高等教育出版社
HIGHER EDUCATION PRESS

神经科学进展（四），80～94

Intrinsic mechanisms that control the specification of mammalian retinal cell types

Mengqing Xiang

Center for Advanced Biotechnology and Medicine and Department of Pediatrics,

UMDNJ-Robert Wood Johnson Medical School

【作者简介】Mengqing Xiang, Ph.D., born in 1965, is Associate Professor at the University of Medicine and Dentistry of New Jersey (UMDNJ)-Robert Wood Johnson Medical School. While serving as a faculty member in the Department of Pediatrics, the Center for Advanced Biotechnology and Medicine (CABM) and the Cancer Institute of New Jersey (CINJ), he directs the Molecular Neurodevelopment Laboratory at CABM. He earned his Ph.D. degree from the University of Texas M.D. Anderson Cancer Center and conducted his postdoctoral studies at the Johns Hopkins University School of Medicine. Dr. Xiang received a number of honors including a China-U.S. Government Graduate Study Fellowship (CUSBEA Program), a Howard Hughes Medical Institute Postdoctoral Fellowship, a Basil O'Connor Starter Scholar Research Award, a Sinsheimer Scholar Award, and a Wolf, Block, Schorr and Solis-Cohen, LLP Award. His work on the molecular mechanisms of neural development and diseases has over the years been funded by the National Institutes of Health, March of Dimes Birth Defects Foundation, Alexandrine and Alexander L. Sinsheimer Fund, New Jersey Commission on Spinal Cord Research, UMDNJ Foundation, Glaucoma Foundation, and the National Organization for Hearing Research Foundation. Dr. Xiang has published about 40 research articles in frontline scientific journals. He is also a member of the Society for Neuroscience, the American Association for the Advancement of Science, and the Association for Research in Vision and Ophthalmology.

Representative publications

1. **Xiang, M.**, Zhou, L., Peng, Y.-W., Eddy, R. L., Shows, T. B., and Nathans, J. Brn-3b: a POU-domain gene expressed in a subset of retinal ganglion cells. Neuron, 1993, 11:689-701.

2. Liu, W., Mo, Z., and **Xiang, M.** The Ath5 proneural genes function upstream of Brn3 POU domain transcription factor genes to promote retinal ganglion cell development. Proc. Natl. Acad. Sci., 2001, USA 98:1649-1654.
3. Li, S., Price, S. M., Cahill, H., Ryugo, D. K., Shen, M. M., and **Xiang, M.** Hearing loss caused by progressive degeneration of cochlear hair cells in mice deficient for the Barhl1 homeobox gene. Development, 2002, 129**:**3523-3532.
4. Li, S., Mo, Z., Yang, X., Price, S. M., Shen, M. M. and **Xiang, M.** Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron , 2004, 43:795-807.
5. Li, S., Misra, K., Matise, M. and **Xiang, M.** Foxn4 acts synergistically with Mash1 to specify subtype identity of V2 interneurons in the spinal cord. Proc. Natl. Acad. Sci. USA, 2005, 102:10688-10693.

Abstract

The generation of diverse neuronal types and subtypes from multipotent progenitors during development is a prerequisite for establishing functional neural circuits in the central nervous system (CNS). The retina has long served as an excellent model system in which to study the mechanisms of CNS neuronal specification and diversification due to its accessibility and relative simplicity. During mammalian retinogenesis, seven classes of cells are specified from multipotent progenitors by the action of various intrinsic and extrinsic factors. Recent molecular genetic studies involving loss-of-function and gain-of-function approaches have uncovered a number of transcription factors, in particular, several homeodomain and basic helix-loop-helix (bHLH) factors, as pivotal intrinsic regulators of mammalian retinogenesis. These factors are found to act at different developmental processes to establish progenitor multipotency, define progenitor competence, determine cell fates, and/or specify cell types and subtypes. These findings have provided an important framework for a full understanding of gene regulatory networks involved in the generation of neuronal diversity.

Introduction

The mammalian retina is a highly organized sensorineural tissue composed of six classes of neurons and one type of glial cells that are interconnected in a laminar structure. These include the rod and cone photoreceptor cells in the outer nuclear layer; the horizontal, bipolar, and amacrine interneurons plus the Müller glial cells in the inner nuclear layer; and the ganglion and displaced amacrine cells in the ganglion cell layer. In addition, a small number of displaced amacrine and ganglion cells are located in the inner plexiform layer. Apart from these seven major cell classes, some classes of retinal neurons contain many diverse subtypes that differ in morphologies, physiological properties and functions[1,2].

Owing to its accessibility, well-characterized cell classes and neural tube origin, the retina has provided an excellent model system in which to study the mechanisms of neuronal determination and

differentiation in the CNS. During embryogenesis, the retina develops from the optic vesicle, a protrusion of the diencephalic neuroepithelium of the neural tube. A central question in retinal development is how those diverse retinal cell types and subtypes are specified and differentiated from an initially seemingly uniform neuroepithelium. Birthdating analysis by tritiated thymidine labeling has revealed a loose and yet fixed temporal order for the genesis of each cell type[3,4] **(Fig. 1)**. For instance, during murine retinogenesis, ganglion cells are the first cell type to exit the cell cycle, followed closely by horizontal, amacrine and cone cells, while rod, bipolar and Müller cells are the last cell types to be produced (Fig. 1). Despite this loose temporal order, multiple cell types can be generated at any given age of retinal development (Fig. 1). Moreover, lineage tracing studies have clearly demonstrated that retinal progenitors are multipotent, suggesting that retinal cells are determined without following strict cell lineages, but rather depending on local environment and cell-cell interactions[5-8]. However, intrinsic properties of progenitors must also contribute to the choice of cell fate since the progenitors must first become competent to respond to extrinsic cues to generate appropriate cell types. In this review, we will focus on recent advances in the identification of intrinsic transcriptional regulators that control fate commitment and differentiation of various retinal cell types and subtypes.

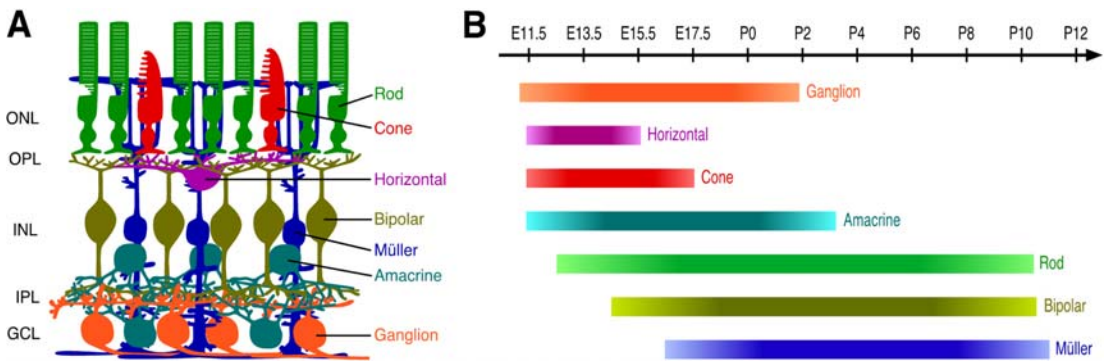

Fig. 1. Retinal structure and development. (A)Schematic of the retinal structure. (B) Order of birth of mouse retinal cells. Birthdating analysis by tritiated thymidine labeling has revealed a loose temporal order of generation of the six neuronal and one glial cell types in the mouse retina. In spite of this loose birth order, multiple cell types can be generated at any given age of retinogenesis

Current model of retinal cell specification

As discussed above, there exists some inconsistency between the results obtained from birthdating and lineage tracing studies. To accommodate these sometimes contradictory findings, a recent model of retinogenesis suggests that intrinsic and extrinsic factors together determine the choice of cell fate[9-11]. In this model, it is hypothesized that during retinogenesis, progenitors pass through

successive and distinct states of competence to respond to temporally varying environmental cues for the ordered generation of different cell types (Fig. 2). Each state of competence is transient and probably defined by a network of transcription factors. The progression of competence states appears to be unidirectional and these states may not be skipped. Once committed by extrinsic cues, the progenitor cell moves forward to form a proper cell type following a particular differentiation program.

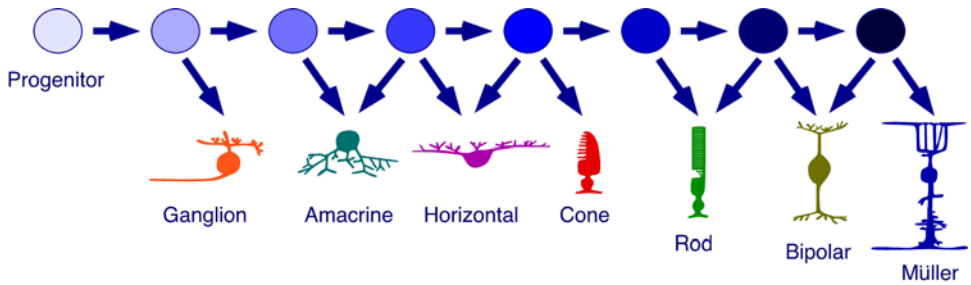

Fig. 2. Model of retinal development. Retinal progenitors may undergo a series of changes in competence to respond to environmental cues for producing different cell types. Each progenitor cell is thought to be controlled by a network of transcription factors that define its competence to make a particular retinal cell type, or a small set of cell types. Retinal progenitors may progress from one state of competence to another only in one direction. Early progenitor cells may be unable to jump ahead to later stages of competence. The commitment of a competent cell to become a particular cell type is controlled by extrinsic signals. Once committed, the progenitor differentiates into the appropriate cell type.

This model can elegantly reconcile most of the data accumulated to date on retinogenesis as well as can nicely explain the ordered and yet overlapping phases of generation of different cell types. However, one should keep in mind that this is a working model that is still evolving as new data emerge. For instance, a recent report suggests that the progression of competence states may be reversible since a subset of late progenitors can generate early born ganglion cells at conditions conducive for their differentiation[12]. Furthermore, the current model does not consider the relative importance of intrinsic and extrinsic factors in a particular cell fate decision or deal with the mechanism regulating the total number of each cell type generated. There is currently limited evidence implicating intrinsic factors as the major determinants of cell fate choice in the developing retina[13].

Transcriptional regulators as intrinsic factors controlling retinal development

Retinal development involves both intrinsic and extrinsic factors for cell fate commitment and differentiation. It is generally assumed that a competence state of progenitor cells is defined by the

expression of a particular combination of transcription factors. Similarly, extracellular signals may affect the choice of cell fate by programming the expression and activity of transcription factors in competent progenitors. The transcription factors in turn modulate the expression of specific sets of target genes, which ultimately specify cell fates and program differentiation. Therefore, transcription factors play essential roles in controlling cell determination and differentiation during retinogenesis. To identify transcription factors and subsequently elucidate their functions in the retina will be an important first step toward understanding the molecular events that lead to this highly specialized sensorineural tissue. In the past decade or so, experiments that perturb normal expression of transcription factor genes have provided fundamental insights into the molecular mechanisms of cellular fate commitment and differentiation during retinogenesis. A variety of transcription factors have been found to control intrinsic properties of retinal progenitors and/or participate in differentiation processes. These include two classes of factors, the homeodomain and bHLH transcription factors that have emerged as some of the major molecular players.

Multipotent state of progenitors. Prior to the initiation of retinogenesis, neuroepithelial cells in the optic vesicle must first acquire and then maintain multipotency for the generation of the full range of retinal cell types. How this comes about still remains to be determined but two transcription factors, Pax6 and Foxn4, have been demonstrated to play a crucial role in this process (Figs. 3, 4). Pax6 is a paired-type homeodomain factor that is required for patterning early eye development. Its mutations or overexpression result in a range of ocular phenotypes including small eyes, absence of eyes, cataract, or aniridia in the mouse or human[14-18]. Although Pax6 is expressed by all retinal progenitor cells, its essential early patterning role has made it difficult to gauge a possible later function during retinal development. The advent of the conditional gene knockout technique has resolved this problem and we now know that all retinal cell types except for γ-aminobutyric acid (GABA)ergic amacrine cells disappear when Pax6 is ablated specifically from retinal progenitor cells[19]. Thus, Pax6 appears to be required by retinal progenitors to acquire and/or maintain the multipotent state. Moreover, it appears to do so by regulating the expression of multiple retinogenic bHLH factors which are key intrinsic regulators controlling cell type specification[19,20].

Foxn4, a winged-helix/forkhead transcription factor that is expressed by a large subset of retinal progenitors, appears to be required by progenitors to acquire competence states for the generation of amacrine and horizontal cells[21]. Loss of *Foxn4* function in mice causes a loss of all horizontal cells as well as the great majority of amacrine cells, while overexpression of Foxn4 biases most progenitors into an amacrine cell fate. Similar to Pax6, Foxn4 appears to control the genesis of amacrine and horizontal cells by activating retinogenic bHLH and homeodomain factors that are involved in the specification of these two cell types[21]. It is conceivable that Pax6 may act together with Foxn4 and other homeodomain and bHLH factors to confer retinal progenitors with the full potential for the genesis of all seven cell classes (Figs. 3, 4).

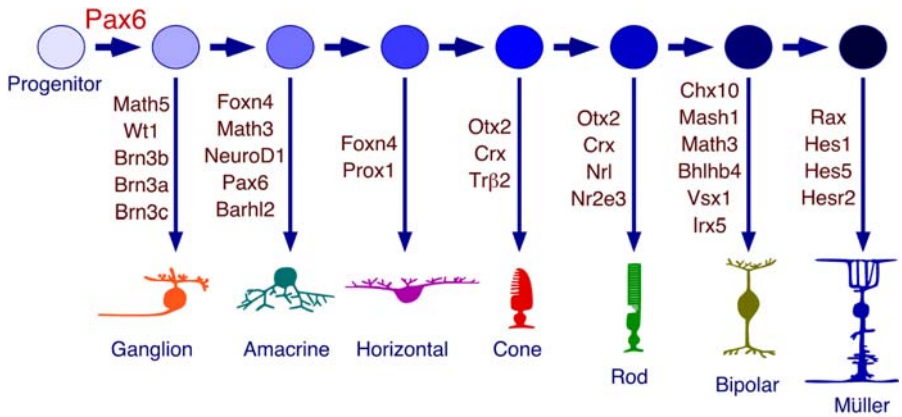

Fig. 3. Transcription factors involved in the specification and differentiation of different retinal cell types.

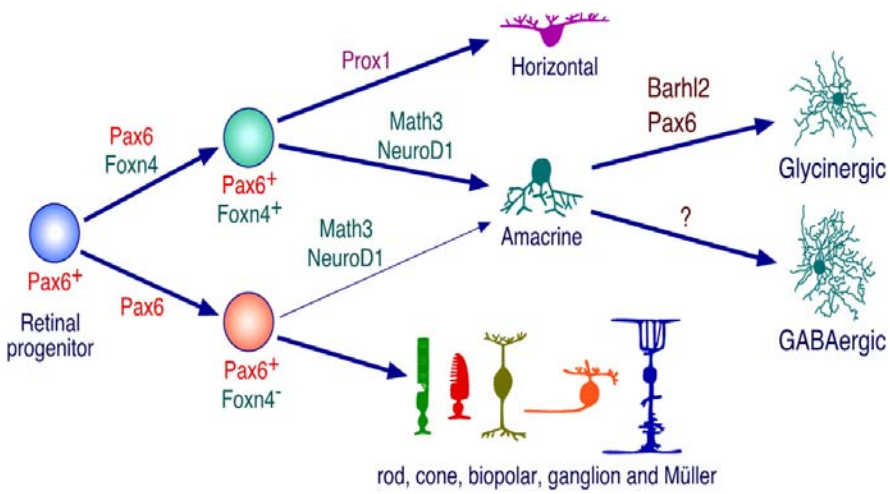

Fig. 4. Mechanism that controls the specification of amacrine and horizontal cells by retinal progenitors. Retinal progenitors with the activation of both Foxn4 and Pax6 ($Pax6^{+}Foxn4^{+}$) are competent for the genesis of all horizontal cells and the great majority of amacrine cells. Only a small number of amacrine cells are generated from the $Pax6^{+}Foxn4^{-}$ progenitors. Some of the newly generated amacrine cells may differentiate into glycinergic cells by the action of Barhl2 and Pax6. Foxn4 confers progenitors with the competence for the genesis of amacrine and horizontal cells by activating the expression of Math3, NeuroD1 and Prox1, three retinogenic factors involved in the specification of these two cell types. The rod, cone, bipolar, ganglion, and Müller cells are likely to be derived largely from the $Pax6^{+}Foxn4^{-}$ progenitors via activation of other retinogenic factors[21,52].

Photoreceptors. Although retinal progenitor cells can exist in multiple competent states as development progresses, they require the function of particular intrinsic factors to commit to a particular cell fate. For photoreceptor cells, two homeodomain transcription factors, Otx2 and Crx,

are required for fate commitment and differentiation, respectively (Fig. 3). Conditional gene targeting of *Otx2* results in a complete absence of rod and cone cells while causing a fate-switch in progenitors to generate more amacrine cells; whereas its overexpression promotes a photoreceptor cell fate[22]. Otx2 controls photoreceptor development partly by activating the expression of *Crx*[22], which has been shown by gene targeting and overexpression analyses to be essential for differentiation but not for determination of photoreceptor cells[23,24]. Microarray and other studies have revealed that Crx programs photoreceptor differentiation by regulating the expression of a network of photoreceptor-specific/enriched genes. These include genes involved in the phototransduction pathway such as those encoding opsins, transducins, phosphodiesterases, and arrestins[24,25]. In the human, mutations in *Crx* are associated with retinal diseases including cone-rod dystrophy, retinitis pigmentosa, and Leber congenital amaurosis[26-29]. As also demonstrated by gene targeting and overexpression experiments, the terminal differentiation and survival of photoreceptors require the critical function of another transcription factor, the NeuroD1 bHLH factor[30,31] (Fig. 3).

The mechanism of photoreceptor subtype specification remains to be defined but thus far three key intrinsic transcription factors have already been identified that play such a role. These are the Nrl basic motif-leucine zipper (bZIP) factor, the Nr2e3 orphan nuclear receptor, and the thyroid hormone receptor 2 (Tr 2) (Fig. 3). In the human, missense mutations in NRL are associated with autosomal dominant retinitis pigmentosa[32,33]. In the mouse, the absence of *Nrl* leads to a switch in the rod fate[34], generating supernumerary S (blue)-cones with resemblance to the symptom of enhanced S-cone syndrome (ESCS), which is caused by mutations in *NR2E3* in humans[35]. A null *Nr2e3* mutation in mice results in the rd7 retinal degeneration characterized by the abundant presence of a morphologically hybrid photoreceptor and a modest increase of S-cones[36-39]. Nr2e3 is expressed exclusively in rods and appears to influence rod differentiation primarily by repressing a large number of cone-specific/enriched genes in rods, as demonstrated by microarray and several other techniques[37-39]. Similar approaches have revealed that Nrl functions to determine rods from progenitor cells by activating numerous rod-specific genes as well as by suppressing cone-specific genes in part by regulating *Nr2e3* expression[34,40]. Interestingly, both Nrl and Nr2e3 control downstream gene expression by interacting with Crx at their target DNA-binding sites[39,41]. The specification of M (green)-cones has been shown to be critically dependent on the function of Tr 2 as the absence of its function causes a complete loss of M-cones and a concomitant increase of S-cones[42].

Bipolar cells. Fate determination of bipolar cells relies on the synergistic activities between the Chx10 homeodomain factor and the two bHLH factors Mash1 and Math3 (Fig. 3). Mutations in *Chx10* block bipolar cell specification as well as retinal progenitor proliferation, and have been shown to be responsible for the mouse mutation *ocular retardation*[43]. Retinas deficient for both *Mash1* and *Math3* lack bipolar cells and display a fate change to Müller cells[44,45]. However, albeit necessary, none of the three factors appear to be sufficient on their own for the selection of a bipolar fate.

When each of them is overexpressed in retinal progenitor cells, they do not exert any effect on bipolar cell formation. Only when Chx10 is co-expressed with Mash1 or Math3 are more bipolar cells formed[44], indicating that commitment to a bipolar cell fate requires a combinatorial action of Chx10 and Mash1 or Math3 in progenitor cells.

In the mouse retina, there exist one type of rod bipolar cells and at least nine types of morphologically and physiologically distinct cone bipolar cells[46]. At present, it is entirely unclear how each of these subtype identities is specified but strides have been made to identify some of the intrinsic factors involved in their differentiation (Fig. 3). For instance, the bHLH factor Bhlhb4 is expressed by all developing rod bipolar cells and loss of *Bhlhb4* function causes a near complete loss of these cells due to a failure in their terminal differentiaton[47]. As for cone bipolar cells, two homeodomain factors Vsx1 and Irx5 are involved in the maturation of cone bipolar subtypes[48-50]. They are expressed by overlapping subsets of cone bipolar cells and have been found by gene targeting to constitute two distinct genetic pathways that together regulate the differentiation of Type 2 and Type 3 OFF cone bipolar cells[48-50].

Amacrine and horizontal cells. The specification of amacrine cells involves the concerted activities of Foxn4 and NeuroD1 or Math3 (Figs. 3,4). As discussed above, Foxn4 is required by retinal progenitors to establish the competence state for the generation of amacrine cells. It appears to do so in part by activating the expression of *Math3* and *NeuroD1*[21]. In compound knockout mice deficient for both *NeuroD1* and *Math3*, a complete loss of amacrine cells is accompanied by a fate-switch of progenitors to ganglion and Müller cells[51]; whereas, the formation of amacrine cells is essentially normal in single mutants null for either *NeuroD1* or *Math3*[30,51], indicating that *NeuroD1* and *Math3* are redundantly required for fate determination of amacrine cells. Overexpression experiments have shown that NeuroD1 alone or in combination with Pax6 is capable of promoting amacrine cell differentiation[30,51]. Similarly, Math3 together with Pax6 can promote amacrine cell differentiation but Math3 alone lacks this activity[51]. Therefore, besides its role in the establishment of progenitor multipotency, Pax6 may also be involved in the specification of amacrine cells even though it is not necessary for their generation. The absence of *Pax6* has been shown not to alter *NeuroD1* expression during retinogenesis[19], thereby allowing the differentiation of amacrine cells to occur. However, Pax6 and another homeodomain factor, Barhl2, are expressed by differentiating and mature amacrine cells and have been implicated as playing a role in the specification and/or differentiation of glycinergic amacrine cells[19,52] (Figs. 3,4). Despite our current knowledge of amacrine cell development, it remains to be determined what factors are involved in the specification of amacrine subtypes, numbered at least 26 in the mouse retina[53].

Only until recent 2-3 years have we begun to understand the intrinsic molecular basis of horizontal cell development. Similar to amacrine cells, Foxn4 is clearly required for the genesis of horizontal cells as retinas deficient for *Foxn4* fail to generate any of them[21]. However, contrary to its potent activity in the promotion of amacrine cell formation, Foxn4 is unable to promote horizontal

cell differentiation when overexpressed in progenitors[21]. Thus, Foxn4 is required only for competence acquisition but not for fate specification of horizontal cells. On the other hand, Prox1, a homeodomain factor expressed by a small set of progenitors, has been shown to act as a crucial intrinsic factor that controls fate commitment of this cell type[54] (Figs. 3,4). Targeted *Prox1* deletion results in loss of horizontal cells accompanied by a fate-switch of progenitors to rod and Müller cells while its overexpression strongly promotes a horizontal cell fate[54]. Foxn4 appears to confer retinal progenitors with the competence for a horizontal cell fate in part by directly or indirectly activating *Prox1* expression[21].

Ganglion cells. Two key intrinsic transcriptional regulators, Math5 and Brn3b, have been identified that control retinal ganglion cell development (Fig. 3). Math5 is a bHLH factor that is expressed by a subpopulation of progenitor cells and has been shown to confer progenitors with the competence for a ganglion cell fate[55-57]. Mouse retinas deficient for *Math5* fail to generate the great majority of ganglion cells and instead overproduce amacrine and cone cells[56,57]. Since not all progenitors expressing Math5 are committed to a ganglion cell fate as demonstrated in a lineage study, Math5 is thought to be essential only for competence acquisition but not for fate commitment to ganglion cells[58]. Thus, the intrinsic factor(s) that acts to specify ganglion cells from competent progenitors remains to be determined. Acting downstream from Math5, Brn3b is a POU-homeodomain transcription factor expressed by differentiating and mature ganglion cells[59-61]. Loss of *Brn3b* function leads to a loss of most ganglion cells as a result of inappropriate early and terminal differentiation while its overexpression can weakly promote ganglion cell differentiation[59,60,62-65].

Microarray and other analyses have determined the network of genes regulated by Math5 and Brn3b during ganglion cell development[58,60,66-68]. Both factors control the expression of a large set of downstream genes and the set downstream of Brn3b constitutes only one branch of the Math5 gene regulatory network[68]. Notably, Math5 is found to activate *Brn3b* expression in differentiating ganglion cells, which in turn activates the expression of *Brn3a* and *Brn3c*, two *Brn3b* homologs with a role redundant with that of *Brn3b* during ganglion cell differentiation[64,69-71]. The continued expression of *Brn3b* in differentiated ganglion cells appears to be maintained in part via feedback autoregulation and cross-activation by Brn3a and Brn3c[69] (Fig. 3). Wt1, a zinc-finger transcription factor encoded by the Wilms' tumor gene, appears to regulate ganglion cell differentiation also by directly activating *Brn3b* expression[72]. However, the relationship between Math5 and Wt1 is not clear at present.

Müller cells. Thus far, several transcription factors have been identified that influence the specification of Müller glial cells (Fig. 3). Hes1, Hes5 and Hesr2 are three closely related bHLH transcriptional repressors whose expression is found early in retinal progenitors but

later restricted to Müller cells[73-75]. When overexpressed in progenitor cells, all three factors strongly promote a Müller cell fate at the expense of neurons[73-75]. Conversely, loss of *Hes5* function causes a decrease in the production of Müller cells[74]. The paired-type homeodomain factor Rax

exhibits an expression pattern similar to that of the Hes factors and it too has a potent activity to induce Müller cell development[73]. Rax may promote Müller cell formation in part by directly activating *Hes1* gene expression[73].

Conclusion remarks

Recent years have witnessed rapid advances in our understanding of the intrinsic molecular mechanisms governing retinal cell type specification and differentiation. Molecular genetic studies involving loss-of-function and gain-of-function approaches have unraveled a number of transcription factors, in particular, several homeodomain and bHLH factors, as pivotal intrinsic regulators of mammalian retinogenesis. These findings have provided significant insights into the molecular bases that define progenitor competence states and program cell type specification. Despite these exciting advances, however, our understanding of the relationship between various intrinsic factors and the gene regulatory networks in which they participate still remain rudimentary. Continued microarray and other studies such chromatin immunoprecipitation promise to fill some of the knowledge gaps. Another issue is the intrinsic mechanism, of which little is known at present, that underlies subtype specification of different retinal cell classes. Given the extreme diversity of some cell classes such as amacrine and ganglion cells[2,53], the mechanism may prove to be exceedingly complex. For instance, there are at least 26 morphological subtypes of amacrine cells in the mouse retina[53]. Research aimed at a molecular elucidation of the mechanism that specifies subtype identities such as these will undoubtedly lead to many more exciting discoveries in years to come.

As a crucial part of our visual system, the retina is not only scientifically intriguing but also clinically important. Identification and functional characterization of transcription factors involved in retinal development have provided fundamental insights into the molecular mechanisms that govern mammalian neurogenesis. In the long term, understanding the regulatory molecules involved in retinogenesis may provide the foundation for improved treatment of some blinding diseases, since defects in these molecules are often associated with retinal disorders in both human and experimental animal models. In this regard, mutations in several transcription factors cause eye abnormalities as described above. Furthermore, given the potential applications of retinal stem cells in treating retinal degenerative diseases[76,77], it is clearly of considerable interest to understand the molecular basis of normal retinogenesis so that we may better manipulate stem cells to achieve controlled regeneration of desired retinal cell types as a possible stem cell-based therapy.

Acknowledgements

I thank Dr. Feng Qiu for his helpful comments on this manuscript. This work was supported by the National Institutes of Health and New Jersey Commission on Spinal Cord Research.

References

1. Vaney, D.I. Retinal neurons: cell types and coupled networks. Prog Brain Res, 2002, 136: 239-54
2. Wassle, H. & Boycott, B.B. Functional architecture of the mammalian retina. Physiol Rev, 1991, 71: 447-80
3. Sidman, R.L. Histogenesis of mouse retina studied with thymidine-3H, 1961, 487-506 Academic Press, New York
4. Young, R.W. Cell differentiation in the retina of the mouse. Anat Rec, 1985, 212: 199-205
5. Holt, C.E., Bertsch, T.W., Ellis, H.M. & Harris, W.A. Cellular determination in the Xenopus retina is independent of lineage and birth date. Neuron, 1988, 1: 15-26
6. Turner, D.L. & Cepko, C.L. A common progenitor for neurons and glia persists in rat retina late in development. Nature, 1987, 328: 131-6
7. Turner, D.L., Snyder, E.Y. & Cepko, C.L. Lineage-independent determination of cell type in the embryonic mouse retina. Neuron, 1990, 4: 833-45
8. Wetts, R. & Fraser, S.E. Multipotent precursors can give rise to all major cell types of the frog retina. Science, 1988, 239: 1142-5
9. Cepko, C.L. The roles of intrinsic and extrinsic cues and bHLH genes in the determination of retinal cell fates. Curr Opin Neurobiol, 1999, 9: 37-46
10. Cepko, C.L., Austin, C.P., Yang, X., Alexiades, M. & Ezzeddine, D. Cell fate determination in the vertebrate retina. Proc Natl Acad Sci USA, 1996, 93: 589-95
11. Livesey, F.J. & Cepko, C.L. Vertebrate neural cell-fate determination: lessons from the retina. Nat Rev Neurosci, 2001, 2: 109-18
12. James, J. In vitro generation of early-born neurons from late retinal progenitors. J Neurosci, 2003, 23: 8193-203
13. Cayouette, M., Barres, B.A. & Raff, M. Importance of intrinsic mechanisms in cell fate decisions in the developing rat retina. Neuron, 2003, 40: 897-904
14. Glaser, T. PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. Nat Genet, 1994, 7: 463-71
15. Glaser, T., Walton, D.S. & Maas, R.L. Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. Nat Genet, 1992, 2: 232-9
16. Hill, R.E. Mouse small eye results from mutations in a paired-like homeobox- containing gene. Nature, 1991, 354: 522-5
17. Jordan, T. The human PAX6 gene is mutated in two patients with aniridia. Nat Genet, 1992, 1: 328-32
18. Schedl, A. Influence of PAX6 gene dosage on development: overexpression causes severe eye abnormalities. Cell, 1996, 86: 71-82
19. Marquardt, T. Pax6 is required for the multipotent state of retinal progenitor cells. Cell, 2001, 105: 43-55
20. Akagi, T. Requirement of multiple basic helix-loop-helix genes for retinal neuronal subtype specification. J Biol Chem, 2004, 279: 28492-8

21. Li, S. Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron, 2004, 43: 795-807
22. Nishida, A. Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development. Nat Neurosci, 2003, 6: 1255-63
23. Furukawa, T., Morrow, E.M. & Cepko, C.L. Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. Cell, 1997, 91: 531-41
24. Furukawa, T., Morrow, E.M., Li, T., Davis, F.C. & Cepko, C.L. Retinopathy and attenuated circadian entrainment in Crx-deficient mice. Nat Genet, 1999, 23: 466-70
25. Livesey, F.J., Furukawa, T., Steffen, M.A., Church, G.M. & Cepko, C.L. Microarray analysis of the transcriptional network controlled by the photoreceptor homeobox gene Crx. Curr Biol , 2000, 10: 301-10
26. Freund, C.L. Cone-rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (CRX) essential for maintenance of the photoreceptor. Cell, 1997, 91: 543-53
27. Freund, C.L. De novo mutations in the CRX homeobox gene associated with Leber congenital amaurosis. Nat Genet, 1998, 18: 311-2
28. Sohocki, M.M. A range of clinical phenotypes associated with mutations in CRX, a photoreceptor transcription-factor gene. Am J Hum Genet, 1998, 63: 1307-15
29. Swain, P.K. Mutations in the cone-rod homeobox gene are associated with the cone- rod dystrophy photoreceptor degeneration. Neuron, 1997, 19: 1329-36
30. Morrow, E.M., Furukawa, T., Lee, J.E. & Cepko, C.L. NeuroD regulates multiple functions in the developing neural retina in rodent. Development, 1999, 126: 23-36
31. Pennesi, M.E. BETA2/NeuroD1 null mice: a new model for transcription factor-dependent photoreceptor degeneration. J Neurosci, 2003, 23: 453-61
32. Bessant, D.A. A mutation in NRL is associated with autosomal dominant retinitis pigmentosa. Nat Genet, 1999, 21: 355-6
33. Martinez-Gimeno, M. Mutations P51U and G122E in retinal transcription factor NRL associated with autosomal dominant and sporadic retinitis pigmentosa. Hum Mutat, 2001, 17: 520
34. Mears, A.J. Nrl is required for rod photoreceptor development. Nat Genet, 2001, 29: 447-52
35. Haider, N.B., Naggert, J.K. & Nishina, P.M. Excess cone cell proliferation due to lack of a functional NR2E3 causes retinal dysplasia and degeneration in rd7/rd7 mice. Hum Mol Genet,2001, 10: 1619-26
36. Akhmedov, N.B. A deletion in a photoreceptor-specific nuclear receptor mRNA causes retinal degeneration in the rd7 mouse. Proc Natl Acad Sci U S A, 2000, 97: 5551-6
37. Chen, J., Rattner, A. & Nathans, J. The rod photoreceptor-specific nuclear receptor Nr2e3 represses transcription of multiple cone-specific genes. J Neurosci, 2005, 25: 118-29
38. Corbo, J.C. & Cepko, C.L. A hybrid photoreceptor expressing both rod and cone genes in a mouse model of enhanced s-cone syndrome. PLoS Genet 1, 2005, e11

39. Peng, G.H., Ahmad, O., Ahmad, F., Liu, J. & Chen, S. The photoreceptor-specific nuclear receptor Nr2e3 interacts with Crx and exerts opposing effects on the transcription of rod versus cone genes. Hum Mol Genet, 2005, 14: 747-64
40. Yoshida, S. Expression profiling of the developing and mature Nrl-/- mouse retina: identification of retinal disease candidates and transcriptional regulatory targets of Nrl. Hum Mol Genet, 2004, 13: 1487-503
41. Mitton, K.P. The leucine zipper of NRL interacts with the CRX homeodomain. A possible mechanism of transcriptional synergy in rhodopsin regulation. J Biol Chem, 2005, 275: 29794-9
42. Ng, L. A thyroid hormone receptor that is required for the development of green cone photoreceptors. Nat Genet, 2001, 27: 94-8
43. Burmeister, M. Ocular retardation mouse caused by Chx10 homeobox null allele: impaired retinal progenitor proliferation and bipolar cell differentiation. Nat Genet, 1996, 12: 376-84
44. Hatakeyama, J., Tomita, K., Inoue, T. & Kageyama, R. Roles of homeobox and bHLH genes in specification of a retinal cell type. Development, 2001, 128: 1313-22
45. Tomita, K., Moriyoshi, K., Nakanishi, S., Guillemot, F. & Kageyama, R. Mammalian achaete-scute and atonal homologs regulate neuronal versus glial fate determination in the central nervous system. EMBO J, 2000, 19: 5460-72
46. Ghosh, K.K., Bujan, S., Haverkamp, S., Feigenspan, A. & Wassle, H. Types of bipolar cells in the mouse retina. J Comp Neurol, 2004, 469: 70-82
47. Bramblett, D.E., Pennesi, M.E., Wu, S.M. & Tsai, M.J. The transcription factor Bhlhb4 is required for rod bipolar cell maturation. Neuron, 2004, 43: 779-93
48. Cheng, C.W. The Iroquois homeobox gene, Irx5, is required for retinal cone bipolar cell development. Dev Biol, 2005, 287(1): 48-60
49. Chow, R.L. Control of late off-center cone bipolar cell differentiation and visual signaling by the homeobox gene Vsx1. Proc Natl Acad Sci USA, 2004, 101: 1754-9
50. Ohtoshi, A. Regulation of retinal cone bipolar cell differentiation and photopic vision by the CVC homeobox gene Vsx1. Curr Biol, 2004, 14: 530-6
51. Inoue, T. Math3 and NeuroD regulate amacrine cell fate specification in the retina. Development,2002, 129: 831-42
52. Mo, Z., Li, S., Yang, X. & Xiang, M. Role of the Barhl2 homeobox gene in the specification of glycinergic amacrine cells. Development, 2004, 131: 1607-18
53. MacNeil, M.A. & Masland, R.H. Extreme diversity among amacrine cells: implications for function. Neuron, 1998, 20: 971-82
54. Dyer, M.A., Livesey, F.J., Cepko, C.L. & Oliver, G. Prox1 function controls progenitor cell proliferation and horizontal cell genesis in the mammalian retina. Nat Genet, 2003, 34: 53-8
55. Brown, N.L. Math5 encodes a murine basic helix-loop-helix transcription factor expressed during early stages of retinal neurogenesis. Development, 1998, 125: 4821-33

56. Brown, N.L., Patel, S., Brzezinski, J. & Glaser, T. Math5 is required for retinal ganglion cell and optic nerve formation. Development, 2001, 128: 2497-508
57. Wang, S.W. Requirement for math5 in the development of retinal ganglion cells. Genes Dev, 2001, 15: 24-29
58. Yang, Z., Ding, K., Pan, L., Deng, M. & Gan, L. Math5 determines the competence state of retinal ganglion cell progenitors. Dev Biol, 2003, 264: 240-54
59. Gan, L. POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells. Proc Natl Acad Sci U S A, 1996, 93: 3920-5
60. Xiang, M. Requirement for Brn-3b in early differentiation of postmitotic retinal ganglion cell precursors. Dev Biol, 1998, 197: 155-69
61. Xiang, M. Brn-3b: a POU domain gene expressed in a subset of retinal ganglion cells. Neuron, 1993, 11: 689-701
62. Erkman, L. Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development. Nature, 1996, 381: 603-6
63. Gan, L., Wang, S.W., Huang, Z. & Klein, W.H. POU domain factor Brn-3b is essential for retinal ganglion cell differentiation and survival but not for initial cell fate specification. Dev Biol, 1999, 210: 469-80
64. Liu, W. All Brn3 genes can promote retinal ganglion cell differentiation in the chick. Development, 2000, 127: 3237-47
65. Wang, S.W., Gan, L., Martin, S.E. & Klein, W.H. Abnormal polarization and axon outgrowth in retinal ganglion cells lacking the POU-domain transcription factor Brn-3b. Mol Cell Neurosci, 2000, 16: 141-56
66. Erkman, L. A POU domain transcription factor-dependent program regulates axon pathfinding in the vertebrate visual system. Neuron, 2000, 28: 779-792
67. Mu, X. Discrete gene sets depend on POU domain transcription factor Brn3b/Brn-3.2/POU4f2 for their expression in the mouse embryonic retina. Development, 2004, 131: 1197-210
68. Mu, X. A gene network downstream of transcription factor Math5 regulates retinal progenitor cell competence and ganglion cell fate. Dev Biol, 2005, 280: 467-81
69. Liu, W., Mo, Z. & Xiang, M. The Ath5 proneural genes function upstream of Brn3 POU domain transcription factor genes to promote retinal ganglion cell development. Proc Natl Acad Sci USA, 2001, 98: 1649-54
70. Pan, L., Yang, Z., Feng, L. & Gan, L. Functional equivalence of Brn3 POU-domain transcription factors in mouse retinal neurogenesis. Development, 2005, 132: 703-12
71. Wang, S.W. Brn3b/Brn3c double knockout mice reveal an unsuspected role for Brn3c in retinal ganglion cell axon outgrowth. Development, 2002, 129: 467-77
72. Wagner, K.D. The Wilms' tumor gene Wt1 is required for normal development of the retina. EMBO J, 2002, 21: 1398-405
73. Furukawa, T., Mukherjee, S., Bao, Z.Z., Morrow, E.M. & Cepko, C.L. rax, Hes1, and notch1 promote the formation of Müller glia by postnatal retinal progenitor cells. Neuron, 2000, 26: 383-94
74. Hojo, M. Glial cell fate specification modulated by the bHLH gene Hes5 in mouse retina. Development, 2000, 127: 2515-22

75. Satow, T. The basic helix-loop-helix gene hesr2 promotes gliogenesis in mouse retina. J Neurosci, 2001, 21: 1265-73
76. Ikeda, H. Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. Proc Natl Acad Sci USA, 2005, 102: 11331-6
77. Tropepe, V. Retinal stem cells in the adult mammalian eye. Science, 2000, 287: 2032-6