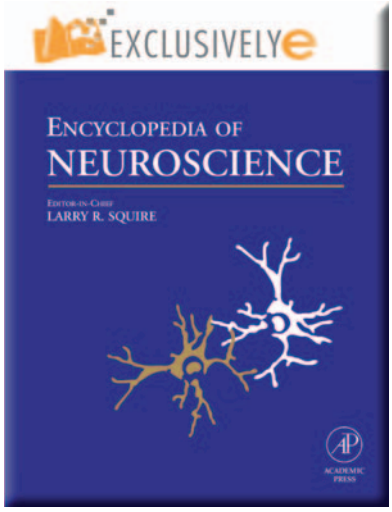

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Visual System Development: Invertebrates

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Introduction

Visual system development in most invertebrate groups commences with photoreceptor differentiation in an ectodermal epithelium. Photoreceptors generally differentiate independently of the nerve centers they innervate, and peripherally derived visual interneurons are exceptional. This is in contrast to vertebrates, in which the 'retina' arises from an embryological outgrowth of the forebrain, which generates multiple classes of interneurons as well as photoreceptors. Most of what we know about the genetic and molecular bases of visual system development in invertebrates is derived from studies on the compound eyes of the fruit fly *Drosophila melanogaster*. While this focus on a single model system rewards us with the depth of our understanding of developmental mechanisms, it has given rise to a substantial gap in our often rudimentary knowledge of other deserving groups: cephalopods, pectinid scallops, spiders, cubomedusans, and salps, to name but a few, all with advanced eyes. The same is true for the development of neuronal connections within the visual centers in the brain (Figure 1). Here, too, *Drosophila* is the invertebrate model of choice to elucidate underlying molecular mechanisms. Our understanding of many aspects of invertebrate visual system development must, however, encompass knowledge not only of the compound eyes of arthropods, but also of eyes as diverse as, for example, the highly developed single-lens eyes of cephalopods, or ancestral eyecup eyes in forms such as planaria.

Evolution of Invertebrate Eyes

Different eye types are distinguished by substantial differences in morphogenesis as well as their embryonic origin. Two lines of photoreceptor evolution, rhabdomeric and ciliary, had been thought the exclusive domains of, respectively, protostomes and deuterostomes. However, various invertebrate groups have both types of photoreceptors, and in some cases (e.g., *Pecten*) both types are found in one eye. Various examples of developing rhabdomeric photoreceptors (which bear visual pigment upon microvilli) illustrate the transient appearance of ciliary structures. Such differences in form, development, and photoreceptor organelles among the different eye types have been taken to imply an independent origin for the eyes of the three main animal groups that have advanced visual systems: arthropods, mollusks, and vertebrates. Based on classical anatomical and developmental criteria, this view has been challenged by the advent of molecular genetics and the discovery of ancestral conserved genes such as *Pax6*. *Pax6* is essential for eye development in numerous species so far tested. Conversely, its overexpression induces ectopic eyes, as shown in flies and frogs. The *Pax6* gene – like its fly homologs *eyeless* and *twin of eyeless* – encodes a homeodomain transcription factor that regulates a cascade of downstream genes. In *Drosophila*, this cascade involves around 2500 genes that are required to make an eye. The *eyeless* gene and several other genes that are essential to specify eye development, as well as sufficient to induce ectopic eye development in other tissues, are therefore sometimes referred to as 'master control genes.' These are found throughout the animal kingdom, even in ancestral forms such as the freshwater sponge *Ephydatia*. In *Drosophila*, at least six such genes have been characterized to date: *eyeless* (*ey*), *twin of eyeless* (*toy*), *sine oculis* (*so*), *eyes absent* (*eya*), *dachshund* (*dac*), and *optix*; removal of any one results in reduction or deletion of the eye, while ectopic expression (except for *so*) can lead to ectopic eye formation (Figure 2). Genes of the *six* family are transcription factors with a six domain and a six-type homeodomain that share homology with *Drosophila so*. Tentacular eyes of the jellyfish *Cladonema radiatum* express orthologs of *six1/2* and *six3/6* in the eyecup, and during eye regeneration upregulate their expression, suggesting conservation of upstream regulatory mechanisms in eye development since prebilaterian ancestors. Additional molecular evidence supporting the relatedness of all animal eyes includes the primary signal for patterning, *hedgehog*; the *atonal*-related basic helix–loop–helix transcription factors that specify the first retinal neurons; and the highly conserved visual pigment protein, opsin. Most *opsin* genes contain putative *Pax6*-binding sites, for which reason *opsin* is proposed to form part of a conserved ancestral *Pax6–opsin* regulation module. Although the evolutionary relations between eye types are still unresolved, it seems likely that a common ancestral unit existed which may have been a simple photoreceptive cell using *Pax6* to regulate *opsin* expression. From there, gene regulatory regions may have diverged and different molecular pathways recruited in different groups. This scheme thus implies the independent evolution of different eye

types that nevertheless share a remote common ancestor. The widespread role of *Pax6* is consistent with its action in the last common ancestors of insects and vertebrates in simple pigment-cup ocelli, as represented by cerebral eyes in today's errant polychaetes, such as *Platyneiris*. In early developing *Platyneiris*, *six1/2* expression covers the complete visual system and *atonal* (*ath*) expression appears in its first photoreceptor cells. The same is true in insects, but not vertebrates. This sequence has suggested that *Pax6*-, *six1/2*-, and *ath*-positive larval eyes, as found in living trochophore larvae, were already present in the last common ancestor of all bilaterians, vertebrate and invertebrate.

Invertebrate Eye Types and Their Development

Most invertebrate eyes arise from an ectodermal epithelium in one of two main ways: (1) by the formation of a fold and invagination to create either a pit, open to the outside, or a vesicle that subsequently becomes internalized, and (2) by delamination, the thickening of a region as cells divide, grow, and differentiate, followed by the formation of the cell layers that eventually constitute the adult retina. These can either remain as part of the epithelium, or detach and move into the body cavity. The original topography of the epithelium is conserved in the retinal layer of photoreceptors following invagination and delamination *in situ*, whereas it must be reconstituted secondarily following the inward migration of surface epithelial cells. Unlike vertebrate groups, different groups of invertebrates use different modes of eye development and have varying numbers of eyes. Both developmental origins (invagination and delamination) are described in some invertebrate groups and even, in some cases, in the different eyes of a single species.

Evolutionary Divergence among Invertebrate Eyes

The flagellate protozoans, *Erythropsis* and *Warnowia*, have elaborate ocellar organelles with a dioptric hyalosome and pigmentary melanosome. These two components dissociate and duplicate during cell division, the daughter organelles recombining thereafter. In Cnidaria, the ocellus of the medusa of *Cladonema* arises from an ectodermal primordium from which photoreceptor, corneal, and pigment cells differentiate,

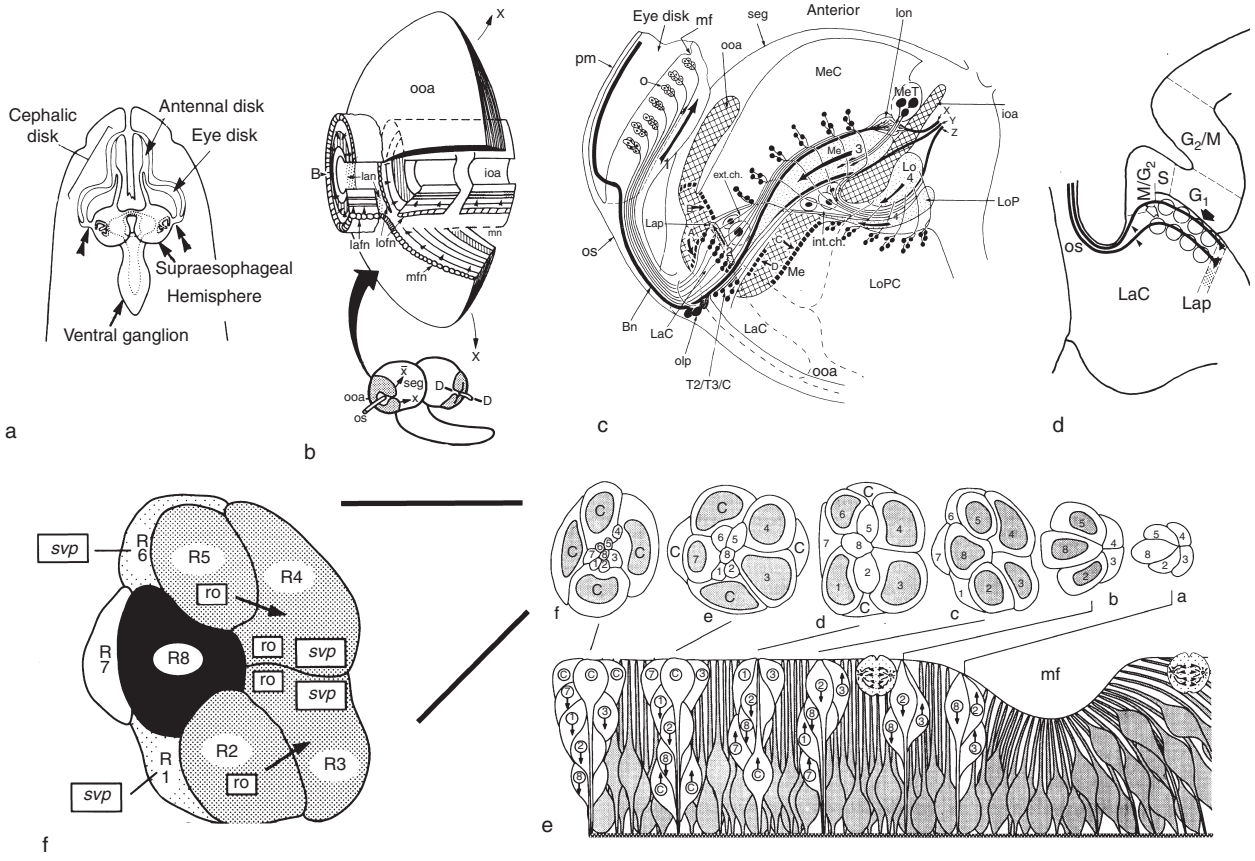

Figure 1 Continued

the lens arising in the latter. In turbellarians with direct development, eyespots appear in the ectoderm and then sink into the mesoderm, enlarge, and divide to provide multiple pairs. In annelids, eyes are usually simple eyecup ocelli, frequently with one or few photoreceptors arising, as in the prostomial eyecups in leeches, from epidermal thickenings. Only errant polychaetes have elaborate eyes, which are variably said to arise either from the epidermis, without invagination, or by delamination from an anlage, apparently of ectodermal origin, lying between ectoderm and pharynx and containing a special lens-secreting cell. Differentiation occurs in a wave – first in the inner, retinal layer. By contrast, the pair of ocelli in Onycophora invaginate from epidermal placodes, which pinch off to form a vesicle lined by everted microvillar photoreceptors. Echinoderms have multiple, simple ocelli on optic cushions that form when relatively few cells invaginate from the epidermis. Among prevertebrate chordates, interesting eyes are found in salps, which have a single, dorsal eye pushed out in vertebrate fashion from a crescent-shaped optic ridge of the cerebral ganglion, to form an ocular disk. In the chain form, the asexual part of the life cycle, the ocular disk folds up, flips over, and produces a layer of inverted photoreceptors abutting a layer of everted ones and then an everted secondary eye.

Mollusks and Arthropods

Clear accounts of eye ontogeny are generally restricted to groups with elaborate eyes, especially

Figure 1 Summary of development in the visual system in *Drosophila melanogaster*, viewed in steps of progressively finer cellular resolution. (a) The eye develops from an eye disk connected by an optic stalk (double arrowheads) to the developing optic lobe in the supraesophageal hemisphere, or ganglion, of the larval brain. (b) The optic stalk (os) penetrates the supraesophageal ganglion (seg) at the center of the crescent-shaped outer optic anlage (ooa). With later development, the arms of the anlage open in the direction marked 'X,' converting the cortices to which it gives rise from circular to rectangular forms. The outer anlage and the concentric inner optic anlage (ioa) are shown in relation to the point of entry of the optic stalk to the posterolateral surface of the right hemisphere. Neuroblasts and other progenitor cells in the anlagen proliferate in the directions of the arrowheads, contributing time-sorted strata of cells to the lamina (lamina-forming neuroblasts; lafn), medulla (mfn), and third optic neuropil (lobula; lofn). One stratum (shown cross-hatched in each cortex) was produced at the same time and has been displaced by newer strata in its respective cortex (lan, lamina neuropil; mn, medulla neuropil). The relationships between these cell populations is clearest in cross section (in diagram c). (c) Relationship between the generation of cell cortices in diagram b and the axonal pathways growing between them, in a horizontal plane of diagram b, that illustrates the close relationship between the waves of imaginal photoreceptor innervation and the path of the larval Bolwig's nerve (Bn). This runs from the inner face of the peripodial membrane (pm) to the lamina plexus (Lap) and medulla (Me) via the optic stalk (os). It and/or the axons of three optic lobe pioneers (olp), postulated interneurons, innervate a larval optic neuropil (lon), which is connected to the central brain by a pathway (X) that anticipates the posterior optic tract. New ommatidial clusters (o) accumulate behind the morphogenetic furrow (mf), contributing new axon bundles that fasciculate on previously extended axons in the optic stalk with the anterior expansion of the retinal field (arrow 1). Underlying cell populations expand in corresponding directions: arrow 2, lamina cortex (LaC); arrow 3, medulla (Me) and medulla cortex (MeC); arrow 4, lobula (Lo) and lobula plate cortex (LoPC). Cells add to the lamina cortex from one side of the outer optic anlage (ooa), the lateral proliferation zone (A), and to the medulla cortex from the other side, the medial proliferation zone (B). The first progeny from B are precocious medulla tangentials (MeT), growth cones from which weave across the medulla and intersect new columnar elements growing at the leading edge. Other tangential pathways correspond to the later paths of the anterior optic tract (Y) and of lobula tangential cells (Z). The inner optic anlage (ioa) proliferates in two directions: in direction C, to generate cells into the cortex of the lobula plate, and in direction D, to generate cell types T2, T3, or C, as judged only with reference to the positions of somata in the adult optic lobe. Axons of cellular progeny, and their growth cones, generate a plexus for each cortex, which will eventually form the adult neuropil. Crossing of fiber bundles between lamina and medulla results from the selective fasciculation of fiber pathways in a sequence of innervation like a conveyer belt and from the direction of approach between bundle and plexus. (Bundles penetrate the lamina cortex to innervate its plexus but grow along the inner margin of the medulla cortex to innervate the medulla.) Large glial cells lie along the paths of fibers in the external chiasma (ext.ch) and internal chiasma (int.ch); sg, subesophageal ganglion. (d) Proliferation from the lateral proliferation zone of the outer anlage (see diagram c). Cells are progressively displaced from neuroblasts in the outer anlage in a succession of stages in their cell cycle (G_2/M, G_1, S, G_2/M) around the lip of the anlage. Postmitotic cells lie in the lamina cortex (LaC), where they are innervated by photoreceptor axon bundles (arrowheads) from the optic stalk (os), which trigger the transition from G_1 to S in cells of the adjoining anlage (filled arrow) as well as the onset of differentiation and axonogenesis in cells already postmitotic (open arrow). (e) Ommatidial clusters mature in the eye disk, behind the morphogenetic furrow (mf), seen in elevation (clear profiles) and corresponding cross-sections in which nuclei are shaded. Arrows indicate directions of nuclear migration, matched in pairs of photoreceptors R1–R8 (labeled 1–8). Cross-sections from a (youngest) to f (oldest) are of preclusters (a, b), of immature (c) and symmetrical (d) eight-cell clusters, and of two-cone cell (e) and four-cone cell (f) stages. Cone cells are labeled 'C.' (f) R1–R8 in ommatidial cluster (corresponding to cross-section c in diagram e) comprise two central cells (R8, R7) and three pairs (R2/R5, R3/R4, R1/R6). Induction in R1–R6 involves a signal from R2/R5 that depends on expression of *rough* (*ro*) to induce development in R3/R4; *rough* product also appears in R3 and R4. The four cells defining the second line of symmetry (R3, R4, R1, and R6) all require the expression of *sevenup* (*svp*) to acquire their normal fates. (c) Modified from Meinertzhagen IA (1973) Development of compound eye and optic lobe in insects. In: Young D. (ed.) *Developmental Neurobiology of Arthropods*, pp. 51–104. Cambridge, UK: Cambridge University Press. (d) Modified from Selleck SB, Gonzales C, Glover DM, et al. (1992) Regulation of the G1-S transition in postembryonic neuronal precursors by axon ingrowth. *Nature* 355: 253–255. (e, f) Modified from Wolff T and Ready DF (1993) Pattern formation in the *Drosophila* retina. In: Bate M and Martinez Arias A (eds.) *The Development of Drosophila melanogaster*, pp. 1277–1325. Plainview, NY: Cold Spring Harbor Laboratory Press, with permission of the authors and Cold Spring Harbor Press.

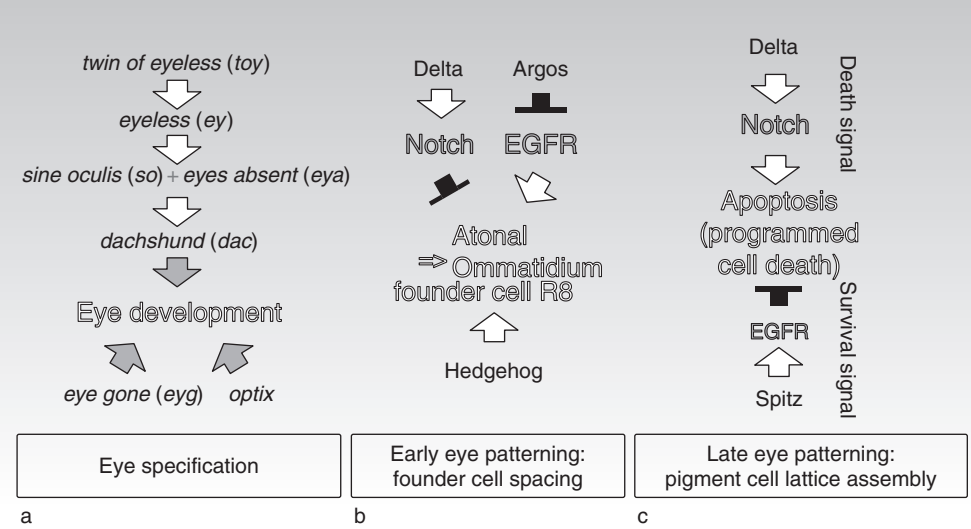

Figure 2 Selected signaling pathways during eye specification, founder cell spacing via lateral inhibition, and assembly of the pigment cell lattice via apoptosis in *Drosophila*. (a) Several nuclear factors are reported as so-called master control genes of eye development. Of these, all but two (*eyg* and *optix*) are part of a complex signaling network that has been proposed to be induced by Notch signaling and inhibited by epidermal growth factor receptor (EGFR) signaling. (b) Atonal expression determines the R8 founder cell and prepatterns the eye. Positive signals for R8 formation include Hedgehog and EGFR signaling, whereas lateral inhibition acts through the Notch pathway to establish correct founder cell spacing. (c) After ommatidial assembly, excess interommatidial cells are removed by apoptosis. Notch and EGFR signaling again exhibit antagonistic roles, providing death and survival signals, respectively.

mollusks and arthropods. Large, single-lens cephalic eyes of gastropod and cephalopod mollusks, which project directly to the brain, generally arise from an invaginating ectodermal placode, either *de novo* or during regeneration (*Strombus*, *Cardium*, *Helix*, etc.). Details vary depending on whether development is direct or via a veliger larva, and on the complexity of nonneural ocular components. Mantle eyes in bivalves have diverse origins and include both single-lens eyes and the compound eyes of arcid clams. In *Pecten* they arise by delamination of an epithelial placode that is secondarily reconstituted after migrating from the epidermis. All show spectacular regenerative capabilities. Arthropods generally have ocelli that arise by invagination, delamination, or both in some insect groups. Spiders have single-lens frontal eyes that arise by invagination and lateral eyes that form by delamination. In contrast, compound eyes form by similar means in all groups (by delamination *in situ* from an embryonic epidermal placode) to form a patterned array of modular ommatidia. In holometabolous insects, the retinal epithelium is secondarily internalized within specialized imaginal disks borne inside a postembryonic larva (**Figure 1(a)**). Reminiscent of these additions, the ocelli of diplopod myriapods arise from individual anlagen and accumulate in rows with each molt.

In ametabolous insects such as silverfish and bristletails, as well as in hemimetabolous insects (dragonflies, grasshoppers, cockroaches, etc.), eye development is a continuous process that starts during embryogenesis. In contrast, in holometabolous insects (such as *Drosophila*) visual system development consists of separate phases: an early phase during embryogenesis and a second phase during metamorphosis, separated by a 'developmental silence' during early larval stages. Compound eyes arise during the passage of a wave of development. In hemimetabolous insects and many Crustacea, the eye generated in the embryo is augmented by ommatidial increments added in adulthood; the wave is interrupted at each molt, its front contributing a succession of vertical strips of ommatidia at the eye's anterior margin. Regional specializations of the eye, such as foveae in dragonflies or mantids, therefore shift in the visual field with each molt, or are successively reconstructed. Intermolt growth in eye size generally results mostly from increased cell numbers rather than hypertrophy of existing cells, but species differ. The anterior eye margin in hemimetabolous insects is said to be a budding zone, harboring ommatidial precursor cells. In holometabolous insects, retinal development lays down ommatidial rudiments during a double postero-anterior wave of mitoses (e.g., *Drosophila* and *Ephestia*) over the largely two-dimensional epithelium of undifferentiated cells in the imaginal disk. These possibly overlap within the single wave of mitoses seen in the dragonfly.

Compound Eye Development in *Drosophila*

Compound eye development in *Drosophila* is well characterized and has become a model system to study a wide range of events in neural development, including cellular and molecular mechanisms of both tissue patterning and growth control, and the determination of cell fate. The adult eye comprises about 750 ommatidia, each in turn containing eight photoreceptor neurons, R1–R8. The rigid geometrical organization of these arises through iterated cell-fate decisions mediated by cell–cell communication within the undifferentiated imaginal disk. These occur during and after a wave of development, morphologically visible as a morphogenetic furrow initiated at the posterior margin of the disk, that progresses anteriorly (Figures 1(e) and 3(a)).

The first photoreceptor to differentiate from a field of uncommitted cells is the photoreceptor cell R8. Selection of R8 cells occurs initially to establish the correct spacing and positioning of future ommatidia (Figure 3(a)). The differentiation of R8 is also crucial because the cells of one ommatidium do not derive from a single clone, but are instead recruited by R8 and subsequent downstream events. Such recruitment occurs in pairs from the existing field of undifferentiated cells, with the first pair, R2/R5, followed by R3/R4 and R1/R6 (Figure 1(f)). Last, R7 is recruited. The initial steps of R8 selection and spacing are therefore crucially instructive for the patterned development of the eye and involve a well-characterized signaling network (Figure 2(b)). R8 differentiates under the control of the transcription factor encoded by *atonal*, which is initially expressed in all cells during the passage of the morphogenetic furrow. Behind the furrow *atonal* expression becomes restricted to the exact number of required R8 founder cells. In *atonal* mutants, ommatidial assembly fails entirely. Thus, the developing eye is prepatterned by selecting cells that express *atonal* behind the morphogenetic furrow (Figure 3(a)). Initially, all cells have the potential to become R8 and to repress others, but through lateral inhibition of their neighbors' fates only a few finally do so. Many genes encoding products required to signal this lateral inhibition are known from mutants with altered numbers of R8 cells, most prominently those involved in signaling via the Notch and epidermal growth factor (EGF) receptors (Figure 2(b)).

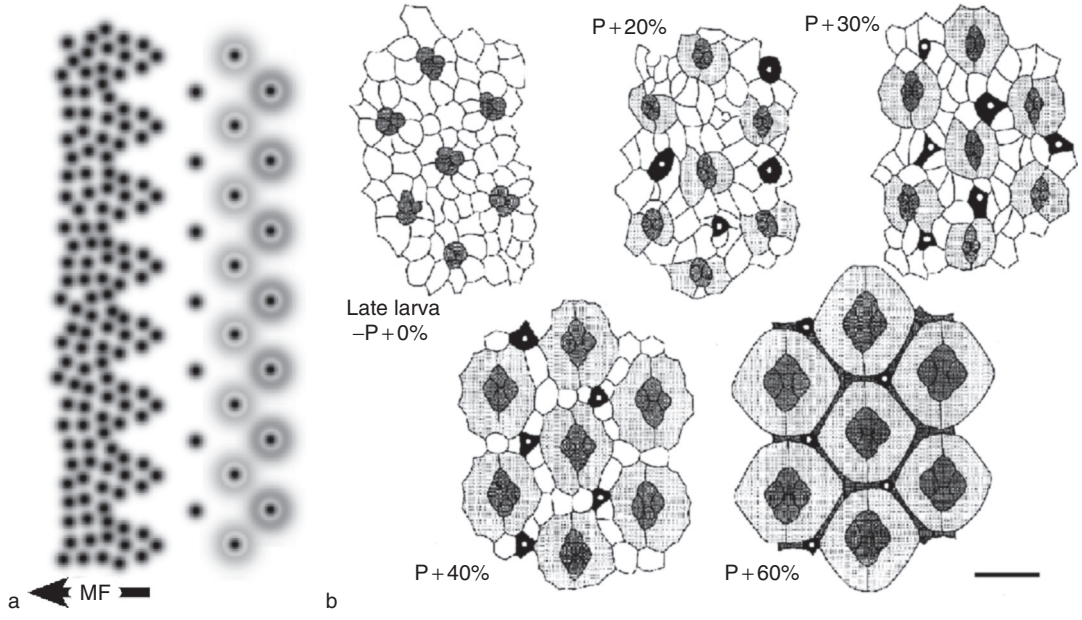

Figure 3 Maturation of *Drosophila* ommatidia. (a) As the morphogenetic furrow (MF) passes over the eye imaginal disk epithelium (arrow), the ommatidial array emerges with the differentiation of ommatidial founder R8 cells, which express *atonal* (filled circles) and prepattern the eye. (b) Pattern formation in the *Drosophila* eye. At late larval stages and the beginning of pupation (P + 0%), the eye disk exhibits the four-cone-cell stage (stippled) in its posterior region. In the 20-h pupal eye disk (P + 20%), cone cells are surrounded by two primary pigment cells (light stippling); bristle cells (black) appear between ommatidia. At P + 40%, approximately one-third of all interommatidial cells have been removed by apoptosis. Unfilled profiles between ommatidia are prospective secondary pigment cells and (at vertices) tertiary pigment cells. The P + 60% pupal eye exemplifies adult organization. Secondary (dark stippling) and tertiary pigment cells (at vertices) surround ommatidia. Scale bar = 10 μm; anterior to right. Modified from Wolff T and Ready DF (1993) Pattern formation in the *Drosophila* retina. In: Bate M and Martinez Arias A (eds.) *The Development of Drosophila melanogaster*, pp. 1277–1325. Plainview, NY: Cold Spring Harbor Laboratory Press, with permission of the authors and Cold Spring Harbor Press.

Molecules that have been proposed to act as inhibitors include Delta and Scabrous, both of which interact with Notch. Hedgehog and ligands of the EGF receptor (EGFR) are positive signals for founder cell specification (Figure 2(b)).

The subsequent recruitment of R2/R5 and R3/R4 creates preclusters containing five cells, evenly spaced behind the morphogenetic furrow (Figure 3(b)). Now postmitotic, the cells of these preclusters commence differentiation, whereas all other undifferentiated cells reenter the cell cycle in the second mitotic wave. Also born during that wave are the remaining 14 cells required to constitute an ommatidium. The differentiation of these cells as well as of all photoreceptors other than R8 is controlled by different signaling pathways, again by the EGFR pathway as well as the mitogen-activated protein (MAP) kinase and other receptor-mediated signaling pathways. Secretion of the EGFR ligand Spitz is required for all non-R8 photoreceptor differentiation. The first such pathway to be identified, the signaling cascade leading to the final recruitment and differentiation of R7, is particularly well characterized. A membrane-associated protein encoded solely in R8 by the *boss* locus interacts with the Sevenless receptor tyrosine kinase on the presumptive R7. Internalization of Boss protein in R7 and induction of the downstream Ras/Raf MAP kinase pathway lead to R7 differentiation.

Finally, all ommatidia are positioned within a hexagonal array with shared interommatidial cells in between. The assembly of the secondary and tertiary pigment cell lattice depends on the spatially restricted apoptosis of excess cells, which also involves a complex signaling network implicating the Notch and EGFR pathways (Figure 2(c)). In this late phase of eye development, Notch promotes apoptosis of excess interommatidial cells, while EGFR signaling, initiated by cone or primary pigment cells, opposes Notch (Figure 2(c)). As a result, approximately one-third of the interommatidial cells are eliminated before eye development concludes.

The Eye's Connection with Its Visual Centers and Their Development

Wiring the invertebrate eye to its brain is as diverse a process as are the eye types themselves. A common feature of all is the retinotopy of mapping the optical world upon the visual brain. This is documented at single-cell level in the insect compound eye, but is also a clear feature in, for example, the single-lens eye of cephalopods. Development among invertebrate visual centers is once again best described in the arthropod optic lobe. Visual interneurons are the progeny of neuroblasts that reside in two groups, the outer and inner proliferation centers, or optic anlagen (Figures 1(b) and 1(c)). In insects, the outer anlage generates the first neuropil, the lamina, and the distal part of the second, the medulla, while the inner anlage gives rise to the remaining medulla and deeper optic neuropils (Figure 1(c)). In both anlagen, the cells are born by a sequence of divisions that matches the sequence of ommatidial assembly in the compound eye, but begins first in the medulla. The match between eye and lamina in *Drosophila* is attained by a competition between targeting signals at the N-terminus and C-terminus of the secretory Hedgehog protein: after cleavage, the N-terminal domain is targeted to the retina, while the C-terminal domain overrides this path and transports Hedgehog along the photoreceptor axons, where it induces the development of target neurons in matching numbers.

In Crustacea, the number and arrangement of the optic neuropils are more diverse than in insects, and details of crustacean development are less widely known. In decapod Crustacea, the shape, location, and mitotic patterns of proliferation zone 2 (PZ2) closely resemble the outer optic anlage of insects. In the water-flea *Daphnia*, the counterpart of an outer optic anlage is the sole so-called optic lobe primordium (Figure 4(b)). In *Drosophila*, photoreceptor innervation triggers the final cell cycle of lamina precursor cells, procuring targets for the next wave of innervation and matching the proliferative activities of eye and optic lobe (Figure 1(d)). (Lamina glial cells, by contrast, arise independently from R1–R6 input and from a lineage that is distinct from that of lamina cells.) Photoreceptor cell axons innervate new ganglion cells, so produced, in successive rows corresponding to the wavefront of retinal differentiation. Most lamina cells are thereby enlisted, five per axon bundle, and cell death, presumably among supernumerary cells, concludes the wave of development in the outer neuropils. In *Daphnia*, newly innervated cells are invariably just postmitotic. As ingrowing photoreceptor axons recruit them in final groups of five (Figure 4(b)), each soma displays a stereotypic response in which it envelops the incoming axons, forming gap junctions with them transiently. The same occurs in *Drosophila*, but is less clearly analyzed. In *Daphnia*, all cells contacted exhibit this envelopment. They do so in the exact sequence of contact, but cells from which retinal innervation has been withheld do not, while envelopment is delayed in cells to which innervation arrives late. Envelopment manifests a presumed inductive signal to each lamina cell, after which the latter differentiates, elaborating a neurite that will follow the retinal axon bundle in a secondary wave.

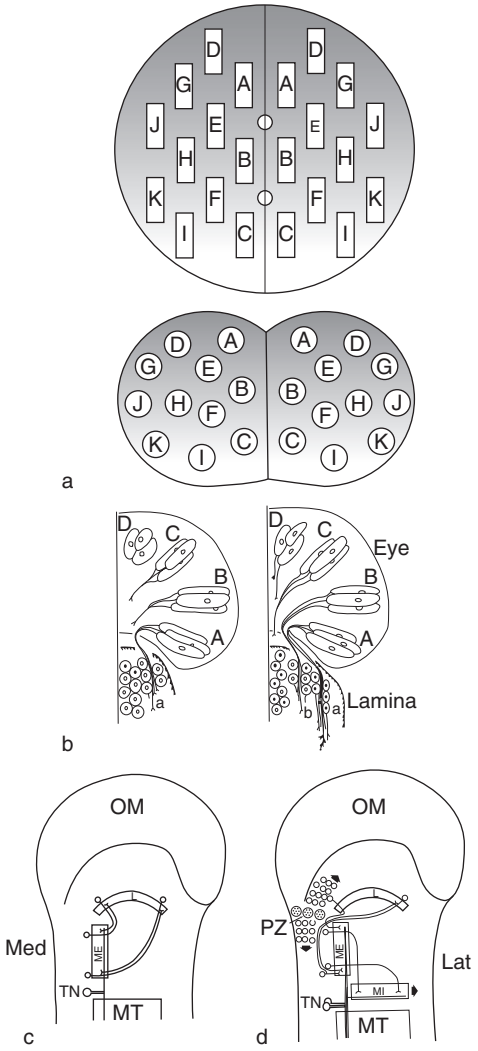

Figure 4 Development of the visual system in representative Crustacea. (a) Schematic diagram of the compound eye (top) and optic lamina (bottom) in *Daphnia*, showing locations of ommatidia (lettered rectangles) and of lamina cartridges (circles with corresponding letters) to which photoreceptor axons of each ommatidium project. Both are viewed in a transverse projection, dorsal side up, with the left eye to the left. The projection is direct, without an intervening chiasma. (b) Schematic representation of the development of the eye–lamina projection in *Daphnia* at an early stage in its development (left) and several hours later (right). Only the right halves of the eye and lamina are depicted, in a view perpendicular to that shown in diagram a. The ommatidia are shown in four rows (A–D), with each row at a different stage of development; cartridges a and b are depicted during assembly. At both stages illustrated the most lateral ommatidium, A, is also the most developed, while D, the most medial, is the least so. (Left) Axons from A have grown along the midline glial bridge that connects eye and lamina anlage and have reached the target region, where they begin to recruit lamina neurons to form cartridge a. (Right) Axons from B have followed the same path as those of A and complete the recruitment of five lamina neurons to form cartridge b, displacing cartridge a laterally. Axons from C and D will subsequently repeat this process until the projection is completed. (c, d) Assembly sequence of neuropil layers and axon projections between them in the optic lobes of a nonmalacostracan (c) and malacostracan (d) crustacean. In both examples, a common neuroblast population, the outer proliferation zone PZ2 (PZ) generates progeny in two antiparallel directions. These assemble the cell cortices of lamina (L) and medulla externa (ME) in chronotopic sequence, like a conveyor belt. After their birth, cells are continuously displaced from PZ2 to await innervation from overlying ommatidia (OM). The lamina and distal medulla in Crustacea both have major structural and developmental similarities to their possible evolutionary homologs in insects; malacostracan Crustacea grow chiasmal interconnecting pathways identical to those found in insects (diagram d). In nonmalacostracans, axons of cell bodies in the lamina cortex penetrate the lamina and enter the medulla externa from its neuropil face, thus traversing directly between corresponding neuropil faces. In Malacostraca, as in insects, on the other hand, a chiasma arises because the corresponding axons enter the medulla from the opposite direction, through its cortex, and in doing so invert their sequence. Tangential neurons (TN) from the medulla terminalis (MT) grow across the neuropil of the medulla externa and interna (MI), advancing to contact the newly grown retinal innervation and the higher order columnar pathways upon which it projects. (a, b) From Macagno E (1984) Formation of ordered connections in the visual system of *Daphnia magna*. *BioScience* 34: 308–312. (c, d) From Elofsson R and Dahl E (1970) The optic neuropiles and chiasmata of Crustacea. *Zeitschrift fur Zellforschung* 107: 343–360, used with permission of the authors and Springer-Verlag.

Axonal Outgrowth and Pathfinding

Photoreceptor axons grow centrally, innervating the lamina and distal medulla in a sequence that mirrors the ordered sequence of retinal differentiation. Outgrowth within the ommatidial axon bundle is asynchronous. The axon from one photoreceptor in *Daphnia* (R1) has a well-defined growth cone and is the first to grow and contact target cells. Axons of other cells following this lead axon (**Figure 4(b)**) have reduced growth cones or none. After R1 is ablated, the axon of another photoreceptor assumes the role of lead fiber, indicating that all photoreceptors are equivalent. In *Drosophila*, axon outgrowth occurs initially in groups of five (presumably R2–5 and 8), later supplemented by the remaining three. In many insect groups, photoreceptor axons grow postembryonically along the pathway provided by larval or nymphal visual systems. Best known is Bolwig's organ, a group of 12 internal photoreceptors at the tip of a fly larva, with optic lobe pioneers (their presumed interneurons in *Drosophila*), a larval nerve, and optic neuropil, all of which preempt the axon pathways of the earliest adult neurons, which assemble around them (**Figure 1(c)**). Bolwig's nerve is dispensable for adult wild-type photoreceptor axons to project normally, and is thus not an essential pioneer of axonal navigation to the lamina. Bolwig's organ later transforms into the four photoreceptors of an extraretinal posterior 'eyelet,' which entrains circadian rhythmicity in the larva. The terminals of Bolwig's nerve metamorphose into those of the eyelet nerve. Their branching parallels the arborization

of neurons immunoreactive to pigment-dispersing hormone, which serve as outputs from the circadian clock. In other insects, there are larval ocelli (in mosquitoes) and stemmatal nerves (in Lepidoptera and Coleoptera). Larval photoreceptor axons and adult axons in insects lacking larval visual centers grow in the embryo, thus initially spanning only short distances to reach their targets, but elongating dramatically with subsequent growth. Those in *Daphnia* grow initially over a glial substrate; later axon bundles follow the preexisting neural pathway to the center, reinforcing the fiber tract in which they grow while simultaneously providing the substrate for yet further growth (**Figure 4(b)**). The sequence in which new axon bundles accrue to existing fiber tracts, and their relative positions within the tract, confer the antero-posterior polarity of the resulting topographical map. Chiasmata form when this polarity inverts, as in the external chiasma of malacostracan Crustacea (**Figure 4(d)**) and insects, as revealed by subsequent rotation of the medulla in *Drosophila* (**Figure 1(c)**). Many arachnid groups also have chiasmata, but non-malacostracan Crustacea, such as *Daphnia*, lack them (**Figure 4(c)**). Eye rotation experiments in locusts indicate that ingrowing receptor axons fail to discriminate the particular column of neuropil in which they terminate. On the other hand, there is evidence that retinotopy is reinforced in *Drosophila* by guidance mechanisms that label both photoreceptor axons and their targets. For example, (1) in the mutants *sine oculis* and *Ellipse*, axons project to the correct retinotopic location even when not surrounded by axons from neighboring ommatidia, while (2) wild-type axons project normally, even when they neighbor mutant *glass* axons with aberrant projections. At a coarser level in *Drosophila*, mutant chiasmal phenotypes can result from the perturbed axon pathways of the larval optic lobe pioneer neurons (**Figure 1(c)**) caused by the displacement of their cell bodies in the larva.

Several *Drosophila* mutants have been isolated that affect pathfinding, target recognition, or medulla rotation. Photoreceptor axons R1–R6 enter the lamina as rapidly growing ommatidial bundles, where their growth cones stop between layers of glial cells; thus, the primary targets that terminate growth cone guidance are not the synaptic partners, but glia. Mutations in the *brakeless* gene cause the axons of R1–R6 to penetrate their normal lamina targets and project instead to the medulla, where normally only R7 and R8 terminate. A transcription factor encoded by the *runt* gene, which is normally repressed in R1–R6, is derepressed in *brakeless* mutants, and overexpression of *runt* causes the same mistargeting phenotype. Apart from these nuclear factors, genes encoding cell surface receptors, as well as downstream signal transduction proteins, have been characterized in *Drosophila*. Genes encoding cell surface receptors include *rst* (a transmembrane receptor of the immunoglobulin superfamily), *DPTP69D* and *DLar* (both receptor tyrosine phosphatases), *DN-Cadherin* (a classical cadherin), *Flamingo* (a protocadherin), *capricious* (a transmembrane receptor with LRR-repeats), and others. Mutants of all these genes exhibit targeting phenotypes of differing severities. Few studies have so far addressed the spatiotemporally controlled localization of guidance receptors during development. Mutants in the vesicle trafficking gene *sec15* suggest that trafficking of guidance receptors during axon targeting requires highly regulated intracellular trafficking. The best characterized signal transduction pathways required for photoreceptor growth cone guidance include *dreadlocks* (a SH2/SH3 adaptor protein), *pak* (p21-activated protein kinase), *trio* (a Rho family guanine exchange factor that activates Rac), *misshapen* (an Ste20-like serine/threonine kinase), and *bifocal* (a putative cytoskeletal regulator).

Synaptic Partner Selection and Synaptogenesis

Once in the lamina, the growth cone filopodia of R1–R6 in the fly continuously interact with neighboring growth cones in a distinct developmental step that follows initial target recognition until each gradually draws away from its parent ommatidial bundle. The growth cones then pass through a succession of shifting geometric arrays, apparently driven by filopodial interactions that are initially directed exclusively between R1–R6, but eventually directed to the growth cones of their monopolar cell targets, L1 and L2, around which R1–R6 assemble to form nascent cartridges. Among the previously mentioned genes, mutants in several (e.g., *DPTP69D*, *DLar*, *DN-Cadherin*, *Flamingo*) cause severe defects during this lateral sorting of photoreceptor terminals into the correct cartridges, whereas the protocadherin *flamingo* is required for the initial orientation of the R1–R6 bundle. In the medulla, the axon terminals of R7 and R8 initially occupy temporary strata relative to terminals of other cells, but then grow down deeper to their permanent stratum, R7 below R8. Synaptogenesis has been analyzed in detail only in the lamina, where it commences only in the second half of pupal development, after the R1–R6 terminals have grown centripetally, when neurites of lamina cells contact photoreceptor presynaptic sites. Starting with a neurite contact from L1 or L2, each synaptic site selectively accumulates other postsynaptic elements to constitute a final tetrad synapse (a synapse with four postsynaptic targets). Up to 50% of all contacts formed are lost. Although synaptic transmission

functions early, while synaptic junctions mature, pupal photoreceptors generate only small, slow responses to light and are not normally exposed to bright light anyway. In contrast to vertebrate visual systems, neither spontaneous nor evoked neuronal activity is required to form a normal visual map in the fly's brain.

The Morphogenetic Interdependence of Eye and Visual Centers

Development in the visual centers generally exhibits some dependence on innervation from the eye, but this is quantitatively variable; eye development is usually autonomous. In *Drosophila*, clones of mutant tissue with perturbed organization generated in either the eye or optic lobe reveal that abnormal pattern is communicated from the retina to the lamina, but not in the opposite direction. This refines conclusions from the coarser effects of deafferentation in many species. More central neuropils are volumetrically less affected by deafferentation than are peripheral ones. In congenitally eyeless mutant *Drosophila*, second-order neurons in the lamina never differentiate, but some medulla neurons grow axons, albeit their morphogenesis is abnormal, whereas many lobula neurons look quite normal and establish a regular array of neuropil columns. Tangential cells of all neuropils show complete autonomy from afferent innervation. Qualitatively similar results follow lesions in the embryonic *Daphnia* eye. Differentiation in the fly's lamina and medulla is largely unaffected by the absence of target cells on the lobula, indicating the relative absence of retrograde influences of central visual neuropils upon distal ones. On the other hand, ommatidia eventually degenerate after disconnection from the lamina, indicating that the optic lobe has a retrograde influence on the long-term maintenance of the retina.

Plasticity

Insect visual systems exhibit various forms of plasticity, many at a behavioral level. The developmental emergence of pattern discrimination and visual preference behavior in flies, for example, depends on prior visual experience, the flies preferring a pattern to which they were selectively exposed during a critical period as young adults. Various neurochemical correlates exist. Functional binocular vision, as revealed by prey-catching behavior in mantids, matures after emergence, and its development also exhibits a critical period. Praying mantises that are monocularly occluded as emerging nymphs nevertheless have normal monocular visual fixation and binocular distance estimation when they attain adulthood, indicating that binocular visual experience is not essential. Even so, monocular rearing initially impairs distance estimation, which then only gradually recovers; such recovery is progressively lost in older nymphs or adults. Bees reared in ultraviolet light are less sensitive to light of longer wavelengths; terminals of photoreceptors that peak in the green, in which the bees were deprived, also have fewer synapses. Other examples of structural plasticity are reported. In flies, lamina feedback synapses are more numerous in young dark-reared adults and, during subjective night, in flies held under constant darkness; these synapses also exhibit reactive synaptogenesis after losing their targets, R1–R6. Photoreceptor input synapses are not fixed synaptic sites: fly tetrads can both form and disappear rapidly (within minutes) in the adult, especially in response to functional reversals (e.g., light exposure after dark rearing; warm recovery after cold exposure). Central optic lobe neurons sprout after congenital deprivation of their visual inputs, procured either by lesion or mutation. The volume of the optic neuropils increases in *Drosophila* reared under 'enriched' conditions, relative to solitary rearing, and with various other rearing conditions. At the cellular level, L1 and L2 change the caliber of their lamina axons during a cycle of day/night changes.

See also: Animal Models of Inherited Retinal Degenerations; Learning and Memory in Invertebrates: *Drosophila*; Olfaction in Invertebrates: *Drosophila*; Photoreceptor Adaptation; Photoreceptors: Physiology; Retinal Horizontal Cells; Retinal Pharmacology: Inner Retinal Layers; Visual System: Invertebrates.

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