A conserved blueprint for the eye?

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Summary

Although the eyes of all organisms have a common function, visual perception, their structures and developmental mechanisms are quite diverse. Recent research on eye development in *Drosophila* has identified a set of putative transcription factors required for the earliest step of eye development, specification of the field of cells that will give rise to the eye. These factors appear to act in a hierarchy, although cross-regulation may amplify the eye fate decision or promote progression to the next step. Surprisingly, homologous proteins are also involved in vertebrate eye development, suggesting that this regulatory network was present in a primitive common ancestor and that it has been adapted to control visual organ formation in multiple species. The identification of genes acting upstream and downstream of these transcription factors will contribute to our understanding of the establishment of a developmental field, as well as of the divergence of regulatory pathways controlling the formation of eye structures. *BioEssays* 21: 843–850, 1999.

Introduction

The question of how a field of cells becomes determined to form a particular organ has long intrigued developmental biologists. The eye is a particularly challenging example, as it contains multiple cell and tissue types that must coordinate their development to form a functional unit. Recent advances in molecular genetics have identified some of the critical determinants of eye formation; they have also indicated a surprising conservation at the molecular level between structurally unrelated eyes that have evolved independently. This review will discuss what we have learned about the early events leading to eye specification in the fruit fly *Drosophila* and in vertebrates, as well as describing some of the questions still outstanding.

A set of transcription factors specifies the *Drosophila* eye

The *Drosophila* compound eye develops from the eye-antennal imaginal disc, which invaginates from the ectoderm during embryogenesis and grows inside the larva. In the third larval instar, photoreceptors begin to differentiate in the eye disc. These begin at the posterior margin and spread anteriorly, led by a depression in the disc known as the morphogenetic furrow.[1] Early determination of the eye primordium requires six genes encoding nuclear proteins that are likely to act as transcriptional regulators (Table 1). The *eyeless* (*ey*) and *twin of eyeless* (*toy*) genes encode Pax-6 proteins, containing paired domain and homeodomain DNA-binding motifs.[2,3] *eye gone* (*eyg*) encodes a Pax-like protein[4] and *sine oculis* (*so*), a homeodomain protein,[5,6] while *eyes absent* (*eya*)[7] and *dachshund* (*dac*)[8] encode novel nuclear proteins. No mutations in *toy* are available, but mutations in any of the other genes can result in the absence of the eye due to the lack of any photoreceptor differentiation. *so* is also closely linked to a highly related gene, *optix*,[9] although no mutations have as yet been described in this gene. Recent research has focused on the order in which these genes act to specify eye development and on their interactions with each other. During normal development, it seems that the expression of each gene in the eye primordium is activated in a sequential and hierarchical manner, as described below.

The embryonic primordium of the eye-antennal disc is first recognized by its expression of *toy*, *ey*, and *eyg*; as *ey* and *eyg* do not require each other for their expression, they must be independently activated by other factors.[2–4,10] Toy, which

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TABLE 1. Genes Implicated in the Specification of the Eye in *Drosophila* or Vertebrates and Their Homologs

***Drosophila* gene**	**Expression pattern**	**Function**	**Vertebrate gene**	**Expression pattern**	**Function**
eyeless (Pax-6)	Embryonic eye-antennal primordium; throughout early eye disc; anterior to morphogenetic furrow	Eye disc growth, furrow initiation, rhodopsin expression; induces ectopic eyes in most discs	*Small eye* (Pax-6)	Anterior neural plate, retina; lens placode, lens, cornea	Required for optic cup invagination and lens formation; induces ectopic lenses in *Xenopus*
eyes absent (novel nuclear protein)	Late second instar eye disc, highest at posterior margin; in furrow and posterior	Eye disc growth, furrow movement, photoreceptor differentiation; induces ectopic eyes more strongly in combination with *so* or *dac*	*Eya1, Eya2, Eya3*	*Eya1* earliest in lens placode, *Eya2* in retina, *Eya3* in optic vesicle	Not known in mouse; mutated in human branchio-oto-renal syndrome
sine oculis (homeodomain)	Embryonic visual system primordium; late second instar disc, highest at posterior margin; in furrow and posterior	Eye disc growth, furrow movement, photoreceptor differentiation; induces ectopic eyes only in combination with *eya*	*Six1, Six2, Six3*	*Six3* in anterior neural plate, retina; lens placode, lens	Induces ectopic lenses in medaka fish
dachshund (novel nuclear protein)	Late second instar eye disc, highest at posterior margin; in furrow and posterior	Furrow initiation; induces ectopic eyes more strongly in combination with *eya*	*Dach*	Retina, surrounding mesenchyme	Unknown
DRx (homeodomain)	Brain but not eye primordium or eye disc	Unknown	*Rx*	Anterior neural plate, retina	Required for optic cup formation; induces ectopic retina in *Xenopus*
eyegone (Pax-like)	Embryonic eye-antennal primordium; anterior part of early eye disc; anterior to furrow	Eye disc growth, furrow initiation; represses *wingless*	*Unknown*		

The expression patterns and functions inferred from mutations or ectopic expression are listed. Where the names of the genes differ between vertebrate species, the names of the mouse genes are given. Genes corresponding to *Pax-6, Six3,* and *Rx* have also been described in *Xenopus* and zebrafish, and *Pax-6* and *Six3* in chicken. See text for references.

is present in the early embryonic primordium of the entire visual system and can bind to essential sites in the *ey* enhancer,[3,10] is a good candidate for such a factor. Expression of *eya*, *so* and *dac* in the eye disc begins in the second larval instar, prior to the onset of photoreceptor differentiation,[5,7,8] although *so* has an earlier role, independent of *toy* and *ey*, in the embryonic development of the whole visual system.[5,6,10] Each of these genes is expressed at the highest levels at the posterior margin of the eye disc, where differentiation will later initiate. At this stage of development, the genes appear to act in the ordered sequence shown in Figure 1A. *ey* is required for the expression of *eya* and *so* but not *toy,* while *eya*, *so*, and *dac* are not required for *ey* expression.[3,10–13] *dac* expression requires both *eya* and *so*, and its function is not necessary for *eya* expression.[12,14] *eya* and *so* probably act at the same level; both are independently activated by *ey*, but each is required for full expression of the other.[10]

Some additional complexity has been revealed by experiments in which these genes have been misexpressed in other tissues. One striking result is the ability of misexpressed *ey* or *toy* to induce eye development in other imaginal discs that would normally develop as wings, legs, or antennae.[3,15] This suggests that *Pax-6* can act as a ''master regulator'' capable of inducing all the downstream genes required for eye development. Consistent with this, it has been shown that ectopic expression of *ey* induces the expression of *eya*, *so*, and *dac*.[10,11,13] Further evidence that *toy* acts upstream of *ey* is that ectopic *toy* induces *ey* expression and requires *ey* for its function, while ectopic *ey* does not induce *toy* expression.[3] However, the ability to induce ectopic eye development is not limited to *Pax-6* homologs; *eya* and *dac* have been shown to have the same effect, albeit with a much lower efficiency and predominantly in the antennal disc.[11,12,13] Combinations of two genes are in general more effective than individual genes; for example, a combination of *ey* and *eya* induces eye development even on the genitalia.[11] Similarly, a combination of *eya* with either *so* or *dac* induces eye development with a higher frequency and in a wider range of tissues than *eya* or *dac* alone.[12,14] Interestingly, misexpression of *eya* or *dac* can induce ectopic *ey* expression,[11–13] suggesting that there is some feedback regulation, as shown in Figure 1B.

This feedback could reflect a normal positive, though nonessential, regulation of *ey* by its downstream targets, thus setting up an autoregulatory loop that locks in the eye fate[16]; a similar process appears to operate in muscle determination.[17] Alternatively, it is possible that, in this case, *ey* expression is relevant to a later function of the gene. Ectopic expression may initiate the process that normally occurs downstream, activating a second, independently regulated phase of gene expression. Many of these genes indeed function at several stages of eye development, including growth of the early eye disc (*ey*, *eya*, *so*, *eyg*)[4,5,7,10]; initiation of the morphogenetic furrow (*ey*, *eya*, *so*, *eyg*, *dac*)[4,8,10,12]; progression of the morphogenetic furrow (*eya*, *so*)[12]; photoreceptor differentiation (*eya*, *so*)[12]; and rhodopsin gene expression during pupal eye development (*ey*).[18] *eya* and *dac* only activate *ey* in the restricted range of tissues in which they induce eye development, lending support to the second model and suggesting that other factors contribute to this activation. It has been shown that *ey* function is essential for *eya* or *dac* to induce full ectopic eye development,[11,14] but this may not be true of intermediate stages; for example, *eya* and *so* together can induce the expression of *dac* without inducing *ey*.[19] It would be interesting to determine whether *ey* is necessary for *eya* and *so* to induce the proneural gene *atonal*,[20] an early marker of photoreceptor differentiation.

The requirement for multiple nuclear proteins to regulate the same processes may be partially explained by the formation of protein complexes. This concept is supported as Eya and So have been shown to interact in vitro. As So contains a DNA-binding homeodomain and Eya a transcriptional activation domain, and the phenotypes of loss of either gene from the eye appear identical, an attractive hypothesis is that Eya and So are two subunits of a single transcription factor.[12] Similarly, Dac also interacts with Eya in vitro. As Dac contains a transcriptional activation domain but no DNA-binding domain and has a more restricted function than Eya, it may modify the specificity of the So/Eya complex on a subset of its target promoters[14] (Fig. 1C).

Additional factors must create a context for eye formation

Despite these recent advances in the field, we still do not have a complete understanding of the process of eye determination. The question of how the eye field is specified has now become the question of how *toy* is activated in a specific region of the embryo, and how its initially broad expression is refined precisely to the eye-antennal primordium.[3,10] It seems likely that a complex combination of positional cues defines this region, in a process analogous to segmentation of the embryonic trunk.

Additional factors must also act in parallel to the genes described above. All of these genes have other sites of expression in addition to the eye disc, including the embryonic central nervous system (*toy*, *ey, dac*), the embryonic gonad (*eya*), the optic lobes (*toy, so, dac*), the embryonic salivary duct (*eyg*) and the intermediate leg segments (*dac*).[2,3,5,6,8,21–23] Clearly, their expression is not sufficient to trigger eye development in these sites. *toy, ey, eya, so*, and *dac* only induce ectopic eye development in other imaginal discs, suggesting that additional factors present in imaginal tissues are required to establish a permissive context for eye induction. The antennal disc is the most sensitive of the imaginal discs to misexpression of *eya, so*, or *dac*, possibly because expression of *toy* and *ey* during embryonic stages leads to the activation of additional contributing genes. Development of an ectopic eye in the antennal disc is also triggered by misexpression of *teashirt*, a regulator of homeotic gene expression that is not essential for normal eye development[24]; this might occur through the inappropriate maintenance of *ey* expression. Furthermore, even when *ey* is misexpressed ubiquitously, ectopic eye development is restricted to specific positions in the imaginal discs.[10] These positions correspond to sites where the BMP family member *decapentaplegic* (*dpp*) is present and the *Wnt* family member *wingless* (*wg*) is absent; co-misexpression of *dpp* suggests that it may be an important factor contributing to eye development.[19] Initiation of the morphogenetic furrow in the eye disc itself likewise requires the presence of *dpp* and the absence of *wg*.[25–28]

Finally, the targets that these genes regulate remain to be defined. In addition to regulating each other's expression early in eye development, they must also control downstream genes involved in eye differentiation. So far the clearest example of this is the activation of *rhodopsin 1* gene expression through Ey binding sites present in its promoter.[18] Direct activation of a late target gene by *ey* argues against the idea that the genes act in a strict hierarchy in which each gene regulates only the level immediately below. At an intermediate stage of eye development, secreted proteins encoded by the *hedgehog* (*hh*), *dpp*, and *wg* genes organize the pattern of differentiation in the eye disc. *hh* expression at the posterior margin of the early eye disc is required for morphogenetic furrow initiation,[29,30] and its later expression in the differentiating photoreceptors drives furrow progression.[31–33] *dpp* is expressed at the posterior and lateral margins of the eye disc and is required for furrow initiation,[25,28] while *wg* expression at the lateral margins inhibits ectopic furrow initiation in this region.[26,27] One role of the eye specification genes could be to control the expression patterns of *dpp*, *wg*, and *hh*. Indeed, a critical function of *eyg* is to prevent *wg* expression at the posterior margin, thus allowing morphogenetic furrow initiation.[4,34] As *eya* and *so* act as positive regulators of *dpp* expression,[12,34] but *dpp* is also a positive regulator of *eya* and *so*,[19] it is not clear which is normally activated first. Hh, Dpp, and Wg also function to pattern the other imaginal discs, where they elicit very different responses; presumably the

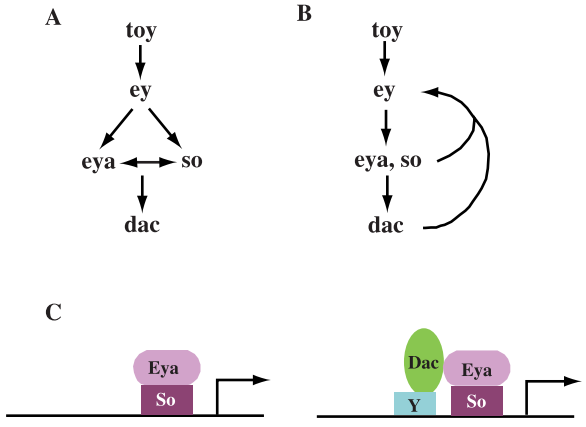

Figure 1. A: Hierarchy of eye specification gene action during normal development. *toy* is the first gene to be expressed in the eye disc primordium; it probably activates *ey*, which is required for the expression of later genes. *dac* expression is dependent on *eya* and *so*. *eyg* neither requires nor is required for *ey* expression, and is not included in this diagram. **B:** Feedback regulation revealed by ectopic expression. Misexpression of downstream genes can induce ectopic expression of *ey*. **C:** Interactions at the protein level. Eya and So form a complex in which So contributes a DNA-binding domain and Eya a transcriptional activation domain. Dac also interacts with Eya on the promoters of genes required for morphogenetic furrow initiation. Dac may be brought to the DNA by another factor, Y, and may enhance Eya-dependent transcriptional activation.

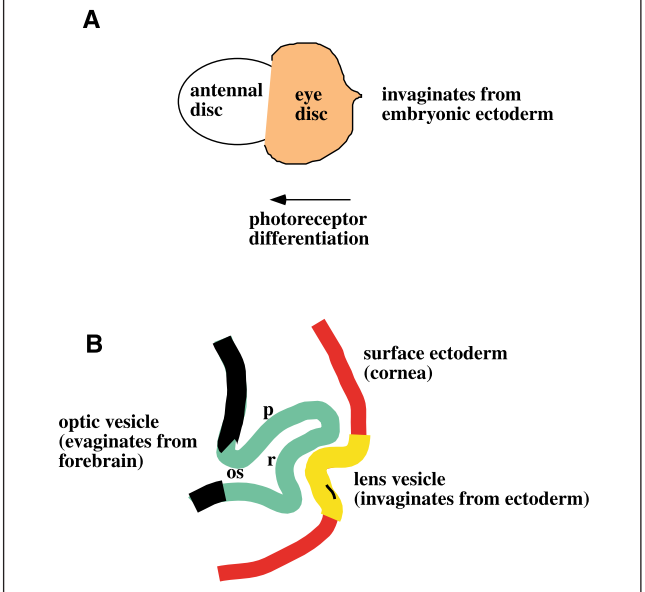

Figure 2. Comparison of the derivation of the *Drosophila* eye (**A**) and the parts of the vertebrate eye (**B**). The *Drosophila* eye-antennal disc (**A**) invaginates from the embryonic ectoderm during embryonic head morphogenesis. In the third larval instar a wave of photoreceptor differentiation proceeds from posterior to anterior across the eye field. In vertebrates (**B**) the optic vesicle (green) evaginates from the forebrain and then invaginates to form the optic stalk (os), retina (r) and RPE (p). The overlying surface ectoderm invaginates to form the lens (yellow) and the remaining ectoderm (red) then forms the cornea.

eye specification genes are also required downstream of Hh, Dpp, and Wg to make photoreceptor differentiation the outcome of the interaction between these signals.

Conservation of eye specification mechanisms

The discovery that the *ey* gene encoded a Pax-6 protein[2] first raised the possibility that the mechanism of eye specification might be conserved. The *Small eye* (*Sey*) phenotype in mouse and aniridia in humans both result from mutations in Pax-6,[35,36] and mouse *Pax-6* is able to induce ectopic eye development in *Drosophila*.[15] *Pax-6* expression has now been found in the eyes of many other species, including those with very primitive eyes.[37–41] Thus, despite the striking structural and developmental differences between the insect compound eye and the vertebrate single-lens eye, it has been suggested that they both evolved from a common precursor structure in which Pax-6 was used to regulate the light-sensitive properties of a primitive photoreceptor cell.[42,43] As the eye became more complex, additional genes required to build it could have come under the transcriptional control of Pax-6; other direct targets may have been lost, as *Pax-6* is not expressed in fully differentiated photoreceptors in vertebrates[44] (although Pax-6 binding sites are present in the promoters of vertebrate opsin genes[45]). However, *Pax-6* expression is never restricted to the eye, and is also present in species completely lacking eyes, such as sea urchins and the nematode *C. elegans*.[46,47] It is not clear whether its original function in head development was expanded to include a role in the eye, or whether some lineages have lost both the eye and the corresponding domain of *Pax-6*.[48]

Further research has led to the identification of vertebrate homologs of other genes that play a role in specifying fly eye development. Three *eya* homologs, one *so* homolog, and a *dac* homolog are expressed during mouse eye development, while two *so* genes are expressed in the zebrafish eye,[49–53] probably reflecting an extra polyploidization event in the lineage leading to zebrafish[54] (Table 1). An additional homeobox gene, *Rx*, is required for vertebrate eye development,[55] although its *Drosophila* homolog is not expressed in the embryonic or larval eye disc.[56]

A comparison between the roles of these genes in flies and vertebrates is complicated because the fly eye develops from a single imaginal disc epithelium but the vertebrate eye forms from the conjunction of two tissue types (Fig. 2). An evagina-

tion from the forebrain, the optic vesicle, grows out to approach the surface ectoderm, which forms a thickened lens placode at this point. The optic vesicle then invaginates to form the optic cup, while the lens placode invaginates and pinches off to form the lens; the remaining surface ectoderm differentiates into the cornea. The inner layer of the optic cup forms the neural retina, while the outer layer forms the retinal pigment epithelium (RPE). The *Rx* gene appears to act only in the neurally derived components of the eye; it is first expressed at the neural plate stage and expression continues in differentiating retina.[55,57,58] *Rx* loss- and gain-of-function phenotypes are also consistent with a role in the neural portion of the eye; it is required in the mouse for optic vesicle formation, and its ectopic expression in the frog induces duplications of the retina and RPE.[55]

Pax-6 is expressed in the neural eye field from the neural plate stage and also in a broad ectodermal domain that later becomes refined to the lens placode; its expression persists in the retina, lens, and cornea.[44,59] *Pax-6* function is autonomously required in the surface ectoderm for lens placode formation and subsequent lens development.[59–61] In homozygous *Sey* -/- mice, the neural component of the eye is affected at a later stage than in *Rx* mutant mice; the optic vesicle still forms and is capable of inducing lens development in wild-type ectoderm, but it fails to invaginate.[59,60] Misexpression of *Pax-6* in *Xenopus* has been shown to induce ectopic lens development[62] and under certain conditions it can promote the formation of complete ectopic eyes (R. Lang and A. Hemmati-Brivanlou, pers. comm.) The mouse *so* homolog *Six3* and two related zebrafish genes are expressed both in the neural eye field at the neural plate stage and in the lens placode[50,51]; loss-of-function mutants of these genes have not yet been described, but *Six3* misexpression in medaka fish induces lens development in the otic vesicle[63] or retinal development in the midbrain.[64] The otic placode may share factors with the lens placode that make it a permissive site for lens induction, just as other imaginal discs are permissive sites for eye induction in flies.

The functions of the vertebrate homologs of *eya* and *dac* have not yet been evaluated. The three mouse *eya* homologs have partially overlapping expression patterns. *Eya1* is expressed initially in both the optic vesicle and the lens placode, but is later restricted to peripheral retina and corneal ectoderm. *Eya2* is present in central retina, and *Eya3* is expressed first in the optic vesicle and later in the retina and lens vesicle.[52] Additional sites of expression are observed outside the eye, and heterozygous mutations in human *Eya1* cause branchio-oto-renal syndrome, affecting ear and kidney development.[65] All three *Eya* genes retain the ability to rescue a fly *eya* mutant and are therefore likely to have related functions[11] (N. Bonini, pers. comm.). *Dach*, a mouse homolog of *dac*, is expressed in the retina, as well as in the neural crest-derived mesenchyme surrounding the eye.[49] This relatively late and restricted expression is reminiscent of the requirement for fly *dac* in morphogenetic furrow initiation but not at earlier stages of eye development.

The conservation of expression in the vertebrate eye of homologs of four genes required for early eye development in *Drosophila* is striking. As only *Pax-6* has been examined in intermediate species, it is unclear whether this full set of transcription factors is always associated with photoreceptive organs. Homologs of *so*, *eya*, and *dac* (though not *Rx*) appear to be present in the *C. elegans* genome; if they are found to be co-expressed with *Pax-6* in this eyeless species, then their role in the eye might be an adaptation of a more general sensory or anterior function. *Pax-6* is also expressed in the olfactory organs of several species,[39,40,59] including the embryonic *Drosophila* antennal disc,[2,3,66] indicating that it may be an oversimplification to consider it an eye specification gene. The observations that *Drosophila* eye development does not require the retina-specific gene *Rx*, but does require *Pax-6* which, in vertebrates, is more critical for lens development, suggests that the fly eye may have more in common with the vertebrate lens than with the eye as a whole. This may be related to the developmental origin of the eye imaginal disc as an evagination of the embryonic ectoderm, like the lens vesicle, rather than an outgrowth from the central nervous system, like the optic vesicle.[66] Perhaps the original function of the ''eye specification'' network was to determine an ectodermal sensory placode. In this regard it would be useful to examine the roles of these genes in cephalopod species, in which both the lens and the retina are ectodermally derived. *Pax-6* expression in the squid is restricted to the early eye primordium and to some of the lens- and cornea-forming cells,[40] but homologs of *so* and *Rx* have not yet been described.

Regulation and function of vertebrate eye specification genes

Again, many questions remain concerning the functions of and interactions between the genes involved in vertebrate eye formation. There is little information on whether these genes regulate each other's transcription in the eye as their homologs do in flies. *Eya1* and *Six3* expression in the lens-forming ectoderm require *Pax-6*, while expression of *Six3* in the optic vesicle and forebrain, like *so* expression in the optic lobe primordium, does not[50,52] (G. Oliver, pers. comm.). Ectopic expression of *Six3* induces the expression of both *Rx* and *Pax-6* in the brain of medaka fish,[64] but it is not known whether *Six3* is required for the normal retinal expression of these genes. Few of the factors upstream or downstream of this set of genes are known. In zebrafish, the hh family members Sonic hedgehog (Shh) and Tiggy-winkle hedgehog (Twhh) are present in the ventral forebrain, where they appear to restrict *Pax-6* expression to the optic cup, and to promote *Pax-2* expression in the optic stalk.[67,68] Lack of

this inhibitory signal in the *cyclops* mutant leads to fusion of the two eye fields into a single *Pax-6* expressing region extending across the midline. In the quail retina, it has been suggested that c-Myb activates *Pax-6*.[69] Induction of *Pax-6* expression in the ectodermal lens placode requires a signal arising from the future retinal region of the neural plate, but its molecular nature is unknown[70,71]; the recent discovery of an element in the *Pax-6* promoter specific for lens and corneal ectoderm may contribute to its identification.[72] Interestingly, an enhancer from the *Drosophila ey* gene sufficient to drive expression in the eye disc also directs reporter gene expression in the retina and peripheral optic cup in transgenic mice,[73] suggesting that some upstream regulators of *Pax-6* may be conserved.

Finally, Pax-6 binding sites have been found in the promoters of several lens crystallin genes,[74] suggesting that, as in flies, Pax-6 may activate genes with a late function in the eye. Future work will probably identify earlier targets, predicted to affect such processes as lens vesicle invagination or differentiation of cell types in the retina and RPE. Some possible candidates that are expressed in the lens and promote its differentiation are the genes encoding the HMG box proteins Sox1–3[75,76] and the bZIP protein L-Maf.[77] *Xath-5,* the *Xenopus* homolog of *atonal*, the proneural gene for photoreceptor formation in *Drosophila*, is expressed in the differentiating retina and could be a target of Rx or Pax-6.[78]

Conclusions

The early stages of eye development appear to involve many of the same gene products, even in species in which the final structure of the eye is quite different. It will be interesting to see whether these genes form a regulatory network in vertebrates and in intermediate species as well as in flies, or whether the different developmental strategies used to produce an eye have altered the relationships between members of the group while preserving their involvement. For the moment, the identification of these transcription factors has shed some light on the mechanism by which cells are set aside to form an organ that will later contain multiple cell types. Further study of their regulation and functions will lead to both evolutionary and developmental insights into eye specification.

Acknowledgments

I thank Kevin Moses and Claude Desplan for their roles in the inception of this review, and Claude Desplan, Richard Lang, Guillermo Oliver, Jeff Lee, and Gord Fishell for insightful comments on the manuscript.

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