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Determination of vertebrate retinal progenitor cell fate by the *Notch* pathway and basic helix-loop-helix transcription factors

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Abstract. The retina is an excellent system in which to study neural cell fate decision mechanisms. It is an organized laminated structure with a limited array of cell types. During the last 5 years, experiments that perturb normal gene expression have highlighted some molecular mechanisms involved in cellular fate choice in the retina. By controlling when a retinoblast is allowed to differentiate, *Delta-Notch* signaling plays a critical role in the generation of neuronal diversity in the vertebrate retina. When cells are released from the inhibition mediated by the *Delta-Notch* pathway, basic helix-loop-helix (bHLH) transcription factors act as intrinsic factors that bias neuroblasts towards particular fates. In this review, we present an overview of the data leading to these conclusions on the role of the *Delta-Notch* pathway and the bHLH proteins on cell fate decisions during vertebrate retinogenesis.

Key words. Retina; neurogenesis; bHLH transcription factors; proneural genes; *Delta-Notch* pathway; neurogenic genes; cell determination.

Introduction

The process of retinal cell differentiation leads to the subdivision of the retina into different layers. The vertebrate retina contains two synaptic layers intercalated between three nuclear layers: the outer nuclear layer, the inner nuclear layer, and the ganglion cell layer. The outer nuclear layer consists of two major classes of photoreceptors, the rods and the cones. The inner nuclear layer contains horizontal, bipolar, and amacrine neurons, as well as a few displaced retinal ganglion cells, and Müller glia cells. The ganglion cell layer contains predominantly retinal ganglion cells and some displaced amacrine cells. The different cell types of the retina are generated in a rough sequential order from proliferative neuroepithelial cells. Retinal ganglion cells, cone photoreceptors and horizontal cells are born first, followed by amacrine and rod photoreceptor cells, while bipolar and Müller cells are born last [reviewed in ref. 1]. These cell-type gradients of retinal histogenesis are largely conserved in vertebrate retinas. Even in the rapidly developing retina of *Xenopus*, retinal cells differentiate in a sequential fashion [2, 3]. The mechanisms that allow this succession and cellular diversity are not well understood, although previous work has implicated cell-cell interactions. For example, ablation [4, 5] and lineage tracing [2, 6] experiments have shown that retinoblasts are multipotent and these precursors acquire a particular fate by local cell interactions. Cocultures of mouse and rat retinal cells, from different developmental stages, showed that the particular fate of a cell depends on surrounding cells, suggesting that cell-cell interactions are important for cell fate decision [1]. Photoreceptor differentiation is promoted by cell-cell interactions in vitro: uncommitted cells from *Xenopus* retinas produce photoreceptor cells only when grown in a clump [7]. Several experiments have emphasized the importance of a succession of cell-cell interactions that progressively restrict the fate of a cell. For example, using molecular markers two cellular inductions were shown to be involved in photoreceptor determination [7]. One interpretation of these data is that the

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first induction promotes a photoreceptor fate, while later in development, another inductive event restricts it to a rod fate. Several specific cone markers were used to show that the earliest cones are born before the first rods [3]. Using a reaggregate culture system with rat retinal cells, postnatal amacrine cells were shown to inhibit the production of additional amacrine cells from embryonic progenitors, suggesting that amacrine cell number is controlled by feedback inhibition [8]. A similar feedback loop has been suggested for ganglion cells in the developing chick retina, where early differentiating retinal ganglion cells appear to produce a factor that inhibits newly postmitotic cells from generating ganglion cells [9]. Therefore, it is probable that both positive and negative interactions between cells work together to influence the proper succession of cell types.

Generation of retinal cell diversity by *Delta-Notch* signaling

Expression patterns in retinogenesis

The *Delta-Notch* pathway is known to play a role in the development of disparate tissues in multiple organisms [reviewed in ref. 10]. Its function has been investigated during vertebrate retinogenesis because of its role in lateral inhibition during *Drosophila* neurogenesis, and especially during *Drosophila* retinal development [11, 12]. The prediction that this pathway is active during vertebrate eye development has been strengthened by the expression pattern analysis of *Delta* and *Notch* homologs during vertebrate retinogenesis. The expression patterns of *Notch-1* and *Notch-2*, and their ligands *Delta* and *Jagged*, were examined in the developing rat eye [13, 14]. *Notch-1* and *Delta* are expressed in largely overlapping patterns in the neural retina during the period of cell fate determination and differentiation, whereas *Notch-2* is expressed in the nonneuronal derivatives of the optic cup. *Jagged* is expressed in distinct regions within the optic vesicle, ciliary body, and lens. These observations suggest that the ligand/receptor couple Notch1/Delta is likely involved in retinal cell differentiation. Consistent with this, *C-Delta-1* is coexpressed spatially and temporally with *C-Notch-1*, and their expression is associated with the temporal aspects of cell differentiation in the developing chick retina [15].

In fish and amphibians, the retina grows throughout life by adding new cells of all types from the ciliary marginal zone (CMZ), a region at the peripheral edge of the retina [16–18]. The CMZ is spatially ordered with respect to cellular development and differentiation, with the least determined cells closest to the periphery, and cells in the process of differentiation at the central edge [17, 19, 20]. In *Xenopus*, *X-Notch-1* is expressed in undifferentiated precursor cells of the CMZ but not in the most peripheral region where multipotent cells are present [19]. *X-Delta-1* expression overlaps that of *X-Notch-1* in the CMZ [21]. These expression patterns are therefore consistent with a role for this pathway in retinal cell fate determination.

The gene *hairy* and genes of the *Enhancer of Split* [*E*(*spl*)] complex are downstream components of the *Notch* pathway in *Drosophila*, and also act as negative regulators of neurogenesis [reviewed in ref. 22]. Notch signaling activity controls *E*(*spl*) gene expression positively and directly [23]. This activation requires the activity of Suppressor of Hairless [reviewed in ref. 24], and the nuclear translocation of the intracellular domain of Notch [25–27]. Some *E*(*spl*) vertebrate homologs have been isolated, such as *HES1* and *HES3* in rodents [28], or *ESR1* and *ESR3* in *Xenopus* (D. Turner, personal communication). In cells expressing *Notch-1*, an *HES-1*-derived promoter construct is transactivated in response to Delta-1 stimulation [29]. In *Xenopus* animal cap assays, *ESR1* expression is induced by Notch signaling [30]. These data suggest that vertebrate *E*(*spl*) homologs are also targets of the *Notch* pathway. Retinal progenitor cells that express *X-Notch-1* and *X-Delta-1* also express *HES1* in mouse and rat, or *ESR1* and *ESR3* in *Xenopus*, suggesting that this signaling pathway is active during retinogenesis [20, 31].

Misexpression experiments

Misexpression studies have addressed the function of the *Delta-Notch* pathway during retinogenesis in several vertebrate species (table 1). In *Xenopus*, transfection of an activated form of *X-Notch-1* into retinoblasts caused them to retain a neuroepithelial morphology, and inhibited their development into mature neurons, whereas cells transfected with green fluorescent protein differentiated into a variety of retinal cells [19]. By inhibiting differentiation, the *Notch* pathway has also been suggested to regulate ganglion cell number. When *Notch* activity was diminished in vivo in chick retina using antisense oligonucleotides, or augmented either by incubating chick eye cups with *Drosophila* cells expressing *Delta*, or using a retrovirally transduced active allele of *Notch*, the number of ganglion cells produced was inversely related to the level of *Notch* activity [15, 32]. In contrast, blocking the *Notch* pathway by overexpression of a dominant negative form of *Delta* pushes cells to differentiate prematurely as neurons of diverse types, not only ganglion cells [33]. Another member of the *Notch* gene family has also been implicated in retinogenesis. Inhibition of *G-Notch-3* expression by injection of antisense oligonucleotides into eyes of juvenile goldfish causes the premature differentiation of multiple

retinal cell types [34]. Together these data suggest that the *Notch* pathway normally inhibits cell differentiation during retinogenesis.

In *Xenopus*, the role of *Delta* has been investigated by overexpressing either *X-Delta-1* or a dominant negative form of *X-Delta-1*, *Delta*^{STU} [21]. When X-Delta-1 signaling was blocked by overexpressing Delta^{STU}, all Delta^{STU}-positive cells exhibit a bias towards early differentiation, consistent with the idea that releasing cells from X-Delta-1 inhibitory signaling pushes them to premature differentiation. When *X-Delta-1* was overexpressed, cells surrounding overexpressing *X-Delta-1* cells expressed a low level of endogenous X-Delta-1 compared to nonneighbor cells. This suggests that a cell expressing *X-Delta-1* inhibits expression of *X-Delta-1* in neighbor cells. This negative feedback loop probably allows one cell to escape the inhibition while the others remain inhibited from differentiating. Similarly in *Drosophila*, an amplification of subtle fluctuations in the amount of Delta by negative feedback interactions has been suggested to allow one cell to escape the lateral inhibition while inhibiting its neighbor cells [reviewed in ref. 35]. Such a mechanism may also occur in vertebrate retinogenesis. For example, *Notch-1* is expressed throughout the proliferative zone of the chick retinal neuroepithelium, whereas *Delta-1* is expressed in scattered cells [33]. Consistent with this hypothesis, in the *Xenopus* retina, cells overexpressing *X-Delta-1* with wild-type neighbors showed a bias towards early differentiation, probably because they no longer received X-Delta-1 signaling from their wild-type neighbors [21]. On the other hand, cells within a large clone of X-Delta-1-positive cells remained neuroepithelial. They do not differentiate, probably because they receive the inhibitory X-Delta-1 signaling from their X-Delta-1-positive neighbor cells.

Furthermore, the importance of timing in the experimental perturbation of *Delta-Notch* signaling has been demonstrated. The particular fates adopted by the cells were different, in a small misexpressing clone, depending on the stage at which *X-Delta-1* was overexpressed [21]. In all cases, however, the effect was to force cells to differentiate too early. This suggests that depending on the developmental stage at which a cell is released from X-Delta-1 inhibitory signaling, it will take on a particular fate, dictated by the specific environmental cues present at the time. Together, these results suggest that *Delta-Notch* signaling controls neuronal diversity in the

Table 1. Summary of gain- and loss-of-function analyses of *Notch*/*Delta* and bHLH genes in vertebrates.

Experiment	Effects on cell fate							References
	neuroepithelial	ganglion	amacrine	bipolar	horizontal	photoreceptor	Müller	
Notch or *Delta* gain of function	+	–	–	–	–	–	–	15, 19, 21, 32, 33
Delta or *Notch* loss of function		+	nd	–	nd	+ (cone), – (rod)	–	15, 32
HES1 gain of function	+	–	–	–	–	–	–	31
HES1 loss of function		+	+	*	+	+ (rod)	nd	31
Xath5 gain of function		+	–	–	nc	nc	–	45
Ath3 gain of function		+	nc	–	nc	+	–	52
X-ngnr-1 gain of function		nc	nc	–	nc	+	–	52
NeuroD gain of function (*Xenopus*)		nc	nc	–	nc	+	–	Perron et al., unpublished data
NeuroD gain of function (chick)		nc	nc	nc	nd	+	nd	56
NeuroD gain of function (mouse)		nd	+	–	nd	+	–	57
NeuroD loss of function		nd	delay	+	nd	–* (rod)	+	57

–, decrease; +, increase; nc, no change; *, apoptosis; nd, not determined.

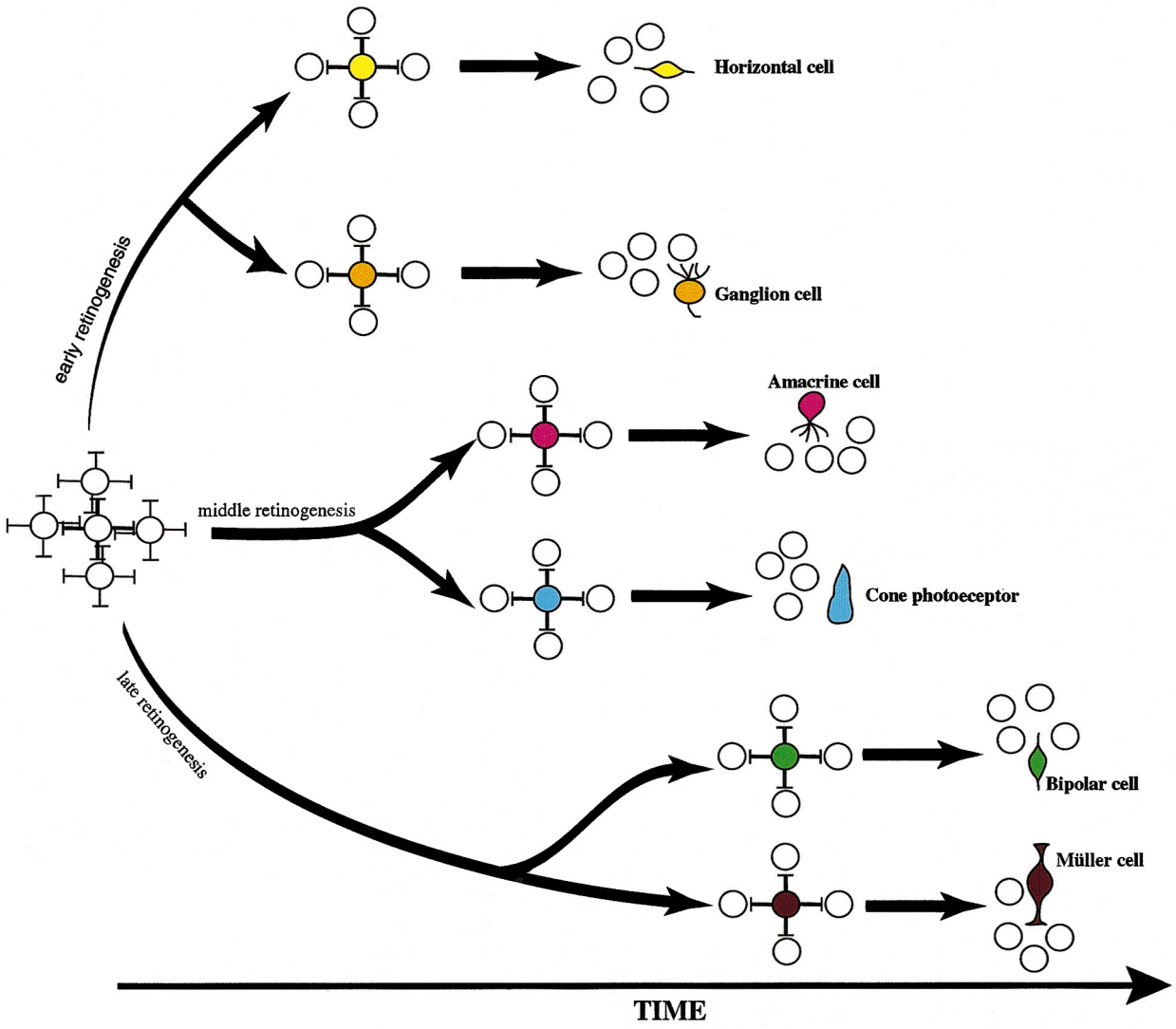

Figure 1. Model of the role of *Notch*/*Delta* signaling and bHLH proteins during retinogenesis. Multipotent progenitors express *Delta* and *Notch* genes, and therefore inhibit each other from differentiating (flat-headed arrows). A negative feedback loop allows a cell that expresses more *Delta* (the middle cell on the left with bold flat-head arrows) to reduce expression of *Delta* in neighboring cells and therefore to escape lateral inhibition and differentiate according to cues in the cellular environment and the combination of bHLH genes it expresses (filled color). The timing of escape from lateral inhibition influences the cell fate. For example, a progenitor differentiates into a ganglion cell (orange) or a horizontal cell (yellow) during early retinogenesis, into an amacrine cell (pink) or a cone photoreceptor cell (blue) later on, and into a bipolar (green) or a Müller cell (brown) during late retinogenesis.

retina by regulating the competence of precursors to respond to signals that promote different cell types. Similar results were obtained in the compound eye of *Drosophila* [12]. Reducing Notch activity at different times, using a *Notch* temperature-sensitive allele, produced defects in specific cells. Early temperature shifts affected photoreceptor and cone cell development, while bristles and then pigment cells were affected by later shifts. From all these data one can conclude that Delta signaling, both in *Drosophila* and in vertebrate retina, is a basic regulatory mechanism that can be used to generate neuronal diversity (fig. 1). This mechanism may operate throughout the vertebrate central nervous system, enabling neurons to be produced sequentially and adopt different fates in response to cues in the cell environment.

Gain- and loss-of-function studies have been carried out in mouse to study the role of an *E*(*spl*)/*hairy* homo-

logue, *HES1* [31]. Persistent expression of *HES1* blocks retinal cell differentiation, and this effect mimics the overexpression of a constitutively active form of *Notch*. The effect of loss-of-function was analyzed in an *HES1*-null mouse. Because these mice die during gestation or soon after birth, retinal cultures that develop in a manner similar to retinas in vivo were examined. In *HES1*-null mutant mice, neural markers were expressed earlier than in wild-type retinas suggesting that neuronal differentiation was accelerated. This mimics the phenotype observed in *Xenopus* retina when cells no longer receive Delta signaling [21]. These similarities in phenotypes when *Notch-Delta* and *HES1* functions are altered suggest that *Notch-Delta* and *HES1* indeed act in the same pathway during retinogenesis.

Involvement of bHLH proneural genes in cell fate decision

Expression patterns in retinogenesis

When a neuroblast escapes Delta-Notch lateral inhibition, intrinsic factors are already expressed that bias it to adopt a particular fate. Proneural genes are bHLH transcription factors that promote neural cell differentiation both in *Drosophila* neurogenesis and in primary vertebrate neurogenesis [reviewed in refs 35, 36]. In *Drosophila*, a proneural function has been attributed to four bHLH genes of the *achaete scute* complex (*AS-C*) [reviewed in ref. 37]. Interestingly, these genes are required for the differentiation of different types of neurons, suggesting that they are also involved in cell fate decisions [38]. Perturbing the expression of *AS-C* genes changes the identity of the neural cells produced, consistent with a role for these genes in establishing neural precursor identity in the *Drosophila* central nervous system [39]. Another *Drosophila* proneural bHLH protein is encoded by the *atonal* gene, which, unlike the *AS-C* genes, is involved in the differentiation of chordotonal sense organs and photoreceptor cells in the eye disc [40, 41]. Are proneural genes also involved in cell fate decisions during vertebrate eye development?

In vertebrates, unlike in *Drosophila* where the only proneural bHLH gene expressed in the eye disc is *atonal*, both *achaete scute*-like (such as *Xash1* and *Xash3* in *Xenopus*) and *atonal*-like genes (such as *Xath3*, *Xath5*, *X-ngnr-1*, and *NeuroD* in *Xenopus*) are expressed in the developing eye [42–46]. In *Xenopus* retina, proneural genes, including *Xash1*, *Xash3*, *X-ngnr-1* [47], *Xath3*, *NeuroD*, and *Xath5* are expressed in the CMZ, where retinal precursors are present [20, 42, 45, 48; M. Perron, K. Opdecamp, K. Butler, W. A. Harris and E. J. Bellefroid, unpublished data]. *Math5*, a homolog of *Xath5* in mouse, as well as *Cash1* and *Mash1*, chick and mouse homologues of *Xash1*, respectively, are also restricted to a subset of retinal progenitor cells [49–51]. In mouse, early appearing retinal cells, such as ganglion, horizontal and cone photoreceptor cells, start being generated prior to the onset of *Mash1* expression, suggesting that this gene is not involved in specification of these cell fates [51].

In *Xenopus* CMZ, proneural genes are expressed sequentially during retinogenesis, with *Xash1*, *Xash3*, and *X-ngnr-1* preceding *Xath5*, *Xath3*, and *NeuroD* [20, 52]. In the chick retina, two *atonal*-like genes are also expressed sequentially, *NeuroM* expression preceding *NeuroD* [53]. These results suggest that the sequential expression of proneural genes might define different stages of retinal precursor development, which may in turn reflect changes in the specific environmental cues that regulate cell fate.

In *Xenopus*, *Xath3* and *NeuroD* are also expressed in central retina outside the CMZ in certain cells of the mature neural retina [20]. For example, *Xath3* is expressed in the outer part of the inner nuclear layer, whereas *NeuroD* is expressed in the outer nuclear layer (i.e., photoreceptor layer) and transiently in the outer part of the inner nuclear layer [20, 54]. *Math3*, the mouse homolog of *Xenopus Xath3*, is also maintained in differentiated cells of the inner nuclear layer, as revealed with a reporter gene under the control of the *Math3* promoter [55]. In chick, human, and monkey retinas, *NeuroD* is expressed in the outer nuclear layer, which contains the photoreceptors [56, 57]. Another study described a low level of *NeuroD* expression in the ganglion cell layer of chick retina, in addition to expression in the photoreceptor layer [53]. In rodent, *NeuroD* expression was also detected both in undifferentiated retinal cells and in developing photoreceptors and developing amacrine cells [58]. The implications of these differences in expression of *NeuroD* between different species are discussed below. Taken together, these expression patterns suggest that proneural genes are involved in retinogenesis, and some of them continue to function in differentiated retinal cells.

Misexpression experiments

Misexpression experiments highlight the role of proneural genes in cell fate decisions during retinogenesis (table 1). In *Xenopus*, targeted expression of *Xath5* by lipofection in retinal progenitor cells biased the differentiation of these cells towards a ganglion cell fate at the expense of later-born cells, such as bipolar and Müller cells [45]. Since ganglion cells are the first cells born during normal retinogenesis, this effect could result from action of bHLH proneural transcription factors promoting differentiation prematurely, or from a specific action of *Xath5* in promoting ganglion cell fate. In a similar lipofection assay, the effects of other *atonal*-

like genes on cell fate decision were compared [52]. Whereas *Xath5* promotes only ganglion cells at the expense of later-born cells, *Xath3* promotes both ganglion and photoreceptor cells. In contrast, *X-ngnr-1* promotes only photoreceptors at the expense of late-born cells and *NeuroD* also promotes photoreceptors, at the expense of late-born cells, albeit not very strongly [M. Perron et al., unpublished data]. These results show that when overexpressed under similar conditions, *atonal*-like proneural genes act differently on cell fate decisions, suggesting that they play distinct roles in promoting particular neuronal fates in the retina.

The effect of *NeuroD* on photoreceptor fate decision has also been clearly shown in chick retina using retrovirus-mediated gene misexpression [57]. Overexpression of *NeuroD* in retinal neuroepithelium produced a photoreceptor layer three cell bodies deep, instead of the normal two. Furthermore, the number of cells that expressed visinin (a marker for cone photoreceptors) increased over 50% compared to control embryos misexpressing a control gene (green fluorescent protein). No significant changes were observed in the numbers of ganglion, amacrine, or bipolar cells. Retroviral-driven misexpression of *NeuroD* in cultures of retinal pigment epithelium also yielded de novo production of photoreceptor cells with no other types of retinal neurons [57]. These studies suggest that *NeuroD* is important for photoreceptor cell production in the vertebrate retina.

Analysis of *NeuroD* loss and gain of function in mouse showed that photoreceptors are not the only cells affected in a retina misexpressing *NeuroD* [58]. Retinal explants derived from *NeuroD*-null mice demonstrated an increase in Müller glia and bipolar cells, whereas the number of rod photoreceptors was moderately reduced and amacrine cell differentiation was delayed. In contrast, forced expression of *NeuroD* in retinal progenitors in rat using retrovirus completely blocked gliogenesis in vivo and reduced the number of bipolar cells, whereas it favored amacrine cells and moderately enhanced the number of rod cells. The effects on Müller glia cells suggest that *NeuroD* regulates neuron versus glial cell fate decisions. This result is consistent with the decrease in Müller glia cells observed in *Xenopus* retina when *NeuroD* was transfected by lipofection [M. Perron et al., unpublished data]. However, an increase in amacrine cells has not been observed when *NeuroD* is overexpressed in chick or *Xenopus* [57; M. Perron et al., unpublished data]. This difference might be explained by species differences in expression of *NeuroD*, which is found in amacrine cells only in rodents. An increase in apoptosis was also detected in the photoreceptor layer in *NeuroD*-null mice retina, suggesting that *NeuroD* is also involved in the survival of a subset of rod photoreceptors. Taken together, these results suggest that *atonal*-like genes are involved in a variety of retinal cell fate decisions and might also have a broader function, for example in neuron survival.

The role of only one *achaete-scute*-like gene, *Mash1*, has been studied so far during retinogenesis in mouse. Although *Mash1* is expressed during retinal development, no apparent abnormalities were found in the retina during embryogenesis or at birth in *Mash1*-null mouse [59]. Since the differentiation of ganglion, amacrine, horizontal and cone receptor cells starts before birth [60], the absence of phenotype suggests either that these early differentiating cells do not require *Mash1* or that there is some redundancy among proneural genes. The first hypothesis is more consistent with the fact that *Mash1* expression is restricted to the late phase of retinal neurogenesis [51]. Because *Mash1*-null mice die soon after birth, retinal explants were examined in order to study postnatal retinal development in the *Mash1*-null mouse [61], during the period when rod photoreceptor, bipolar, and Müller cells are born [60]. In these retinas, differentiation of late-appearing cells such as rod and bipolar cells was delayed, and the final number of bipolar cells was significantly reduced [61]. A delay in horizontal cell differentiation was also observed even though no horizontal cells are born postnatally [60], suggesting that *Mash1* affects the rate of horizontal cell production before birth or a postnatal phase of horizontal cell differentiation. Vimentin-positive cells (probably Müller glial cells) were increased in *Mash1*-null retina. Thus, *Mash1* was suggested to promote neuronal differentiation during retinal development.

A more direct effect of proneural genes on photoreceptor differentiation has also been proposed [62]. Using an electrophoretic mobility shift assay, Mash1, together with E12 (a ubiquitous bHLH transcription factor required for Mash1 dimerization) was shown to specifically bind the E-box element present in the opsin promoter. This result suggests that *Mash1* regulates opsin expression during retinogenesis and therefore plays a role in rod specification. However, since rods differentiate in *Mash1*-null mouse retina, although with a delay, other factors might compensate for the absence of *Mash1* function. *NeuroD* is a good candidate, since it is expressed during rod differentiation and also has the ability to bind the E-box element in the opsin promoter [63]. It would be helpful to study retinal development in mice in which several proneural genes have been knocked out simultaneously in order to counteract functional redundancy.

Relationships between neurogenic and proneural genes

In *Drosophila*, proneural and neurogenic genes interact in a feedback loop. For example, the *Notch* pathway negatively regulates the expression of proneural genes [23, 64, 65]. Conversely, proneural gene activity promotes the expression of the neurogenic gene *Delta* [66, 67]. Similar interactions have been demonstrated during vertebrate primary neurogenesis, where, for example, overexpression of *X-ngnr-1* or *Xath3* leads to an expansion of the domain of *Delta* expression, whereas *Delta-Notch* signaling inhibits *X-ngnr-1* and *Xath3* expression [47, 52]. However, very little is known about these interactions during retinogenesis. One study in mouse showed that the *Notch* pathway might regulate *Mash1* expression. The loss of function of *HES1* up-regulates *Mash1* expression in the retina [68]. Since *HES1* is probably downstream of Notch signaling, this result suggests that *Notch* signaling may inhibit *Mash1* expression during normal retinogenesis.

It has recently been proposed that in addition to the *Notch* pathway, the intracellular cascade triggered by epidermal growth factor (EGF)-mediated cell interactions could also negatively regulate *Mash1* expression during rat retinogenesis [63]. Signaling through the EGF receptor is known to be essential for development of the *Drosophila* compound eye [69–73]. In vertebrates, EGF can stimulate cell proliferation in dissociated retinal cell cultures [1, 74] and in retinal explant culture [63]. Based on the location of ^{3}H-thymidine incorporation in explant cultures, EGF was proposed to promote proliferation of cells that are likely to be rod precursors [63]. Treatment of the explant with EGF significantly decreases both Mash1 and opsin immunoreactivity [63]. When EGF was subsequently removed from the culture media, both Mash1 and opsin immunoreactivity were restored. These results imply that *Mash1* expression is regulated by the EGF pathway and support the hypothesis that *Mash1* is involved in rod differentiation. Together, these results lead to the suggestion that EGF and Notch act in concert to control proneural gene expression and cell fate determination in the retina.

Other less well studied signaling pathways are probably also involved in controlling cell fate decisions during retinogenesis by interacting with the *Notch* pathway and by regulating proneural gene expression. For example the *hedgehog* pathway has recently been shown to control *atonal* expression in *Drosophila* eye disc [75, 76]. In zebrafish, the activity of *neurogenin* is dependent on *hedgehog* signaling during early neurogenesis [77]. In the vertebrate retina, members of the *hedgehog* gene family are expressed either in the neural retina or in the retinal pigment epithelium [78, 79; M. Perron et al., unpublished data], and culturing rat retinal cells in the presence of Sonic hedgehog increases the number of photoreceptors [77]. These data are consistent with a role of *hedgehog* during retinogenesis. Placing the proneural and neurogenic genes into the context of these other regulatory pathways will enable us to reconstitute the mechanisms leading to cell fate decision.

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