Title

A Conserved Developmental Mechanism Builds Complex Visual Systems in Insects and Vertebrates

Journal

Current Biology, 26(20)

ISSN

0960-9822

Authors

Joly, Jean-Stéphane
Recher, Gaelle
Brombin, Alessandro
et al.

Publication Date

2016-10-01

DOI

10.1016/j.cub.2016.08.017

Peer reviewed

HHS Public Access

Author manuscript

Curr Biol. Author manuscript; available in PMC 2017 October 24.

Published in final edited form as:

Curr Biol. 2016 October 24; 26(20): R1001–R1009. doi:10.1016/j.cub.2016.08.017.

A Conserved Developmental Mechanism Builds Complex Visual Systems in Insects and Vertebrates

Jean-Stéphane Joly[1,*], **Gaelle Recher**[2,4], **Alessandro Brombin**[1,5], **Kathy Ngo**[3], and **Volker Hartenstein**[3,*]

[1]INRA CASBAH group, Neuro-PSI Institute, CNRS, University Paris-Saclay, Gif-sur-Yvette, France

[2]BioEmergences, Multilevel Dynamics in Morphogenesis, CNRS, Gif-sur-Yvette, France

[3]Department of Molecular Cell and Developmental Biology, University of California Los Angeles, Los Angeles, USA

Abstract

The visual systems of vertebrates and many other bilaterian clades consist of complex neural structures guiding a wide spectrum of behaviors. Homologies at the level of cell types and even discrete neural circuits have been proposed, but many questions of how the architecture of visual neuropils evolved among different phyla remain open. In this review we argue that the profound conservation of genetic and developmental steps generating the eye and its target neuropils in fish and fruit flies supports a homology between some core elements of bilaterian visual circuitries. Fish retina and tectum, and fly optic lobe, develop from a partitioned, unidirectionally proliferating neurectodermal domain that combines slowly dividing neuroepithelial stem cells and rapidly amplifying progenitors with shared genetic signatures to generate large numbers and different types of neurons in a temporally ordered way. This peculiar ‘conveyor belt neurogenesis’ could play an essential role in generating the topographically ordered circuitry of the visual system.

Introduction

Vertebrates and flies possess an image forming eye whose photoreceptors project onto a multi-layered visual neuropil. Already in the early 20th century, Santiago Ramon y Cajal noted the striking similarities between the neuronal organization of the visual systems of vertebrates and flies [1]. More recently, a wealth of molecular studies demonstrated that conserved transcription factors such as Pax6/Eyeless and Six/Sine oculis form a central part of the gene network that controls the development of the visual system of vertebrates and flies [2,3]. This conservation led to the proposal that invertebrate and vertebrate eyes share homologous cytological and neuroanatomical features already present in their common bilaterian ancestor [4]. Focusing on the first steps of visual processing, performed by retina

*Correspondence: joly@inaf.cnrs-gif.fr (J.-S.J.), volkerh@mcdb.ucla.edu (V.H.).

[4]Current address: Bioimaging and Optofluidics Group, IOGS, CNRS and University of Bordeaux, Talence, France

[5]Current address: GMM South Building, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh, UK

and optic tectum in vertebrates, or by lamina, medulla and lobula in insects, it has been argued that vertebrate and insect neuronal networks use similar design principles which could be explained by a common ancestry [5–9]. In this review we survey the commonalities between visual system development in bilaterian animals, with a special focus on *Drosophila* and zebrafish. We discuss the genetic and cellular aspects of visual system development and explore the extent to which basic morphogenetic mechanisms, rather than the complex organs *per se*, are shared among vertebrates and insects.

Synopsis of the Vertebrate and Fly Visual Systems

The vertebrate eye is composed of multiple tissues derived from the neural ectoderm (neural retina, optic stalk, pigment epithelium), the epidermal ectoderm (lens), and the ocular mesenchyme or neural crest (corneal epithelium; iris). The neural retina is formed by a large array of ciliated photoreceptors (rods and cones) that project their short axons towards a central layer (inner plexiform layer) formed by first order visual interneurons, the bipolar cells (Figure 1). Bipolar cells target the basally located layer of 2nd order visual interneurons, the ganglion cells. Several types of local interneurons, including amacrine cells and horizontal cells, laterally connect bipolar cells and ganglion cells. Ganglion cell axons leave the eye through the optic stalk and project in a homotopic (i.e., retinotopic) order to one of the superficial layers of the contralateral optic tectum and dorsal thalamus. Different afferents are segregated in separate layers [10]. Several classes of local interneurons form connections vertically and laterally within the tectum.

The fly eye, which develops from the epidermal ectoderm, is composed of an outer layer of cuticle and lens tissue. The retina underneath contains stereotyped clusters (ommatidia) of photoreceptor neurons which project in a somatopic order to the optic lobe, the part of the brain that processes exclusively visual information [11]. The optic lobe has four main neuropil compartments, called lamina, medulla, lobula and lobula plate, each of which is further subdivided into multiple layers (Figure 1). Photoreceptors involved in motion detection (R1–6) terminate in the lamina; R7 and R8, responsible for color vision, project to the distal layers of the medulla. Columnar visual interneurons targeted by photoreceptors vertically interconnect the layers of the lamina and medulla and project to the lobula and lobula plate. From here, processed visual information is relayed by higher order visual interneurons to the central brain. Tangential neurons, similar to vertebrate amacrine and horizontal cells, laterally interconnect the vertical columns of the medulla and lobula/lobula plate (Figure 1).

A number of different hypotheses have been advanced in regard to the comparison between different layers of the visual system in flies and vertebrates. These include the idea that the peripheral layer of photoreceptors of an ancestral invertebrate diversified into the multiple neural layers of the vertebrate retina [12]; conversely, it has been proposed that the photoreceptor layer of the insect eye, in addition to the first neuropils of the optic lobe (part of the central nervous system), can be homologized to the multilayered retina [5,6]. Continuing along this line of thought, the vertebrate tectum, the target neuropil of retinal ganglion cells, might correspond to the deeper layers of the insect optic lobe (i.e., lobula and lobula plate). Unfortunately, evidence (in the form of anatomical/functional neural properties

or the expression of molecular markers) is ambiguous and no generally accepted model has been worked out yet. In this review we will follow the proposal of Sanes and Zipursky [6], that the neuropil layers of the fly optic lobe correspond to the intermediate and deep layer of the vertebrate retina, in addition to the tectum.

In conclusion, fly and fish visual systems share certain fundamental design principles that form the structural basis for feature detection and coverage of the visual field. Among these principles, aside from a number of conserved cell types, are: a layered and modular architecture; the inclusion of many vertically and horizontally projecting neuronal cell types within each module and layer; a tightly controlled connectivity of neurons within and in between modules; and strictly homotopically ordered connections between layers.

There exists a longstanding, unresolved debate about to what extent these conserved design principles point at a homology versus analogy between discrete elements of the visual system. It seems likely that at the cell type level, photoreceptor cells and certain classes of central target neurons receiving direct photoreceptor input were present in the bilateran ancestor and are thereby homologous [13]. In the following, we review evidence that a distinct conserved neurogenetic mechanism, optimized for the generation of an extended, modular neural network interconnected by homotopic projections such as those encountered in the visual system, exists in vertebrates and arthropods alike, and we propose that this mechanism could have been present at the root of bilaterian evolution.

Conserved Embryonic Origin and Genetic Specification of the Fish and Drosophila Visual Systems

The neurectoderm of the early embryo is subdivided along the anterior–posterior (AP) and medio-lateral axis by a group of conserved transcriptional regulators. Genes expressed along the AP axis include the Hox genes, which define the posterior brain and spinal cord (vertebrates) or ventral cord (insects), and a set of genes that are expressed in domains of the anterior brain (Otx, Emx, Tlx and their insect homologs) [14–18]. Mediolaterally the neurectoderm comprises three columnar domains defined by the expression of the genes Nkx-2 (medial), Gsx (intermediate), and Msx (lateral) [19,20] in vertebrates and their homologs in *Drosophila*.

In vertebrates and insects alike, the visual system develops from the anterior domain of the neurectoderm, characterized by the (partially) overlapping expression of Orthodenticle (Otx/Otd) and Tailless (Tlx/Tll), and the absence of genes of the Hox complex (Figure 2A,B). This domain, in vertebrates, includes both the anlagen of the neural retina and, posteriorly adjacent, the tectum (Figure 2A). Pax6 and other transcriptional regulators, including Six1/3/6 and Rx, are expressed in domains that are nested within the Otx/Tlx domain. In particular, Pax6 and its *Drosophila* homolog, *twin of eyeless* (*toy*), define the eye field that gives rise to the neural retina in vertebrates and the eye plus optic lobe in *Drosophila* [21,22] (Figure 2A,B). The second Pax6 homolog, *eyeless (ey)*, appears in the *Drosophila* eye imaginal disc but does not coincide with the eye field in the early embryo [21]. The same applies to the Six family of transcriptional regulators, in which Six3/6 defines the boundaries of the eye field in the vertebrate embryo, whereas the *Drosophila* Six3/6 homolog, *optix*

(*opt*), is expressed at later stages in the eye disc [23]. Interestingly, *sine oculis* (*so*), the *Drosophila* homolog of Six1, whose expression outlines the placodal ectoderm in vertebrates, fully overlaps with the eye field of the *Drosophila* embryo [21,22].

In vertebrates, the eye field of either side initially occupies a dorso-lateral position in the alar plate of the forebrain, in between the anlagen of the ventral forebrain (septum, hypothalamus and optic stalk) and the dorsal forebrain (pallium; Figure 2A). The optic tectum maps posteriorly adjacent to the eye field, in the alar part of the midbrain vesicle. The alar plate coincides with the intermediate column, as defined by molecular markers [24]. Indeed, tectal lateral progenitors (but not retinal progenitors) express the intermediate column determinant Gsx [25]. The eye field in *Drosophila*, similar to that of the vertebrate embryo, is also surrounded anteriorly and laterally by the anlage of the protocerebrum (the part of the fly brain likened to the forebrain [16,21,22] (Figure 2B). The expression domain of the *Drosophila* Gsx homolog, *ind*, overlaps with the most posterior part of the eye field that gives rise to the lobula complex (K.N. and V.H., unpublished), indicating that both vertebrate optic tectum and fly lobula complex arise from the intermediate column within the neurectoderm.

During neurulation, when the neurectoderm is folded into the interior of the embryo, the eye field of vertebrates becomes fully incorporated into the forebrain vesicle of the neural tube, from which it then evaginates as the optic cup, composed of the multilayered neural retina and the pigment epithelium (Figure 3A–F). The tectum forms in the dorso-lateral domain (alar plate) of the midbrain vesicle [26] (Figure 3A,G). In *Drosophila*, the anlagen of the optic lobe and the eye also internalize as two invaginating neuroepithelia, a mechanism that sets these regions apart from the remainder of the fly brain. The canonical mechanism by which the fly brain develops involves stem-celllike neuroblasts that delaminate from the neurectoderm [27]. By contrast, the *Drosophila* optic lobe anlage invaginates and forms an epithelial vesicle, which subsequently breaks up into two sheets, the inner and outer optic anlage (Figure 1D). These give rise to the lobula complex and medulla/lamina, respectively [28–30] (Figure 1E). The *Drosophila* eye also develops from an invaginating neuroepithelium, the eye imaginal disc, located anteriorly adjacent to the optic lobe anlage [22,31] (Figure 1D). Following a phase of growth and differentiation that takes place in this invaginated state, the eye disc everts during metamorphosis to become the compound eye (Figure 1E). Development of the eye from an invaginated disc is a derived feature seen in dipterans; in other insects, as well as arthropods with compound eyes in general, the growing epithelium giving rise to the eye forms part of the externally located head epidermis.

In conclusion, progenitors of the photoreceptors and their layers of target neurons derive from a genetically and topologically conserved domain, the anterior intermediate column of the neurectoderm. From within this domain, the primordia of the eye and its target structure (optic tectum/optic lobe) develop as invaginating neuroepithelia. As will be discussed in the following section, proliferation of these primordia follows a peculiar, highly structured pattern, termed ‘conveyor belt neurogenesis’. We will first introduce this mechanism for the optic tectum and retina in zebrafish, before attempting a side-by-side comparison with its *Drosophila* counterpart.

The Conveyor Belt Neurogenesis in the Fish Optic Tectum and Retina

At early stages of the development of vertebrates, proliferating progenitors of retinal neurons are found throughout the alar plate of the presumptive forebrain [32,33] (Figure 3A,D). Subsequently, neural retina progenitors become located in a lens-facing domain all around the optic vesicle. From there, they flow to their destinations in the ciliary marginal zone [34] (Figure 3B,E). Hence, progenitors lie at a hinge (ciliary marginal zone; CMZ) between two cortical structures, the retinal pigment epithelium and the neural retina [35] (Figure 3C,F). At later stages, most of the neuroepithelium differentiates into an ependymal layer and becomes semi-quiescent. However, active proliferation or proliferation potentials persist in a peripheral zone of the retina, resulting in a continuous growth of these structures [36–38]. Similarly, dividing tectal progenitors become restricted to a peripheral hinge (tectal marginal zone; TMZ) between the optic tectum and torus semicircularis (Figure 3G–I). This hinge is formed when the torus semicircularis invaginates into the brain and comes to lie below the tectum, which occupies the dorsal and lateral parts of the brain [39] (Figure 3H,I).

The CMZ and TMZ exhibit a peculiar mode of proliferation, which has been termed conveyor belt neurogenesis, as mentioned above [40]. This process includes several key features. Firstly, the external edge of the CMZ and TMZ (CMZe, TMZe) is formed by a ribbon of slowly dividing neuroepithelial stem cells [39,41,42] (Figure 4A,B) which accumulate transcripts for nucleotide and ribosome synthesis enzymes [39]. These genes form a synexpression group that provides a useful signature to compare neuroepithelial stem cells of the retina and tectum, as well as other stem cell types. Secondly, the progeny of the peripheral stem cells form an intermediate layer (CMZi/TMZi) of rapidly dividing progenitor cells (‘amplifying progenitors’; Figure 4A,B). These cells accumulate proliferation-associated genes [39] that represent another large synexpression group between the CMZ and TMZ, as already noted by Ramialison *et al.* [43]. Proliferation of cells within the CMZi/TMZi is limited to a few rounds [39] of divisions before cells exit the mitotic cycle. And thirdly, postmitotic neural precursor cells gather at the central edge of the CMZ/TMZ. In this region cell cycle exit proteins (Kip, Insm1) are expressed as in many other proliferative domains [44,45].

As already suggested by Cerveny *et al.* [46], the synexpression groups discussed in the preceding section might reflect the tight functional link between eye and optic tectum, which need spatially and temporally coordinated developmental processes to establish a precise connectivity map. We propose that the convergence of the molecular signatures with the morphogenetic features (conveyor belt neurogenesis) found in the primordia of eye and optic tectum supports a serial homology between these two primordia. Both emanate from neighboring domains within the alar plate of the forebrain/midbrain, forming an anterior morphological unit located in front of the midbrain– hindbrain boundary. The chordate ancestor probably possessed a simple, tube-shaped brain [47]. Photoreceptors and their direct target neurons developed in the wall of this tube, forming a ‘proto-retina’. Neurons posteriorly adjacent to the proto-retina formed a ‘proto-tectum’. Directed proliferation of progenitors of photoreceptors and target neurons from a peripheral growth zone (conveyor belt neurogenesis) could have already been present in the ancestral state. Subsequently, the increase in the size of the eyes in vertebrates led to the evagination of the optic cup from the

telencephalon. Evolutionary scenarios propose that to colonize deeper waters, where the light levels are lower, the animal's photosensitivity was increased by expansion of the photosensitive region. For craniates, there may have been distinct advantages if such expansion occurred by lateral ballooning, so that the light-sensitive region was not shadowed by the protective cranium [48]. Along with the increase in retinal size, the tectum also expanded to accommodate the growing number of retinal afferents.

Pattern of Growth of the Fly Eye and Optic Lobe

The development of the visual system in insects and other arthropods is also characterized by a mechanism of directed proliferation within a neuroepithelial growth zone. In hemimetabolous insects and crustaceans the eye originates from a narrow, vertically oriented growth zone in the anterior head epidermis from where rows of differentiating ommatidia are 'budded off' posteriorly [49] (Figure 5A). In *Drosophila*, the eye is derived from an invaginated epithelium, the eye imaginal disc. Following a period of symmetric cell divisions during the early larval stages (Figure 5B,D), a dorso-ventrally oriented growth zone becomes apparent within the disc (Figure 5C,E); progenitors within this zone maintain their mitotic activity throughout larval development. Whether within this growth zone one can differentiate between a spatially ordered subpopulation of more slowly dividing 'stem cells' and faster dividing 'progenitors', as in the case of the vertebrate retina/tectum or the optic lobe (see below), is not clear. Posterior to the growth zone, differentiation sets in. The first cells that become postmitotic differentiate into the R8 photoreceptors, forming a highly regular pattern, with one cell defining the center of each protoommatidium [50,51] (Figure 5C). Other cells surrounding the R8s undergo one final mitosis before exiting the mitotic cycle. To this first vertical row of nascent ommatidia are then added, one after another, more anterior rows of ommatidia. Decapentaplegic (Dpp) signaling, Hedgehog (Hh) signaling and Notch (N) signaling are responsible for the orderly progression and spacing of R8 production. Subsequently, the nascent R8 cells serve as 'organizers' for the ommatidia, recruiting surrounding cells to adopt specific cellular fates. Both Notch and epidermal growth factor signaling play dominant roles in this process [52].

Proliferation of the optic lobe, whose neurons are the targets of retinal photoreceptors, is also characterized by spatiotemporally directed growth. During early larval stages, the inner (IOA) and outer (OOA) optic anlagen that derive from the invaginated embryonic head neurectoderm form expanding sheets of neuroepithelial cells that grow by symmetric cell division [11,28,30,53,54] (Figure 5B,D). By the beginning of the third larval instar, the OOA is subdivided into two domains, visibly separated by the lamina furrow (Figure 5C,E). Cells lateral to the furrow (OOAl) form the lamina, while cells medial to the furrow form the distal medulla (OOAm). Note that the polarity of the neuroepithelium giving rise to the visual system is inverted in *Drosophila* compared to vertebrates. Thus, the apical membrane of the fly neuroepithelia faces outwards, while it is directed internally, towards the ventricle, in zebrafish (Figure 4D). This inversion can be viewed as a result of the invagination of the neurectoderm in vertebrates: the primordia of the eye and optic tectum form part of the neural tube, whose apical membrane faces towards the ventricular lumen. Progenitor cells and neurons are given off at the basal side of the neuroepithelium, which faces the outer surface of the neural tube. In flies, the apical membrane of the eye and optic lobe

neuroepithelia face outward (dotted lines in Figure 4D); neural progeny is pushed basally, as in vertebrates, but this direction is oriented inward.

By the mid-third larval instar, cytological and proliferative characteristics of the OOAm change towards a mode that closely resembles the conveyor belt morphogenesis outlined above for the zebrafish optic tectum. Slowly cycling neuroepithelial cells of the OOAm, comparable to the TMZe of the zebrafish tectum, convert into rapidly dividing progenitors, called neuroblasts in insects, following a tightly regulated temporal sequence ('proneural wave') that begins at the medial edge and moves slowly laterally towards the lamina furrow [51,54,55] (Figure 5E, inset). As a result, neuroblasts born first occupy a position medially within the OOAm, whereas the lastborn neuroblasts are located laterally (Figure 5E, inset; arrow 'a' indicates gradient).

Neuroblasts divide asymmetrically, thereby creating a second gradient (Figure 5E, inset, arrow 'b') that reflects both the time of birth and differentiative fate of neurons. Each mitosis produces a large, peripherally located daughter cell that continues to cycle as a neuroblast, and a small internal cell, called ganglion mother cell (GMC), that divides once before differentiation [54,56,57]. Thus, neurons born early are located deep within the layer of cell bodies (cortex), whereas neurons born late are superficial, close to the neuroblast.

Neurons derived from the OOAl (lamina) and the IOA (proximal medulla, lobula, lobula complex) are also born in a temporally graded manner [52,58] (Ngo and Hartenstein, unpublished observations). However, compared to the OOAm, little is known about detailed aspects of proliferation of the OOAl and IOA, such as the orientation of mitotic spindles, or the relationship between birthdate and cell fate.

A Similar Growth Mode in the *Drosophila* and Fish Visual Systems

In both vertebrate and *Drosophila* visual systems, photoreceptors and their target neuropils originate from neuroepithelial stem cell layers. Neuroepithelial cells are polarized, express apical markers and establish contacts with collagen-rich basal membranes. These cells undergo interkinetic movements and perform their symmetric mitoses at the most apical part of the neuroepithelium [39,56]. Epithelial stem cells convert into asymmetrically dividing progenitors that give off their fast-amplifying progeny. In the conveyor belt mode of neurogenesis, resulting global cell movements are tangential, because early-born cells are pushed away from the progenitor population by later-born cells (Figure 4A–C).

Molecular signatures of the different zones within the fish eye and tectal marginal zone and the fly optic lobe also show similarities. As described in Recher *et al.* [39], retina and tectum neuroepithelium in zebrafish are characterized by the expression of factors involved in nucleotide and ribosome turnover. Importantly, Mycn appears as a central player in the neuroepithelium network of insects [59] and vertebrates [60]. Following an siRNA screen in *Drosophila*, Neumüller *et al.* [61] demonstrated that a network of 39 genes involved in ribosome biogenesis are crucial for the survival of *Drosophila* neural stem cells. Analogies between neurogenesis in fly and mouse adult forebrains have previously been reported in several reviews [61–64], mainly on the basis of the succession of slow-activated stem cells,

generating fast transitory amplifying progenitors. In addition, Strausfeld and Hirth [65] proposed deep homology between insect central complex and mammal basal ganglia. Here, based on previously proposed theoretical grounds [9,66], we propose that the observation of so many similarities between the conveyor belt neuroepithelial-based morphogenesis in *Drosophila* and fish, in terms of molecular signatures, ontogeny and cytology, constitute strong arguments for deep homologies of the *Drosophila* eye/optic lobes and the vertebrate eye and tectum.

Conclusion

Comparative developmental and genetic studies support the hypothesis that the anterior brain (the domain specified by Otx/Otd) and the central nervous system of the trunk (expressing Hox genes) have separate evolutionary origins that date farther back than the appearance of bilaterian animals. The anterior nervous system (‘apical nervous system’ [67]) included sensory receptors and neuroendocrine elements that might have functioned in orienting the organism relative to light, gravity, and chemical composition of the substrate. The nervous system of the trunk (‘blastoporal nervous system’ [67]) consisted of reflexive sensori-motor neural networks controlling the locomotor apparatus. Both apical and blastoporal components had likely merged in the bilaterian ancestor, setting the stage for a course of evolution where enlarging, modular arrays of sensory receptors could exert a much more elaborate control over locomotion. According to the currently prevailing view, the bilaterian ancestor most likely did not have a large, image-forming visual system. However, it may have possessed a visual system formed by a considerable number of ciliary photoreceptors developing in the Six/Rx positive domain, as described for the annelid *Platynereis* [68]. These photoreceptors (ancestral eye) projected their axons on neighboring target interneurons which, as a result, adopted the role of processing visual input. Extant chordate species such as the amphioxus [68] also obviously suggest that early chordates already possessed axonal projections resembling the basic photosensory–motor circuits of the vertebrate forebrain. Moreover, vertebrate fossils from the early Cambrian [69] seem to suggest foraging behaviors already at this time.

We speculate that the generation of large neuron numbers and (more importantly) the formation of highly ordered, topologically specific neural connections was made possible by the advent of improved neurogenetic mechanisms, such as the conveyor belt neurogenesis discussed in this review (Figure 6). Conveyor belt neurogenesis took advantage of generally available ‘elementary’ components that formed part of the urbilaterian neurogenetic ‘tool kit’, such as the invagination of neuroepithelia and the delamination and oriented division of neural progenitors [27], and combined them in a manner that allowed for the temporally protracted generation of photoreceptors and their target neuropils. A specialized domain with conveyor belt characteristics may also exist in other parts of the neurectoderm, such as the cortical hem of the vertebrate forebrain [70,71]. The conveyor belt combines a neuroepithelial, slowly dividing part with an adjacent, fast dividing part; it can generate large numbers of neurons of different types in a temporally coordinated way, which is likely to be important for the formation of precise, homotopically ordered (e.g., retinotopic) projections. We propose that conveyor belt neurogenesis was a plesiomorphic trait in bilaterians and

became increasingly more complex as imaging vision assumed greater importance in the different bilaterian groups.

A hypothesis of homology between visual systems has important implications for more applied fields, such as biomedical research. By providing genetically tractable models for studying visual system biology, developmental studies of the insect optic lobe may enhance our understanding of congenital malformations at the cellular and molecular levels. Genetic pathways affected in microcephaly and microphtalmia are indeed active in the mammalian embryonic brain and retina neuroepithelial progenitors [72]. In mammals, these progenitors proliferate during embryonic development and become dormant in adults but are importantly remobilized in regenerative processes [73]. Studies of visual system evolution can be used as a powerful tool to identify key conserved pathways that are at the basis of pathologies in mammalian systems.

Acknowledgments

We wish to thank Sophie Creuzet and Muriel Perron for fruitful discussions and Franck Bourrat for helpful comments on the manuscript.

REFERENCES

1. Cajal SR, Sanchez D. Contribucion al conocimiento de los centros nerviosos del los insectos. Trab. Lab. Invest. Biol. 1915; 13:1–167.
2. Gehring WJ. Chance and necessity in eye evolution. Genome Biol. Evol. 2011; 3:1053–1066. [PubMed: 21979158]
3. Steinmetz PR, Urbach R, Posnien N, Eriksson J, Kostyuchenko RP, Brena C, Guy K, Akam M, Bucher G, Arendt D. Six3 demarcates the anterior-most developing brain region in bilaterian animals. Evodevo. 2010; 1:14. [PubMed: 21190549]
4. De Robertis E, Sasai Y. A common plan for dorsoventral patterning if Bilateria. Nature. 1996; 380:37–40. [PubMed: 8598900]
5. Erclik T, Hartenstein V, McInnes RR, Lipshitz HD. Eye evolution at high resolution: the neuron as a unit of homology. Dev. Biol. 2009; 332:70–79. [PubMed: 19467226]
6. Sanes JR, Zipursky SL. Design principles of insect and vertebrate visual systems. Neuron. 2010; 66:15–36. [PubMed: 20399726]
7. Wernet MF, Huberman AD, Desplan C. So many pieces, one puzzle: cell type specification and visual circuitry in flies and mice. Genes Dev. 2014; 28:2565–2584. [PubMed: 25452270]
8. Rutishauser R, Moline P. Evo-devo and the search for homology (“sameness”) in biological systems. Theory Biosci. 2005; 124:213–241. [PubMed: 17046357]
9. Strausfeld NJ, Hirth F. Homology versus convergence in resolving transphyletic correspondences of brain organization. Brain Behav. Evol. 2013; 82:215–219. [PubMed: 24296550]
10. Robles E, Filosa A, Baier H. Precise lamination of retinal axons generates multiple parallel input pathways in the tectum. J. Neurosci. 2013; 33:5027–5039. [PubMed: 23486973]
11. Fischbach KF, Hiesinger PR. Optic lobe development. Adv. Exp. Med. Biol. 2008; 628:115–136. [PubMed: 18683642]
12. Nilsson DE, Arendt D. Eye evolution: the blurry beginning. Curr. Biol. 2008; 18:R1096–R1098. [PubMed: 19081043]
13. Arendt D. Evolution of eyes and photoreceptor cell types. Int. J. Dev. Biol. 2003; 47:563–571. [PubMed: 14756332]
14. Sprecher SG, Reichert H. The urbilaterian brain: developmental insights into the evolutionary origin of the brain in insects and vertebrates. Arthropod Struct. Dev. 2003; 32:141–156. [PubMed: 18089000]

15. Hirth F, Kammermeier L, Frei E, Walldorf U, Noll M, Reichert H. An urbilaterian origin of the tripartite brain: developmental genetic insights from Drosophila. Development. 2003; 130:2365–2373. [PubMed: 12702651]
16. Reichert H, Simeone A. Developmental genetic evidence for a monophyletic origin of the bilaterian brain. Philos. Trans. R. Soc. Lond. B. Biol Sci. 2001; 356:1533–1544. [PubMed: 11604121]
17. Lichtneckert R, Reichert H. Anteroposterior regionalization of the brain: genetic and comparative aspects. Adv. Exp. Med. Biol. 2008; 628:32–41. [PubMed: 18683636]
18. Steinmetz PR, Kostyuchenko RP, Fischer A, Arendt D. The segmental pattern of otx, gbx, and Hox genes in the annelid Platynereis dumerilii. Evol. Dev. 2011; 13:72–79. [PubMed: 21210944]
19. Cornell RA, Ohlen TV. Vnd/nkx, ind/gsh, and msh/msx: conserved regulators of dorsoventral neural patterning? Curr. Opin. Neurobiol. 2000; 10:63–71. [PubMed: 10679430]
20. Urbach R, Technau GM. Dorsoventral patterning of the brain: a comparative approach. Adv. Exp. Med. Biol. 2008; 628:42–56. [PubMed: 18683637]
21. Chang T, Mazotta J, Dumstrei K, Dumitrescu A, Hartenstein V. Dpp and Hh signaling in the Drosophila embryonic eye field. Development. 2001; 128:4691–4704. [PubMed: 11731450]
22. Hartenstein V, Reh TA. Homologies between vertebrate and invertebrate eyes. Results Probl. Cell Differ. 2002; 37:219–255. [PubMed: 25707078]
23. Seimiya M, Gehring WJ. The Drosophila homeobox gene optix is capable of inducing ectopic eyes by an eyeless-independent mechanism. Development. 2000; 127:1879–1886. [PubMed: 10751176]
24. Arendt D, Nübler-Jung K. Comparison of early nerve cord development in insects and vertebrates. Development. 1999; 126:2309–2325. [PubMed: 10225991]
25. Deschet K, Bourrat F, Chourrout D, Joly JS. Expression domains of the medaka (Oryzias latipes) Ol-Gsh 1 gene are reminiscent of those of clustered and orphan homeobox genes. Dev. Genes Evol. 1998; 208:235–244. [PubMed: 9683739]
26. Nakamura H. Regionalization of the optic tectum: combinations of gene expression that define the tectum. Trends Neurosci. 2001; 24:32–39. [PubMed: 11163885]
27. Hartenstein V, Stollewerk A. The evolution of early neurogenesis. Dev. Cell. 2015; 32:390–407. [PubMed: 25710527]
28. Hofbauer A, Campos-Ortega JA. Proliferation pattern and early differentiation of the optic lobes in Drosophila melanogaster. Roux's Arch. Dev. Biol. 1990; 198:264–274.
29. Green P, Hartenstein AY, Hartenstein V. The embryonic development of the Drosophila visual system. Cell Tissue Res. 1993; 273:583–598. [PubMed: 8402833]
30. Meinertzhagen, IA.; Hanson, TE. The development of the optic lobe. In: Bate, M.; Martinez-Arias, A., editors. The Development of Drosophila melanogaster. Cold Spring Harbor, New York: Cold Spring Harbor Press; 1993. p. 1363-1491.
31. Daniel A, Dumstrei K, Lengyel JA, Hartenstein V. The control of cell fate in the embryonic visual system by atonal, tailless and EGFR signaling. Development. 1999; 126:2945–2954. [PubMed: 10357938]
32. Adler R, Canto-Soler MV. Molecular mechanisms of optic vesicle development: complexities, ambiguities and controversies. Dev. Biol. 2007; 305:1–13. [PubMed: 17335797]
33. El Yakoubi W, Borday C, Hamdache J, Parain K, Tran HT, Vleminckx K, Perron M, Locker M. Hes4 controls proliferative properties of neural stem cells during retinal ontogenesis. Stem Cells. 2012; 30:2784–2795. [PubMed: 22969013]
34. Heermann S, Schütz L, Lemke S, Krieglstein K, Wittbrodt J. Eye morphogenesis driven by epithelial flow into the optic cup facilitated by modulation of bone morphogenetic protein. Elife. 2015; 4:e05216.
35. Amato MA, Arnault E, Perron M. Retinal stem cells in vertebrates: parallels and divergences. Int. J. Dev. Biol. 2004; 48:993–1001. [PubMed: 15558490]
36. Scheib D. Recent research on regeneration of the crystalline lens in vertebrates. Ergeb. Anat. Entwicklungsgesch. 1965; 38:46–114. [PubMed: 5319095]
37. Creuzet S, Vincent C, Couly G. Neural crest derivatives in ocular and periocular structures. Int. J. Dev. Biol. 2005; 49:161–171. [PubMed: 15906229]

38. Stenkamp DL. Neurogenesis in the fish retina. Int. Rev. Cytol. 2007; 259:173–224. [PubMed: 17425942]
39. Recher G, Jouralet J, Brombin A, Heuzé A, Mugniery E, Hermel JM, Desnoulez S, Savy T, Herbomel P, Bourrat F, et al. Zebrafish midbrain slow-amplifying progenitors exhibit high levels of transcripts for nucleotide and ribosome biogenesis. Development. 2013; 140:4860–4869. [PubMed: 24198278]
40. Devès M, Bourrat F. Transcriptional mechanisms of developmental cell cycle arrest: problems and models. Semin. Cell Dev. Biol. 2012; 23:290–297. [PubMed: 22464972]
41. Alunni A, Hermel JM, Heuzé A, Bourrat F, Jamen F, Joly JS. Evidence for neural stem cells in the medaka optic tectum proliferation zones. Dev. Neurobiol. 2010; 70:693–713. [PubMed: 20506557]
42. Ito Y, Tanaka H, Okamoto H, Ohshima T. Characterization of neural stem cells and their progeny in the adult zebrafish optic tectum. Dev. Biol. 2010; 342:26–38. [PubMed: 20346355]
43. Ramialison M, Reinhardt R, Henrich T, Wittbrodt B, Kellner T, Lowy CM, Wittbrodt J. Cis-regulatory properties of medaka synexpression groups. Development. 2012; 139:917–928. [PubMed: 22318626]
44. Nguyen V, Deschet K, Henrich T, Godet E, Joly JS, Wittbrodt J, Chourrout D, Bourrat F. Morphogenesis of the optic tectum in the medaka (Oryzias latipes): a morphological and molecular study, with special emphasis on cell proliferation. J. Comp. Neurol. 1999; 413:385–404. [PubMed: 10502247]
45. Candal E, Nguyen V, Joly JS, Bourrat F. Expression domains suggest cell-cycle independent roles of growth-arrest molecules in the adult brain of the medaka, Oryzias latipes. Brain Res. Bull. 2005; 66:426–430. [PubMed: 16144625]
46. Cerveny KL, Varga M, Wilson SW. Continued growth and circuit building in the anamniote visual system. Dev. Neurobiol. 2012; 72:328–345. [PubMed: 21563317]
47. Lacalli TC. Basic features of the ancestral chordate brain: a protochordate perspective. Brain Res. Bull. 2008; 75:319–323. [PubMed: 18331892]
48. Lamb TD, Collin SP, Pugh EN Jr. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. Nat. Rev. Neurosci. 2007; 8:960–976. [PubMed: 18026166]
49. Harzsch S, Benton J, Dawirs RR, Beltz B. A new look at embryonic development of the visual system in decapod crustaceans: neuropil formation, neurogenesis, and apoptotic cell death. J. Neurobiol. 1999; 39:294–306. [PubMed: 10235683]
50. Cagan R. Cell fate specification in the developing Drosophila retina. Dev. Suppl. 1993; 1993:19–28.
51. Treisman JE. Retinal differentiation in Drosophila. Wiley Interdiscip. Rev. Dev. Biol. 2013; 2:545–557. [PubMed: 24014422]
52. Flores GV, Duan H, Yan H, Nagaraj R, Fu W, Zou Y, Noll M, Banerjee U. Combinatorial signaling in the specification of unique cell fates. Cell. 2000; 103:75–85. [PubMed: 11051549]
53. Egger B, Gold KS, Brand AH. Regulating the balance between symmetric and asymmetric stem cell division in the developing brain. Fly (Austin). 2011; 5:237–241. [PubMed: 21502820]
54. Sato M, Suzuki T, Nakai Y. Waves of differentiation in the fly visual system. Dev. Biol. 2013; 380:1–11. [PubMed: 23603492]
55. Yasugi T, Umetsu D, Murakami S, Sato M, Tabata T. Drosophila optic lobe neuroblasts triggered by a wave of proneural gene expression that is negatively regulated by JAK/STAT. Development. 2008; 135:1471–1480. [PubMed: 18339672]
56. Egger B, Boone JQ, Stevens NR, Brand AH, Doe CQ. Regulation of spindle orientation and neural stem cell fate in the Drosophila optic lobe. Neural Dev. 2007; 2:1. [PubMed: 17207270]
57. Ngo KT, Wang J, Junker M, Kriz S, Vo G, Asem B, Olson JM, Banerjee U, Hartenstein V. Concomitant requirement for Notch and Jak/Stat signaling during neuro-epithelial differentiation in the Drosophila optic lobe. Dev. Biol. 2010; 346:284–295. [PubMed: 20692248]
58. Apitz H, Salecker I. A region-specific neurogenesis mode requires migratory progenitors in the Drosophila visual system. Nat. Neurosci. 2015; 18:46–55. [PubMed: 25501037]
59. Southall TD, Gold KS, Egger B, Davidson CM, Caygill EE, Marshall OJ, Brand AH. Cell-type-specific profiling of gene expression and chromatin binding without cell isolation: assaying RNA Pol II occupancy in neural stem cells. Dev. Cell. 2013; 26:101–112. [PubMed: 23792147]

60. Xue XY, Harris WA. Using myc genes to search for stem cells in the ciliary margin of the Xenopus retina. Dev. Neurobiol. 2012; 72:475–490. [PubMed: 21465669]
61. Neumüller RA, Richter C, Fischer A, Novatchkova M, Neumüller KG, Knoblich JA. Genome-wide analysis of self-renewal in Drosophila neural stem cells by transgenic RNAi. Cell Stem Cell. 2011; 8:580–593. [PubMed: 21549331]
62. Huttner WB, Brand M. Asymmetric division and polarity of neuroepithelial cells. Curr. Opin. Neurobiol. 1997; 7:29–39. [PubMed: 9039800]
63. Götz M, Huttner WB. The cell biology of neurogenesis. Nat. Rev. Mol. Cell. Biol. 2005; 6:777–788. [PubMed: 16314867]
64. Homem CC, Knoblich JA. Drosophila neuroblasts: a model for stem cell biology. Development. 2012; 139:4297–4310. [PubMed: 23132240]
65. Strausfeld NJ, Hirth F. Deep homology of arthropod central complex and vertebrate basal ganglia. Science. 2013; 340:157–161. [PubMed: 23580521]
66. Arendt D. Genes and homology in nervous system evolution: comparing gene functions, expression patterns, and cell type molecular fingerprints. Theory Biosci. 2005; 124:185–197. [PubMed: 17046355]
67. Tosches MA, Arendt D. The bilaterian forebrain: an evolutionary chimaera. Curr. Opin. Neurobiol. 2013; 23:1080–1089. [PubMed: 24080363]
68. Vopalensky P, Pergner J, Liegertova M, Benito-Gutierrez E, Arendt D, Kozmik Z. Molecular analysis of the amphioxus frontal eye unravels the evolutionary origin of the retina and pigment cells of the vertebrate eye. Proc. Natl. Acad. Sci. USA. 2012; 109:15383–15388. [PubMed: 22949670]
69. Raup DM, Seilacher A. Fossil foraging behavior: computer simulation. Science. 1969; 166:994–995. [PubMed: 17758062]
70. Grove EA, Tole S, Limon J, Yip L, Ragsdale CW. The hem of the embryonic cerebral cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-deficient mice. Development. 1998; 125:2315–2325. [PubMed: 9584130]
71. Liddelow SA, Dziegielewska KM, Vandeberg JL, Saunders NR. Development of the lateral ventricular choroid plexus in a marsupial, Monodelphis domestica. Cerebrospinal Fluid Res. 2010; 7:16. [PubMed: 20920364]
72. Novorol C, Burkhardt J, Wood KJ, Iqbal A, Roque C, Coutts N, Almeida AD, He J, Wilkinson CJ, Harris WA. Microcephaly models in the developing zebrafish retinal neuroepithelium point to an underlying defect in metaphase progression. Open Biol. 2013; 3:130065. [PubMed: 24153002]
73. Sabelström H, Stenudd M, Réu P, Dias DO, Elfineh M, Zdunek S, Damberg P, Göritz C, Frisén J. Resident neural stem cells restrict tissue damage and neuronal loss after spinal cord injury in mice. Science. 2013; 342:637–640. [PubMed: 24179227]
74. Wilson SW, Houart C. Early steps in the development of the forebrain. Dev. Cell. 2004; 6:167–181. [PubMed: 14960272]

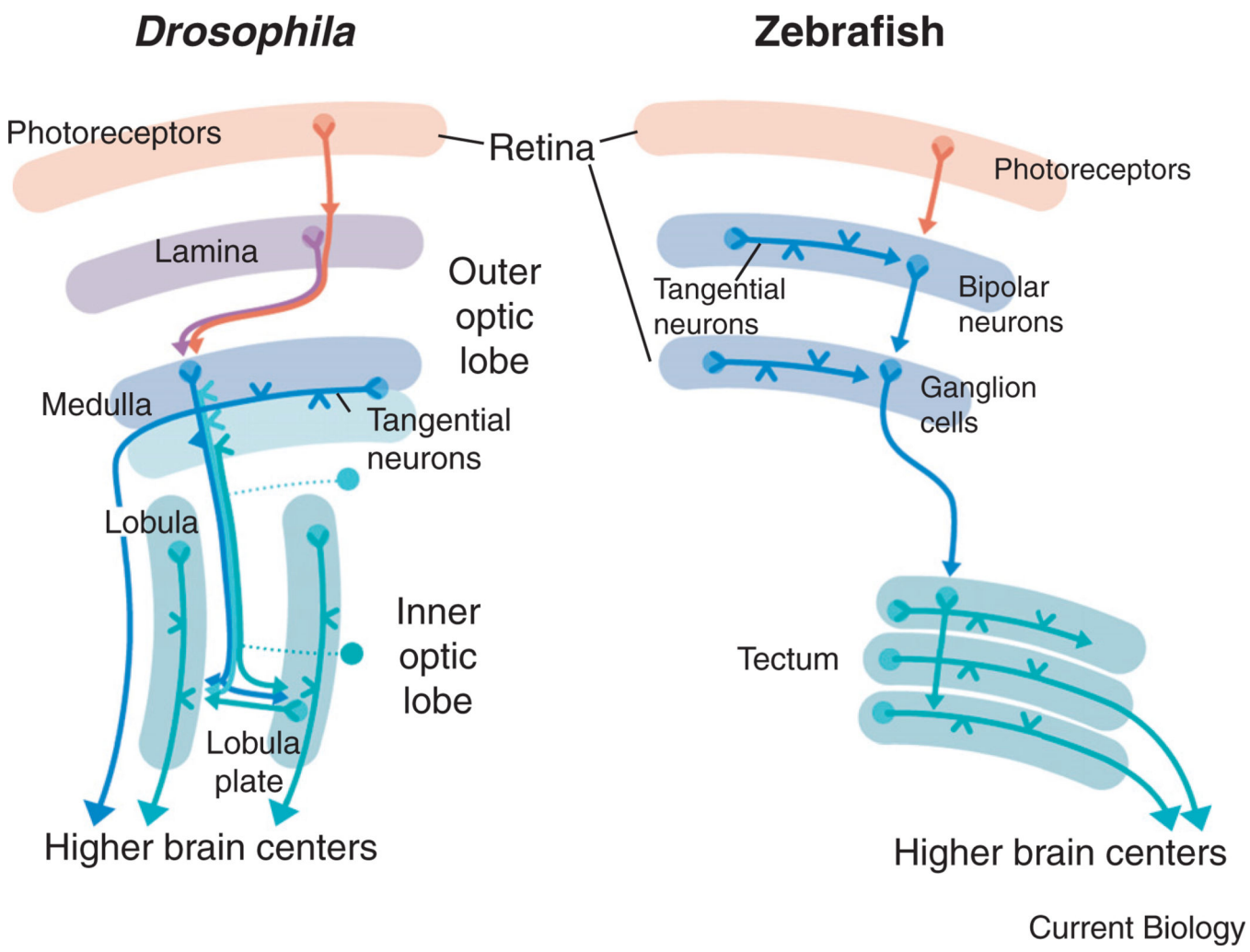

Figure 1. Schematic representation of the visual neuropil layers and their connectivity in zebrafish and *Drosophila*

A strict retinotopic organization of the visual neuropils is maintained throughout all layers of the retina to the optic tectum in vertebrates, and throughout all neuropils of the optic lobe in *Drosophila*.

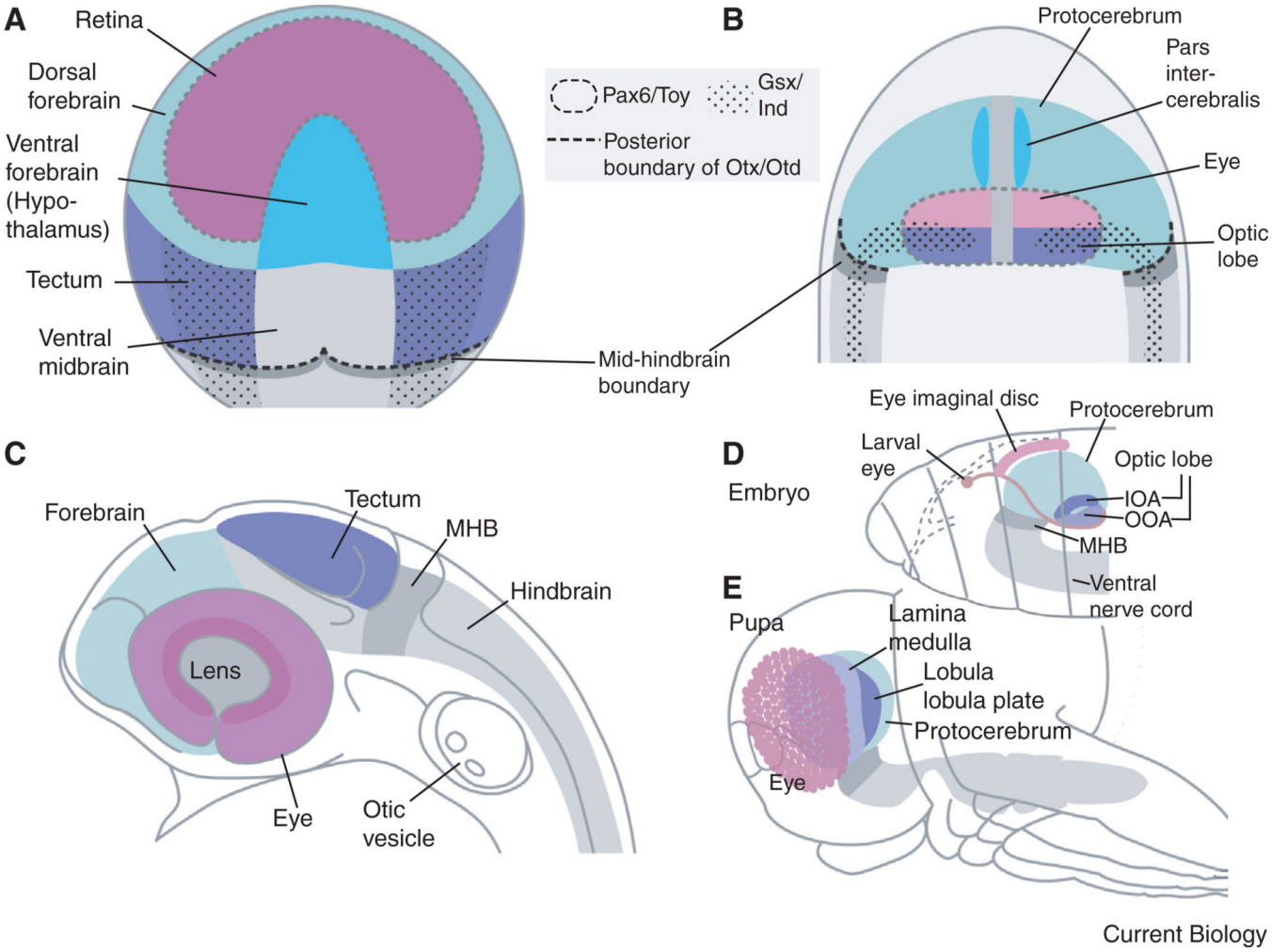

Figure 2. Embryonic origin and morphogenesis of the visual system in zebrafish and *Drosophila*

(A) Schematic of zebrafish late gastrula/early neurula embryo, dorsal view (redrawn from [74]). The domains giving rise to the optic tectum and neural retina fall within the Otx-positive anterior neural plate. The anlage of the retina (eye field) is characterized by the expression of Pax6, Six1/3/6 and Rx. Grey transverse stripe posterior to the tectum indicates the midbrain–hindbrain boundary, which expresses Pax2/5/8 and Engrailed (En). (B) Fatemap of the *Drosophila* visual system at the gastrula stage. Otd defines a large domain within the dorso-anterior neurectoderm that gives rise to the protocerebrum and visual system. The Six1 homolog Sine oculis (So) and the Pax6 homolog Twin of eyeless (Toy) are expressed in the anlage of the visual system, which includes the eye and optic lobe. Expression of the Pax2/5/8 homologs Poxn and Dpax2 are observed in a narrow stripe of neurectoderm likened to the vertebrate midbrain– hindbrain boundary [21]. Similar medio-lateral systems (medial: Vnd/Nkx; intermediate: Ind/Gsx (stippled); lateral: Msh/Msx) subdivide the neurectoderm in fish and flies. *Drosophila* Ind expression overlaps with the anterior lip of the optic lobe anlage, which gives rise to the lobula complex, while the zebrafish Ind homolog, Gsx, is expressed in the optic tectum. (C) Zebrafish brain and visual system at larval stage, lateral view (anterior to the left). (D,E) Lateral view of late

Drosophila embryo (D) and 24 h pupa (E), depicting the protocerebrum and associated visual system. Consistent color code used throughout (A–E) illustrates the relationship between early embryonic anlagen and their derivatives.

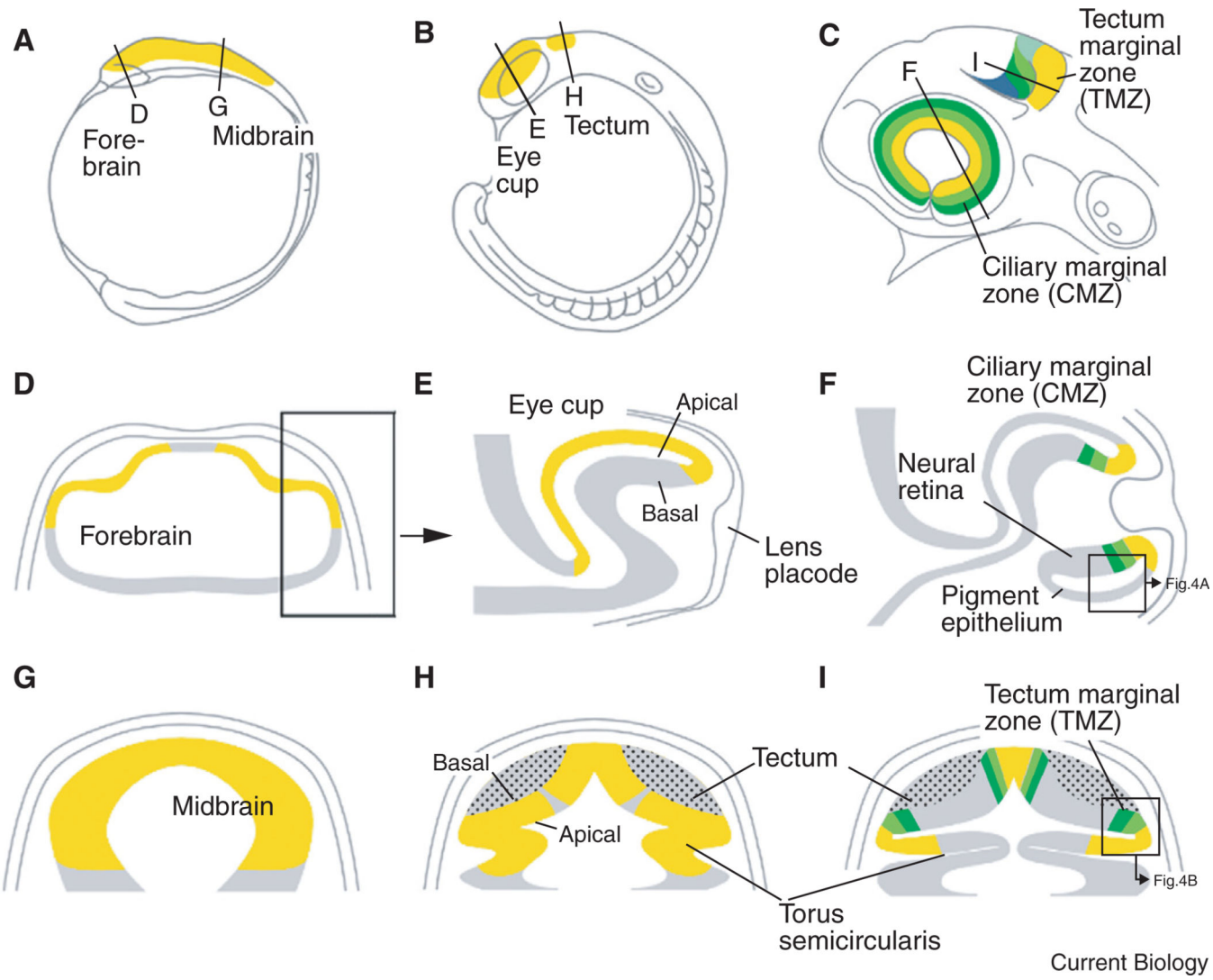

Figure 3. The tectal marginal zone (TMZ) and the ciliary marginal zone (CMZ) are serially homologous structures

Schematic lateral views (A–C) and cross sections (D–I) of zebrafish embryos; yellow shading marks expression of proliferation genes (e.g., *impdh2*; [45]). These genes are first expressed in the entire alar part of the forebrain/midbrain, but then expression retreats to the stem cell zones of the tectal marginal zone (TMZ) and the ciliary marginal zone (CMZ). (A,D,G) At the 3-somite stage, expression of proliferation genes is in the dorsal part of the anterior neural tube. (B,E,H) At the 15-somite stage, the primordia of the tectum and retina become separated. The retina evaginates, forming the eye cup. Expression of proliferation genes becomes confined to the dorsal eye cup. Complex morphogenetic movements change spatial relationships within the midbrain. Here, proliferation genes retreat towards the mid-dorsal and the ventral part of the alar plate, which invaginates to form the torus semicircularis. (C,F,I) At the 25-somite stage, expression of proliferation genes become restricted to the TMZ and CMZ. The CMZ forms a transitional domain between the neural retina and pigmented epithelium, encircling the lens. Similarly, the TMZ forms a narrow, hinge-like region encircling the lateral and posterior tectum.

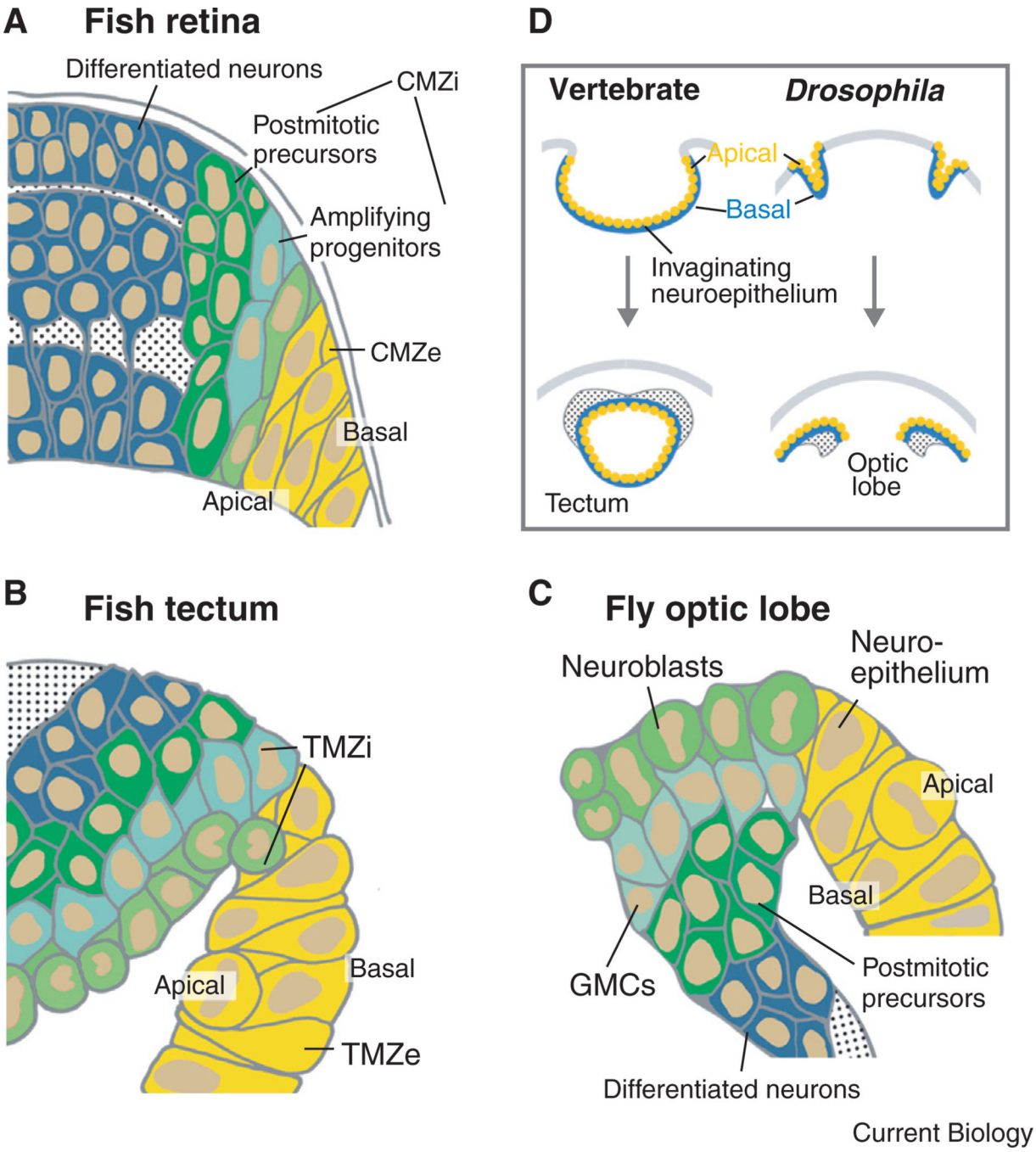

Figure 4. Conveyor belt neurogenesis in the visual system of vertebrates and *Drosophila*

(A,B) Magnified view of schematic sections of the ciliary marginal zone (A; redrawn from [64]) and tectum marginal zone (B; redrawn from [45]). Both CMZ and TMZ can be further subdivided, which is indicated by color coding. At their peripheral edge, the TMZ and CMZ contain stem cells (yellow). Away from this edge one finds the intermediate TMZ (TMZi) and intermediate CMZ (CMZi), both of which have fast amplifying progenitors (light green). Dark green indicates neural precursors exiting the cell cycle. In dark blue are differentiated neurons. (C) Magnified view of schematic section of *Drosophila* outer optic

anlage, generating medulla neurons in a conveyor belt mechanism (for spatial orientation, see Figure 5E). Stippling indicates neuropil. (D) Simplified depiction of neurulation, illustrating inverse apico-basal axis of neuroepithelium in vertebrates and *Drosophila*. In vertebrates, after invagination of neural tube (bottom panel), apical surface of neuroepithelium containing neural progenitors (yellow) faces inward (ventricular lumen). Postmitotic neurons (blue) and neuropil (stippled) accrete at outer (=basal) surface of neural tube. In *Drosophila*, optic lobe neuroepithelium, following invagination, does not form a lumen, and apical surface points outward.

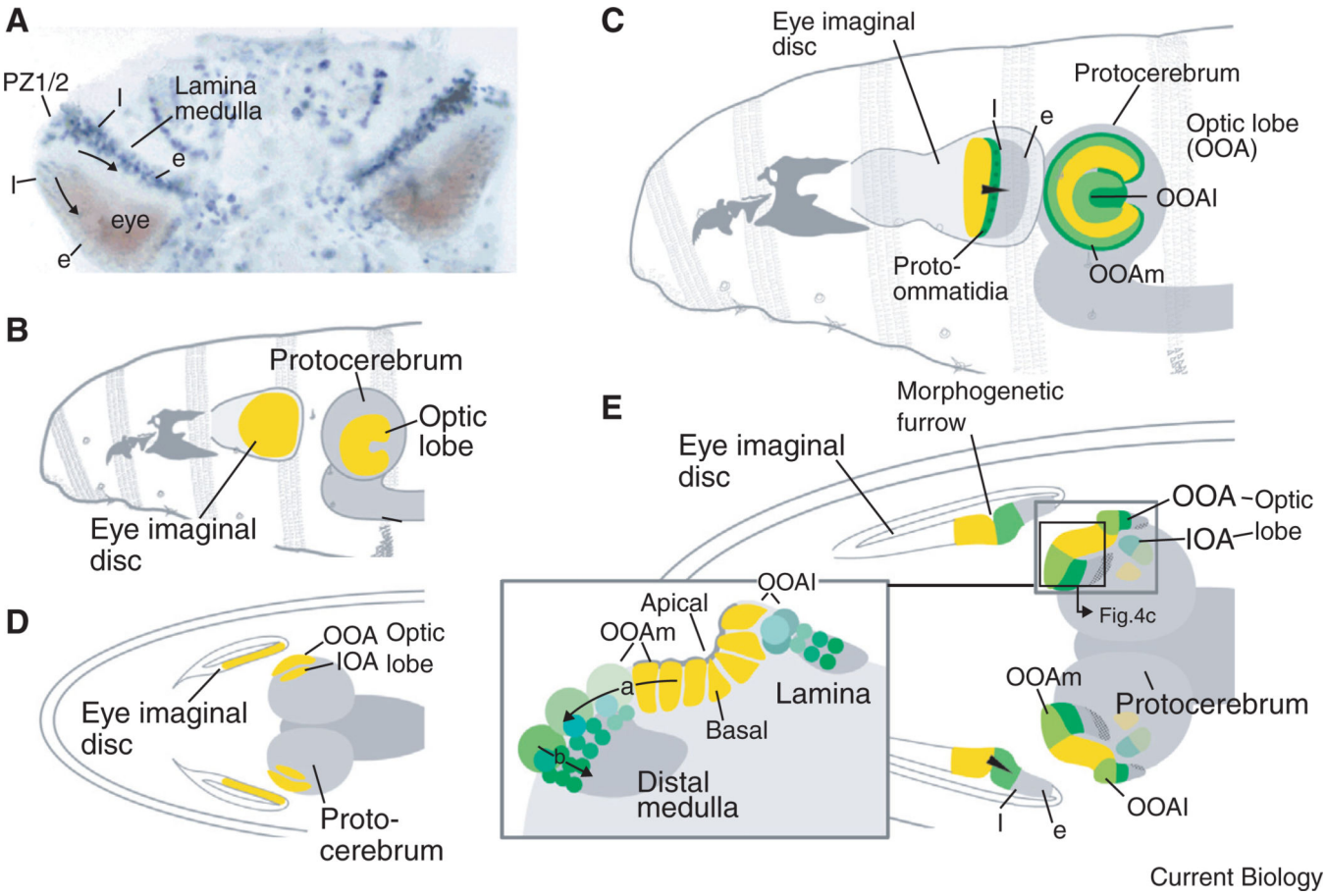

Figure 5. Development of the visual system in insects

(A) Microphotograph showing frontal view of embryonic head of the crustacean *Hyas araneus* (from [54], with permission). An integrated growth zone (PZ1/2; blue label demarcates proliferating cells) generates the eye and outer optic lobe (lamina, medulla), following a temporal gradient. Early born cells ('e') are located posteriorly, late born cells ('l') anteriorly. (B,C) Schematic lateral views of *Drosophila* early larva (B) and late larva (C), showing growth zones in eye imaginal disc and optic lobe. (D,E) Schematic section of optic lobe of early larva (D) and late larva (E; based on [36,60–62]). In (B–E), epithelial optic anlagen and eye disc (giving rise to retina) rendered in *yellow*; neuroblasts forming from anlagen in *light green*, neural progeny dark green. Optic anlagen of the early larva are formed by symmetrically dividing neuroepithelia (B,D). In late larva, epithelia convert directionally into asymmetrically, rapidly dividing neuroblasts (E; arrow 'a' in inset indicates directionality of epithelium>neuroblast conversion). Neuroblasts produce ganglion mother cells (GMCs)/neurons (arrow 'b' in inset to E). The eye disc also undergoes directed growth (arrowheads in C,E). e, early born cells; IOA, inner optic anlage; l, late born cells; OOA, outer optic anlage; OOAl, lateral domain of outer optic anlage; OOAm, medial domain of outer optic anlage.

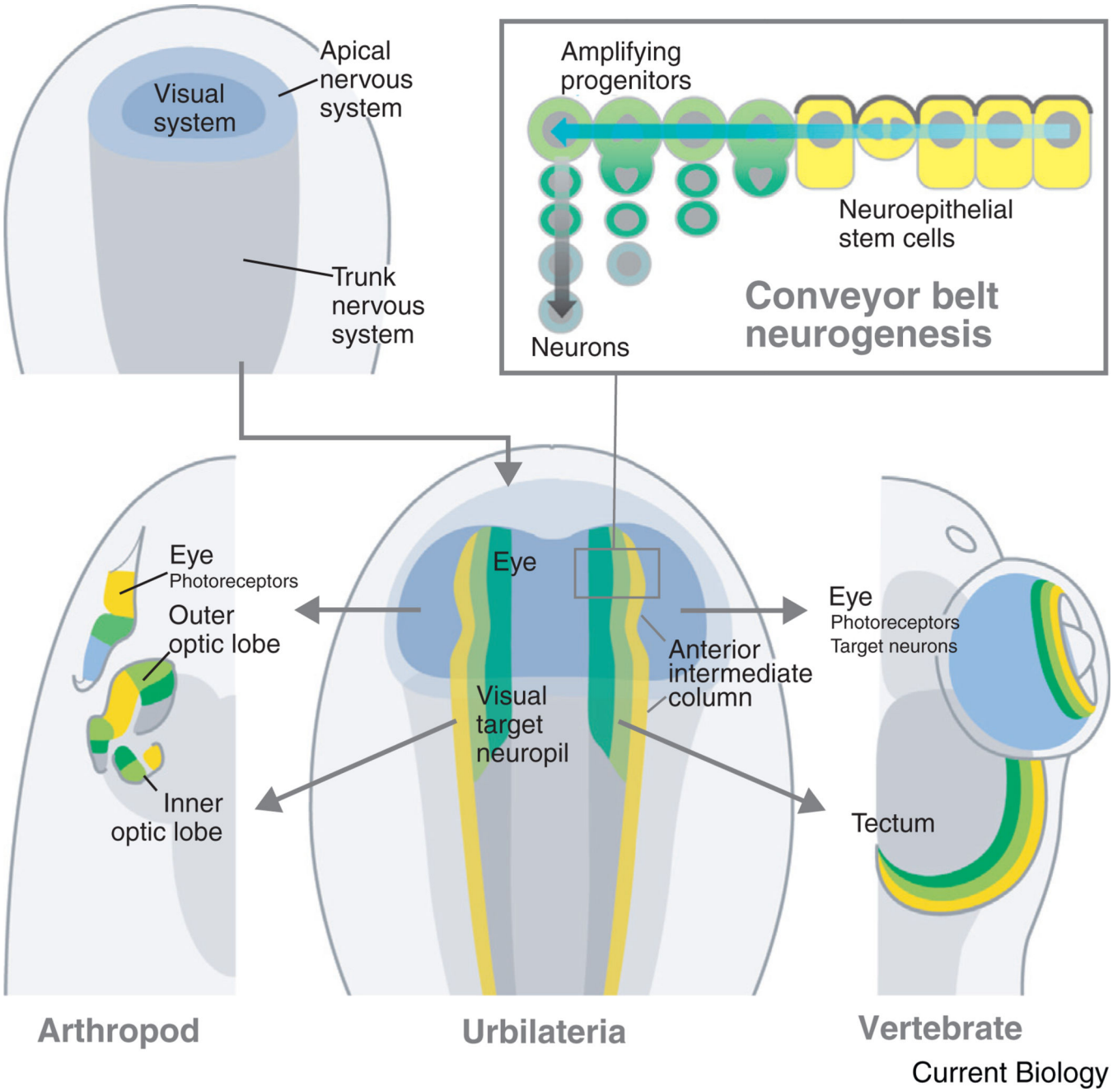

Figure 6. Conveyor belt neurogenesis in the bilaterian ancestor

Schematic representation of the neuroectoderm of hypothetical primitive bilaterans (top left, bottom center). The inception of the conveyor belt-mode of neurogenesis (top right) in discrete domains of the neurectoderm of the last common ancestor of chordates and arthropods allowed for a more efficient, protracted and temporally coordinated generation of photoreceptors and their target neuropils. The resulting evolution of complex visual systems in the arthropod clades (bottom, left) and chordate clades (bottom, right) greatly enhanced

the role of visual input in controlling the locomotion, and thereby the spectrum of visually guided behaviors.