Pax6 lights-up the way for eye development

Ruth Ashery-Padan* and Peter Gruss†

Recent reports have exposed the temporal and spatial functions of the transcription factor Pax6 in the developing vertebrate eye. Pax6 is demonstrated to play essential roles in successive steps triggering lens differentiation while in the retina it functions to maintain multipotency and proliferation of retinal progenitor cells. These findings, together with the identification of Pax6 protein partners and downstream targets, pave the way for future work aimed to understand the molecular mechanism of eye development.

Addresses
*Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University Ramat Aviv, Tel Aviv 69978, Israel; e-mail: ruthash@post.tau.ac.il
†Max Planck Institute of Biophysical Chemistry, Department of Molecular Cell Biology, Am Fassberg 11, D-37077 Göttingen, Germany; e-mail: pgruss@gwdg.de
Correspondence: Ruth Ashery-Padan

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Abbreviations

bHLH	basic helix–loop–helix
FGF	fibroblast growth factor
NR	neuroretina
OV	optic vesicle
RPC	retinal progenitor cell
RPE	retinal pigmented epithelium
SE	surface ectoderm
Shh	Sonic hedgehog

Introduction

Eye development in vertebrates has been an excellent model system to investigate fundamental processes in developmental biology from tissue induction to the formation of highly specialized structures such as the lens and the retina. This complex optic system develops primarily from three embryonic parts: the optic vesicle (OV), which is a lateral evagination from the wall of the diencephalon, the surrounding mesenchyme and the overlying surface ectoderm (SE). Successive signals between these tissue components are thought to coordinate their development (Figure 1a). The OV contacts the SE and triggers a response that leads to a thickening of the ectoderm, the lens placode, which later develops into the mature lens. While the lens placode internalizes to form the lens vesicle, the distal OV invaginates to form the optic cup with the inner layer developing into the neuroretina (NR) and the outer layer forming the retinal pigmented epithelium (RPE). The proximal regions of the OV form the optic stalk that connects the retina to the brain.

Recently, the expression and function of numerous genes have been correlated with defined cell types and stages of eye development. Comparison of gene expression, function and regulation in development of the fly and vertebrate eyes has revealed a surprising conservation of molecular mechanisms. In particular, the study of the transcription factor Pax6 promoted our understanding of the development of ocular tissues. Pax6 is a member of the Pax family of transcription factors. It contains two DNA-binding motifs the paired domain and paired-type homeodomain [1]. In vertebrates this factor is essential for normal development of several organs including the brain, pancreas and the eye [2]. Pax6 has been reported to be a key regulator of eye development as it is both essential for eye formation in different organisms as well as sufficient to induce ectopic eyes in flies and frogs upon misexpression [3,4•]. Interestingly, the correct dosage of Pax6 is essential for normal eye development: overexpression of Pax6 in mice results in a severe eye phenotype [5], whereas reduction of Pax6 activity in heterozygotes for Pax6 mutation results in ocular phenotypes such as *Aniridia* in humans [6] and *Small eye* in mice and rats [7,8]. The conserved expression pattern of Pax6 in the developing and adult vertebrate eye and recent functional studies of Pax6 by conditional mutagenesis document the involvement of this factor in a whole spectrum of events essential for normal eye development.

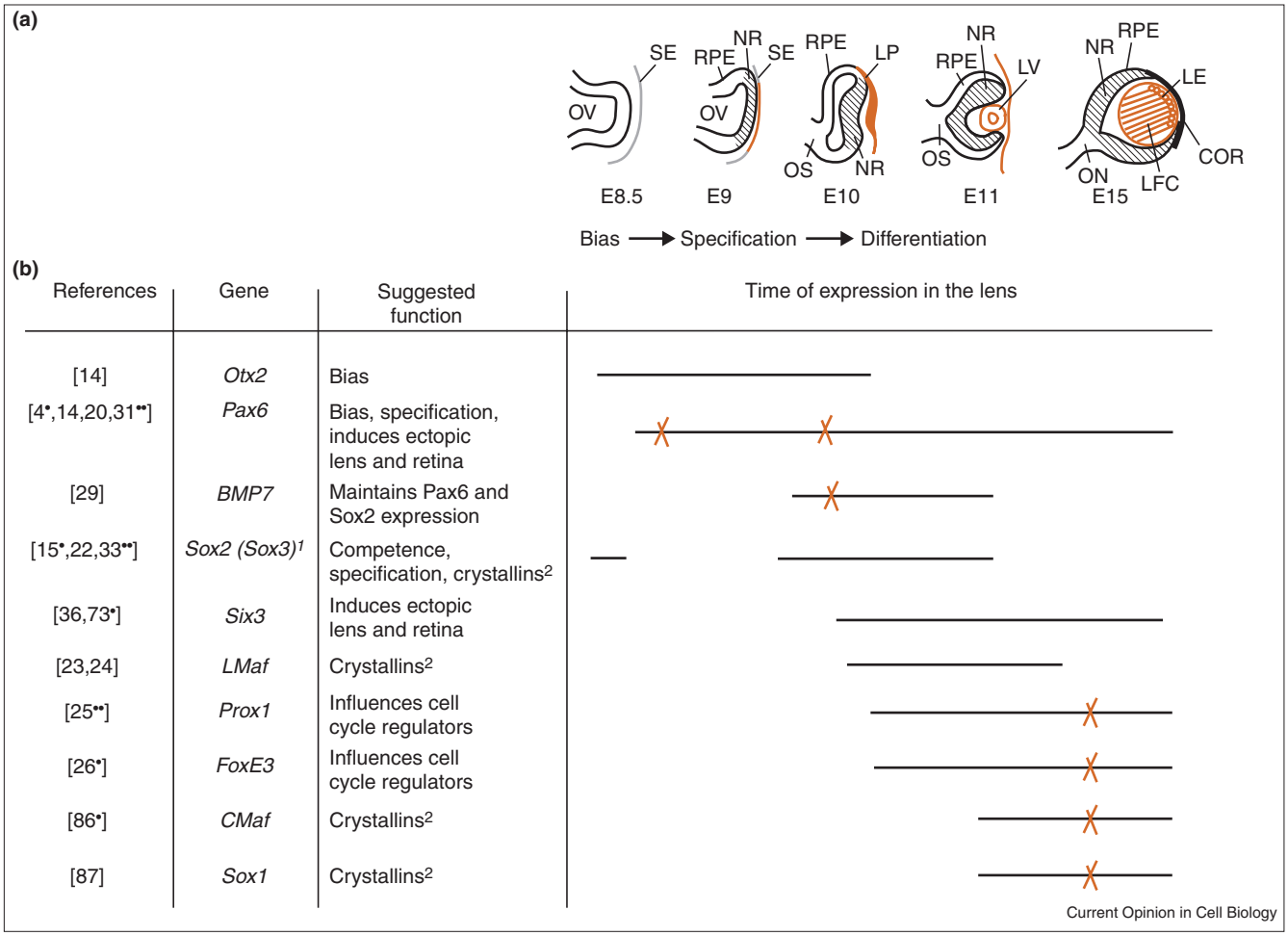
In this review we highlight the recent results on the molecular mechanisms underlying the development of the eye as unraveled by the study of this gene. Readers are directed to recent comprehensive reviews for further discussion on evolution of eyes [9,10] and on cell proliferation and differentiation in the retina [11].

Lens induction from experimental embryology to molecular mechanisms

The early, pioneering work of Spemann [12] described lens induction as a single step process in which the OV influences the development of the SE. Today, lens induction is conceived as a multi-step process (Figure 1a) [13,14]. The competence of the SE to respond to lens inductive signals is acquired during gastrulation. Subsequently, at the neural plate stage, planar signals from the adjacent neural folds further bias the ectoderm enhancing its lens-forming capacity. The expression pattern and function of several genes correspond to these early events (Figure 1b). Among them, the Sox3 transcription regulator is implicated to confer lens competence [14,15•], in fish and frogs, while Otx2 and Pax6 are associated with the lens bias stage [14].

Only after the newly formed OV contacts the overlying ectoderm is the small region juxtaposed to the OV specified to a lens fate. In most vertebrates, lens specification is dependent on the OV as ablation of the OV or arrest in OV development (e.g. *Lhx2* and *Rx* mutants; [16,17]) prevents the formation of lens structures. Recently, the secreted

Figure 1



Development of the vertebrate eye. **(a)** Schematic illustration of eye development in the mouse. At embryonic day 8.5 (E8.5) the evagination that will give rise to the optic vesicle (OV, black) is extending laterally from the brain. In response to inductive signals from the OV the overlying surface ectoderm (SE, orange) thickens, forming the lens placode (LP), which then internalizes (E10) and detaches from the ectoderm (lens vesicle, LV) (E11). The posterior cells of the lens vesicle differentiate to lens fiber cells (LFC) while the anterior cells become the lens epithelial cells, a layer that maintains mitotic potential (E15). The corresponding embryonic stages according to [13] are indicated. **(b)** The sequential expression of factors during early stages of lens development. Several genes that play a role in the corresponding stages of lens development and the timing of their expression in the lens are presented. The developmental stage at which gene function is essential based on mutant analysis is marked by X. COR, cornea; LE, lens epithelium; NR, neuroretina; ON, optic nerve; OS, optic stalk; RPE, retinal pigmented epithelium. [1]Sox2 in mouse, Sox2 and Sox3 in chicken and Sox3 in frog and fish. [2]Suggested to regulate the expression of crystallins.

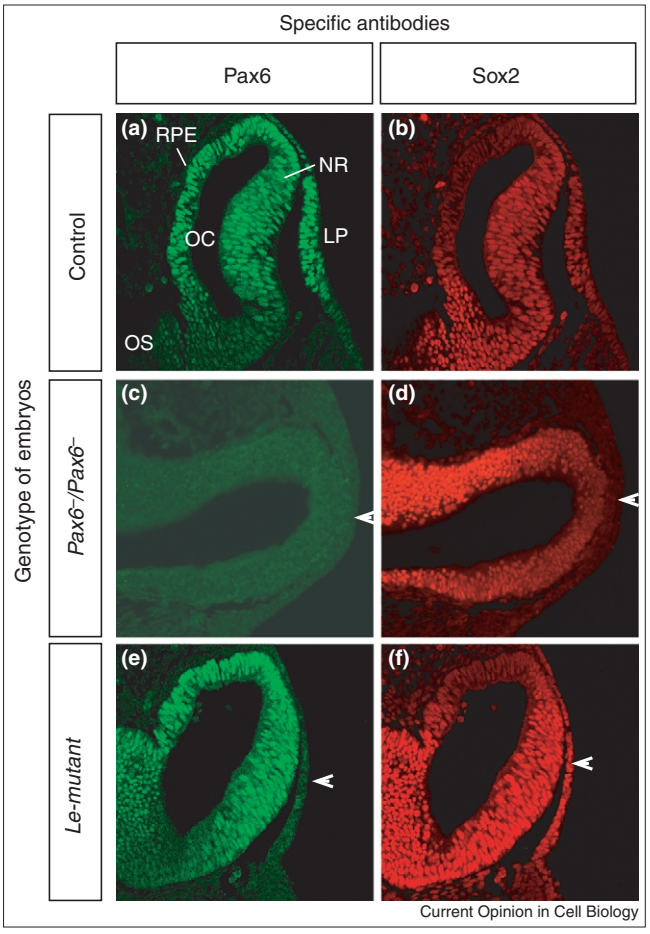
factor BMP4 has been associated with the inductive activity of the OV in mice [18]. In chick, however, probably other BMPs mediate this function as neither BMP4 nor BMP7 are expressed in the OV during lens induction [19].

The contact with the OV is followed by abrupt changes in gene expression profile in the SE, which reflects lens specification (Figure 1b). Specifically, the expression of some genes is downregulated (e.g. *Otx2*) whereas the expression of others (e.g. *Pax6*) is maintained [14,20]. Finally, upregulation of transcription factor expression in the SE (e.g. Six3, Sox2/Sox3, Lamf, Prox1 and FoxE3) is evident during lens placode formation [21–24,25••,26•]. Some of these proteins also play a role during later stages of lens differentiation in controlling the expression of crystallins and cell cycle regulators (Figure 1b; reviewed in [27]). However, the regulatory mechanisms that mediate the initiation of lens differentiation have been only recently addressed by molecular and functional studies.

Pax6 in early lens development

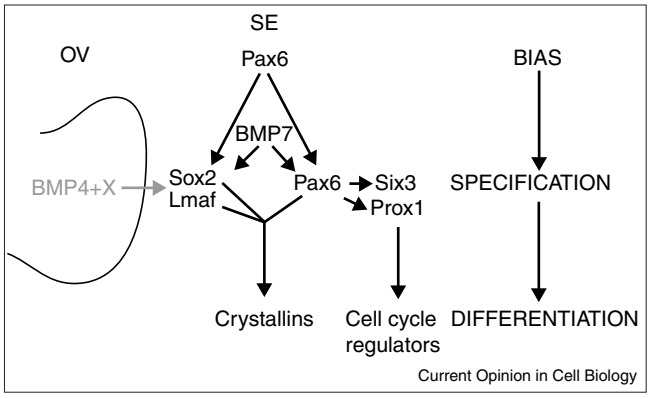
Several findings document an essential role of Pax6 during early stages of lens induction: first, *Pax6*$^{-}$/*Pax6*$^{-}$ cells are excluded from the SE of chimeric embryos [28], second, the expression of the lens-specification marker Sox2 fails in *Pax6*$^{-}$/*Pax6*$^{-}$ embryos (Figure 2d) [18,29], and third, tissue recombination between OV and SE from *Pax6*$^{-}$/*Pax6*$^{-}$ and wild-type rat embryos suggested that Pax6 is not essential

Figure 2



The lens phenotype of *Pax6*$^{-}$/*Pax6*$^{-}$ and *Le-mutant* points to an essential function for Pax6 in two successive stages prior to the onset of lens differentiation. At embryonic day 10 transverse sections of **(a,b)** wild-type control, **(c,d)** *Pax6*$^{-}$/*Pax6*$^{-}$ and **(e,f)** *Le-mutant* embryos were immunolabeled with specific antibodies to (a,c,e) Pax6 or (b,d,f) Sox2. (a,b) In wild-type embryos both Pax6 and Sox2 are detected in the lens placode. (d) In *Pax6*$^{-}$/*Pax6*$^{-}$ Sox2 is not detected in the SE (white arrow heads), whereas (f) in the *Le-mutant* expression of Sox2 in the SE is evident. LP, Lens placode; OC, optic cup; OS, optic stalk.

Figure 3



A model of the regulatory interactions during early stages of lens development in vertebrates. Early expression of Pax6 during neural plate stages (bias) is required for the upregulation of the high mobility group transcription factor Sox2 and for maintaining of Pax6 expression in the SE in the next step of lens specification. BMP4 in mice and yet unknown factors (gray) [18] secreted from the OV elicit the upregulation of Sox2 and the expression of the basic leucine zipper transcription factor Lmaf [24]. During this stage Pax6 is essential for the expression of Six3 and Prox1 but not for maintenance of Sox2 expression [31••]. The maintenance of Sox2 and Pax6 expression is dependent, however, on BMP7 [29]. The combined function of Pax6, Sox2 and Lmaf seems to trigger the expression of structural proteins, while expression of Prox1 primarily influences the expression of cell cycle regulators [25••,33••].

for the inductive activity of the OV, but rather has a cell autonomous function in the SE [30]. These results, however, could not define the step in which Pax6 is required during the successive events preceding lens differentiation.

To address the molecular function of Pax6 in the SE Ashery-Padan *et al.* [31••] employed the Cre/*loxP* approach to somatically delete Pax6 exclusively from the SE of the *Pax6*^{flox}*Pax6*$^{-}$*;Le-Cre* (*Le-mutant*) embryos. In the *Le-mutant*s Pax6 protein was eliminated from the ectoderm after the lens bias stage during lens specification (Figure 2). This somatic mutation resulted in absence of all lens structures. Comparison of the lens phenotype of *Pax6*$^{-}$/*Pax6*$^{-}$ mice with the *Le-mutant* revealed that Pax6 function is essential in each of the two successive stages of lens induction (Figures 2 and 3). Initially, Pax6 is essential for the activation of Sox2 in the ectoderm, thus implying a role for Pax6 in maintaining lens-bias of the SE. Then Pax6 activity is essential for the initiation of lens differentiation. During this stage, Pax6 controls the expression of other regulatory genes such as the homeobox genes *Six3* and *Prox1* but is not required for maintaining *Sox2* expression (Figure 3) [31••]. Sox2 alone, however, cannot support lens formation in the absence of Pax6. This is in agreement with a recent finding that Pax6 binds cooperatively with Sox2 to the δcrystallin enhancer forming a ternary complex that mediates δcrystallin expression in the lens placode in chick embryos [32•,33••]. It has also been suggested that the basic leucin zipper Maf transcription factor synergizes with Pax6 and Sox2 in activating crystallin expression [27].

Other candidates that function with Pax6 in conferring lens specification are homologs of the *Drosophila* eye specification genes: *Six3*, *cSix4* and *Eya1* [21,34,35]. From these, Six3 has been demonstrated to induce ectopic lenses in fish [36]. Interestingly, Pax6 and Six3 seem to positively regulate each other. Pax6 is required for Six3 expression [31••] while Six3 can induce Pax6 expression reminiscent of the regulatory interaction between the fly homologs *eyeless* and *sine oculis* (G Goudrou, personal communication). Furthermore, members of the Six family have been suggested to activate transcription by cooperative interaction with Eya proteins [37•,38••]. In contrast, the Six proteins, in particular Six3, have been recently shown

to interact with the co-repressor Groucho to repress transcription of target genes in fish [39•] and to repress the murine γFcrystallin promoter in cell lines [40]. Further functional studies are required to determine the *in vivo* function of Six3 in triggering lens differentiation.

Pax6 in early retina development

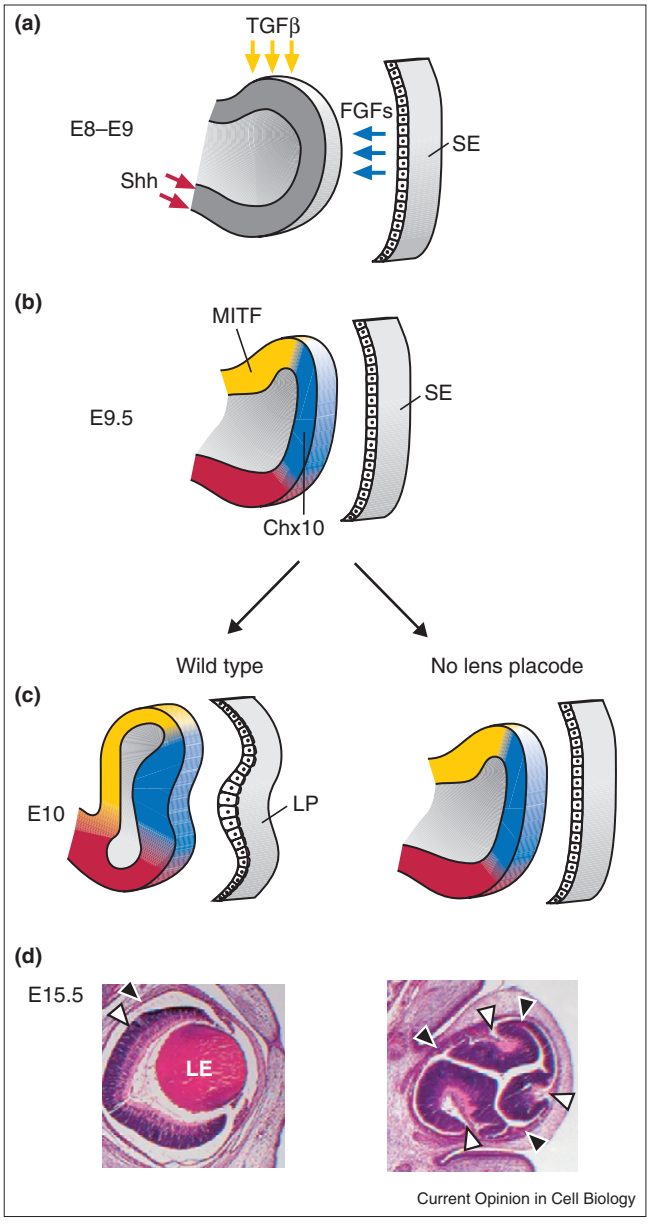
The growing OV contains bipotential progenitors that could give rise to both RPE and NR cell types. Separation of these progenitors to NR and RPE domains is mediated by external cues. Fibroblast growth factors (FGFs) secreted from the SE promote NR cell fate, whereas the ocular mesenchyme directs RPE formation (Figure 4a) [41,42,43•,44•]. Finally, Sonic hedgehog (Shh) secreted from the ventral forebrain seems to influence the patterning of the OV [45•]. These early positional cues impose regionalization of the OV and early optic cup as manifested by the distribution of factors, which are instrumental during later stages of retinogenesis (Figure 4b) [43•–45•].

Pax6 is expressed in the anterior neural plate in the cells that will give rise to the OV. Surprisingly Pax6 function seems to be dispensable for the formation of OV and the establishment of NR and RPE domains, as indicated by the expression of early retinal markers in the *Pax6*$^{-/-}$ optic rudiment ([20,46]; T Marquardt, personal communication). Possibly other transcription regulators compensate for the loss of Pax6 by initiating retinal specification.

Following the establishment of RPE and NR domains, the OV invaginates to form the optic cup (Figure 4c). This step is completely dependent on the development of a lens placode as demonstrated by analysis of the *Le-mutant* embryos where the loss of Pax6 activity in the SE resulted in genetic ablation of the lens placode (Figure 4c,d). In *Le-mutants* the optic cup did not form. Instead, several neuroretina folds separated by patches of RPE evolved from the OV (Figure 4d). Hence, the early lens structures provide the molecular and mechanical cues required for the invagination of the optic vesicle to an optic cup. This step is probably essential for the lens to be perfectly positioned with respect to the retina. Remarkably in each fold neurons differentiated in a central to peripheral pattern similar to the pattern of neuronal differentiation in the normal retina, and at postnatal stages all neuronal subtypes were detected in the *Le-mutant* eyes [31••]. Thus, the subsequent steps of retinal development and differentiation seem to be independent of the lens. Indeed ablation of the lens during later stages of development in chick and mice revealed that after the optic cup and lens vesicle are formed the lens is no longer required for either retinal survival or differentiation [47–50]. In some fish species, however, the lens might play a more essential role for retinal survival [51•].

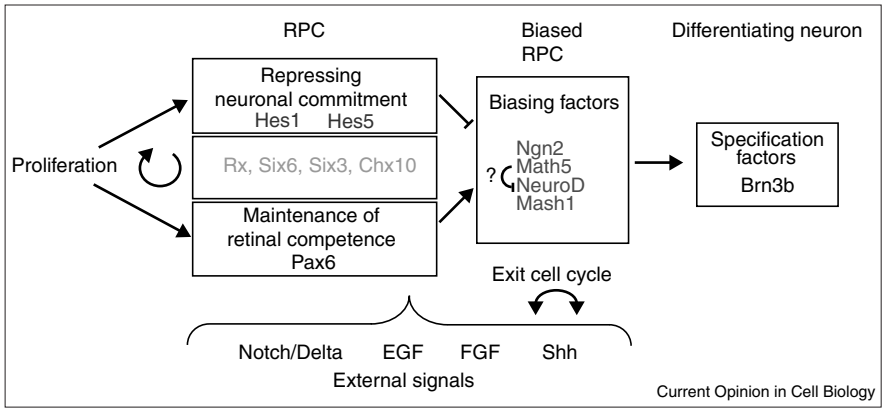
Although Pax6 is not required for optic vesicle formation, it does play a role in subsequent steps of retinogenesis. At the optic cup stage, Pax6 seems to be required for cell

Figure 4



The influence of the lens ectoderm on the development of the optic vesicle and optic cup. **(a)** The initial patterning of the optic vesicle to distal NR and proximal RPE domains is mediated by the head surface ectoderm (SE) and surrounding mesenchyme. FGFs secreted from the SE (blue arrows) promote NR differentiation while a transforming growth factor β (TGFβ) family member secreted from the mesenchyme (yellow arrows) is a candidate for promoting RPE cell fate. Finally sonic hedgehog (Shh) emanating from the ventral forebrain (red arrows) promotes formation of the optic stalk from the ventral portion of the OV. **(b)** The external cues instruct the early regionalization of the optic vesicle and optic cup. Several transcription factors are expressed in restricted manner in response to these external signals. For example, Chx10 is upregulated in the NR and Mitf expression is restricted to prospective RPE [43•–45•]. **(c)** The earliest lens structure, the lens placode (LP), is essential for instructing the formation of an optic cup with a single retina fold facing the lens. In the absence of early lens structures the optic vesicle does not invaginates to form the optic cup **(d)** but after a delay, several folds of retina are formed and these develop to multiple retina folds (white arrows) separated by patches of RPE (black arrows). E, embryonic day of mouse development; LE, lens epithelium.

Figure 5



The possible regulatory pathways leading to neuronal cell differentiation from multipotential retinal progenitor cells (RPCs). Several transcription factors expressed early in retinal development seem to maintain and modulate RPC multipotency and self-renewal. Some of these factors, such as Pax6, maintain retinal multipotency as they are essential for the expression of bHLH transcription factors that bias neuronal cell fate (Math5, Ngn2, Mash1 [80••]), whereas others such as Hes1 repress neuronal commitment by repressing proneural gene expression. The other early retinal determinates (gray) are documented to influence PRC proliferation but their influence on proneural gene expression is not yet known. It is conceivable that the distribution of these early retinal determinates in the RPCs will define the cell sensitivity and response (competence) to the changing external cues. The external signals influence both the onsets of cell differentiation and cell-fate specification. For example; Shh regulates ganglion cell fate while EGF and Notch/Delta seem to influence cell proliferation and to promote Muller glia cell fate [58••,59,60,79••]. It is the expression of proneural genes in the progenitor that bias the cell towards specific cell fate. Proneural genes seem to restrict cell fate both by activating factors that are essential for the differentiation of specific cell type (specification factors), and possibly by restricting expression of other proneural genes. For example, Math5 promotes ganglion cell differentiation by activating Brn3b [85] and Math5 seems to be instrumental in restricting amacrine cell production [70••].

proliferation and differentiation as both are affected in *Pax6*$^{-}$/*Pax6*$^{-}$ retinal rudiment (R Ashery-Padan, unpublished data). The relatively normal retinogenesis in the absence of a lens in the *Le-mutant* points to an autonomous function of Pax6 in the retina, which is further supported by the expression of Pax6 during the ensuing stages of retinogenesis. Following optic cup formation, Pax6 is downregulated in the optic stalk and the RPE, but retained in the neuroretina. Expression in the NR is maintained in the proliferating retinal progenitor cells (RPCs), while it is downregulated in most cells upon differentiation. In the mature retina, Pax6 expression persists in amacrine and ganglion cells. This dynamic expression pattern is conserved among vertebrates thus reflecting a conserved function for Pax6 during retinogenesis and in subtypes of mature neurons [1,52].

Pax6 in retinal progenitors cells

The vertebrate retina is composed of six types of neurons and one type of glia, which are interconnected in a complex, highly ordered cytoarchitecture [53]. During retinogenesis the different retinal cell types are generated in a defined birth order from a population of multipotent retinal progenitor cells (RPCs) residing in the inner layer of the optic cup. Retinal ganglion cells, cone photoreceptors and horizontal cells are born first, followed by amacrine and rod photoreceptor cells, while bipolar and Muller cells appear last [54]. This histogenic order is largely conserved among vertebrates suggesting a conservation of the regulatory mechanisms mediating the onset of differentiation of each cell type [55]. A variety of extrinsic factors have been demonstrated to influence retinogenesis, among them the secreted factors FGFs, Shh, EGFs and contact-mediated regulators of the Notch/Delta signaling pathway [56,57••,58••,59–62]. The cell-extrinsic factors seem to influence intrinsic regulators of retinal cell differentiation. The basic helix–loop–helix (bHLH) transcription factors are important regulators of neurogenesis in invertebrates and vertebrates [63]. In the vertebrate retina the bHLH factors Hes1 and Hes2 (related to *hairy* and *enhancer of split* in *Drosophila*) appear to function downstream of the Notch/Delta signaling pathway as negative regulators of neuronal cell differentiation [64,65,66••]. These factors seem to repress the expression of other bHLH factors, which have been demonstrated to play an essential role in directing progenitor cell fate (e.g. the proneural genes *Math5*, *Mash1*, *Ngn2* [67,68]). Mutational analyses have implicated Math5 in promoting ganglion cell fate while restricting differentiation into amacrine cell fate [69,70••]. Mash1 regulates bipolar cell differentiation and NeuroD promotes amacrine and rod but restricts bipolar cell fates [71,72].

Additional transcription factors that are expressed before and during retinal differentiation are the homeodomain proteins Rx, Lhx2, Pax6, Six3, Six6/Optx2 and Chx10. Several lines of evidence document the involvement of these factors as early retinal determinants and later in cell fate specification of RPCs. First, ectopic expression of *Six3, Six6/Optx2, Pax6* and *Rx* induces retinal tissue [4•,17,73•–75•]. Second, *Pax6*, *Rx/Rax* and *Lhx2* are essential for optic cup formation in mice [16,17,20], and Chx10 is required for RPC proliferation [76]. Third, *Six3, Pax6* and *Rx* are expressed in retinal stem cells in *Xenopus* [77], and Chx10 is expressed and influences the proliferation of mammalian retinal stem cells [78••]. Fourth, *Rx* has been shown to regulate the expression of Notch and Hes1 in the retina [79••].

Marquardt *et al*. investigated the role of one of these early retinal determinants *Pax6* in cell fate specification of RPCs by somatic deletion of this gene from the distal optic cup before onset of cell differentiation [80••]. *Pax6*-deficient RPCs exhibited reduced proliferation, did not acquire early or late neuronal cell fates but differentiated exclusively into amacrine interneurons. Interestingly, *Pax6*-deficient amacrine cells did not give rise to the glycinergic amacrine cell subtype. Taken together, these results suggest that Pax6 is essential for the multipotency of RPCs and for their normal proliferation. Furthermore, Pax6 seems to have a later function in the specification of a subtype of mature amacrine cells. This work further revealed that Pax6 activity in RPCs is directly required for the expression of some of the proneural genes including *Ngn2*, *Mash1* and *Math5*, but not for the expression of *NeuroD*. Thus, the combined loss of several proneural genes appears to account for the inability of *Pax6*-deficient RPCs to acquire all neuronal cell fates.

These observations lead to several suggestions concerning the role of Pax6 in determination of neuronal cell fate in the retina (Figure 5): Pax6, Hes1, Hes5 and possibly the other early retinal determinants maintain the multipotency and proliferation of RPCs. Some act as repressors (Hes1, Hes5) and others as activators (Pax6) of proneural genes. Recent studies revealed heterogeneity between RPCs in respect of gene expression and competence to differentiate to different cell types [81]. The intrinsic determinants seem to change over time and to mediate the competence of the RPC to acquire specific cell fate [82••,83•]. It is therefore conceivable that the distribution and expression levels of the factors that mediate the multipotency of RPCs, modulate the intrinsic competence of RPCs. Finally, Pax6 seems to be necessary for normal proliferation of RPCs and possibly in other cells where it is expressed, including the cerebral cortex [84]. The challenge ahead is to understand how Pax6 function coordinates the two critical processes of proliferation and differentiation, both of which are crucial for normal development.

Conclusions

Recent studies have revealed that Pax6 mediates two sequential steps during early lens development: lens-bias and lens-specification. In contrast to the complete dependence of lens specification on Pax6 activity, during retinal development Pax6 function seems to be partly compensated by factors acting in parallel to confer retinal identity. The combined function of these factors probably confers the competence of RPC to differentiate into the different cell types. Further analysis of the role of Pax6 in different organs, at defined developmental stages and in various species, will unravel on the one hand the conservation of the underlying molecular mechanisms and on the other the mode by which these mechanisms evolved to accommodate tissue-specific functions.

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Conditional knockout of Pax6 designed to study the role of Pax6 during the rapid developmental process leading to the initiation of lens differentiation. The elimination of Pax6 exclusively from the surface ectoderm (SE), one-day after onset of Pax6 expression, reveals the autonomous requirement for Pax6 in the ectoderm during specification in addition to the early requirement of Pax6 during the stage of lens bias [20]. The work further address the influence of lens on retinal development demonstrating that the developing lens placode is essential to instruct the formation of a single retina in the eye, although retinal cell differentiation seems to be mostly independent from the lens.

32. • Kamachi Y, Cheah KS, Kondoh H: **Mechanism of regulatory target selection by the SOX high-mobility-group domain proteins as revealed by comparison of SOX1/2/3 and SOX9.** *Mol Cell Biol* 1999, **19**:107-120.
Study on the mechanisms that enable closely related transcription factors such as the Sox, high-mobility-group proteins to identify different target sequences. The binding specificity of Sox1/2/3, which bind δcrystallin enhancer were compared with the binding specificity of Sox9, which activates col2a1 enhancer. The analysis argues that the C-terminal domain of Sox proteins is important for transactivation and for selection of target enhancer.

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Functional expression screen reveals that Pax6 is the protein partner of Sox1/2/3 for binding the δ crystallin enhancer. The work demonstrates that in chick Pax6 binds cooperatively with Sox2 to the δcrystallin enhancer forming a ternary complex *in vitro* as well as *in vivo*. The combined function of Pax6 and Sox2 accounts for initiation of the lens differentiation program including onset of crystallin expression and thickening of the ectoderm.

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Members of the Six family of transcription factors (Six2, Six4 and Six5) are found to translocate members of the Eya family of transcription regulators (Eya 1, Eya 2, Eya 3) to the nucleus in cotransfected Cos cells. This activity seems to involve formation of a protein complex between the family members. Physical interaction between the fly homologs eya and so has been documented. Taken together, these results suggest that the cooperative interaction between these protein families has been conserved in evolution.

38. •• Heanue TA, Reshef R, Davis RJ, Mardon G, Oliver G, Tomarev S, Lassar AB, Tabin CJ: **Synergistic regulation of vertebrate muscle development by Dach2, Eya2, and Six1, homologs of genes required for *Drosophila* eye formation.** *Genes Dev* 1999, **13**:3231-3243.
This paper demonstrates surprising similarity in the synergistic interactions and genetic hierarchy between *Drosophila* eye determination genes (*eyeless, dachshund, eyes absent, sine oculis*) and members of homologous gene families in vertebrates (*Pax, Dach, Eya, Six*). These parallels suggest that the Dach, Pax, Eya, Six genetic network has been conserved to be redeployed in the context of myogenesis.

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This study provides compelling evidence that Six3 functions as a transcription repressor during fish embryogenesis. First, overexpression of the activator form of Six3 leads to an eye and forebrain hypoplasia opposite to the phenotype observed upon overexpression of wild-type or the transcription repressor form of Six3. Second, Six3 is found to contain transcriptional repression motifs and to physically interact with Grg3, a fish member of the Groucho family of co-repressors.

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See annotation [44•].

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The studies presented in [41,42] provide evidence that FGFs secreted from the surface ectoderm (SE) influence the initial separation of the presumptive neural and retinal pigmented epithelium (RPE) domains in the optic vesicle (OV). Nguyen *et al.* [44•] demonstrate that the bHLH transcription factor Mitf is essential for normal RPE development and that Mitf expression is repressed by external FGFs. Fuhrmann *et al.* [43•] provide evidence that a TGFβ member (possibly activin) secreted from the mesenchyme promotes Mitf expression while restricting the expression of the neuroretina specific factor Chx10.

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This work demonstrates the role of the secreted factor Shh in the dorsoventral patterning of the optic vesicle (OV) in chick embryos. Misexpression of Shh using virus or by blocking Shh activity with antibodies affects the localization of factors implicated to play essential role during subsequent stages of optic cup development including Pax6 (neuroretina), Pax2, Vax1 ventral optic cup, Otx2 (in the RPE development) and BMP4 (dorsal optic cup).

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The authors switched lenses between two forms of *Astyanax mexicanus* fish: the blind cave fish and the surface fish that has functional eyes. Transplanting the surface fish lens to the blind fish optic cup rescued cup formation in the blind fish, whereas ablating the lens of the surface fish led to eye degeneration. These results suggest evolutionary mechanism in which change in one structure imposes a dramatic morphological change on the whole organ.

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See annotation [58••].

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These papers [57••,58••] report striking conservation in the role the secreted factor Hedgehog (Hh in flies and Shh in vertebrate) play in controlling R8 photoreceptor cell differentiation in flies and retinal ganglion cells production in vertebrates. Neumann and Nuesslein-Volhard [57••] demonstrate that in zebrafish, as in fly, Shh is both necessary and sufficient to induce its own expression and to propagate the wave of retinal ganglion cell differentiation wave via activation of the Ras/MAP kinase pathway. Zhang and Yang [58••] further report that in chick high levels of Shh behind the differentiation wave inhibit retinal progenitor cell differentiation. This is similar to the fashion in which different levels of Hh influence R8 photoreceptors genesis in flies.

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In mouse mutants in either Hes1 or Hes5 premature neuronal cell differentiation in the retina is documented. This phenotype is enhanced in the double mutants indicating that Hes1 and Hes5 have an overlapping activity in retinal progenitor cells. Constitutive expression of the active form of Notch (caNotch), which normally inhibits cell differentiation, does not prevent the early neuronal differentiation of cells isolated from the retina of Hes1 and Hes5 deficient embryos. These results support the notion that in the retina Hes1 and Hes5 are downstream mediators of the Notch signaling pathway.

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The retina phenotype of Math5 null mutants is described. In absence of Math5 the retinal progenitor cells (RPCs) destined to form ganglion cells are formed, but these progenitors do not express the POU domain factor, Brn3b, which is required for the differentiation of most ganglion cells [85]. Indeed ganglion cells are mostly not detected in the Math5 deficient retina. Furthermore, the authors report an increase in amacrine cells in the absence of Math5 indicating that Math5 play a role in repressing amacrine cell fate.

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See annotation [75•].

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See annotation [75•].

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Forced expression of Six3 in fish or *Xenopus* [73•,74•] induces ectopic eyes in midbrain and hindbrain. Similarly ectopic expression of Six6 results in enlargement [75•] and formation of ectopic retinae [74•]. These results argue that Six3 and Six6 play pivotal role in retinal specification and growth.

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This work demonstrates for the first time that the adult mammalian retina contains self-renewing multipotential cells. These retinal stem cells were isolated from the pigment cilliary margin of the mammalian retina where normally they remain quiescent. When released from the inhibitory environment of the adult eye these cells have the potential to regenerate retinal cells.

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Rax/Rx a homeodomain containing transcription factor, Notch1 a transmembrane receptor and its effector Hes1, a basic helix–loop–helix transcription factor, are demonstrated in this work to be part of a regulatory pathway that promotes formation of Muller Glia cells in postnatal retina. Furthermore, Rx is indicated to mediate the expression of Notch and Hes1.

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Conditional mutagenesis of Pax6 reveals for the first time the role of this key regulator of eye development during later stages of retinogenesis. Pax6 protein was eliminated before onset of neuronal cell differentiation from the distal optic cup while expression persisted through the proximal portion of the *Pax6flox/Paxflox;aCre* retina, thus providing normal extrinsic cues to the mutant distal cells. This mutation resulted in reduced proliferation of retinal progenitor cells (RPCs) and their exclusive differentiation to amacrine cells. Interestingly amacrine cells devoid of Pax6 mostly do not differentiate to the glycinergic amacrine cells. These results suggest that Pax6 plays a pivotal role in RPCs for maintaining their multi-potency and their proliferation rate, and reveals a possible function of this gene in mature amacrine subtypes.

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See annotation [83•].

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These works [82••,83•] utilize reaggregation approach to evaluate the influence of external factors on retinal progenitor cells (RPCs) isolated from defined developmental stages. Belliveau and Cepko [82••] demonstrate that E16 progenitors do not change their type of progeny even when cultured with access of P0 retinal cells. Extrinsic signals, however, do seem to influence the number of cells generated from each cell type. Using a similar approach Belliveau *et al.* [83•] report that late progenitors cannot acquire early cell fates even if aggregated with access of embryonic progenitors.

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