Embryonic Retinal Progenitor Cells Are Heterogeneic with Respect to Proneural Transcription Factor Expression

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In all species that have been studied, the first cell type in the retina to differentiate is the retinal ganglion cell (RGC). We are specifically interested in understanding how these first neurons arise from the progenitor pool, since there is no predecessor neuron to influence their fate. The current model of retinogenesis proposes that the integration of extrinsic signals, transcriptional regulators and intercellular interactions are necessary for the differentiation of retinal progenitor cells into the various types of retinal neurons and glia.

Evidence suggests that individual members of the proneural basic helix-loop-helix (bHLH) family of transcriptional regulators are critical for the proper acquisition of particular retinal cellular identities, yet evidence also suggests that combinations of bHLH's are necessary for other cellular identities. We hypothesize that subsets of proneural genes are expressed within discrete cell types. We have used in situ hybridizations combined with subsequent immunolabeling for progenitor cells and RGCs to determine which members of the proneural bHLH transcription factors are expressed in mitotically active progenitor cells and/or differentiating RGCs.

Our results demonstrate that even early in retinal development, members of this family of transcription factors are differentially expressed, with some genes restricted to progenitor cells and some to newborn RGCs. Our results also suggest that the progenitor pool itself consists of subpopulations of progenitors that express different proneural bHLH genes. Finally, we have analyzed a previously uncharacterized member of this family, chicken acheate-achute homolog 3 (Cash3), during retinal development.

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Introduction

The retina is an excellent model system for exploring both molecular function and cellular interactions during neuronal development. For example, most of the factors that have been shown to regulate embryonic retinal neurogenesis have also been shown to be key regulators of neurogenesis in other regions of the central nervous system (CNS). Much is known about the type, timing, and ultimate pattern of cells generated during retinogenesis, and certain molecular pathways involved in these processes are being elucidated.[1–3] It has been postulated that the specific cell identities in the retina are determined in part by interactions among the progenitor cells and their previously generated progeny. Several factors have been identified that can direct progenitor cells to specific cell fates.[2,4] In addition to extracellular signaling molecules, there is evidence that specific transcription factors are also critical for the acquisition of particular retinal cell identities.[5,6] Thus, the current model of retinal development proposes that interplay between transcriptional regulators within cells and the interactions between neighboring cells are necessary for the differentiation of retinal progenitor cells into the various types of retinal neurons and glia. This also suggests that the population of the progenitor cell could be more diverse than previously thought.

Our hypothesis was that early in development, the pool of retinal progenitors exhibits diversity with respect to their expression of important regulatory gene families. We chose to investigate the proneural bHLH family of transcription factors, which are important intrinsic regulators.[7] There are five proneural bHLH genes important for retinal development: Cath5, NeuroM, Cash1, Ngn2, and NeuroD.[5,6] These genes have been implicated in influencing retinal development,[8] yet their exact functions remain unclear. We analyzed the expression of these genes at the beginning of the retinal development when the only first neurons and progenitor cells are developed, in order to examine if their expression patterns could contribute to progenitor cell diversity.

Methods

The method used to investigate how the proneural genes are expressed is called in situ hybridization, which detects the mRNA of a particular gene. First we incubated chicken embryos to the right stage, embryonic day E4.5. We windowed the eggs, injected them with a chemical called BrdU, and placed the eggs in the incubator for two hours. After two hours we collected, fixed, embedded in paraffin, and sectioned the embryos. Anti-sense DIG-labeled riboprobes were generated for each proneural gene, hybridized to the sections, detected via standard colorimetric reaction, and subsequently immunostained with BrdU and neurofilament (NF) to reveal progenitor cells and ganglion cells respectively.[9] Individual photographs of the in situs, the BrdU+ cells, and the NF+ cells were taken on a Zeiss microscope. Cash3 in situ's remain to be optimized and are not presented.

Data Analysis

Regions near the front of ganglion cell differentiation, which consist of

only progenitor cells and newborn ganglion cells, were analyzed to determine whether cells expressing a particular proneural gene were also BrdU or NF positive. bHLH+ cells were identified, marked, and counted. BrdU+ and NF+ cells were marked. Then each bHLH+ cell was assayed for BrdU or NF expression. Cell counts were compiled in Excel, and converted to percentage of cells expressing a particular bHLH and BrdU or NF.

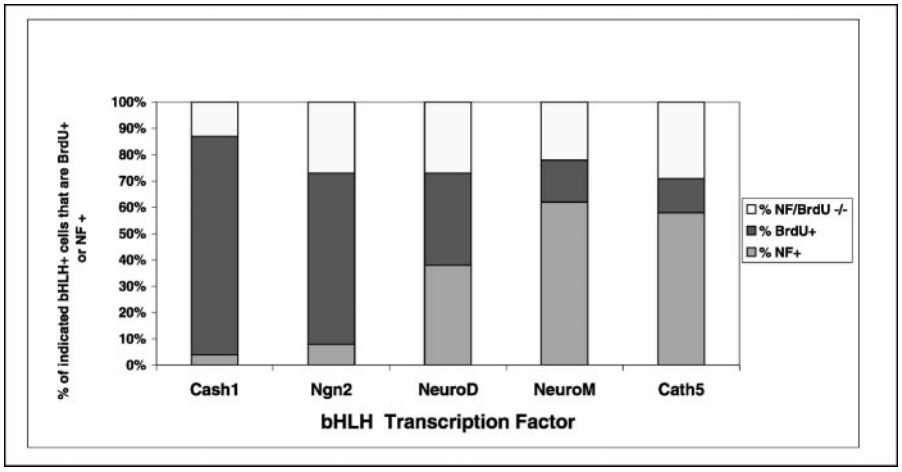

Fig 1. Relationship of proneural bHLH genes to progenitor cells and RGCs

RESULTS

Figure 1 compares proneural bHLH genes to progenitor cells and RGCs. Our results indicate that Cash1 and Ngn2 are predominately expressed in progenitor cells (most likely separate pools of progenitors based on their expression patterns), while NeuroM and Cath5 are predominately expressed in newborn and migrating ganglion cells. Interestingly NeuroD seems to fall into both of these groups.

CONCLUSION

Even early in eye development, retinal progenitor cells are a complex population with respect to expression of proneural transcription factors. These results might help explain how the different cell types in the retina arise through the actions of discrete subpopulations of progenitor cells that can generate different cell types.

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