larva of *S. uniporus*, the paired larval eyespots on both sides of the apical brain divide to give rise to two pairs of adult eyespots (Kato 1940, p. 564). In the direct developing turbellarian *Hoploplana villosa*, the early developing single pair of eyespots after duplication finally gives rise to six pairs of eyes (Kato 1940, p. 557).

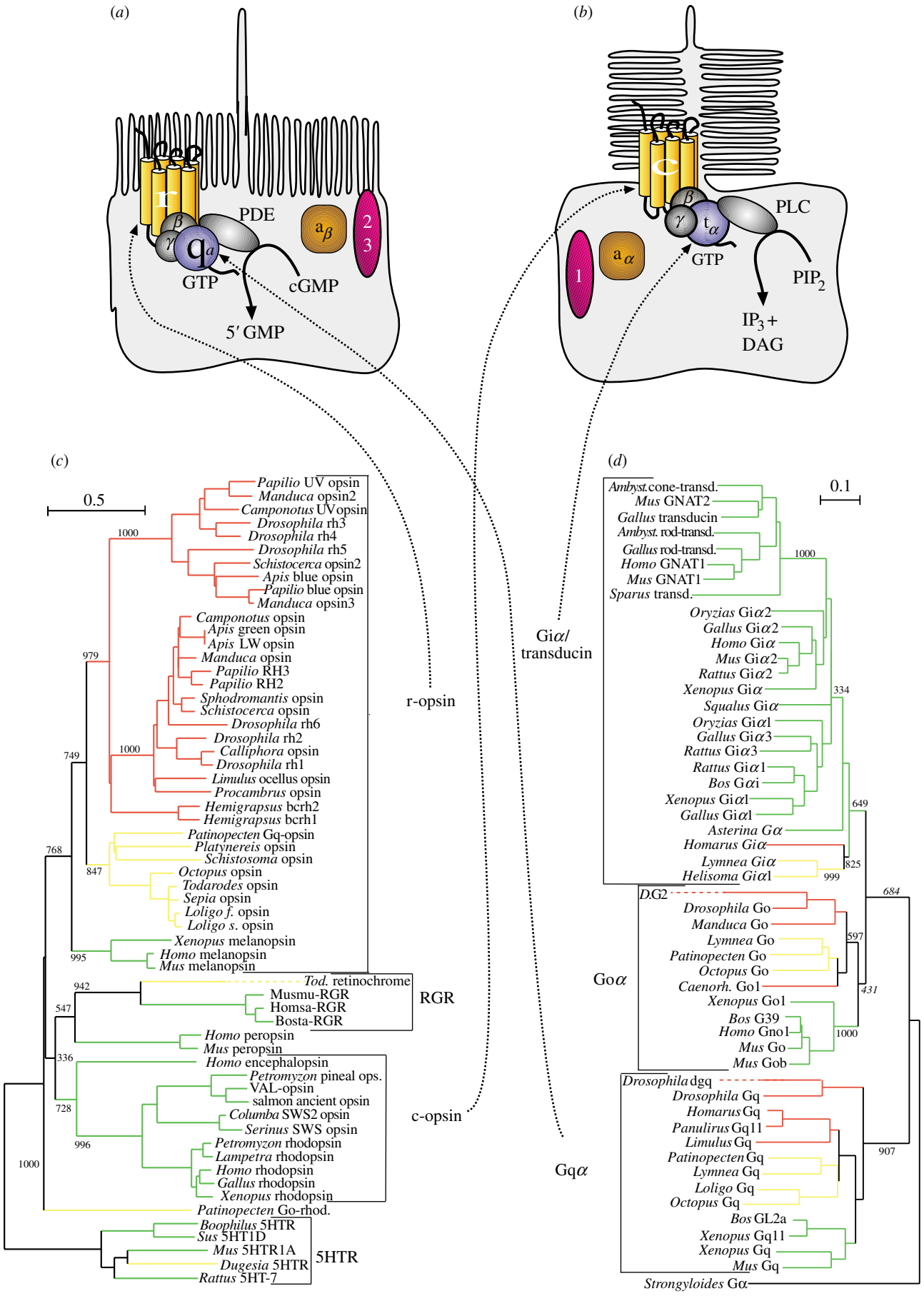
(b) *Everse eyes*

In Polychaeta, everse adult eyes are prototypically present in carnivorous groups (Phyllodocida and Eunicida that belong to the Aciculata/Errantia; Bartolomaeus 1998). Photoreceptive cell bodies are located on the outside of the pigment cell bodies (figure 5*a*; figure 6). They send out sensory processes that traverse the pigment cell layer to finally form photoreceptoral organelles on the apical/inner side of the pigment cells, orientated towards the light. Everse eyes develop in close vicinity to, but separate from, the eyes, to finally replace them, as described for *Anaitides* (Bartolomaeus 1987; Rhode 1991), *Platynereis* (Rhode 1992), *Harmothoë* (Holborow & Laverack 1972) (all: Phyllodocida) and *Capitella* (Capitellida) (Rhode 1993). Everse eyes are often equipped with a lens and can acquire a rather complex structure (Salvini-Plawen & Mayr 1977, p. 239; Verger-Bocquet 1984). Their development has been described in detail for *Vanadis* (Alciopidae, Phyllodocida), or *Platynereis* (Nereidae, Phyllodocida) (Rhode 1992), depicted in figure 6.

In Mollusca, cerebral everse eyes are present in Gastropoda (figure 5*b*), Cephalopoda (figure 5*c*), and filibranchious Bivalvia. Structurally, mollusc everse eyes resemble polychaete everse eyes in that photoreceptor cell processes similarly traverse the pigment cell layer (Yamamoto *et al.* 1965; Hermans & Eakin 1974). However, everse eye homology between molluscs and polychaetes, as proposed for example by Rosen *et al.* (1979) or Hermans & Eakin (1974), is doubtful. First, adult everse eyes of Bivalvia and Gastropoda are persisting larval eyes (Salvini-Plawen & Mayr 1977; Bartolomaeus 1992*b*), in contrast to those of polychaetes. Second, cerebral eyes are absent in basal groups of molluscs (Salvini-Plawen 1982) (although see above).

In Sipunculida, cerebral everse eyes with possible homology to polychaete everse eyes have been described ultrastructurally (figure 5*d*) (Hermans & Eakin 1969; Salvini-Plawen & Mayr 1977). As in the polychaetes, sipunculan everse eyes form separately from the larval inverse eyes.

In Onychophora, the general organization and ultrastructure of everse eyes in *Peripatus* very much resembles that of nereid polychaetes (Eakin & Westfall 1965; Hermans & Eakin 1974). Onychophoran eyes in turn are considered starting points towards the evolution of arthropod compound eyes (Paulus 1972*a*,*b*). Both share the arrangement of microvilli in the rhabdomeric photoreceptor cell, as an 'even array of straight cylindrical projections of the cell membrane' (Eakin & Westfall 1965).


(a)
PDE
GTP
cGMP
5′ GMP
(b)
PLC
GTP
PIP2
IP3 + DAG
(c)
0.5
Papilio UV opsin
Manduca opsin2
Camponotus UV opsin
Drosophila rh3
Drosophila rh4
Drosophila rh5
Schistocerca opsin2
Apis blue opsin
Papilio blue opsin
Manduca opsin3
Camponotus opsin
Apis green opsin
Apis LW opsin
Manduca opsin
Papilio RH3
Papilio RH2
Sphodromantis opsin
Schistocerca opsin
Drosophila rh6
Drosophila rh2
Calliphora opsin
Drosophila rh1
Limulus ocellus opsin
Procambrus opsin
Hemigrapsus bcrh2
Hemigrapsus bcrh1
Patinopecten Gq-opsin
Platynereis opsin
Schistosoma opsin
Octopus opsin
Todarodes opsin
Sepia opsin
Loligo f. opsin
Loligo s. opsin
Xenopus melanopsin
Homo melanopsin
Mus melanopsin
Tod. retinochrome
Musmu-RGR
Homsa-RGR
Bosta-RGR
Homo peropsin
Mus peropsin
Homo encephalopsin
Petromyzon pineal ops.
VAL-opsin
salmon ancient opsin
Columba SWS2 opsin
Serinus SWS opsin
Petromyzon rhodopsin
Lampetra rhodopsin
Homo rhodopsin
Gallus rhodopsin
Xenopus rhodopsin
Patinopecten Go-rhod.
Boophilus 5HTR
Sus 5HT1D
Mus 5HTR1A
Dugesia 5HTR
Rattus 5HT-7
r-opsin
RGR
c-opsin
5HTR
(d)
0.1
Ambyst. cone-transd.
Mus GNAT2
Gallus transducin
Ambyst. rod-transd.
Gallus rod-transd.
Homo GNAT1
Mus GNAT1
Sparus transd.
Oryzias Giα2
Gallus Giα2
Homo Giα
Mus Giα2
Rattus Giα2
Xenopus Giα
Squalus Giα
Oryzias Giα1
Gallus Giα3
Rattus Giα3
Rattus Giα1
Bos Gαi
Xenopus Giα1
Gallus Giα1
Asterina Gα
Homarus Giα
Lymnea Giα
Helisoma Giα1
Giα/ transducin
D.G2
Drosophila Go
Manduca Go
Lymnea Go
Patinopecten Go
Octopus Go
Caenorh. Go1
Xenopus Go1
Bos G39
Homo Gno1
Mus Go
Mus Gob
Goα
Drosophila dgq
Drosophila Gq
Homarus Gq
Panulirus Gq11
Limulus Gq
Patinopecten Gq
Lymnea Gq
Loligo Gq
Octopus Gq
Bos GL2a
Xenopus Gq11
Xenopus Gq
Mus Gq
Strongyloides Gα
Gqα

APPENDIX D. ADDITIONAL PHOTORECEPTOR TYPES?

Photoreceptor cells with multiple photosensitive cilia have been treated as an additional, 'unpleated type' of photoreceptor cells (Salvini-Plawen & Mayr 1977). Such cells, however, can also be considered subtypes of the ciliary type (Eakin 1979).

Salvini-Plawen & Mayr (1977) also distinguish another 'ganglionic type' of photoreceptor cells. These should represent 'modified ganglia cells which never bear cilia', thus deviating from photoreceptor cells of the true rhabdomeric type (which are of epidermal origin and thus bear simple or reduced cilia, see above). Examples should be the Hesse and Joseph cells in *Branchiostoma* (not expressing *Pax6*) and the larval photoreceptors in *Polychaeta* (Salvini-Plawen & Mayr 1977, p. 226). Eakin (1979) already considered the 'ganglionic' type 'not significantly different from his rhabdomeric type', stating that 'if a careful search were made, cilia would be found in the embryo if not in the adult, because cilia are ubiquitous . . . in ectodermally derived organs' (Eakin 1979). Vanfleteren & Coomans (1982, p. 108) also see 'no advantage to such distinctions', neither does Burr (Burr 1984, p. 159). Meanwhile the identification of non-photosensitive cilia or ciliary rudiments next to the rhabdomere in both polychaete larval photoreceptors (Eakin & Westfall 1964; Holborow & Laverack 1972; Brandenburger & Eakin 1981; Bartolomaeus 1987, 1992*a*; Marsden & Hsieh 1987; Rhode 1992) and in Hesse and Jospeh cells of *Branchiostoma* (Ruiz & Anadon 1991*a*) clarified the issue, and demonstrated that these photoreceptor cells are truly rhabdomeric.

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Figure 9. Phylogenetic tree of bilaterian opsins and G-α subunits and their relation to ciliary and rhabdomeric photoreceptors. Schematic of (*a*) rhabdomeric and (*b*) ciliary photoreceptor cells with relevant components of their respective phototransduction cascades. Rhabdomeric (r, orange) and ciliary (c, orange) opsins, G-α subunits (blue), arrestin α and β and rhodopsin kinases (purple). cGMP, cyclic guanosylmonophosphate; DAG, diacylglycerol; GTP, guanosyl trisphosphate; IP_3, inositol 1,3,5-trisphosphate; PDE, phosphodiesterase; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C. The trees were calculated using CLUSTALX (Thompson *et al*. 1997) on opsin protein sequences (*c*) and on G-αDNA 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.

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