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Introduction – from eye field to eyesight

Rachel O. L. Wong
University of Washington, Seattle, USA

Vision begins at the retina, a light-sensitive tissue at the back of the eye that comprises highly organized, laminated networks of nerve cells. Investigating the mechanisms of retinal development is fundamentally important to gaining a basic knowledge of how vision is established. In this book, we present the sequence of developmental events and the mechanisms involved in shaping the structure and function of the vertebrate retina.

1.1 Formation of the eye

The eye is derived from three types of tissue during embryogenesis: the neural ectoderm gives rise to the retina and the retinal pigment epithelium (RPE), the mesoderm produces the cornea and sclera, and the lens originates from the surface ectoderm (epithelium). During embryogenesis (Figure 1.1), the eyes develop as a consequence of interactions between the surface ectoderm and the optic vesicles, evaginations of the diencephalon (forebrain). These optic vesicles are connected to the developing central nervous system by a stalk that later becomes the optic nerve. When the optic vesicles contact the ectoderm, inductive events take place to cause the epithelium to form a lens placode. The lens placode then invaginates, pinches off eventually and becomes the lens. During these events, the optic vesicle folds inwards and forms a bilayered cup, the optic cup. The outer layer of the optic cup differentiates into the RPE whereas the inner layer differentiates into the retina. The iris and ciliary body develop from the peripheral edges of the retina. The sclera is derived from mesenchymal cells of neural crest origin, which also migrate to form the cornea and trabecular meshwork of the anterior chamber of the eye. During early development, the hyaloid artery and vein provide the major blood supply to the eye; these structures is later disassembled, leaving behind the ophthalmic artery and veins. In humans, eye development begins at around 22 days of development and is not completed until several months after birth (Mann, 1964).

1.2 Basic organization of the mature vertebrate retina

Since the early investigations of Cajal in the past century (see Cajal, 1972), it is well established that the vertebrate retina comprises five major classes of nerve cells or neurons

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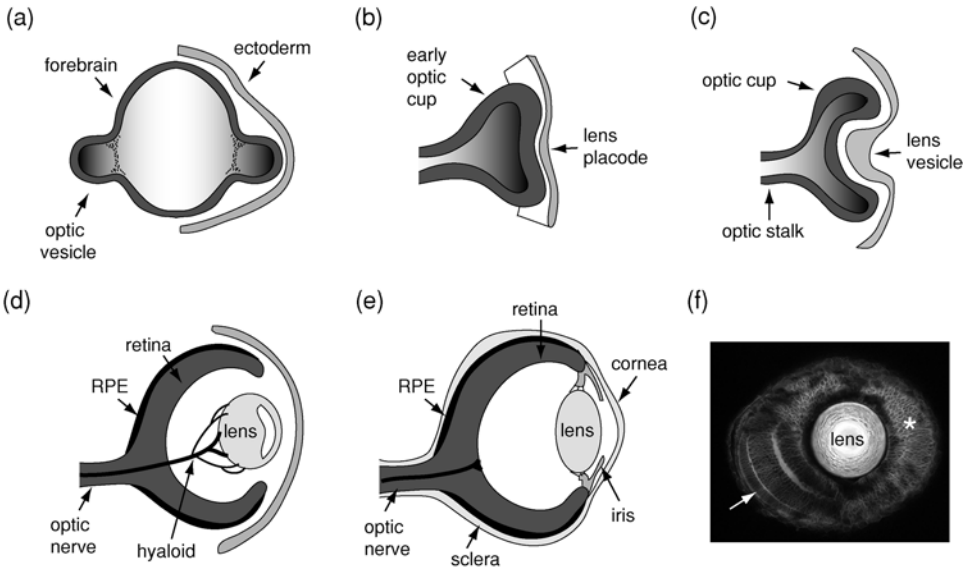

Figure 1.1 Development of the eye. (a) Optic vesicles that form from the neural tube give rise to the two eyes. (b) Contact between the optic vesicles and the surface ectoderm produces a lens placode. (c) The lens placode pushes into the optic vesicle, resulting in the formation of an optic cup and a lens vesicle. (d) The outer surface of the optic cup becomes the retinal pigment epithelium (RPE), and the inner surface becomes the retina. (e) Location of the retina within the mature eye. (f) Example of a developing vertebrate eye (zebrafish), showing the developing lens and the retina. All cell membranes are labelled here by expression of fluorescent protein in a transgenic animal, and imaged in the live animal. The region of the retina deeper within the eye (arrow) shows its characteristic lamination pattern whereas peripheral retina (example, asterisk) is last to differentiate and laminate.

(Figure 1.2; see Wässle, 2004, for review). Rod and cone photoreceptors convert light information to chemical and electrical signals that are relayed to interneurons in the outer retina. Bipolar interneurons are contacted by photoreceptors and convey signals from the outer retina to the inner retina. Transmission from photoreceptors is modulated by horizontal cells that also contact the bipolar cells. In the inner retina, bipolar cells form chemical synapses with their major targets, the retinal ganglion cells and amacrine interneurons. Amacrine cells not only modulate signals from the bipolar cells by providing inhibition directly onto ganglion cells, but also modulate transmitter release from the bipolar cells. Light information leaves the retina via axons of the retinal ganglion cells that collectively form the optic nerve.

The cell bodies and connections of retinal neurons are arranged in layers (Figure 1.2). This laminar organization of the retina is stereotypic across species. Connections are restricted to two major laminae, the outer plexiform layer (OPL) and the inner plexiform layer (IPL). Müller glial cells, which span the depth of the retina, provide important structural and functional support for the retinal neurons. Embedded within this basic organization of the vertebrate retina are many specialized subcircuits, working together in parallel to process

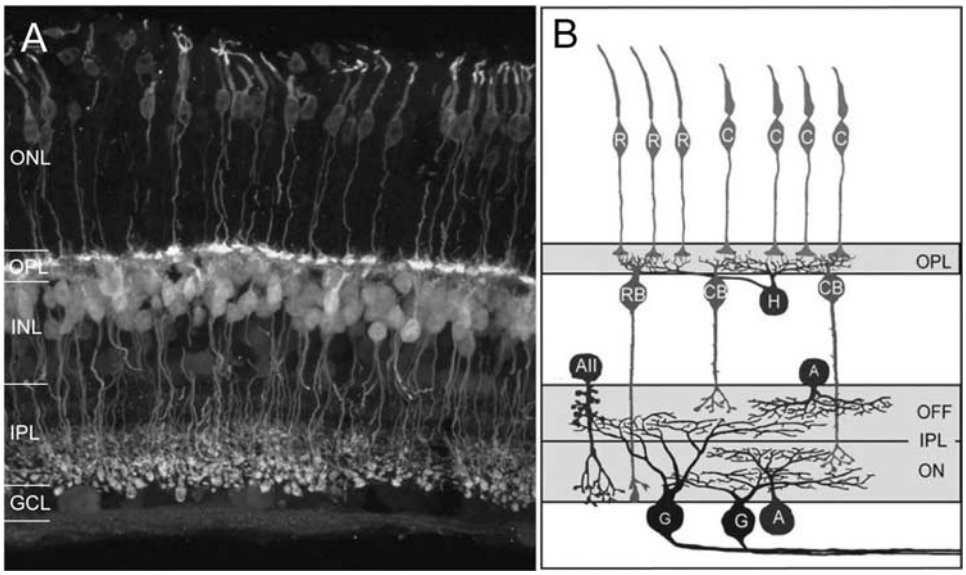

Figure 1.2 Basic circuitry of the vertebrate retina. (A) Cross-section of a mouse retina showing the laminated distribution of cell bodies and neuronal processes visualized by cell-staining methods. ONL, outer nuclear layer comprising photoreceptors; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Image provided by J. Morgan. (B) Schematic representation of the basic wiring diagram of the retina. R, rods; C, cones; RB, rod bipolar cell; CB, cone bipolar cell; H, horizontal cell; AII, AII-type amacrine cell in the rod pathway; A, amacrine cell; G, ganglion cell.

different features of the image. Rods are sensitive to low light levels and a rod-driven circuit exists for visualizing objects under dim light conditions. In vertebrates, this circuit involves connections between rod photoreceptors, rod bipolar cells and a special type of amacrine cell, the AII amacrine cell (Figure 1.2B). Increments (ON) and decrements (OFF) in light intensity are detected and processed along two vertical pathways. Cone photoreceptors contact a variety of cone bipolar cells, some of which are depolarized (ON) and others, hyperpolarized (OFF) by increased illumination. ON- and OFF-cone bipolar cells contact retinal ganglion cells, which respond to changes in illumination according to their bipolar input. Together, the ON and OFF pathways provide contrast information. In addition to these basic features, the retina also has specialized circuits that can compute other features of the visual scene, such as the direction of motion or orientation of edges.

Work to date has identified components of several subcircuits of the retina, demonstrating a high degree of correlation between structure and function. For example, connections involving ON and OFF components are largely confined to distinct sublaminae within the IPL (Figure 1.2). The relationship between structure and function of circuits in the mature retina has facilitated studies aimed at understanding the mechanisms essential to its development, and also studies that wish in general to determine what factors are essential for the development of the central nervous system.

1.3 Development of the vertebrate retina

Vision, of course, relies on the proper development of the retina. Much progress has been made towards our understanding of the cellular and molecular mechanisms underlying retinal development. We will present the major areas of retinal research, beginning at the stage when the eyes form to when the retina performs its mature task, processing the visual scene.

1.3.1 Specification of the eye field and building blocks of the retina

Retinal development begins with specification of the eye primordia during early stages of embryonic life. One major area of investigation thus encompasses studies aimed at elucidating the genes and molecular signalling pathways required for the formation of the two eyes (Chapter 2). Once the eye fields are defined, the next step concerns the generation of the appropriate cell types, their numbers and distributions (Chapter 3). Here, considerations have been given to cell-intrinsic (genetic) and extrinsic (environmental) signals that act in concert to specify cell fate. Decisions to become one or another type of retinal cell appear to depend on many factors, including the time of cell genesis (Chapter 5). Cell division occurs at the retinal surface abutting the pigment epithelium. From this location, postmitotic cells migrate to their final locations within the retina. Although cellular mechanisms underlying neuronal migration are well studied in the central nervous system in general, our understanding of this process in the vertebrate retina is only in its infancy (Chapter 4). One thing that is evident, however, is that, like other parts of the nervous system, there is an overproduction of retinal neurons during development, many of which die before eye opening. The mechanisms regulating naturally occurring cell death in the retina are important for controlling cell number and distribution (Chapter 11). Also, understanding what evokes cell survival or death in the retina is likely to have implications for the regenerative capacity of the vertebrate retina. To date, mammalian retinas show a limited ability to regenerate whereas in other vertebrates, such as the zebrafish, retinas have a tremendous capability to regenerate. Studies comparing the development of different vertebrate species are thus important for the discovery of genes and cellular interactions that support regeneration of the vertebrate retina (Chapter 15).

1.3.2 Wiring cell components of the retina

Following the generation of each cell type, the major sequence of developmental events in the retina pertains to the formation and maintenance of connections between its cellular components, and between the retina and its brain targets. For the latter, the formation of the optic nerve is of primary importance in order to wire the eye to the brain (Chapter 8). Within the retina, organization of its networks occurs progressively and with precision. First, the various cell types need to express their appropriate neurotransmitters for intercellular communication. These transmitters, as well as neurotrophic molecules, play essential roles in the survival and differentiation of the retina (Chapter 6). Second, to communicate with

their neighbours, retinal neurons need to extend processes. The dendritic processes of retinal neurons, their input surface, are contacted by presynaptic cells. Conversely, the axons of retinal neurons, their output processes, synapse onto their target cells. It should be noted, however, that the processes of amacrine cells are both pre- and postsynaptic in nature. One important requirement for dendritic outgrowth of retinal neurons, studied most widely in retinal ganglion cells, is that their arbors overlap by defined amounts, leading to tiling and complete coverage of the retinal surface. Different cell types show different amounts of overlap. How these mosaics of cell territories are established during development is fascinating and important to study because they relate to spatial processing by each cell population (Chapter 10). In fact, retinal ganglion cells that can sample at high acuity have small dendritic arbors that hardly overlap whereas those that detect motion primarily show greater overlap. One idea is that early contact between neighbouring cells of the same type regulates their spacing via adhesion-based signalling. However, there is also evidence for intrinsic factors limiting how large a dendritic arbor retinal ganglion cells, and perhaps other retinal neurons, can grow. Our knowledge of the factors that control the growth of retinal neurons is only just beginning to deepen.

Another essential wiring pattern in the retina is that the processes of ON and OFF bipolar, amacrine and ganglion cells stratify within their appropriate sublaminae. Much work has been focused on determining the role of intrinsic factors as well as cell–cell interactions in shaping the stratification of these cell types. Indeed, the use of state-of-the-art live-imaging techniques, transgenic mice and mutants lacking specific cell types or molecular interactions is beginning to help unravel the mechanisms that regulate neurite patterning in the retina (Chapter 12).

Accurate processing of visual information not only necessitates that the axons and dendrites of retinal neurons target their correct synaptic partners, but importantly, that they form the appropriate balance of excitatory and inhibitory connections. Synapse formation has been studied for many decades in a variety of animals. Traditionally, this developmental event has been investigated using electron microscopy (EM) methods that enable synapses to be visualized at the ultrastructural level (Chapter 13). Photoreceptors and bipolar cells form ribbon synapses, which can be recognized under EM by an electron-dense ribbon-like structure flanked by synaptic vesicles containing neurotransmitter. Amacrine cells form conventional synapses whereby pre- and postsynaptic densities and synaptic vesicles are observed at the contact site, but ribbons are absent. At the EM level, then, it is possible to distinguish photoreceptor (outer retina), bipolar and amacrine synapses. At present, the study of synaptogenesis in the vertebrate retina is restricted to fixed tissue, but modern methods of live-cell labelling using fluorescently tagged synaptic proteins (Morgan *et al.*, 2005) are likely to help us gain a dynamic view of this fundamental developmental process in live tissue.

1.3.3 Properties of early circuits in the retina and the emergence of light sensitivity

It is perhaps surprising that early circuits of the retina are functional and able to generate electrical activity before the retina is sensitive to light. Amacrine cells and the ganglion cells

form the first synaptic circuit in the retina. Photoreceptors develop much later and bipolar cells needed to connect the outer retina to the inner retina form their connections after the retina is wired to visual targets in the brain. The activity produced by the early amacrine–ganglion cell network demonstrates unique spatiotemporal patterns, which is characteristic of many vertebrates studied thus far. These patterns, and their potential function in synaptic wiring, will be discussed in detail (Chapter 13).

Light responses emerge shortly before eye opening in mammals, and in the embryo of turtles and zebrafish. Few studies to date have examined the nature of these responses, and in particular how the region of space encoded by retinal neurons becomes defined is not well understood. With improvements in electrophysiological techniques that allow detailed assessment of the physiological properties of retinal neurons and their early and mature responses to light stimuli, this gap in our knowledge is beginning to fill (Chapter 14).

1.4 Concluding remarks

Although a large part of this book is dedicated to the architecture, connectivity and function of retinal neurons, the maturation of glial cells and their role in retinal development is also considered (Chapter 9). Recent studies certainly demonstrate that glial cells are integral and essential components of the retina, and that they play a significant role in maturation of retinal neurons and their connectivity.

A common theme throughout the book concerns reference to different vertebrates, ranging from zebrafish to primate. Such diversity in the study of vertebrate retinal development has led to the discovery of developmental mechanisms that are unique or common across vertebrates. Moreover, each vertebrate has features that offer investigation of specific developmental events. For example, the rapid development and relative transparency of the embryonic zebrafish eye permits visualization of retinal development in vivo (Chapter 17). In particular, this has enabled cell division and migration in the retina to be followed. The presence of a fovea in monkeys allows us to study the mechanisms underlying the development of this specialized region of the retina, which is necessary for high-acuity vision in human (Chapter 7). Thus, in the future, studies based on different vertebrates are likely to continue to yield basic information of how the retina develops.

A major goal of this book is not only to present the current knowledge of how the vertebrate retina develops, but also to convey a sense of progress in our understanding of the mechanisms involved. This progress has largely been fuelled by advances in several technical areas. First, molecular methods now enable the identification of gene products expressed in specific retinal cell types, and at distinct periods of development. This knowledge should help us better understand the molecular pathways that specify cell identity (Chapter 16). Second, it is now possible to visualize and track retinal cells in live tissue by expression of fluorescent proteins in transgenic fish or mice (Chapter 12). Third, new ways to record physiological responses from not only one, but dozens of retinal neurons simultaneously, has led to the discovery of patterned activity during development (Chapter 13). Such methods

can also be used to study light responses from a multitude of retinal ganglion cells during development. Together with conventional approaches used successfully over the decades, such technological advances will push the frontiers ahead in our quest to understand how the vertebrate retina attains its structure and function.

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2

Formation of the eye field

Michael E. Zuber
SUNY Upstate Medical University, Syracuse NY, USA

William A. Harris
University of Cambridge, Cambridge, UK

2.1 Introduction

Vertebrate eyes originate from a single field of neuroectodermal cells in the anterior region of the neural plate called the eye field (sometimes referred to as the eye anlage, eye primordia or presumptive eye). The origins of the eye field can be traced back to the 32-cell-stage blastula in which a subset of blastomeres is competent, but not yet committed, to form retina. This chapter begins with a discussion of retinal competence and the maternal molecules and cell–cell interactions that take place in and bias early blastomeres toward a retinal fate. Transplantation experiments have shown that the entire presumptive neural plate of midgastrula embryos can form retina, demonstrating the remarkable coordination of neural development with eye formation. Neural induction and the neural patterning events critical for defining where the eye field forms in the developing nervous system will be addressed. Cultured amphibian anterior neural plates form eyes demonstrating that the eye field is specified (committed to form the eye) by the neural plate stage. A conserved set of transcription factors collectively referred to as eye field transcription factors are required for normal eye formation and are expressed in the eye field of the neural plate stage embryo. These genes and their functional interactions, which are required for and under some circumstances sufficient to drive eye field and eye formation will be described. A description of how the single vertebrate eye field separates to form the eye primordia that eventually give rise to the two eyes concludes this chapter.

2.2 Retinal competence

The eye field is not specified until early neurula stages (see below). However, even at early cleavage stages only a subset of blastomeres is competent to contribute to the eye field. Fate-mapping, transplantation and ablation experiments have shown that nine dorsal animal (retinogenic) blastomeres of the 32-cell-stage *Xenopus laevis* embryo normally contribute progeny to each eye (Figure 2.1 and Moody, 1987). A certain level of plasticity is observed during normal development. The proportion of retinal cells derived from any given

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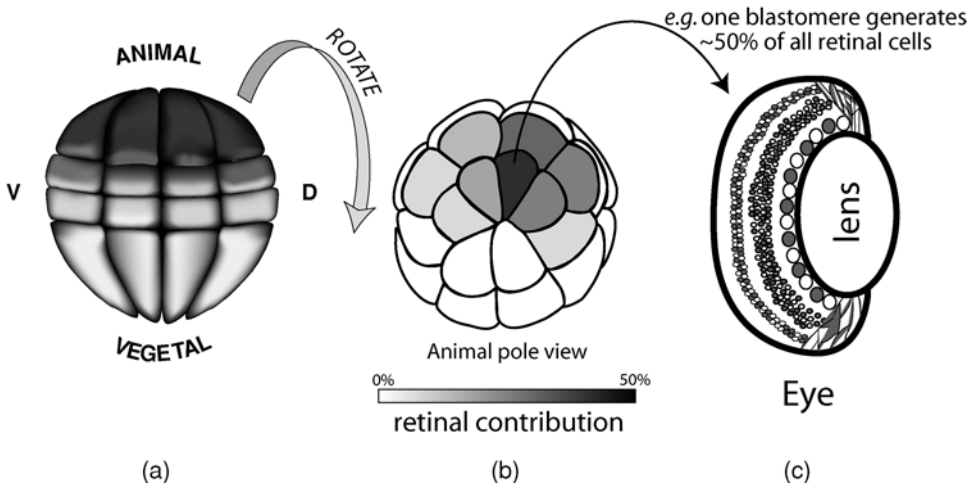

Figure 2.1 Multiple blastomeres generate eye-forming progeny. (a) Three-dimensional schematic diagram of a 32-cell-stage *Xenopus* embryo. (b) Animal pole view shows the subset of blastomeres that normally produce retinal progeny in the left eye. The shaded blastomeres produce fewer than 1% (lightly shaded) to as many as 50% (darkly shaded) of the retinal cells. (c) The progeny of retinogenic blastomeres are distributed throughout the retina (Huang and Moody, 1993).

retinogenic blastomere varies from animal to animal and no significant spatial segregation of their progeny is observed, that is, clones derived from the different blastomeres are found intermixed in the eye (Figure 2.1 and Moody, 1987; Huang and Moody, 1993).

Normally, only dorsal animal blastomeres deposit progeny in the eyes, however, all animal blastomeres are competent to contribute to the eyes. If equatorial or ventral animal blastomeres are transplanted to the retinogenic zone they are reprogrammed in response to interactions with their new neighbours and generate normal sized eyes (Figure 2.2a and Huang and Moody, 1993). If dorsal animal blastomeres in the centre of the retinogenic zone are killed nearby dorsal blastomeres compensate, generating more retinal progeny, resulting in tadpoles with normal eyes (Figure 2.2b). Therefore, cell–cell interactions are important in both determining the location and regulating the size of the retinogenic zone.

Although dorsal animal blastomeres are biased, they are not committed to a retinal lineage at the 32-cell stage. When transplanted to ventral vegetal locations, they retain their neural fate but don't make retina (Figure 2.2c and Gallagher *et al.*, 1991). Conversely, ventral vegetal blastomeres transplanted to retinogenic locations never contribute progeny to the retina (Figure 2.2d and Huang and Moody, 1993). Because zygotic transcription does not begin until later in development, these results show that inherited maternal determinants restrict the location of the retinogenic zone to the animal side of the embryo.

What are the maternal and cell–cell signals that determine whether a given animal blastomere will contribute progeny to the eye field and retina? How do animal blastomeres differ from their vegetal cousins? Suppression of bone morphogenetic protein (BMP) signalling appears to be necessary for animal blastomeres to generate retinal progeny (Moore and

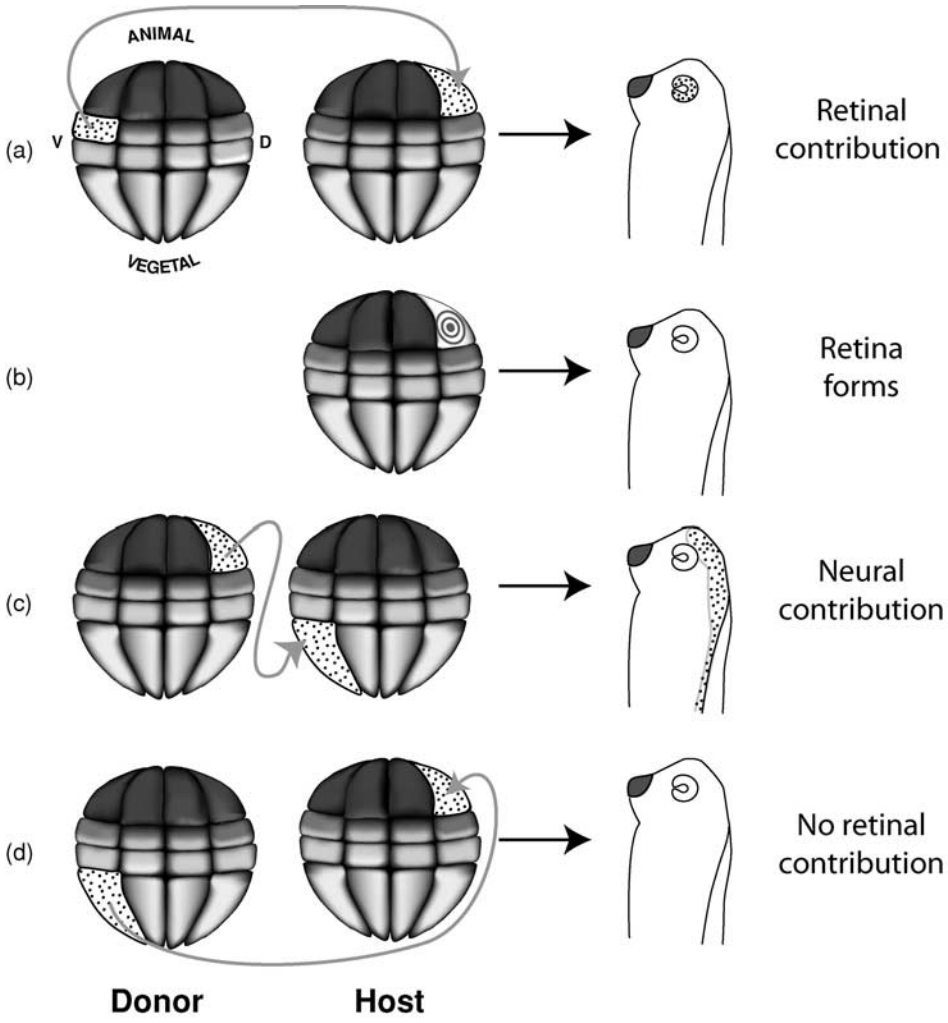

Figure 2.2 Transplantation and ablation experiments highlight the plasticity of blastomeres that contribute to retinal formation. Bull's eye indicates the position of ablated blastomere. See main text for a detailed description.

Moody, 1999). When BMP signalling is activated in retinogenic animal blastomeres, they fail to generate retinal progeny. Blocking BMP signalling in animal blastomeres that normally generate epidermis (by using BMP4 antagonists or dominant negative forms of BMP receptor) alters their fate and they generate retinal progeny. These results are consistent with a model in which eye formation is tightly coupled with neural induction, as inhibition of BMP signalling is required for neural induction (see below). Consistent with this model, the neural inducer noggin can also promote the progeny of animal blastomeres to the retinal lineage.