Published in final edited form as:
Gene Expr Patterns. 2011 ; 11(5-6): 349–359. doi:10.1016/j.gep.2011.03.007.

Genes expressed in Atoh1 neuronal lineages arising from the r1/ isthmus rhombic lip

R Machold[1,*], **C Klein**[2,#], and **G Fishell**[2]

[1] Smilow Neuroscience Program, Department of Otolaryngology, NYU School of Medicine, New York, NY 10016

[2] Department of Cell Biology and Neural Science, NYU School of Medicine, New York, NY 10016

Abstract

During embryogenesis, the rhombic lip of the fourth ventricle is the germinal origin of a diverse collection of neuronal populations that ultimately reside in the brainstem and cerebellum. Rhombic lip neurogenesis requires the bHLH transcription factor Atoh1 (Math1), and commences shortly after neural tube closure (E9.5). Within the rhombomere 1 – isthmus region, the rhombic lip first produces brainstem and deep cerebellar neurons (E9.5-E12), followed by granule cell precursors after E12. While Atoh1 function is essential for all of these populations to be specified, the downstream genetic programs that confer specific properties to early and late born Atoh1 lineages are not well characterized. We have performed a comparative microarray analysis of gene expression within early and later born cohorts of Atoh1 expressing neural precursors purified from E14.5 embryos using a transgenic labeling strategy. We identify novel transcription factors, cell surface molecules, and cell cycle regulators within each pool of Atoh1 lineages that likely contribute to their distinct developmental trajectories and cell fates. In particular, our analysis reveals new insights into the genetic programs that regulate the specification and proliferation of granule cell precursors, the putative cell of origin for the majority of medulloblastomas.

Keywords

rhombic lip; Atoh1; Math1; cerebellum; neurogenesis; rhombomere 1

1. Introduction

Following neural tube closure (approximately embryonic day 9 in the mouse), neurogenesis in the mid-hindbrain region commences within ventricular zone progenitors through the action of intrinsic and extrinsic proneural signaling cues. Within the dorsal isthmic-rhombomere 1 territory, the rhombic lip of the fourth ventricle is located at the caudal boundary of the neuroepithelium, adjacent to the roof plate (Wingate, 2001). This unique neurogenic region gives rise to the glutamatergic cells of the cerebellum as well as specific brainstem nuclei (Gilthorpe et al., 2002; Hevner et al., 2006; Wingate and Hatten, 1999). Throughout the dorsal neural tube, rhombic lip neurogenesis requires the bHLH transcription factor Atoh1 (Math1)(Ben-Arie et al., 1997; Machold and Fishell, 2005; Rose

[*]to whom correspondence should be addressed: robert.machold@nyumc.org Phone: (212) 263-9140 Fax: (212) 263-9170.
[#]Present address: Neuroscience Discovery Research, Abbott GmbH & Co. KG, Ludwigshafen, Germany

et al., 2009; Wang et al., 2005), whose expression in neural progenitors is induced by roof plate derived BMP signaling activity (Alder et al., 1999; Machold et al., 2007; Qin et al., 2006; Timmer et al., 2002; Wine-Lee et al., 2004). Between E9 and E11, neurons born within the rhombic lip express Atoh1 transiently, are post-mitotic, and rapidly migrate into the brainstem, or develop as deep cerebellar neurons (Machold and Fishell, 2005; Rose et al., 2009). After approximately E12, the rhombic lip transitions to producing granule cell precursors (GCP) almost exclusively. Unlike earlier born rhombic lip lineages, GCP maintain expression of Atoh1, terminate their migration on the dorsal surface of the cerebellar primordium to form the external granule layer (EGL), and remain mitotic throughout the first few weeks of postnatal development. The genetic pathways that act in parallel with, or downstream of Atoh1 to specify early (E9-E11) and late (E12-E16) born rhombic lip lineages are not well characterized; thus, in order to gain insights into this developmental transition we performed an unbiased comparison of gene expression in these two pools of rhombic lip derived neurons, using a transgenic labeling methodology combined with microarray analysis.

To obtain selective labeling of Atoh1 expressing lineages arising from the rhombic lip at early and later embryonic stages, we utilized an *Atoh1*^{CreERT2} transgenic line generated and characterized by us previously (Machold and Fishell, 2005), in combination with a cre recombinase-dependent EGFP reporter line (*RCE*; Sousa et al., 2009). Within the early rhombic lip, Atoh1 expression is continuously induced in neural precursors that then rapidly migrate away over the dorsal surface of the cerebellar primordium. These early born populations down-regulate Atoh1 expression immediately after they emerge from the rhombic lip. Thus, a single dose of tamoxifen between E9.5 and E11.5 selectively labels the cohort of rhombic lip neural precursors that expresses high levels of Atoh1 during a temporal window of approximately 12–36 hours following tamoxifen administration to the pregnant transgenic dam (Machold and Fishell, 2005). The schematic in Figure 1 (top) summarizes the neuronal populations that arise during embryogenesis from the rhombic lip within rhombomere 1 and the isthmus: these include projections neurons of the dorsal lateral lemniscus (LL), parabigeminal (PBG), pedunculopontine tegmental (PPTg, or PTg), laterodorsal tegmental (LDTg), and lateral parabrachial (LPB) nuclei, in addition to glutamatergic deep cerebellar (DN) neurons, followed by granule cells (GC) and unipolar brush cells (UBC) after E12 (Englund et al., 2006; Machold and Fishell, 2005; Rose et al., 2009). While the expression of Atoh1 mRNA in early born rhombic lip lineages is restricted to the rhombic lip, as shown in Figure 1 (bottom left), the relative stability of β-galactosidase activity in the Atoh1^{LacZ} knock in mouse line (Ben-Arie et al., 2000; Machold and Fishell, 2005; Wang et al., 2005) can be used to illustrate the rapid migration of early born rhombic lip neurons into the brainstem by E12.5 (Figure 1). Two days later (E14.5), the specification of granule cell precursors (GCP) has resulted in the formation of the EGL. Thus, since E14.5 represents a stage where the majority of rhombic lip lineages have been specified, we chose this as our analysis stage to compare the early (E10.5–E11.5) and later (E13.5–E14.5) Atoh1 dependent genetic programs (Figure 1, lower right panel). Furthermore, the availability of the vast gene expression image collection generated from E14.5 embryos available at Genepaint greatly facilitated our analysis of the microarray results.

2. Results and discussion

2.1 Genes enriched in early born rhombic lip lineages

We first examined the putative transcription factors in E14.5 embryos that are enriched in r1/isthmic Atoh1 lineages labeled by E10.5 tamoxifen administration (‘E10.5 Tm labeled’) in comparison to labeling by E13.5 tamoxifen administration (‘E13.5 Tm labeled’). Figure 2 lists genes that were flagged as DNA binding or transcriptional regulators, from most

enriched to least. In general though, the fold enrichment value is highly sensitive to variability in background signal levels on the microarray chip and thus only provides an approximate measure of differential expression. Furthermore, a comparison of individual probes for the same gene often yielded different fold enrichment values, although the trend was generally consistent. Nevertheless, the overall basis for the observed enrichment was typically apparent upon visual inspection of the *in situ* hybridization data obtained from the public image database Genepaint (http://www.genepaint.org). The images shown in the figures were chosen based on both their availability and the relative specificity of their expression patterns. While Genepaint has its own rigorous internal quality control measures, we avoided genes whose expression appeared to be relatively ubiquitous, since these patterns could not easily be distinguished from background staining. Furthermore, we deliberately avoided genes that were broadly expressed in all post-mitotic neurons in favor of genes whose expression potentially indicated a more selective role in rhombic lip lineages. The reader is encouraged to examine the full set of images available online for their gene of interest by searching Genepaint using the Entrez gene ID number or the Genepaint image series ID (located in the lower left corner of each image panel).

We confirmed the expression of numerous transcription factor genes that had been previously identified in early born rhombic lip derived neurons, including *Lhx9* and *Meis2*. Interestingly, the latter had been identified as being expressed in deep cerebellar neurons (Morales and Hatten, 2006), but is also broadly expressed in other early born Atoh1-dependent lineages (Fig. 2). However, many of the transcription factors we identified as enriched in early rhombic lip neurons are well known from other developmental contexts but have not been characterized in rhombic lip brainstem lineages to date. The transcription factor gene *Nr4a2* (*Nurr1*) is essential for the development of midbrain dopaminergic neurons (Zetterstrom et al., 1997), but its expression at E14.5 indicates a role in other brainstem populations as well. *FoxP2*, a forkhead transcription factor gene that is important for the generation of vocalizations in humans and rodents (Vernes and Fisher, 2009) is broadly expressed in the cerebellar primordium and brainstem, but not in the EGL. The Iroquois homeobox gene family members *Irx3*, *Irx4*, and *Irx5* (Houweling et al., 2001) exhibit overlapping patterns of expression in the brainstem, with *Irx4* being largely restricted to the r1/isthmic region. Both bHLH genes *Olig1* and *Olig2* were enriched by virtue of their expression within deep cerebellar neurons. Likewise, the LIM domain transcription factor gene *Lmo3* was highly expressed in the latter population, with some expression in neighboring rhombic lip derived neurons. The transcription factor genes *Onecut1* and *Onecut2* are important for cell fate determination in the liver (Clotman et al., 2005), and show closely overlapping patterns of expression in brainstem rhombic lip derived lineages. Finally, of particular interest is the Hox-related gene *Evx1*, which is known to direct postmitotic cell fate determination in the spinal cord (Moran-Rivard et al., 2001), and exhibits a very restricted pattern of expression in the brainstem.

The cell surface protein genes whose expression is enriched in early rhombic lip lineages include members of well-known cell adhesion families (e.g., cadherins), as well as some surprising molecules identified previously in other contexts but that were not known to be expressed in the brain (Figure 3). The most striking example of this is *Npnt*, the gene encoding the secreted integrin ligand nephronectin (Brandenberger et al., 2001), whose expression within the E14.5 brain appears to be almost completely restricted to the rhombic lip-derived brainstem nuclei (and choroid plexus). Other enriched genes encoding cell surface molecules include *Unc5D*, which is known to be expressed in migratory multipolar cells of the developing neocortex (Sasaki et al., 2008), the extracellular matrix protein gene *Spon1* (f-spondin), and *Nxph1*, which encodes neurexophilin 1, a secreted binding partner for alpha neurexins. Among the cadherin family member genes, *Cdh4*, *Cdh9*, and *Pcdh7*

showed distinct and restricted patterns of expression within the brainstem, as did members of the semaphorin-plexin families (*Sema3c*, *Plxna1*, *Plxna2*).

2.2 Genes enriched in later born rhombic lip lineages (GCP)

Following early waves of neurogenesis between E9.5 and E12, the rhombic lip of the cerebellar primordium switches primarily to the production of granule cell precursors (GCP). Indeed, our labeling strategy (tamoxifen administration at E13.5) yielded almost exclusively GCP at E14.5 and virtually no labeling of earlier born rhombic lip lineages (illustrated in Figure 1, lower right panel). As expected, examination of the transcription factors enriched in GCP revealed a number of genes that had previously been identified to be important in GCP development. One distinctive change in GCPs compared to earlier born rhombic lip lineages is that the latter maintain high levels of *Atoh1* expression following their specification and migration (Figure 4, top right panel). We also confirmed enrichment of Shh signaling components (*Gli2*, *Gli3*), and *Pax6*, which has previously been shown to influence GCP development (Engelkamp et al., 1999), although the role of also- enriched family member *Pax3* has not yet been characterized. The homeobox protein gene *Otx2*, known to regulate the mid-hindbrain boundary at earlier embryonic stages (Broccoli et al., 1999) exhibits a specific expression pattern in GCP, although its selective removal in the mes/r1 region does not appear to affect the formation of mature granule cell neurons (Puelles et al., 2004). Other genes enriched in our microarray analysis that have been studied in the context of GCP include *Barhl1* (Bulfone et al., 2000), *Zic3* (Aruga et al., 1996), *Eomes* (Tbr2) (Fink et al., 2006), *NeuroD1* (Pan et al., 2009), *Nfia*, *Nfib* (Wang et al., 2007), and *Mxd3* (Yun et al., 2007).

In addition to the transcription factors described above that were known to be expressed in GCP, we identified a large number of novel transcription factor genes that have not been studied in GCP to date. Some of these exhibit unique patterns of expression within the EGL and embryonic brain in general. For example, *Uncx* (*Unc4.1*), a paired homeobox transcription factor gene, is expressed primarily within the medial EGL at E14.5 (Figure 4b), in addition to other scattered neuronal populations throughout the brain, including the olfactory epithelium where it regulates neural progenitor cell fate and proliferation (Sammeta et al., 2010). The forkhead domain transcription factor gene *FoxN2*, expressed dynamically in multiple embryonic tissues throughout development (Tribioli et al., 2002), shows a pronounced enrichment in GCP at E14.5. Interestingly, the transcription factor *Dach1*, also enriched in GCP, has been reported to compete with forkhead transcription factors in binding to their target promoters (Zhou et al., 2010).

One of the interesting questions in rhombic lip neurogenesis is how different lineages arise from a common proneural (Atoh1-dependent) pathway, and how extrinstic cues may direct the specific differentiation programs of early and later born populations. One candidate pathway is the Wnt signaling cascade, which is known to be critical for mid-hindbrain development at earlier developmental stages (Thomas and Capecchi, 1990). Strikingly, we found highly enriched expression of *Sp5* (Figure 4), whose expression is Wnt-responsive, and who encodes a transcription factor that likely represses targets of the transcription factor Sp1 (Weidinger et al., 2005). The Notch pathway has also been proposed to regulate GCP development (Solecki et al., 2001), although recent evidence suggests that canonical Notch signaling is not essential in this context (Julian et al., 2010). Interestingly, we found enriched expression of *Hes6* in GCP, which is consistent with its previous identification as a target of Atoh1 in inner ear hair cells (Scheffer et al., 2007). Hes6 negatively regulates canonical Notch signaling by antagonizing Hes1 and Hes5, and promotes neurogenic differentiation (Bae et al., 2000).

We found that a large proportion of GCP-enriched transcription factors, and genes in general, were involved in cell cycle regulation, which is not surprising given that GCP remain mitotic throughout the early postnatal development of the cerebellum, whereas early born rhombic lip lineages become post-mitotic immediately following their specification. Some GCP enriched transcription factors such as Insm1 have been shown to regulate the formation of basal (i.e., intermediate or SVZ – subventricular zone) progenitors in the cortex (Farkas et al., 2008), which also express *Eomes* (Tbr2). In general, a number of GCP-enriched genes also exhibited an SVZ-like expression pattern in the embryonic cortex, including *Etv5*, *Insm1*, *Srebf1*, *Akna*, *Tmpo*, *Tfdp2*, *Eomes*, *Nhlh1*, *Mxd3*, *NeuroD1*, and *Hmga1* (Figure 4). Thus, based on gene expression, the external granule layer appears to share many of the genetic programs that regulate amplification of neuronal lineages within the cortical SVZ. Interestingly, dysregulation of one or more of these genetic pathways is commonly observed in medulloblastomas (De Smaele et al., 2008), consistent with the proposed GCP origin of many of these tumors.

In addition to transcription factor genes, we found that many of the enriched genes in GCP encoded cyclins (*Ccnb1*, *Ccnd2*, *Ccne2*, *Ccnd1*, *Ccnb2*, *Ccnd3*, *Ccnf*), cell division cycle associated proteins (*Cdca5*, *Cdc2a*, *Cdca2*, *Cdca7*, *Cdc6*, *Cdc20*, *Cdca8*, *Cdca3*, *Cdc45l*, *Cdca7l*), cyclin-dependent kinases (*Cdk4*, *Cdk2*), and mini-chromosome maintenance proteins (*Mcm2*, *Mcm6*, *Mcm5*, *Mcm4*, *Mcm7*, *Mcm10*), among other genes that regulate cell division dynamics, such as *Mki67*, *Aurka*, *Aurkb*, *Hmgb2*, *Smc2*, *Birc5*, and *Prc1* (Figures 5, 5b, 5c, 5d). We also observed enrichment of proto-oncogenes such as *Smo* and *Gas1* (Shh signaling pathway), *Mycn*, *Rb1* (and binding partners encoded by *E2f1*, *E2f3* and *E2f6*), *Brca2*, *Atm*, and *Trp53*, all of which have been implicated in the development of medulloblastoma (Dubuc et al., 2010). Also of interest is the enrichment of genes that regulate sister chromatid cohesion (e.g., *Dscc1*, *Sgol1*, *Esco2*) and of the spindle assembly checkpoint (e.g., *Bub1b*, *Spag5*, *Bub1*, *Spc25*, *Mad2l1*).

As is evident from the E14.5 Atoh1^{LacZ} field shown in Figure 1, GCP cease migration and form a compact EGL immediately following their specification in the rhombic lip, in contrast to earlier born rhombic lip lineages that migrate into the brainstem and deep cerebellar nuclei. We examined the genes encoding cell surface proteins that were enriched in GCP and found a number of interesting candidates not reported previously that could potentially regulate cell adhesion or responsiveness to extracellular cues (Figure 6). *Mfap4* was identified as the gene responsible for Smith-Magenis syndrome, and encodes an extracellular matrix protein that likely serves as a ligand for cell surface expressed integrins (Zhao et al., 1995). Both *Cxcr4* (Zou et al., 1998) and *Unc5c* (Ackerman et al., 1997) genes have been previously shown to be important for cerebellar development, and both were found here to exhibit enriched expression in GCP. In addition, we found enrichment of *EphA3* and *EphA5*, consistent with a proposed role for Ephrin signaling in cerebellar development (Karam et al., 2000). Semaphorin-plexin signaling has also been identified as important for regulating granule cell migration (Renaud et al., 2008), and accordingly we found enrichment of *sema7a*, *Plxnd1*, *Plxnb2*, and *sema6d*, in addition to *sema6a*, whose enrichment in GCP had been reported previously (Kerjan et al., 2005). The diverse cadherin and protocadherin gene families are well known for their roles in nervous system development, and accordingly we found several members of the protocadherin family to be enriched in GCP, including *Pcdh18* and *Pcdh21*. Other cell surface protein genes that were enriched in GCP, such as *Sned1*, *Pde2a*, and *Mpdz* have not been well characterized with regard to brain development to date. The enrichment of genes encoding insulin-like growth factor binding proteins (*Igfbpl1*, *Igfbp5*) is interesting considering that the IGF pathway modulates GCP proliferation that is stimulated by the secreted morphogen Shh (Fernandez et al., 2010). Finally, of interest is the enriched expression of *Fgfr4*, which encodes one of the receptors for fibroblast growth factors. *Fgfr4*, and *Sned1* as well, exhibit a strikingly

restricted expression pattern within the rhombic lip progenitor zone in addition to their expression in GCP (Figure 6).

A small number of GCP enriched genes encoded secreted molecules (Figure 7), such as Reln, which has been shown previously to regulate cerebellar development (D'Arcangelo et al., 1997). We also found enriched expression of *Pdgfc*, which encodes a signaling molecule that was previously found to be highly expressed in medulloblastoma (Whelan et al., 1989), as well as *Gdnf*, whose expression affects GCP survival (Subramaniam et al., 2008). Finally, we examined the expression of genes encoding calcium-binding proteins that were enriched in GCP (Figure 8), and surprisingly, found high levels of *Pvalb* (parvalbumin) expression at E14.5. Parvalbumin is a small calcium binding protein known to be strongly expressed in Purkinje neurons (visibly emerging from the ventricular zone at this stage) as well as stellate and basket cells, but it also appears to be expressed transiently at high levels in GCP during their embryonic development. Several other calcium binding protein genes showed enriched expression in GCP, such as *Rcn1* and *Rcn3*, suggesting that calcium homeostasis may play an important role in GCP development.

3. Experimental Procedures

To isolate cells arising from Atoh1 (Math1) expressing rhombic lip lineages for analysis, we crossed a *Atoh1*^{CreERT2} transgenic line (Machold and Fishell, 2005) with a reporter line (*RCE*) that expresses EGFP from the ubiquitously expressed Rosa locus upon cre dependent removal of a transcriptional stop sequence flanked by loxP sites (Sousa et al., 2009). To activate the CreER^{T2} expressed within Atoh1-expressing lineages in transgenic embryos, tamoxifen (4 mg/30g; Sigma) was administered by oral gavage to pregnant females at either E10.5 or E13.5, and the resulting EGFP labeled transgenic embryos (*Atoh1*^{CreERT2}*; RCE*) were collected at E14.5 and separated from unlabeled littermates by visual inspection under UV light for EGFP fluorescence. The rhombomere 1- isthmus region of the neural tube was then dissected (see Figure 1), and a single cell suspension prepared as described previously (Batista-Brito et al., 2008). Briefly, following removal of the meninges, tissue from 3–4 embryos was pooled, minced, and dissociated in Hank's Balanced Salt Solution (HBSS; Gibco) supplemented with 20 U/mL papain and 2000 U/mL DNase I (Worthington) at 37°C for 30 minutes. Trituration was performed with fire polished glass pipets to complete the dissociation, which was terminated by addition of normal horse serum to 1%. FACS purification of EGFP$^+$ cells was performed at the NYU Cancer Institute Flow Cytometry and Cell Sorting facility using a Beckman-Coulter MoFlo cell sorter to sort positive cells into 1.5 mL tubes containing HBSS. Typical percentages of EGFP$^+$ cells were 0.2–0.5% for E10.5 tamoxifen labeled tissue, and 2–3% for E13.5 tamoxifen labeled tissue. At least 5000 EGFP$^+$ cells were collected for each replicate, and once sorted, cells were pelleted by centrifugation and frozen on dry ice prior to delivery to the Genomics Core Laboratory at the Sloan-Kettering Institute for RNA extraction and microarray analysis using Affymetrix MOE 430 2.0 chips (three replicates for each labeling stage).

The CEL files obtained from the microarray experiments were normalized and further analyzed using Genespring GX11 software (Agilent). Differentially expressed genes across the two pools of data were identified by unpaired t-test with Benjamini-Hochberg multiple testing correction, yielding 5101 of 38908 entities with $p<0.05$. Further criteria included a cut-off in fold change of 2, reducing the entity list to 3142 genes. All gene expression images shown in the figures were selected from Genepaint image collections (http://www.genepaint.org), and the reader is encouraged to examine the full set of images available for their gene(s) of interest using the Entrez Gene numerical identifier listed in the figure table or the Genepaint image series ID number provided in the lower left hand corner of each image. Images of genes highlighted in the text and figures were downloaded from

Genepaint.org as jpegs, and cropped, rotated and contrasted in Photoshop (Adobe). Original CEL files and MIAME 2.0 compliant data from the microarray analysis are available at the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under the study number GSE26355.

References

Ackerman SL, Kozak LP, Przyborski SA, Rund LA, Boyer BB, Knowles BB. The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. Nature. 1997; 386:838–842. [PubMed: 9126743]

Alder J, Lee KJ, Jessell TM, Hatten ME. Generation of cerebellar granule neurons in vivo by transplantation of BMP-treated neural progenitor cells. Nat Neurosci. 1999; 2:535–540. [PubMed: 10448218]

Aruga J, Nagai T, Tokuyama T, Hayashizaki Y, Okazaki Y, Chapman VM, Mikoshiba K. The mouse zic gene family. Homologues of the Drosophila pair-rule gene odd-paired. J Biol Chem. 1996; 271:1043–1047. [PubMed: 8557628]

Bae S, Bessho Y, Hojo M, Kageyama R. The bHLH gene Hes6, an inhibitor of Hes1, promotes neuronal differentiation. Development. 2000; 127:2933–2943. [PubMed: 10851137]

Batista-Brito R, Machold R, Klein C, Fishell G. Gene expression in cortical interneuron precursors is prescient of their mature function. Cereb Cortex. 2008; 18:2306–2317. [PubMed: 18250082]

Ben-Arie N, Bellen HJ, Armstrong DL, McCall AE, Gordadze PR, Guo Q, Matzuk MM, Zoghbi HY. Math1 is essential for genesis of cerebellar granule neurons. Nature. 1997; 390:169–172. [PubMed: 9367153]

Ben-Arie N, Hassan BA, Bermingham NA, Malicki DM, Armstrong D, Matzuk M, Bellen HJ, Zoghbi HY. Functional conservation of atonal and Math1 in the CNS and PNS. Development. 2000; 127:1039–1048. [PubMed: 10662643]

Brandenberger R, Schmidt A, Linton J, Wang D, Backus C, Denda S, Muller U, Reichardt LF. Identification and characterization of a novel extracellular matrix protein nephronectin that is associated with integrin alpha8beta1 in the embryonic kidney. J Cell Biol. 2001; 154:447–458. [PubMed: 11470831]

Broccoli V, Boncinelli E, Wurst W. The caudal limit of Otx2 expression positions the isthmic organizer. Nature. 1999; 401:164–168. [PubMed: 10490025]

Bulfone A, Menguzzato E, Broccoli V, Marchitiello A, Gattuso C, Mariani M, Consalez GG, Martinez S, Ballabio A, Banfi S. Barhl1, a gene belonging to a new subfamily of mammalian homeobox genes, is expressed in migrating neurons of the CNS. Hum Mol Genet. 2000; 9:1443–1452. [PubMed: 10814725]

Clotman F, Jacquemin P, Plumb-Rudewiez N, Pierreux CE, Van der Smissen P, Dietz HC, Courtoy PJ, Rousseau GG, Lemaigre FP. Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. Genes Dev. 2005; 19:1849–1854. [PubMed: 16103213]

D'Arcangelo G, Nakajima K, Miyata T, Ogawa M, Mikoshiba K, Curran T. Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody. J Neurosci. 1997; 17:23–31. [PubMed: 8987733]

De Smaele E, Fragomeli C, Ferretti E, Pelloni M, Po A, Canettieri G, Coni S, Di Marcotullio L, Greco A, Moretti M, Di Rocco C, Pazzaglia S, Maroder M, Screpanti I, Giannini G, Gulino A. An integrated approach identifies Nhlh1 and Insm1 as Sonic Hedgehog-regulated genes in developing cerebellum and medulloblastoma. Neoplasia. 2008; 10:89–98. [PubMed: 18231642]

Dubuc AM, Northcott PA, Mack S, Witt H, Pfister S, Taylor MD. The genetics of pediatric brain tumors. Curr Neurol Neurosci Rep. 2010; 10:215–223. [PubMed: 20425037]

Engelkamp D, Rashbass P, Seawright A, van Heyningen V. Role of Pax6 in development of the cerebellar system. Development. 1999; 126:3585–3596. [PubMed: 10409504]

Englund C, Kowalczyk T, Daza RA, Dagan A, Lau C, Rose MF, Hevner RF. Unipolar brush cells of the cerebellum are produced in the rhombic lip and migrate through developing white matter. J Neurosci. 2006; 26:9184–9195. [PubMed: 16957075]

Farkas LM, Haffner C, Giger T, Khaitovich P, Nowick K, Birchmeier C, Paabo S, Huttner WB. Insulinoma-associated 1 has a panneurogenic role and promotes the generation and expansion of basal progenitors in the developing mouse neocortex. Neuron. 2008; 60:40–55. [PubMed: 18940587]

Fernandez C, Tatard VM, Bertrand N, Dahmane N. Differential modulation of Sonic-hedgehog-induced cerebellar granule cell precursor proliferation by the IGF signaling network. Dev Neurosci. 2010; 32:59–70. [PubMed: 20389077]

Fink AJ, Englund C, Daza RA, Pham D, Lau C, Nivison M, Kowalczyk T, Hevner RF. Development of the deep cerebellar nuclei: transcription factors and cell migration from the rhombic lip. J Neurosci. 2006; 26:3066–3076. [PubMed: 16540585]

Gilthorpe JD, Papantoniou EK, Chedotal A, Lumsden A, Wingate RJ. The migration of cerebellar rhombic lip derivatives. Development. 2002; 129:4719–4728. [PubMed: 12361964]

Hevner RF, Hodge RD, Daza RA, Englund C. Transcription factors in glutamatergic neurogenesis: conserved programs in neocortex, cerebellum, and adult hippocampus. Neurosci Res. 2006; 55:223–233. [PubMed: 16621079]

Houweling AC, Dildrop R, Peters T, Mummenhoff J, Moorman AF, Ruther U, Christoffels VM. Gene and cluster-specific expression of the Iroquois family members during mouse development. Mech Dev. 2001; 107:169–174. [PubMed: 11520674]

Julian E, Hallahan AR, Wainwright BJ. RBP-J is not required for granule neuron progenitor development and medulloblastoma initiated by Hedgehog pathway activation in the external germinal layer. Neural Dev. 2010; 5:27. [PubMed: 20950430]

Karam SD, Burrows RC, Logan C, Koblar S, Pasquale EB, Bothwell M. Eph receptors and ephrins in the developing chick cerebellum: relationship to sagittal patterning and granule cell migration. J Neurosci. 2000; 20:6488–6500. [PubMed: 10964955]

Kerjan G, Dolan J, Haumaitre C, Schneider-Maunoury S, Fujisawa H, Mitchell KJ, Chedotal A. The transmembrane semaphorin Sema6A controls cerebellar granule cell migration. Nat Neurosci. 2005; 8:1516–1524. [PubMed: 16205717]

Machold R, Fishell G. Math1 is expressed in temporally discrete pools of cerebellar rhombic-lip neural progenitors. Neuron. 2005; 48:17–24. [PubMed: 16202705]

Machold RP, Kittell DJ, Fishell GJ. Antagonism between Notch and bone morphogenetic protein receptor signaling regulates neurogenesis in the cerebellar rhombic lip. Neural Dev. 2007; 2:5. [PubMed: 17319963]

Morales D, Hatten ME. Molecular markers of neuronal progenitors in the embryonic cerebellar anlage. J Neurosci. 2006; 26:12226–12236. [PubMed: 17122047]

Moran-Rivard L, Kagawa T, Saueressig H, Gross MK, Burrill J, Goulding M. Evx1 is a postmitotic determinant of v0 interneuron identity in the spinal cord. Neuron. 2001; 29:385–399. [PubMed: 11239430]

Pan N, Jahan I, Lee JE, Fritzsch B. Defects in the cerebella of conditional Neurod1 null mice correlate with effective Tg(Atoh1-cre) recombination and granule cell requirements for Neurod1 for differentiation. Cell Tissue Res. 2009; 337:407–428. [PubMed: 19609565]

Puelles E, Annino A, Tuorto F, Usiello A, Acampora D, Czerny T, Brodski C, Ang SL, Wurst W, Simeone A. Otx2 regulates the extent, identity and fate of neuronal progenitor domains in the ventral midbrain. Development. 2004; 131:2037–2048. [PubMed: 15105370]

Qin L, Wine-Lee L, Ahn KJ, Crenshaw EB 3rd. Genetic analyses demonstrate that bone morphogenetic protein signaling is required for embryonic cerebellar development. J Neurosci. 2006; 26:1896–1905. [PubMed: 16481421]

Renaud J, Kerjan G, Sumita I, Zagar Y, Georget V, Kim D, Fouquet C, Suda K, Sanbo M, Suto F, Ackerman SL, Mitchell KJ, Fujisawa H, Chedotal A. Plexin-A2 and its ligand, Sema6A, control nucleus-centrosome coupling in m igrating granule cells. Nat Neurosci. 2008; 11:440–449. [PubMed: 18327254]

Rose MF, Ahmad KA, Thaler C, Zoghbi HY. Excitatory neurons of the proprioceptive, interoceptive, and arousal hindbrain networks share a developmental requirement for Math1. Proc Natl Acad Sci U S A. 2009; 106:22462–22467. [PubMed: 20080794]

Sammeta N, Hardin DL, McClintock TS. Uncx regulates proliferation of neural progenitor cells and neuronal survival in the olfactory epithelium. Mol Cell Neurosci. 2010; 45:398–407. [PubMed: 20692344]

Sasaki S, Tabata H, Tachikawa K, Nakajima K. The cortical subventricular zone-specific molecule Svet1 is part of the nuclear RNA coded by the putative netrin receptor gene Unc5d and is expressed in multipolar migrating cells. Mol Cell Neurosci. 2008; 38:474–483. [PubMed: 18547816]

Scheffer D, Sage C, Corey DP, Pingault V. Gene expression profiling identifies Hes6 as a transcriptional target of ATOH1 in cochlear hair cells. FEBS Lett. 2007; 581:4651–4656. [PubMed: 17826772]

Solecki DJ, Liu XL, Tomoda T, Fang Y, Hatten ME. Activated Notch2 signaling inhibits differentiation of cerebellar granule neuron precursors by maintaining proliferation. Neuron. 2001; 31:557–568. [PubMed: 11545715]

Sousa VH, Miyoshi G, Hjerling-Leffler J, Karayannis T, Fishell G. Characterization of Nkx6-2-derived neocortical interneuron lineages. Cereb Cortex. 2009; 19(Suppl 1):i1–10. [PubMed: 19363146]

Subramaniam S, Strelau J, Unsicker K. GDNF prevents TGF-beta-induced damage of the plasma membrane in cerebellar granule neurons by suppressing activation of p38-MAPK via the phosphatidylinositol 3-kinase pathway. Cell Tissue Res. 2008; 331:373–383. [PubMed: 18071753]

Thomas KR, Capecchi MR. Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. Nature. 1990; 346:847–850. [PubMed: 2202907]

Timmer JR, Wang C, Niswander L. BMP signaling patterns the dorsal and intermediate neural tube via regulation of homeobox and helix-loop-helix transcription factors. Development. 2002; 129:2459–2472. [PubMed: 11973277]

Tribioli C, Robledo RF, Lufkin T. The murine fork head gene Foxn2 is expressed in craniofacial, limb, CNS and somitic tissues during embryogenesis. Mech Dev. 2002; 118:161–163. [PubMed: 12351180]

Vernes SC, Fisher SE. Unravelling neurogenetic networks implicated in developmental language disorders. Biochem Soc Trans. 2009; 37:1263–1269. [PubMed: 19909259]

Wang VY, Rose MF, Zoghbi HY. Math1 expression redefines the rhombic lip derivatives and reveals novel lineages within the brainstem and cerebellum. Neuron. 2005; 48:31–43. [PubMed: 16202707]

Wang W, Mullikin-Kilpatrick D, Crandall JE, Gronostajski RM, Litwack ED, Kilpatrick DL. Nuclear factor I coordinates multiple phases of cerebellar granule cell development via regulation of cell adhesion molecules. J Neurosci. 2007; 27:6115–6127. [PubMed: 17553984]

Weidinger G, Thorpe CJ, Wuennenberg-Stapleton K, Ngai J, Moon RT. The Sp1-related transcription factors sp5 and sp5-like act downstream of Wnt/beta- catenin signaling in mesoderm and neuroectoderm patterning. Curr Biol. 2005; 15:489–500. [PubMed: 15797017]

Whelan HT, Nelson DB, Strother D, Przybylski C, Figge G, Mamandi A. Medulloblastoma cell line secretes platelet-derived growth factor. Pediatr Neurol. 1989; 5:347–352. [PubMed: 2604798]

Wine-Lee L, Ahn KJ, Richardson RD, Mishina Y, Lyons KM, Crenshaw EB 3rd. Signaling through BMP type 1 receptors is required for development of interneuron cell types in the dorsal spinal cord. Development. 2004; 131:5393–5403. [PubMed: 15469980]

Wingate RJ. The rhombic lip and early cerebellar development. Curr Opin Neurobiol. 2001; 11:82–88. [PubMed: 11179876]

Wingate RJ, Hatten ME. The roe of the rhombic lip in avian cerebellum development. Development. 1999; 126:4395–4404. [PubMed: 10498676]

Yun JS, Rust JM, Ishimaru T, Diaz E. A novel role of the Mad family member Mad3 in cerebellar granule neuron precursor proliferation. Mol Cell Biol. 2007; 27:8178–8189. [PubMed: 17893326]

Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in Nurr1-deficient mice. Science. 1997; 276:248–250. [PubMed: 9092472]

Zhao Z, Lee CC, Jiralerspong S, Juyal RC, Lu F, Baldini A, Greenberg F, Caskey CT, Patel PI. The gene for a human microfibril-associated glycoprotein is commonly deleted in Smith-Magenis syndrome patients. Hum Mol Genet. 1995; 4:589–597. [PubMed: 7633408]

Zhou J, Wang C, Wang Z, Dampier W, Wu K, Casimiro MC, Chepelev I, Popov VM, Quong A, Tozeren A, Zhao K, Lisanti MP, Pestell RG. Attenuation of Forkhead signaling by the retinal determination factor DACH1. Proc Natl Acad Sci U S A. 2010; 107:6864–6869. [PubMed: 20351289]

Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature. 1998; 393:595–599. [PubMed: 9634238]

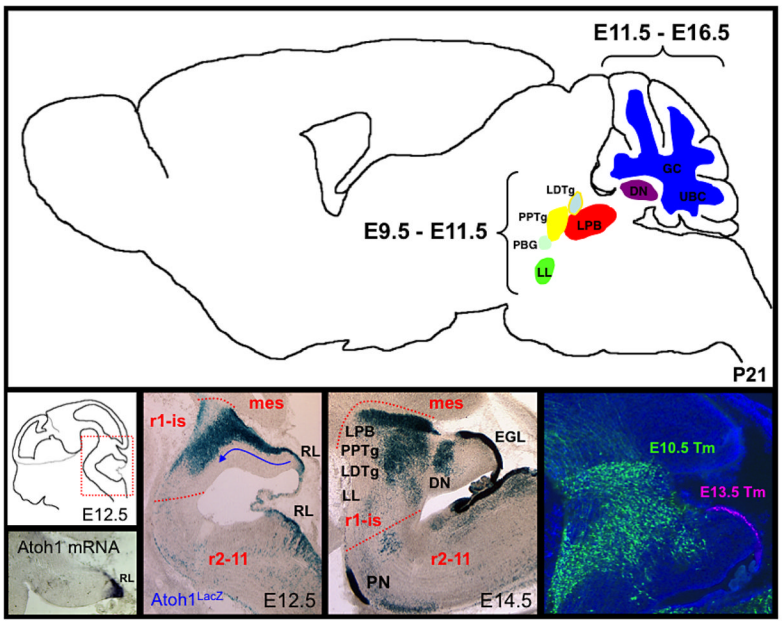


Figure 1.

Neurogenesis within the rhombic lip of the rhombomere 1 – isthmus region. (top) Atoh1 (Math1) dependent lineages that arise in the rhombic lip throughout embryogenesis. *Atoh1* mRNA expression is largely restricted to the rhombic lip prior to the emergence of granule cell precursors at around E12.5 (lower left panel). Sustained β-galactosidase activity in *Atoh1*^{LacZ} embryos illustrates the distinct fates and migration patterns of rhombic lip lineages emerging between E9.5 and E14.5 (bottom middle panels). Selective labeling of early (E10.5 Tm; green) or later (E13.5 Tm; pseudocolored red) born Atoh1 expressing lineages was achieved using a transgenic *Atoh1*^{CreERT2} approach (bottom right panel).

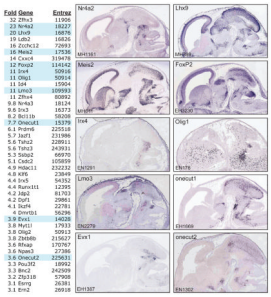


Figure 2.

Transcription factor genes enriched in early born rhombic lip lineages. The table on the left lists the genes in descending order of fold enrichment (E10.5 vs. E13.5 Tm labeled populations), along with their Entrez Gene ID number. Highlighted genes in the list are shown in the panels to the right (images are from Genepaint.org, and the image series ID is shown in the left hard corner of each field).

Fold	Gene	Entrez
30	Npnt	114249
27	Chl1	12661
25	Unc5d	210801
22	Spon1	233744
19	Nxph1	18231
18	Cntnap2	66797
17	Pcdh10	18526
16	Lrrc4c	241568
16	Negr1	320840
9.7	Dpp10	269109
9.5	Cd200	17470
9.5	Sema3c	20348
9.4	Pcdh19	279653
9	Ctxn2	381418
8.9	Lrrtm4	243499
8.7	Nrsn1	22360
8.7	Lrrtm3	216028
7.7	Cadm2	239857
7.6	Cntn4	269784
7.4	Epha4	13838
7	Sema4a	20351
6.1	Dscam	13508
5.8	Lrrtm1	74342
5.1	Nrxn3	18191
4.9	Cdh4	12561
4.9	Ncam2	17968
4.8	Ajap1	230959
4.8	Cdh9	12565
4.8	Pcdh7	54216
4.8	Lsamp	268890
4.5	Amigo2	105827
4.5	Ncam1	17967
4.5	Pcdh9	211712
4.4	Nrsn2	228777
4.4	Fblim1	74202
3.9	Ephb1	270190
3.7	Plxna1	18844
3.7	Plxna2	18845
3.5	Nrxn1	18189
3.4	Slitrk1	76965
3.3	L1cam	16728
3.1	Sema4f	20355
3	Cntnap4	170571

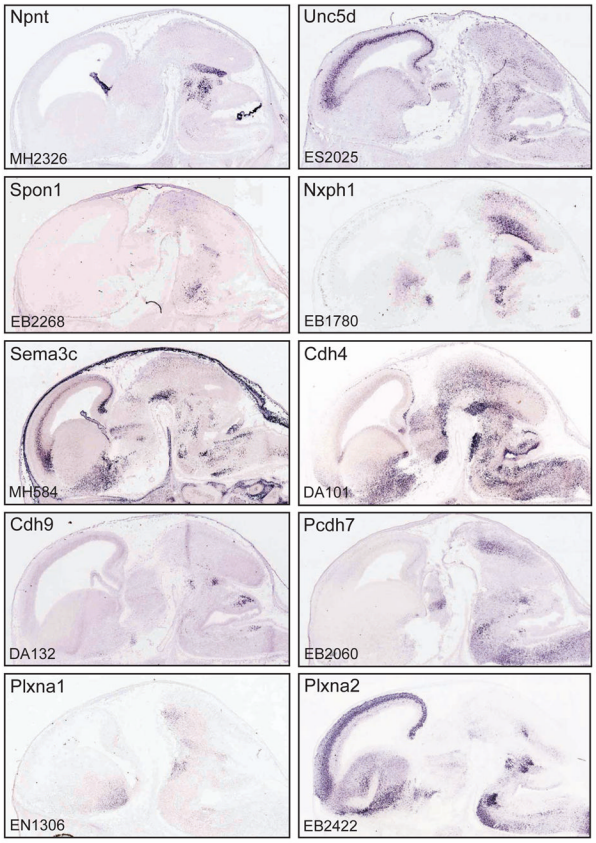


Figure 3.

Cell surface protein genes enriched in early born rhombic lip lineages. The table on the left lists the genes in descending order of fold enrichment (E10.5 vs. E13.5 Tm labeled populations), along with their Entrez Gene ID number. Highlighted genes in the list are shown in the panels to the right (images are from Genepaint.org, and the image series ID is shown in the left hard corner of each field).

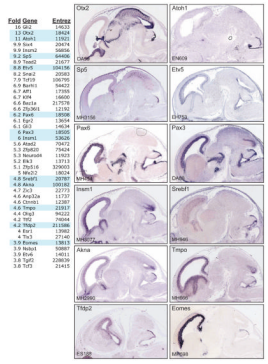


Figure 4b

Fold	Gene	Entrez
3.7	Tgif1	21815
3.7	Hes6	55927
3.6	Etv1	14009
3.6	Nhlh1	18071
3.6	Nfib	18028
3.6	Nfia	18027
3.6	Mxd3	17121
3.4	Hey1	15213
3.4	Six5	20475
3.3	Bhlhe22	59058
3.3	Hbp1	73389
3.3	Uncx	22255
3.2	Sp1	20683
3.1	Nfix	18032
3	Elk4	13714
3	Cbx2	12416
3	Rfc1	19687
2.9	Zeb1	21417
2.9	Stat3	20848
2.7	Ezh2	14056
2.4	Neurod1	18012
2.3	Foxn2	14236
2.1	Dach1	13134
2	Hmga1	15361

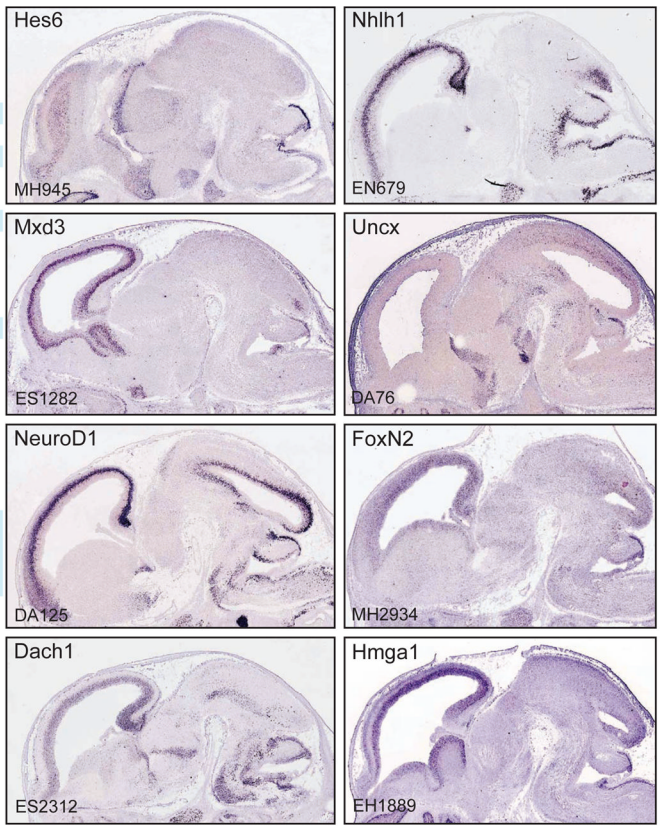
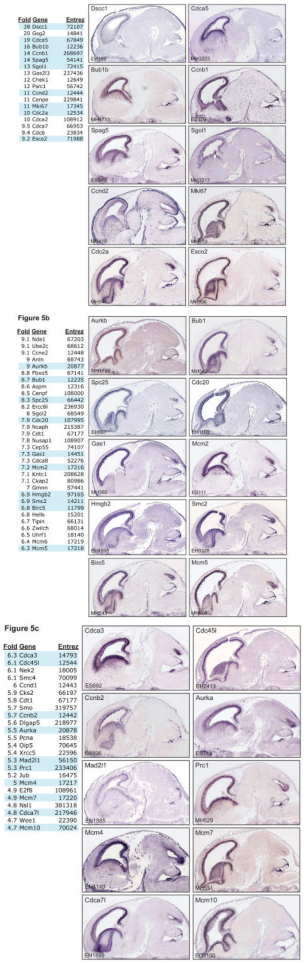


Figure 4.

Transcription factor genes enriched in granule cell precursors. The table on the left lists the genes in descending order of fold enrichment (E13.5 vs. E10.5 Tm labeled populations), along with their Entrez Gene ID number. Highlighted genes in the list are shown in the panels to the right (images are from Genepaint.org, and the image series ID is shown in the left hard corner of each field).

Figure 4b: Transcription factor genes enriched in granule cell precursors (part 2).



Gene Expr Patterns. Author manuscript; available in PMC 2012 June 1.

Figure 5d

Fold	Gene	Entrez
4.3	Skp2	27401
4.2	Nedd1	17997
4	Dbf4	27214
3.6	E2f3	13557
3.6	Ncapd2	68298
3.5	Rbl1	19650
3.3	Mycn	18109
3.3	Ccnd3	12445
3.2	Foxm1	14235
3.1	Cdk4	12567
2.9	E2f1	13555
2.9	Nfatc1	18018
2.8	Cdk2	12566
2.8	Rb1	19645
2.7	E2f6	50496
2.7	Brca2	12190
2.6	Atm	11920
2.6	Ccnf	12449
2.5	Mtbp	105837
2.4	Evi5	14020
2.2	Trp53	22059

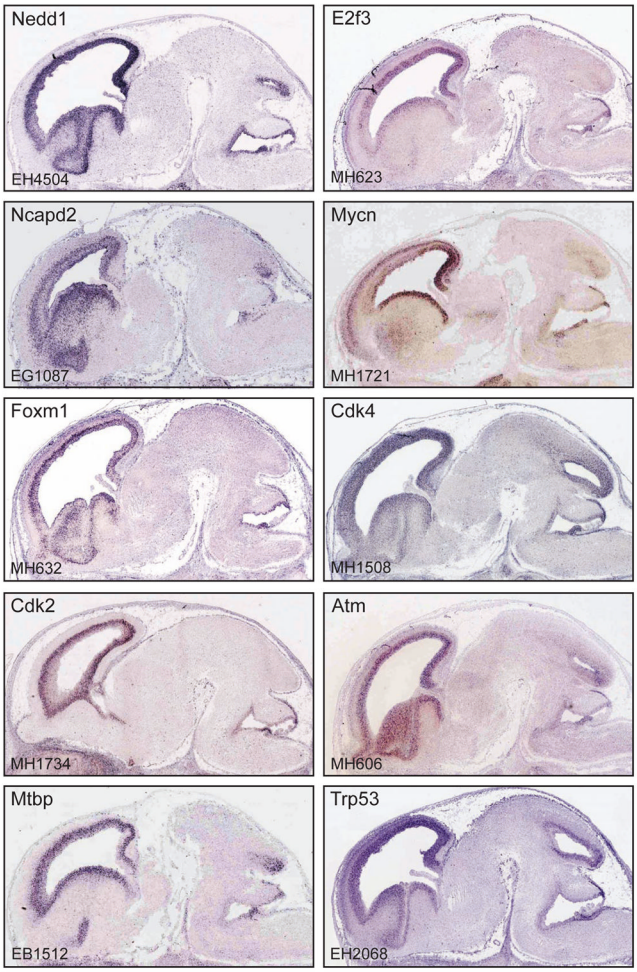


Figure 5.

Cell cycle protein genes enriched in granule cell precursors. The table on the left lists the genes in descending order of fold enrichment (E13.5 vs. E10.5 Tm labeled populations), along with their Entrez Gene ID number. Highlighted genes in the list are shown in the panels to the right (images are from Genepaint.org, and the image series ID is shown in the left hard corner of each field).

Figure 5b: Cell cycle protein genes enriched in granule cell precursors (part 2).

Figure 5c: Cell cycle protein genes enriched in granule cell precursors (part 3).

Figure 5d: Cell cycle protein genes enriched in granule cell precursors (part 4).

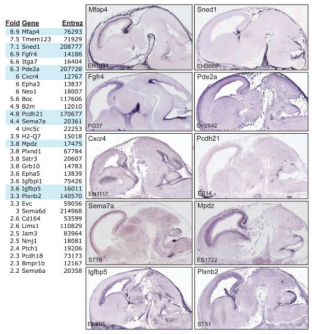


Figure 6.

Cell surface protein genes enriched in granule cell precursors. The table on the left lists the genes in descending order of fold enrichment (E13.5 vs. E10.5 Tm labeled populations), along with their Entrez Gene ID number. Highlighted genes in the list are shown in the panels to the right (images are from Genepaint.org, and the image series ID is shown in the left hard corner of each field).

Fold	Gene	Entrez
7.2	Pdgfc	54635
7.1	Reln	19699
5.5	Gdnf	14573
5.5	Ntf3	18205

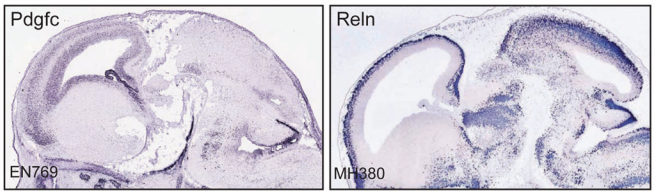


Figure 7.

Genes encoding secreted proteins enriched in granule cell precursors. The table on the left lists the genes in descending order of fold enrichment (E13.5 vs. E10.5 Tm labeled populations), along with their Entrez Gene ID number. Highlighted genes in the list are shown in the panels to the right (images are from Genepaint.org, and the image series ID is shown in the left hard corner of each field).

Fold	Gene	Entrez
11	Pvalb	19293
4.2	Rcn3	52377
3.7	Efemp1	216616
3.7	Rcn1	19672
3.1	Fstl1	14314
2.9	Anxa4	11746

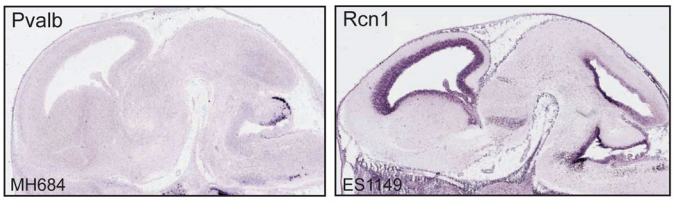


Figure 8.

Calcium binding protein genes enriched in granule cell precursors. The table on the left lists the genes in descending order of fold enrichment (E13.5 vs. E10.5 Tm labeled populations), along with their Entrez Gene ID number. Highlighted genes in the list are shown in the panels to the right (images are from Genepaint.org, and the image series ID is shown in the left hard corner of each field).