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Introduction

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The eye is an exquisitely specialized sensory structure with photosensitive neurons (photoreceptors) and non-neural supporting tissues dedicated to light capture and image formation. The photoreceptors and other neural components of the eye constitute the retina. The four review papers that follow are concerned with the development of the neural retina, and two of them deal with the most specialized type of retinal neuron—the photoreceptor. A common theme threads through all four reviews—an attempt to understand cellular differentiation at the level of molecular mechanisms. Two broad classes of molecular mechanisms are considered—transcription factors that control cascades of gene expression, and signaling molecules that activate intracellular signal transduction pathways, which in turn alter the expression and/or functional activity of transcription factors. Our understanding of how these two levels of regulation are linked is still superficial, and we know only a few of the specific downstream target genes of the regulatory pathways. The endpoint of these developmental signaling cascades is the selective expression of genes that allow a given cell to acquire the distinctive characteristics of a specific type of differentiated retinal neuron.

Three of the papers in this volume examine the origin of neuronal diversity in the vertebrate retina, and the fourth considers the compound eye of the fruit fly, *Drosophila*. Both the vertebrate retina and the insect compound eye have attracted much attention as model systems for studying how the nervous system becomes organized during development. A prime attraction of studying retinal development is the impressive regularity of anatomical pattern created by the orderly placement of specific subtypes of retinal neurons in the neural array. This regularity implies a spatial and temporal precision in the developmental regulation of molecular signals responsible for cellular differentiation and neuronal diversity. Hence the attraction—if the process is so orderly, we can reasonably aspire to decipher its rules.

Recent work from several laboratories has revealed that genes regulating eye formation are conserved across the animal kingdom, a finding that challenged the long-standing belief, based on phylogenetic and embryological considerations, that eyes evolved independently several times. The most dramatic evidence for evolutionary conservation in the genetics of eye formation was provided by the demonstration that the *Drosophila* homeobox gene, *eyeless*, is a structural and functional homolog of the vertebrate gene, *pax6*. Mutations in *eyeless*/*pax6* interfere with proper eye development in both flies and in vertebrates (mice and humans), and ectopic expression of *eyeless* or (astonishingly) the vertebrate *pax6* gene can produce ectopic eyes in *Drosophila*. These results led to the hypothesis that *pax6* is a 'master gene' responsible for initiating the genetic program that produces eyes in all animals, although recently, the universality of this hypothesis has been challenged.

The first paper in this volume, by Mathers and Jamrich, reviews the work on *pax6* and the related, but more recently discovered, homeobox gene, *Rx*, which appears to be more specifically targeted to regulation of retinal development in vertebrates. Both of these genes are members of a large family of transcriptional regulators with paired-type homeodomains including, for example, *six3*, *otx2* and *crx*. Many of these paired-type homeodomain transcription factors are known to influence eye formation at one or more stages in development: first, in establishing and refining distinct regions of gene expression that impart competency to form retinal tissue, later in promoting proliferation of retinal progenitor cells, and finally in specifying the differentiation and/or maintenance of specific classes of retinal neurons. Mathers and Jamrich review the results of recent studies in vertebrates in which the activity of *pax6*, *Rx*,

and *six3* proteins has been altered—either enhanced by overexpression or reduced by a loss-of-function mutation. This work suggests that *pax6*, *Rx*, and *six3* regulate each other's expression and, therefore, the concept of a single master regulator of eye development in vertebrates is not supported by the data. Furthermore, *pax6* is implicated in the development of a widespread array of neural structures in vertebrates (e.g., nasal placodes, cerebellum, spinal cord, neural crest), and *pax6* function is not required for the initial stages of formation of the optic primordium in the vertebrate embryo. Mathers and Jamrich compare the results in vertebrates to work in *Drosophila*, in which powerful genetic tools have been used to investigate hierarchical relationships among *eyeless*, the closely related *twin of eyeless (toy)*, *sine oculis* (in the same family as the vertebrate gene *six3*), and the novel nuclear proteins, Eyes absent and Dachshund, all of which have some capacity to induce ectopic eyes. These studies have revealed that *toy*, not *eyeless*, may be positioned at the top of a complex regulatory network which apparently functions within a limited and defined context, i.e., only during larval stages of development, where it triggers the construction of a compound eye from imaginal disc tissues. Other genes, including *Drosophila* homologs of *Rx* (*DRx*) and *six3* (*Dsix3*), may be implicated at earlier times in development, when imaginal discs (pouches of epithelial tissue) are set aside in the embryo and bestowed with the competence to form adult structures including compound eyes. Putting aside the details and complexities of the networks and hierarchies that link these transcriptional regulators, what remains most compelling is that the same molecular players are involved in eye formation in vertebrates and invertebrates.

The next paper, by Brennan and Moses, takes a more detailed look at how the compound eye of *Drosophila* is constructed and what we know about the molecular mechanisms that control cell specification and patterning of photoreceptors during development. Their title sets the main theme: 'timing is everything.' They lead the reader on a journey through the details of the molecular interactions between cells that shape the forming eye and that give photoreceptor cells their specific identities. There are eight types of photoreceptor in the compound eye—R1 through R8—organized into individual facets, or ommatidia, each containing a single representative of all eight R cell types, arranged in a stereotypic, asymmetric array along with twelve nonneural supporting cells. The molecular players required to construct the compound eye with such crystalline precision include: (i) a transcription factor, Atonal, a member of the basic helix-loop-helix (bHLH) family with 'proneural' activity—proneural genes promote neuronal fate; (ii) the Notch signaling pathway—Notch is a transmembrane receptor whose primary ligand in the eye is Delta and whose function is to control timing of neuronal differentiation; (iii) secreted factors in the transforming growth factor-α (TGF-α) family which bind to the epidermal growth factor receptor (EgfR)—this is a tyrosine kinase receptor which uses the Ras pathway for intracellular signal transduction; (iv) other transcription factors, such as the homeodomain protein, Rough, that confer a potential identity on a specific cell—the fate of the cell is realized only through activation of the Ras signaling pathway, and (v) the secreted factor, Hedgehog—an important morphogen widely involved in organogenesis, whose role in the developing eye is to drive the progression of the morphogenetic furrow—the moving furrow reflects the reiterative molecular signaling events that build the eye, row by row, and that generate the orderly pattern of facets in the adult compound eye.

Brennan and Moses outline the essential concept that underlies development of the compound eye—repeated activation of the Notch and EgfR signaling pathways produces different results in the recipient cell depending on context. The 'context' is understood to be the array of transcriptional regulators present in the cell and the other signals being received simultaneously by the cell, which together will determine the outcome, i.e., the fate of the cell. Another important (and little known) idea reviewed by Brennan and Moses is the dual role of Notch signaling, which promotes neuronal differentiation in some contexts but inhibits it in other situations. In the developing eye, Notch is needed early on to sustain *atonal* expression in the founder R8 cell, which differentiates first and is required to initiate the sequential recruitment of the other seven R cells in the ommatidial cluster. However, Notch is then needed to inhibit *atonal* expression in the non-R8 cells, and thus prevent their differentiation. This latter function represents the classic 'neurogenic' activity of Notch—a somewhat confusing term, derived from the phenotype of mutations that disrupt Notch; these mutations result in excess neurons. This observation implies that functionally intact Notch acts to prevent neuronal differentiation, and the term 'neurogenic' is thus equivalent to 'anti-neural.' Both of these opposing functions of Notch signaling in the eye involve the ligand Delta, although the early requirement of Notch for proneural enhancement is not mediated through the downstream components of the classic neurogenic/anti-neural Notch signaling pathway, which include Hairy and the Enhancer of Split, E(spl) complex, and Suppressor of Hairless, Su(H). In summary, Brennan and Moses point out that many aspects of ommatidial assembly depend on precise timing, although little is known about how the individual signaling events are coordinated.

Some of these neuronal patterning and differentiation genes have vertebrate homologs that are also involved in retinal development, e.g., *atonal*, *Notch* and *Delta*, *hedgehog*, and *EgfR*. The third paper, by Perron and Harris, examines the generation of neuronal diversity in the vertebrate retina and the role of *Notch* and *Delta*, and the bHLH proneural genes related to the *achaete-scute* complex and *atonal* genes in *Drosophila*. The vertebrate retina is organized into strata, with specific subtypes of retinal neurons partitioned into discrete layers. As in the *Drosophila* eye, different types of retinal neurons in vertebrates are generated sequentially, in a reiterative fashion, as a wave of cell differentiation sweeps across the unpatterned epithelium that is the retinal primordium. These spatiotemporal gradients of retinal differentiation are largely preserved in all vertebrates, in that retinal ganglion cells are produced early and rod photoreceptors are produced late, and central retina begins to differentiate before peripheral retina. Lacking the powerful genetic tools available in *Drosophila*, investigations of the molecular mechanisms that generate neuronal diversity in vertebrates have relied primarily on cell culture methods, which have demonstrated the importance of cell-cell interactions (both inhibitory and inductive) in determining cell fate. Recently, techniques have been developed for misexpressing candidate genes that might be involved in retinal cell determination. Among the earliest genes to be investigated were those in the *Notch-Delta* pathway. Consistent with the classic neurogenic/anti-neural action of Notch, activation of Notch signaling in a retinal progenitor cell blocks differentiation, whereas inhibition (expression of dominant negative constructs or blocking with antisense oligonucleotides) promotes premature differentiation. As in *Drosophila*, the outcome of Notch signaling is context dependent—cells forced to differentiate adopt the specific fate appropriate to their current place in the spatiotemporal sequence of differentiation. The early proneural action of Notch signaling described by Brennan and Moses has yet to be observed in vertebrates.

Similar to *Drosophila*, *atonal*-like genes are involved in retinal differentiation in vertebrates, but unlike *Drosophila*, proneural genes related to *achaete-scute* are also involved. In vertebrates, the latter include *NeuroD*, first identified by its ability to produce ectopic neurons when expressed in epithelial cells in the early frog embryo. The various members of the proneural class of genes are expressed sequentially during retinogenesis, suggesting that retinal proneural genes may provide the cellular 'context' in the sense already referred to—the set of transcriptional regulators that define the moment-to-moment differentiation potential of a given progenitor cell and thereby determine its fate in response to a signal to differentiate. Perron and Harris point out that possible interactions between these proneural genes and signaling pathways are beginning to emerge: both Notch and EGF signaling may inhibit expression of *Mash1* (*mouse achaete-scute homolog*).

The last paper, by Levine et al., tackles the complex morass of soluble factors implicated in the differentiation of one subtype of retinal neuron—the rod photoreceptor. These factors can be grouped into those that stimulate rod production and differentiation (retinoic acid, Sonic hedgehog, taurine, and laminin $\beta 2$), those that inhibit differentiation of rods and stimulate proliferation of retinal progenitors (EGF/TGF-α), and several growth factors that have pleiotropic or opposing effects in different vertebrate species (ciliary neurotrophic factor, activin, and fibroblast growth factor). Most of this work has been done with a variety of culture systems, and the challenge now is to link the effects observed in vitro with the actions of proneural genes and other transcriptional regulators that might be involved in rod differentiation in vivo. For example, *NeuroD* and a *neurogenin-related* gene, *ngnr-1*, enhance the production of rods when overexpressed, but it is not clear what downstream target genes these proneural genes influence to generate rod-specific differentiation products, such as rhodopsin and other elements of the visual transduction cascade. Although some of the paired-type homeodomain proteins discussed in the first paper in this volume (i.e., Rx and Crx) can bind to sequences in the rhodopsin promoter and transactivate expression of a reporter gene, it is not known whether a functional relationship exists between *Rx/crx* and *NeuroD/neurogenin*. Another example of a proneural gene that affects rod photoreceptors is *Mash1*, which when 'knocked out,' delays the production of rods. Since treatment of retinal explant cultures with EGF decreases *Mash1* expression while it stimulates cell proliferation, the observed inhibitory effect of EGF on rod photoreceptor production might be mediated through inhibition of *Mash1* expression in retinal progenitors.

Taken together, the work described in these review papers suggests that the pace of research investigating the origin of neuronal diversity in the retina is accelerating. A large and growing number of molecules important for establishing the identity of differentiated neurons and the pattern of retinal organization have been identified. We now know that many of the genes involved in eye formation and retinal development have been conserved through evolution, and this allows informative comparisons to be made across species. Although many of the elements of this developmental jigsaw puzzle have been uncovered, we are only just beginning to assemble the pieces into a unified picture of how an eye is created.