Published in final edited form as:
Mol Cell Neurosci. 2011 February ; 46(2): 548–561. doi:10.1016/j.mcn.2010.12.012.

Transcription factors expressed in olfactory bulb local progenitor cells revealed by genome-wide transcriptome profiling

Gordon R. O. Campbell, **Ariane Baudhuin**, **Karen Vranizan**, and **John Ngai***
Department of Molecular and Cell Biology, Helen Wills Neuroscience Institute & Functional Genomics Laboratory, University of California, Berkeley, California USA 94720-3200

Abstract

The local progenitor population in the olfactory bulb (OB) gives rise to mitral and tufted projection neurons during embryonic development. In contrast, OB interneurons are derived from sources outside the bulb where neurogenesis continues throughout life. While many of the genes involved in OB interneuron development have been characterized, the genetic pathways driving local progenitor cell differentiation in this tissue are largely unknown. To better understand this process, we used transcriptional profiling to monitor gene expression of whole OB at daily intervals from embryonic day 11 through birth, generating a compendium of gene expression encompassing the major developmental events of this tissue. Through hierarchical clustering, bioinformatics analysis, and validation by RNA *in situ* hybridizations, we identified a large number of transcription factors, DNA binding proteins, and cell cycle-related genes expressed by the local neural progenitor cells (NPCs) of the embryonic OB. Further *in silico* analysis of transcription factor binding sites identified an enrichment of genes regulated by the E2F-Rb pathway among those expressed in the local NPC population. Together these results provide initial insights into the molecular identity of the OB local NPC population and the transcription factor networks that may regulate their function.

Keywords

olfactory; olfactory bulb; development; neural precursor; neurogenesis; transcription factor

Introduction

The myriad cell types in the central nervous system (CNS) presents extraordinary challenges for charting the genetic pathways involved in its development. The human cerebral cortex, for example, is estimated to have 1000 types of neurons and supporting cells (Nelson et al., 2006; Stevens, 1998). To unravel the regulatory networks underlying the differentiation of neuronal lineages, we turned to the developing olfactory bulb (OB). Compared to the cerebral cortex, this structure has a more limited number of major cell types: mitral and

*Corresponding author University of California, Department of Molecular and Cell Biology, 269 Life Sciences Addition – 3200, Berkeley, CA 94720-3200, Tel. 510-642-9885, Fax 510-643-2685, jngai@socrates.berkeley.edu.

tufted projection neurons, granule and periglomerular interneurons, and glia, with some heterogeneity within these major cell types (Whitman and Greer, 2009). As in the cortex, OB interneurons and projection neurons are generated from distinct populations of neural progenitor cells (NPCs); we will define NPCs to include both multipotent as well as restricted progenitors (Fasano et al., 2007). Production of interneurons begins embryonically and continues throughout life (Batista-Brito et al., 2008; Whitman and Greer, 2007). During embryogenesis, the majority of their NPCs are located outside the bulb, in the lateral ganglionic eminence and the septum, although there is evidence that some GABAergic originate from local progenitors (Vergano-Vera et al., 2006). OB interneuron precursors are generated postnatally from astrocyte-like stem cells within the subventricular zone (SVZ) of the forebrain (Doetsch et al., 1999; Garcia et al., 2004; Merkle et al., 2007; Merkle et al., 2004). In both cases, interneuron precursors migrate tangentially in long chains to the OB as part of the rostral migratory stream (RMS). A growing list of transcription factors (TFs) and RNA splicing factors involved in their development has been identified (Lim et al., 2006; Lledo et al., 2008; Long et al., 2007; Whitman and Greer, 2009).

In contrast to OB interneurons, the projection neurons arise from local NPCs in the OB germinal zone and then migrate radially to their appropriate locations. Mitral cells are born roughly between embryonic days (E)11 and E13, while tufted cells originate between E13 and E18. As neurons differentiate, the germinal zone becomes smaller and disappears by the time of birth (Hinds, 1968a, b). A small number of genes involved in OB projection neuron development has been identified. The TF Pax6 is highly expressed in the NPC's of the OB (Long et al., 2007) and may play a role in neuronal identity and radial migration (Brill et al., 2008; Haubst et al., 2004; Kohwi et al., 2005; Nomura and Osumi, 2004). The TF Tbr1 is required for the post mitotic development of projection neurons (Bulfone et al., 1995; Bulfone et al., 1998), and Dlx5 plays a non-cell autonomous role in the morphogenesis of mitral cells (Long et al., 2003). Igf signaling is involved in local NPC proliferation and in mitral cell development. (Otaegi et al., 2006; Vicario-Abejon et al., 2003). Little else is known, however, about the genetic networks that regulate local NPC development in the OB.

In the present study we used genome-wide transcriptome profiling to generate a compendium of gene expression in the developing OB. We then used hierarchical clustering and bioinformatics analysis to identify TFs, DNA binding proteins, and cell cycle-related genes expressed by the local NPC population. Further *in silico* analysis identified an enrichment of genes regulated by the E2F-Rb pathway among those expressed in the NPC population. These results provide initial insights into the molecular identity of the local NPC population in the OB and the TF networks regulating their development.

Materials and Methods

Microarray analysis of gene expression during OB development

OBs from CD1 mice (Charles River Laboratories, Wilmington, MA), were isolated from embryos daily between E11 and postnatal day zero (P0), for a total of nine time points. RNA was purified with TRIzol reagent (Invitrogen, Carlsbad, CA), subjected to two rounds of amplification, labeled, and hybridized to Affymetrix Mouse Genome 430.2 GeneChip microarrays (Affymetrix Inc., Santa Clara, CA, USA) using Affymetrix reagents and protocols (http://www.affymetrix.com). One microarray for each time point using RNA from the OBs of one individual embryo. In this experimental paradigm, correlations between adjacent time points serve as surrogate replicates for neighboring data points. Our overall approach (which also included several statistical filtering steps as described below) is validated by the relative smoothness of the curves shown in Figures 1, 4 and 5 – i.e., for any given gene, large jumps in expression levels are not observed. Moreover, as we are

interested in overall patterns in expression, rather than expression levels at individual time points, the present experimental design is well suited for our purposes (Yang and Speed, 2002). Microarray data were normalized using the RMA algorithm (Bolstad et al., 2003; Irizarry et al., 2003a; Irizarry et al., 2003b). Normalized microarray data will be submitted to the Gene Expression Omnibus (GEO) public functional genomics data repository upon acceptance of this manuscript.

Statistical and bioinformatic analysis of gene expression data

Our experimental design is predicated on the logic that changes in the abundance of a cell type in the OB during embryogenesis will be reflected in changes in the relative abundance of transcripts expressed by that cell in the intact tissue. We therefore filtered the microarray probeset-derived data for transcripts that changed significantly in relative abundance over time. A probeset is a set of oligonucleotides that together are used to measure expression of a given transcript. To be significant, the changes for a probeset had to meet three criteria: (1) at least one time point had an intensity value (A value) greater than 6, where A is a measure of transcript level and can range between 0 – 16 on a $\log_2$ scale (Bolstad et al., 2003; Irizarry et al., 2003a; Irizarry et al., 2003b); (2) the $\log_2$ ratio of the A values between at least one time point and E11 was greater than 0.75; (3) p value < 0.02 for differential expression over the time course, where p values were calculated based on a moderated F statistic from a cubic regression model using the Limma package in Bioconductor (Gautier et al., 2004). Seven thousand nine hundred and ninety (7990) probesets (out of a total of 45,102 on the microarray) representing 5570 met these criteria and were operationally defined as differentially expressed.

The differentially expressed genes were clustered using Hierarchical Ordered Partitioning and Collapsing Hybrid (HOPACH) (Pollard and van der Lann, 2003), an algorithm for clustering expression profiles exhibiting similar patterns. HOPACH is an improvement over traditional hierarchical clustering methods in that it also provides statistically defined cluster divisions at each sub-level, and each node can have up to nine splits with the order within a node being directional (Pollard and van der Lann, 2003). The resulting clusters were visualized as heat maps using Mapletree (http://rana.lbl.gov/EisenSoftware.htm). The GenMapp-MappFinder (Dahlquist et al., 2002; Doniger et al., 2003) and Onto Express (Khatri et al., 2002) programs were used to assign biological functions (as defined by Gene Ontology or GO terms) to the genes within the clusters.

TF binding site analysis

The differentially expressed genes coding for TFs were identified by filtering for those present in the RIKEN Transcription Factor Database (Kanamori et al., 2004). They were then clustered using HOPACH and visualized using Mapletree (http://rana.lbl.gov/EisenSoftware.htm). This subset of genes was further characterized by assigning Interpro domain descriptions using the Ensembl mouse database (Flicek et al., 2010).

The presence of binding sites for TFs in the 1000 bp upstream of each differentially expressed gene was determined using the database from the web based program rVista (Loots et al., 2002), which was filtered for genes present on the Affymetrix Mouse Genome 430.2 array. This database contains all the TRANSFAC database binding sites (Matys et al., 2003; Matys et al., 2006) upstream of each gene that are conserved between mouse and human genomes. For each of the 9 main HOPACH clusters, we plotted the average number of binding sites per gene, as well as by the proportion of genes with upstream binding sites in each cluster.

To verify the overrepresentation of E2F sites in certain clusters we carried out a similar meta analysis using two published datasets of E2F binding sites detected by chromatin immunoprecipitation. One dataset contains a core set of approximately 450 genes immunoprecipitated across seven primary mouse tissues and a mouse cell line using E2F4 antibody (Conboy et al., 2007). The other dataset is a list of the 100 most highly represented genes immunoprecipitated by E2F1, E2F4, and E2F6 antibodies from chromatin of five normal and tumorous cell types (Xu et al., 2007).

RNA *in situ* hybridizations

RNA *in situ* hybridizations were performed on 16–18 μm sections prepared from fresh frozen tissue or tissue fixed in 4% paraformaldehyde, essentially as described previously (Lin et al., 2004). Template DNA for generating probes was obtained either from the RIKEN 19K mouse cDNA cloneset (Miki et al., 2001), or by PCR amplification of cDNA generated from mouse head RNA isolated from E15 embryos. In the former case, antisense digoxigenin-labeled RNA probes were synthesized using either T3 or T7 RNA polymerase (Roche) depending on the orientation of the clone insert. In the latter case, probes were prepared by PCR in a similar fashion, using either SP6 (Roche) or T7 polymerase via a promoter incorporated into the 3' PCR primer.

Double-label RNA *in situ* hybridizations were performed using a modification of the protocol from (Mavropoulos et al., 2005). Fluorescein-UTP-labeled probe (250 ng, Roche) was applied to each tissue section and hybridized overnight at 58°C. After washing in 0.2× SSC at 65°C the slide was treated with 1:500 HRP-conjugated anti-fluorescein antibody (Roche). For detection, the slides were washed, incubated for 10 minutes in tyramide-Cy3 (Perkin Elmer TSA Plus kit), followed by 30 minutes in 30% H_2O_2 to inactivate the peroxidase. The second probe, labeled with digoxigenin-UTP (Roche), was similarly detected using HRP-conjugated anti-digoxigenin and tyramide-FITC (Roche). Probes for *Blbp* and *Vim* were labeled with fluorescein and visualized with Cy3. Probes for *Hmgb2*, *Dek*, *Tmpo*, and *Cdk* were labeled with digoxigenin and visualized with FITC. Tissue sections were scanned with a Nikon confocal fluorescence microscope. FITC was visualized with an excitation wavelength of 488 nm, and Cy3 at wavelength 543 nm. Images were processed with SimplePCI software and Adobe Photoshop.

Results

Transcriptome profiling of the developing olfactory bulb reveals stage-specific clusters of gene expression

The overall goal of the present study is to identify the complement of genes expressed by local NPCs in the embryonic OB. Relative to other cells in the structure, they are most abundant at the earliest stages of development, and decrease over time as they differentiate and as other mature cell types accumulate (Hinds, 1968a, b). Accordingly, transcripts of genes expressed by the progenitor cell population should be relatively abundant early on and decrease as embryogenesis proceeds. Conversely, levels of transcripts associated with the terminal differentiation of OB cell types should increase over developmental time.

RNA from OBs collected at one-day intervals from E11 to postnatal day 0 (P0) was hybridized to Affymetrix Mouse Genome 430 2.0 microarrays. Of approximately 34,000 genes represented by 45,102 probesets, 5570 (7990 probesets) meet relatively stringent criteria for being differentially expressed. Hierarchical clustering using the HOPACH algorithm sorted them into nine main groups with six sublevels of clustering based on their expression profiles (Figure 1; see also Figure 3A for median expression levels of each cluster). The differentially expressed genes can be assigned to two main groups: clusters 1–

4, whose members exhibit an increase in expression during OB development, and clusters 5–9, whose members exhibit a decrease.

To relate biological functions to individual clusters, the GO terms for each gene in a cluster were tabulated. The most significant terms are listed in Figure 1A. Figure 2 shows the most frequent GO categories overrepresented in clusters 6–9 and clusters 2–3. Clusters 6–9 are overrepresented in GO terms associated with proliferation including *cell cycle*, *cell division, DNA replication*, and *mitosis*. A substantial proportion of the known genes for the cell cycle regulation and DNA synthesis are present in these clusters. Figure 3B shows the HOPACH clustering data superimposed onto a diagram of the genetic pathways for these processes, highlighting the substantial representation of cell cycle regulatory genes in clusters 6–9. In addition, several of the most significant terms are related to transcription and RNA processing. The decreasing expression profiles together with the prevalence of genes involved in cell cycle regulation support the interpretation that clusters 6–9 represent genes expressed by actively dividing NPCs.

In contrast, clusters 2–3, whose members exhibit an increase in expression over time (Figures 1 and 3A), are enriched for genes expressed in terminally differentiating cells and neurons. The GO terms *nervous system development, axon guidance, negative regulation of axon extension and cell differentiation* are overrepresented in cluster 2, *synaptic transmission, calcium ion transport, regulation of exocytosis*, and *behavioral fear response* in cluster 3, and *neurotransmitter secretion* in both clusters. These clusters also contain many recognized neuronal markers, including the genes encoding microtubule associated proteins Tau/mapt and Map2/Mtap2 (Dehmelt and Halpain, 2005), synaptic vesicle protein synaptotagmin (Syt1) (Rizo and Rosenmund, 2008), calcium/calmodulin dependent kinase 2 (Camk2/Camk2a) (Colomer and Means, 2007), calcium binding proteins S100beta, and S100a6 (Calcyclin) (Heizmann and Cox, 1998) and voltage dependent ion channel subunits.

Cluster 4 has an eclectic set of GO terms (Figure 1A), with none as highly overrepresented as the top-ranked categories in clusters 2–3 or clusters 6–9, suggesting that the genes in cluster 4 are expressed in a diversity of cell types. Its expression profile shows a lag before increasing (Figures 1A and 3A). Clusters 1 and 5 are small and not significantly associated with any GO categories. These groups were not investigated further.

Identification and analysis of differentially expressed genes for TFs and DNA binding proteins

Specification and differentiation of neuronal lineages is largely regulated at the transcriptional level (Flames and Hobert, 2009; Guillemot, 2007; Isshiki et al., 2001; Ross et al., 2003). Indeed, neurons may be most reliably classified by the combination of TFs expressed in their developmental histories (Yuste, 2005). Considering the central role they play in neuronal specification, we were particularly interested in identifying the TFs present in our dataset of differentially expressed genes. Of the 5570 differentially expressed genes identified in the OB time course, we found that 507 encode TFs (Figure 1B, Table S2). We then used the Ensembl mouse database to identify the Interpro protein domains contained by the encoded TFs. The TFs we identified match to 545 Interpro domain descriptions for a total of 1696 matches, with many TFs containing multiple domains. The 22 classes with the most members are listed in Table 1. Znf proteins represent the largest class of TF, containing 120 members, followed by Pro-rich containing 60 members, homeobox with 41 members, and helix-loop-helix proteins with 33 members. The TFs remaining either mapped to other miscellaneous domain names or were not documented by Interpro (Table 1).

Clusters 6–9, which comprise genes exhibiting decreasing transcript levels in the bulb over developmental time, contain 320 TF genes, more than 60 % of the total present in the

dataset. Strikingly, these clusters also contain all of the differentially expressed genes coding for TFs with MCM, E2F, and AAA ATPase domains; these domains are associated with cell cycle regulation. Moreover, 19 out of 24 HMG domain-containing transcripts and 4 out of 5 chromodomain-containing transcripts present in the dataset reside in these clusters. These domains are associated with chromatin modification and remodeling (Hall and Georgel, 2007; Hock et al., 2007), processes involved in but not limited to stem cell self-renewal (Keenen and de la Serna, 2009). Clusters 2–3 contain 108 TFs including 14 out of 33 of the TFs present with HLH domains, and all of the TFs present with PAS domains (Table 1).

Genes from different clusters are transcribed in distinct OB cell populations

To verify that clusters 2–3 and clusters 6–9 are respectively enriched for genes associated with terminally differentiating cells and mitotic progenitor cells, we performed RNA *in situ* hybridizations for representative transcripts on OBs from E15 embryos. Figure 4 A–C shows the expression patterns of three genes from the downward trending clusters 6–9: *Vim*, a radial glial marker (cluster 7), *Pax6*, encoding a TF essential to the development of the rostral telencephalon (cluster 8), and *Ki67*, a proliferation marker (Gatter et al., 1986) (cluster 7). *Vim* and *Pax6* are expressed by NPCs in the germinal zone of the OB (Bailey et al., 1999; Hebert et al., 2003; Long et al., 2007). Probes for all three genes localize to this region of the embryonic OB in the present analysis (Figure 4 A–C). Interestingly, these probes also hybridize to the OB nerve layer where olfactory receptor axons and ensheathing glia are located. We found this to be a fairly common phenomenon for transcripts expressed in the OB germinal region (Table S2).

In contrast, transcripts belonging to the upward trending clusters 2–3 are expressed in a variety of patterns distinct from that observed for the progenitor cell markers (Figure 4 D–I, Table S2). For example, *Mafb* and *Stmn2* (cluster 2) are expressed in a peripheral cell layer in the OB in both E15 and E18 embryos (Figure 4 D and E). This pattern most likely corresponds expression in the mitral cell layer since *Id2* is expressed here at this developmental stage (Neuman et al., 1993) (Figure 4F). Three other genes from cluster 3, *Nr2f1*, *Foxo1*, and *Heyl*, are expressed in the olfactory nerve layer (Figure 4 G–I). None the genes that we tested from clusters 2–3 are expressed in the OB germinal region.

RNA *in situ* hybridization analysis confirms that most genes of clusters 6–9 are specifically expressed in OB progenitor cells

To assess the extent to which clusters 6–9 are enriched for genes expressed in NPCs, we performed RNA in situ hybridizations on 60 genes from these clusters. Among these 60 representative genes were 55 genes from the list of 320 differentially expressed TF genes found in these clusters, with choices spread among a range of p values and informed by the identities of the genes themselves. Using this approach we confirmed that 50 genes from clusters 6–9, including 45 of the 55 TF genes tested, are expressed in the OB germinal region (Figure 5, Table 2, Table S2). This list of validated genes includes many genes associated with proliferation, including cdk2 and cdk4, which are involved in cell cycle regulation, and mcm2 and orc2l from the DNA replication pathway (Figure 3). The Mcm2 protein has both MCM, and AAA ATPase domains, both of which are overrepresented in these clusters. Moreover, a large collection of genes containing the overrepresented E2F and HMG domains are contained in clusters 6–9 and localize to the germinal zone. These include E2F1, E2F5, E2F6, and E2F7, as well as Hmgb2 and Hmgb3. We also verified that of a number of additional cortical NPC markers are expressed in the bulb germinal zone, including Neurog1 (Fode et al., 2000), and Id4 (Yun et al., 2004). Many of the genes are expressed specifically, but not exclusively in the germinal region, with a subset also exhibiting expression in the nerve layer (Figure 5, Table S2). Genes tested from clusters 6–9 that do not have specific expression in the germinal layer predominantly exhibit low level

staining throughout the OB. The exception was Bclaf1, which in addition to this non-specific staining exhibits stronger specific expression in the mitral layer.

To confirm that transcripts localizing to the OB germinal region are indeed expressed in the NPC population, we performed double-label RNA *in situ* hybridizations on four genes – *Dek, Hmgb2, Tmpo*, and *Cdk2* – exhibiting particularly strong staining in the OB germinal region using the NPC markers *Vim* and *Blbp*. In each case, the activity of the candidate gene co-localized with the NPC markers (Figure 6). Together these results suggest that clusters 6–9 are highly enriched for genes that are preferentially expressed in OB progenitor cells. For simplicity, we shall refer to these clusters as the NPC clusters.

Genes from NPC clusters are expressed in the OB prior to RMS migration

Neurons and glia in the OB arise from either the local progenitors that are present in the bulb or from precursors that migrate into the OB from the ganglionic eminences. In the latter case, these cells migrate to the bulb through the RMS in a Dlx1/Dlx2-dependent fashion, arriving in the OB by E14.5 (Hinds, 1968a, b; Lledo et al., 2008). To determine whether genes from the NPC clusters are expressed in local OB progenitor cells and not the migrating precursors, we performed whole-mount RNA *in situ* hybridizations on 37 candidate genes from these clusters at E12 and/or E11, several days before the first migratory OB interneuron precursor cells arrive in the OB. All of these genes are expressed in the rostral tip of the telencephalon (the location of the developing OB), prior to the arrival of cells from the RMS (Figures S1 and S2).

To further verify that the genes from the NPC clusters are expressed in local OB progenitors, we carried out RNA *in situ* hybridizations on parasagittal sections of E13.5 heads, and compared the expression patterns of representative genes to those of *Dlx1, Dlx2* and *Dlx5* (markers of progenitors migrating to the OB in the RMS), the more general NPC marker *Nes*, and the radial glial marker *Vim* (Figure 7). Robust expression of *Dlx1, Dlx2* and *Dlx5* is restricted to the ganglionic eminences and septum at E13.5, with little or no detectable expression in the OB (Figure 7A–C). In contrast, both *Nes* and *Vim* exhibit strong staining at E13.5 in the germinal region of the nascent OB, as well as in the dorsal telencephalon, septum, and ganglionic eminences (Figure 7 D–E). Representative genes from the NPC clusters – *Dek, Hmgb2, Tmpo* and *FoxM1* – all show expression patterns similar to those of *Nes* and *Vim*, with unambiguous expression in the nascent OB (Figure 7F–I). *Ngn1* shows a somewhat different pattern, with expression in the dorsal telencephalon and nascent OB but exclusion from the septum and ganglionic eminences (Figure 7J). Thus, although the genes we tested from the NPC cluster are not excluded from expression in the RMS, they are expressed by the local NPC population in the developing OB, prior to the arrival of the migratory OB interneuron precursor cells.

Identification of the E2F family of transcription factors as candidate upstream regulators of genes expressed in OB local progenitor cells

The rather neat parsing of the genes in our dataset by function as well as cell type suggests that these gene clusters represent synexpression groups. This term refers to collections of co-regulated genes functioning in common processes, a widespread phenomenon both prokaryotes and eukaryotes (Niehrs and Pollet, 1999). Since a major mechanism for coordinated gene expression is the binding of TFs to common sites in the promoter regions of multiple genes, we looked to see whether the genes of any cluster exhibited an enrichment for a particular TF binding site(s). To this end, we mapped the putative binding sites for 296 TFs identified in the rVista program database (Loots et al., 2002) to the upstream regions of each differentially expressed gene in our dataset. We then plotted the number of binding sites per cluster for each TF. Those for E2F1 and E2F4 protein

complexes are enriched in the NPC clusters (Figure 8 A–C; Table S3). Consistent with the notion that members of the E2F family may regulate gene expression in OB progenitor cells, many of the E2F genes also localize to the NPC clusters (Table 2, Figure 3B, Figures S1 and S2).

To confirm that the overrepresented E2F consensus sites correspond to *bona fide* E2F binding sites, we carried out a similar analysis using two published E2F datasets obtained by chromatin immunoprecipitation (ChIP) that include binding sites for E2F1, E2F4, and E2F6 identified in a number of different tissues and cell types (Conboy et al., 2007; Xu et al., 2007). We found enrichment of these genes in the NPC clusters to be even greater than what we observed for the rVista sites (Figure 8D, E).

Consistent with these results, E2F regulatory elements have been found to be over-represented in genes expressed in the proliferative phase of each of 21 stem cell populations (Joung et al., 2006). Moreover, combinations of E2F motifs significantly correlate with the G1/S phase in expression data from synchronized HeLa cells (Das et al., 2006). Together these observations implicate the E2F family of transcription factors as a regulator of gene expression in OB progenitor cells. No binding sites are significantly over-represented in other HOPACH clusters (including clusters 2–3), probably reflecting the heterogeneity of the cell types they represent.

Gene expression in embryonic OB local progenitors is distinct from that of embryonic and postnatal interneuron precursors

Previous studies have identified genes preferentially expressed by the cells of the adult SVZ and the inner, neurogenic region of the adult OB (referred to as the olfactory bulb core or OBC), as well as genes shared between these regions (referred to as the SO region), presumed to represent those genes expressed in the RMS, the route of migratory neuroblasts from the SVZ to the OB (Lim et al., 2006). While the local NPC population in the embryonic OB only generates projection neurons during a specific time window of embryogenesis, OB interneurons continue to be generated after birth for the life of the animal. We were therefore interested in determining the extent to which the patterns of gene expression overlap between the embryonic OB local progenitors (which give rise to the projection neurons) and adult OB interneuron progenitors. Compared to other brain regions, Lim et al. identified 67 genes enriched in the adult SVZ, 171 genes in the OBC and 65 genes in the SO (Lim et al., 2006). Of these genes, 26 of the SVZ-enriched genes (39%), 32 of the OBC-enriched genes (19%), and 24 of the SO genes (35%) are also found in the NPC clusters of the embryonic OB dataset (Table S4); the remaining genes are either not differentially expressed in the embryonic OB time course or are grouped in other clusters. Similarly, a comprehensive analysis of gene expression ascribed to telencephalic interneuron precursors identified 130 TFs expressed in these cells (Long et al., 2009), of which 31 (24%) localize to clusters 6–9 of the embryonic OB dataset and 73 (56%) show no differential expression (Table S5). Thus, although some genes are commonly expressed between the embryonic OB local progenitor cells and interneuron precursors from both embryonic and postnatal sources, based on this meta-analysis the patterns of gene expression manifested by these progenitor cell populations are distinct.

Discussion

A compendium of gene expression in olfactory bulb local progenitor cells

We have identified 5570 genes that are differentially expressed during the embryonic development of the OB using genome-wide transcription profiling followed by hierarchical cluster analysis. These genes fall into 9 clusters based on their expression patterns. We were

able to identify four clusters, 6–9, whose genes are predominantly expressed in the NPC population. Several lines of evidence support this interpretation. First, the median expression level of genes in these “NPC clusters” decreases over time (Figures 1A and 3A), following a similar trend to the decreasing proportion of NPCs present in the OB during its maturation (Hinds, 1968a, b). Second, they contain known NPC markers, including *Hes1, Pax6, Nes*, and *Vim* (Figure 1A, Table 2). Third, the NPC clusters are significantly enriched for genes belonging to GO categories representing pathways involved in regulating the cell cycle and cell division – hallmarks of proliferating progenitor cells. Indeed, the majority of genes comprising the canonical genetic pathways for the G1 to S transition and DNA replication are located in these clusters (Figures 1 and 2). Fourth, the expression patterns of NPC genes were verified by an extensive series of RNA in situ hybridization experiments. Over 80% (50/60) of the genes tested by RNA in situ hybridization are expressed in the germinal region of the embryonic OB at E15. Double-label fluorescent RNA *in situ* hybridizations combined with RNA *in situ* hybridizations conducted at earlier time points verified that these genes are expressed by local OB NPCs. (Figures 5, 6 and 7, Figure S1 and S2, Table 2).

Based on a similar analysis we conclude that clusters 2–3 (Figures 1A and 3A) are enriched for genes expressed in differentiating or mature neurons of the OB. Their transcripts increase with developmental time and include known neuronal markers. RNA *in situ* hybridizations for a sample of these genes exhibited expression in cell layers of the OB containing mature cells, including the mitral cell layer and olfactory nerve layer (Figure 4, Table S2). Moreover, genes of these clusters are overrepresented for GO annotation terms typical for differentiated cell types, including neurons (Figures 1A and 2).

The foregoing conclusions should be tempered by the following considerations. First, while we have indeed succeeded in identifying a large number of genes expressed preferentially in the OB local prognitor cell population, our studies cannot rule out the likely possibility that other neuronal progenitors and other cell types – for example the precurors of cortical projection neurons – also express many of these genes. Thus, the genes identified in this study help to characterize the OB local progenitors, but at the present time cannot necessarily be used to define a unique OB precursor cell signature. Second, since our analysis was conducted on complex tissue samples containing many cell types, the gene groupings generated by hierarchical clustering of gene expression data serve as a rough guide to gene expression in the bulb, and not as a substitute for a direct analysis of purified cell populations. Indeed, classification of gene expression patterns based on temporal profiling can oversimplify the complexity of the underlying expression pattern. For example, clustering of genes for the T-box transcription factors, Tbr2/Eomes, and Tbr1 (Bulfone et al., 1998; Englund et al., 2005), which are known to be expressed in glutamatergic progenitors (in proliferative and postmitotic cells, respectively) serve as a case in point. Tbr1 is re-expressed in differentiated glutamatergic projection neurons throughout the brain (Bedogni et al., 2010; Hevner et al., 2001), and Tbr2/Eomes is expressed in intermediate neural progenitors as well as Cajal-Retzius cells (Englund et al., 2005), complicating their inclusion in a single group of genes. With respect to the time course data, Tbr1 appears in cluster 9 (down-regulated during development), while Tbr2/Eomes appears in Clusters 1 and 2 (up-regulated during development).

Transcription factors and chromatin remodeling proteins expressed in OB local progenitor cells: candidate regulators of OB projection neuron precursors

The NPC clusters include 320 genes encoding TFs, many of which are involved in cell cycle regulation and chromatin structure and remodeling. Their known functions in other cell types and cancers, as well as their involvement in the E2F-Rb pathway, suggest a potential

role in regulating the proliferating local progenitor cells of the embryonic OB. Several examples are highlighted below.

High mobility group (HMG) proteins—The HMG proteins are a group of chromatin architectural proteins highly represented in the NPC clusters, which contain *Hmga1*, *Hmga2*, *Hmgb1*, *Hmgb2*, *Hmgb3*, and *Hmg20b*; we verified by RNA *in situ* hybridizations that *Hmgb2* and *Hmgb3* are specifically expressed in the OB germinal region (Figures 5–7, Table 2). HMG proteins bind to chromatin in a highly dynamic fashion, altering its structure and allowing the binding of additional factors that regulate the expression of specific target genes (Catez and Hock). HMG proteins are involved in many biological processes including transcription, cell proliferation and differentiation, and DNA repair. Their misexpression has been associated with a number of types of cancer (Hock et al., 2007). Some tumors may arise from alterations in the transcriptional activity of cell cycle control genes or enhanced E2F1 activity (Fedele et al., 2006).

Dek—The proto-oncogene *Dek* (Figures 5–7) was originally identified as a fusion with the nucleoprotein Nup214/Can in acute myeloid leukemia patients (von Lindern et al., 1992). DEK is a chromatin architectural protein that plays a role in RNA processing and the regulation of transcription. It also mediates DNA replication, inhibition of cell death, and cell differentiation. It is frequently up-regulated in many types of malignancies including retinoblastomas and gliomas and its expression is regulated by the E2F-Rb pathway (Sherr, 1996) (Carro et al., 2006).

Tmpo/Lap2—Tmpo/Lap2 (Figures 5–7) is another TF that interacts with chromatin and is involved in cell cycle regulation. It affects cell cycle progression and differentiation through the binding of Rb, and its expression is under the direct control of E2F transcription factors (Dorner et al., 2006). Its expression is up regulated in a large number of cancers and neuroblastoma lines (Dorner et al., 2006; Johnson et al., 2004; Markiewicz et al., 2002; Parise et al., 2006; Wagner and Krohne, 2007).

Tardbp—In humans, TARDBP (Figure 5) plays a major role in neurodegenerative disorders. Mutations in this gene are the main cause of ALS and frontotemporal lobar dementia. Moreover the protein it encodes, TDP-43, is the primary component of cytoplasmic inclusions formed in these diseases (Davidson et al., 2007;Neumann et al., 2006). TDP-43 positive inclusions have also been identified in Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies (Gendron et al.). In mice, null mutations are lethal, but heterozygotes for the null allele exhibit a loss of motor function (Kraemer et al., 2010;Sephton et al., 2010;Wu et al., 2010). It is also involved in Rb and Rb2 phosphorylation thereby possibly playing a role in cell proliferation and survival (Ayala et al., 2008).

The E2F family of transcription factors: candidate regulators of gene expression in OB NPCs

Using bioinformatics analysis we identified an over-representation of predicted binding sties for the E2F family of transcription factors in the upstream regions of genes localized to the NPC clusters. The E2F-Rb pathway is a central regulator of the G1 to S phase transition and is disrupted in virtually all human cancers, underscoring its crucial role in cell proliferation (Nevins, 2001). E2F comprises an eight-member family that regulates the expression of many of the genes necessary for cell-cycle progression. E2F activity is regulated at the post-translational level through the binding of Rb protein family members. In a hypophosphorylated state, these proteins can bind to E2F1-5 and sequester their transactivation domain as well as recruit chromatin modification proteins to repress

transcription. In the hyperphosphorylated state Rb family members no longer interact with E2F. Rb phosphorylation during cell cycle progression is mediated by various cyclins and cyclin-dependant kinases (Nevins, 2001; Swiss and Casaccia, 2010).

The eight E2F family members exhibit overlapping DNA recognition motifs and expression patterns, making the relative contribution of individual members notoriously difficult to ascertain. E2F1, E2F2, and E2F5 are all expressed in the germinal region of the mouse telencephalon (Dagnino et al., 1997; Kusek et al., 2001). Mice lacking E2F4 exhibit a reduction in overall telencephalon size, an absence of its ventral structures and impaired renewal of neural precursor cells (Ruzhynsky et al., 2007). This protein is expressed in all tissues and its activity is dependent on which Rb family member to which it is bound (Moberg et al., 1996). E2F1 knockout mice have reduced neurogenesis in adult OB and elsewhere (Cooper-Kuhn et al., 2002). Rb is expressed ubiquitously in the embryonic nervous system, and knockouts die embryonically at E15 and display prominent neuronal defects (Ferguson et al., 2002). Together with previous data linking members of the E2F family with neurogenesis, telencephalic development, and expression by NPCs in the telencephalic germinal zone, our analysis strongly suggests an important role of this TF family in regulating the genes expressed by proliferating OB progenitor cells during OB development. Future studies directly analyzing E2F binding sites in OB tissue and utilizing either E2F gain- or loss-of-function approaches should clarify the role of this family of transcription factors in OB progenitor cell function and the downstream genes whose expression they regulate.

In summary, this study represents a comprehensive analysis of gene expression in the OB during embryonic development, and provides a compendium of genes that are expressed by OB local progenitor cells. Among these are over 320 genes encoding transcription factors, with 45 out of 50 tested verified through RNA *in situ* hybridizations to be expressed by the local progenitor cells of the embryonic OB. These transcription factors comprise a promising and extensive set of candidates for future investigations aimed at identifying the genes and pathways that regulate gene expression in this class of NPCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

OB	olfactory bulb
NPC	neural p rogenitor cell
RMS	rostral migratory stream
TF	transcription factor
E	embryonic day
P	postnatal day
GEO	Gene Expression Omnibus
HOPACH	Hierarchical Ordered Partitioning and Collapsing Hybrid
SVZ	subventricular zone
OBC	olfactory bulb core.

Acknowledgments

We thank Simon Minovitsky and Inna Dubchak for kindly providing the rVista binding site data, John Campbell for carefully reading the manuscript, and the Ngai Lab for their support. This work was supported by grants from the National Institute on Deafness and Other Communication Disorders to GROC and JN. This paper is dedicated to Karen Vranizan (1954 –2009), cherished friend and colleague, statistician extraordinaire.

References

Ayala YM, Misteli T, Baralle FE. TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. Proc Natl Acad Sci U S A 2008;105:3785–3789. [PubMed: 18305152]

Bailey MS, Puche AC, Shipley MT. Development of the olfactory bulb: evidence for glia-neuron interactions in glomerular formation. J Comp Neurol 1999;415:423–448. [PubMed: 10570454]

Batista-Brito R, Close J, Machold R, Fishell G. The distinct temporal origins of olfactory bulb interneuron subtypes. J Neurosci 2008;28:3966–3975. [PubMed: 18400896]

Bedogni F, Hodge RD, Elsen GE, Nelson BR, Daza RA, Beyer RP, Bammler TK, Rubenstein JL, Hevner RF. Tbr1 regulates regional and laminar identity of postmitotic neurons in developing neocortex. Proc Natl Acad Sci U S A 2010;107:13129–13134. [PubMed: 20615956]

Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003;19:185–193. [PubMed: 12538238]

Brill MS, Snapyan M, Wohlfrom H, Ninkovic J, Jawerka M, Mastick GS, Ashery-Padan R, Saghatelyan A, Berninger B, Gotz M. A dlx2- and pax6-dependent transcriptional code for periglomerular neuron specification in the adult olfactory bulb. J Neurosci 2008;28:6439–6452. [PubMed: 18562615]

Bulfone A, Smiga SM, Shimamura K, Peterson A, Puelles L, Rubenstein JL. T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. Neuron 1995;15:63–78. [PubMed: 7619531]

Bulfone A, Wang F, Hevner R, Anderson S, Cutforth T, Chen S, Meneses J, Pedersen R, Axel R, Rubenstein JL. An olfactory sensory map develops in the absence of normal projection neurons or GABAergic interneurons. Neuron 1998;21:1273–1282. [PubMed: 9883721]

Carro MS, Spiga FM, Quarto M, Di Ninni V, Volorio S, Alcalay M, Muller H. DEK Expression is controlled by E2F and deregulated in diverse tumor types. Cell Cycle 2006;5:1202–1207. [PubMed: 16721057]

Catez F, Hock R. Binding and interplay of HMG proteins on chromatin: lessons from live cell imaging. Biochim Biophys Acta 1799:15–27.

Colomer J, Means AR. Physiological roles of the Ca2+/CaM-dependent protein kinase cascade in health and disease. Subcell Biochem 2007;45:169–214. [PubMed: 18193638]

Conboy CM, Spyrou C, Thorne NP, Wade EJ, Barbosa-Morais NL, Wilson MD, Bhattacharjee A, Young RA, Tavare S, Lees JA, Odom DT. Cell cycle genes are the evolutionarily conserved targets of the E2F4 transcription factor. PLoS ONE 2007;2:e1061. [PubMed: 17957245]

Cooper-Kuhn CM, Vroemen M, Brown J, Ye H, Thompson MA, Winkler J, Kuhn HG. Impaired adult neurogenesis in mice lacking the transcription factor E2F1. Mol Cell Neurosci 2002;21:312–323. [PubMed: 12401450]

Dagnino L, Fry CJ, Bartley SM, Farnham P, Gallie BL, Phillips RA. Expression patterns of the E2F family of transcription factors during mouse nervous system development. Mech Dev 1997;66:13–25. [PubMed: 9376316]

Dahlquist KD, Salomonis N, Vranizan K, Lawlor SC, Conklin BR. GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. Nat Genet 2002;31:19–20. [PubMed: 11984561]

Das D, Nahle Z, Zhang MQ. Adaptively inferring human transcriptional subnetworks. Mol Syst Biol 2006;2 2006 0029.

Davidson Y, Kelley T, Mackenzie IR, Pickering-Brown S, Du Plessis D, Neary D, Snowden JS, Mann DM. Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. Acta Neuropathol 2007;113:521–533. [PubMed: 17219193]

Dehmelt L, Halpain S. The MAP2/Tau family of microtubule-associated proteins. Genome Biol 2005;6:204. [PubMed: 15642108]

Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999;97:703–716. [PubMed: 10380923]

Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol 2003;4:R7. [PubMed: 12540299]

Dorner D, Vlcek S, Foeger N, Gajewski A, Makolm C, Gotzmann J, Hutchison CJ, Foisner R. Lamina-associated polypeptide 2alpha regulates cell cycle progression and differentiation via the retinoblastoma-E2F pathway. J Cell Biol 2006;173:83–93. [PubMed: 16606692]

Englund C, Fink A, Lau C, Pham D, Daza RA, Bulfone A, Kowalczyk T, Hevner RF. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J Neurosci 2005;25:247–251. [PubMed: 15634788]

Fasano CA, Dimos JT, Ivanova NB, Lowry N, Lemischka IR, Temple S. shRNA knockdown of Bmi-1 reveals a critical role for p21-Rb pathway in NSC self-renewal during development. Cell Stem Cell 2007;1:87–99. [PubMed: 18371338]

Fedele M, Visone R, De Martino I, Troncone G, Palmieri D, Battista S, Ciarmiello A, Pallante P, Arra C, Melillo RM, Helin K, Croce CM, Fusco A. HMGA2 induces pituitary tumorigenesis by enhancing E2F1 activity. Cancer Cell 2006;9:459–471. [PubMed: 16766265]

Ferguson KL, Vanderluit JL, Hebert JM, McIntosh WC, Tibbo E, MacLaurin JG, Park DS, Wallace VA, Vooijs M, McConnell SK, Slack RS. Telencephalon-specific Rb knockouts reveal enhanced neurogenesis, survival and abnormal cortical development. EMBO J 2002;21:3337–3346. [PubMed: 12093735]

Flames N, Hobert O. Gene regulatory logic of dopamine neuron differentiation. Nature 2009;458:885–889. [PubMed: 19287374]

Flicek P, Aken BL, Ballester B, Beal K, Bragin E, Brent S, Chen Y, Clapham P, Coates G, Fairley S, Fitzgerald S, Fernandez-Banet J, Gordon L, Graf S, Haider S, Hammond M, Howe K, Jenkinson A, Johnson N, Kahari A, Keefe D, Keenan S, Kinsella R, Kokocinski F, Koscielny G, Kulesha E, Lawson D, Longden I, Massingham T, McLaren W, Megy K, Overduin B, Pritchard B, Rios D, Ruffier M, Schuster M, Slater G, Smedley D, Spudich G, Tang YA, Trevanion S, Vilella A, Vogel J, White S, Wilder SP, Zadissa A, Birney E, Cunningham F, Dunham I, Durbin R, Fernandez-Suarez XM, Herrero J, Hubbard TJ, Parker A, Proctor G, Smith J, Searle SM. Ensembl's 10th year. Nucleic Acids Res 2010;38:D557–D562. [PubMed: 19906699]

Fode C, Ma Q, Casarosa S, Ang SL, Anderson DJ, Guillemot F. A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. Genes Dev 2000;14:67–80. [PubMed: 10640277]

Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MV. GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. Nat Neurosci 2004;7:1233–1241. [PubMed: 15494728]

Gatter KC, Dunnill MS, Gerdes J, Stein H, Mason DY. New approach to assessing lung tumours in man. J Clin Pathol 1986;39:590–593. [PubMed: 3013945]

Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004;20:307–315. [PubMed: 14960456]

Gendron TF, Josephs KA, Petrucelli L. Review: transactive response DNA-binding protein 43 (TDP-43): mechanisms of neurodegeneration. Neuropathol Appl Neurobiol 2010;36:97–112. [PubMed: 20202122]

Guillemot F. Cell fate specification in the mammalian telencephalon. Prog Neurobiol 2007;83:37–52. [PubMed: 17517461]

Hall JA, Georgel PT. CHD proteins: a diverse family with strong ties. Biochem Cell Biol 2007;85:463–476. [PubMed: 17713581]

Haubst N, Berger J, Radjendirane V, Graw J, Favor J, Saunders GF, Stoykova A, Gotz M. Molecular dissection of Pax6 function: the specific roles of the paired domain and homeodomain in brain development. Development 2004;131:6131–6140. [PubMed: 15548580]

Hebert JM, Lin M, Partanen J, Rossant J, McConnell SK. FGF signaling through FGFR1 is required for olfactory bulb morphogenesis. Development 2003;130:1101–1111. [PubMed: 12571102]

Heizmann CW, Cox JA. New perspectives on S100 proteins: a multi-functional Ca(2+)-, Zn(2+)- and Cu(2+)-binding protein family. Biometals 1998;11:383–397. [PubMed: 10191501]

Hevner RF, Shi L, Justice N, Hsueh Y, Sheng M, Smiga S, Bulfone A, Goffinet AM, Campagnoni AT, Rubenstein JL. Tbr1 regulates differentiation of the preplate and layer 6. Neuron 2001;29:353–366. [PubMed: 11239428]

Hinds JW. Autoradiographic study of histogenesis in the mouse olfactory bulb. I. Time of origin of neurons and neuroglia. J Comp Neurol 1968a;134:287–304. [PubMed: 5721256]

Hinds JW. Autoradiographic study of histogenesis in the mouse olfactory bulb. II. Cell proliferation and migration. J Comp Neurol 1968b;134:305–322. [PubMed: 5721257]

Hock R, Furusawa T, Ueda T, Bustin M. HMG chromosomal proteins in development and disease. Trends Cell Biol 2007;17:72–79. [PubMed: 17169561]

Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 2003a;31:e15. [PubMed: 12582260]

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003b;4:249–264. [PubMed: 12925520]

Isshiki T, Pearson B, Holbrook S, Doe CQ. Drosophila neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. Cell 2001;106:511–521. [PubMed: 11525736]

Johnson BR, Nitta RT, Frock RL, Mounkes L, Barbie DA, Stewart CL, Harlow E, Kennedy BK. A-type lamins regulate retinoblastoma protein function by promoting subnuclear localization and preventing proteasomal degradation. Proc Natl Acad Sci U S A 2004;101:9677–9682. [PubMed: 15210943]

Joung JG, Shin D, Seong RH, Zhang BT. Identification of regulatory modules by co-clustering latent variable models: stem cell differentiation. Bioinformatics 2006;22:2005–2011. [PubMed: 16899491]

Kanamori M, Konno H, Osato N, Kawai J, Hayashizaki Y, Suzuki H. A genome-wide and nonredundant mouse transcription factor database. Biochem Biophys Res Commun 2004;322:787–793. [PubMed: 15336533]

Keenen B, de la Serna IL. Chromatin remodeling in embryonic stem cells: regulating the balance between pluripotency and differentiation. J Cell Physiol 2009;219:1–7. [PubMed: 19097034]

Khatri P, Draghici S, Ostermeier GC, Krawetz SA. Profiling gene expression using onto-express. Genomics 2002;79:266–270. [PubMed: 11829497]

Kohwi M, Osumi N, Rubenstein JL, Alvarez-Buylla A. Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. J Neurosci 2005;25:6997–7003. [PubMed: 16049175]

Kraemer BC, Schuck T, Wheeler JM, Robinson LC, Trojanowski JQ, Lee VM, Schellenberg GD. Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. Acta Neuropathol 2010;119:409–419. [PubMed: 20198480]

Kusek JC, Greene RM, Pisano MM. Expression of the E2F and retinoblastoma families of proteins during neural differentiation. Brain Res Bull 2001;54:187–198. [PubMed: 11275408]

Lim DA, Suarez-Farinas M, Naef F, Hacker CR, Menn B, Takebayashi H, Magnasco M, Patil N, Alvarez-Buylla A. In vivo transcriptional profile analysis reveals RNA splicing and chromatin remodeling as prominent processes for adult neurogenesis. Mol Cell Neurosci 2006;31:131–148. [PubMed: 16330219]

Lin DM, Yang YH, Scolnick JA, Brunet LJ, Marsh H, Peng V, Okazaki Y, Hayashizaki Y, Speed TP, Ngai J. Spatial patterns of gene expression in the olfactory bulb. Proc Natl Acad Sci U S A 2004;101:12718–12723. [PubMed: 15304640]

Lledo PM, Merkle FT, Alvarez-Buylla A. Origin and function of olfactory bulb interneuron diversity. Trends Neurosci 2008;31:392–400. [PubMed: 18603310]

Long JE, Cobos I, Potter GB, Rubenstein JL. Dlx1&2 and Mash1 transcription factors control MGE and CGE patterning and differentiation through parallel and overlapping pathways. Cereb Cortex 2009;19 Suppl 1:i96–i106. [PubMed: 19386638]

Long JE, Garel S, Alvarez-Dolado M, Yoshikawa K, Osumi N, Alvarez-Buylla A, Rubenstein JL. Dlx-dependent and -independent regulation of olfactory bulb interneuron differentiation. J Neurosci 2007;27:3230–3243. [PubMed: 17376983]

Long JE, Garel S, Depew MJ, Tobet S, Rubenstein JL. DLX5 regulates development of peripheral and central components of the olfactory system. J Neurosci 2003;23:568–578. [PubMed: 12533617]

Loots GG, Ovcharenko I, Pachter L, Dubchak I, Rubin EM. rVista for comparative sequence-based discovery of functional transcription factor binding sites. Genome Res 2002;12:832–839. [PubMed: 11997350]

Markiewicz E, Dechat T, Foisner R, Quinlan RA, Hutchison CJ. Lamin A/C binding protein LAP2alpha is required for nuclear anchorage of retinoblastoma protein. Mol Biol Cell 2002;13:4401–4413. [PubMed: 12475961]

Matys V, Fricke E, Geffers R, Gossling E, Haubrock M, Hehl R, Hornischer K, Karas D, Kel AE, Kel-Margoulis OV, Kloos DU, Land S, Lewicki-Potapov B, Michael H, Munch R, Reuter I, Rotert S, Saxel H, Scheer M, Thiele S, Wingender E. TRANSFAC: transcriptional regulation, from patterns to profiles. Nucleic Acids Res 2003;31:374–378. [PubMed: 12520026]

Matys V, Kel-Margoulis OV, Fricke E, Liebich I, Land S, Barre-Dirrie A, Reuter I, Chekmenev D, Krull M, Hornischer K, Voss N, Stegmaier P, Lewicki-Potapov B, Saxel H, Kel AE, Wingender E. TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. Nucleic Acids Res 2006;34:D108–D110. [PubMed: 16381825]

Mavropoulos A, Devos N, Biemar F, Zecchin E, Argenton F, Edlund H, Motte P, Martial JA, Peers B. sox4b is a key player of pancreatic alpha cell differentiation in zebrafish. Dev Biol 2005;285:211–223. [PubMed: 16055112]

Merkle FT, Mirzadeh Z, Alvarez-Buylla A. Mosaic organization of neural stem cells in the adult brain. Science 2007;317:381–384. [PubMed: 17615304]

Merkle FT, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A. Radial glia give rise to adult neural stem cells in the subventricular zone. Proc Natl Acad Sci U S A 2004;101:17528–17532. [PubMed: 15574494]

Miki R, Kadota K, Bono H, Mizuno Y, Tomaru Y, Carninci P, Itoh M, Shibata K, Kawai J, Konno H, Watanabe S, Sato K, Tokusumi Y, Kikuchi N, Ishii Y, Hamaguchi Y, Nishizuka I, Goto H, Nitanda H, Satomi S, Yoshiki A, Kusakabe M, DeRisi JL, Eisen MB, Iyer VR, Brown PO, Muramatsu M, Shimada H, Okazaki Y, Hayashizaki Y. Delineating developmental and metabolic pathways in vivo by expression profiling using the RIKEN set of 18,816 full-length enriched mouse cDNA arrays. Proc Natl Acad Sci U S A 2001;98:2199–2204. [PubMed: 11226216]

Moberg K, Starz MA, Lees JA. E2F-4 switches from p130 to p107 and pRB in response to cell cycle reentry. Mol Cell Biol 1996;16:1436–1449. [PubMed: 8657117]

Nelson SB, Sugino K, Hempel CM. The problem of neuronal cell types: a physiological genomics approach. Trends Neurosci 2006;29:339–345. [PubMed: 16714064]

Neuman T, Keen A, Zuber MX, Kristjansson GI, Gruss P, Nornes HO. Neuronal expression of regulatory helix-loop-helix factor Id2 gene in mouse. Dev Biol 1993;160:186–195. [PubMed: 8224536]

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314:130–133. [PubMed: 17023659]

Nevins JR. The Rb/E2F pathway and cancer. Hum Mol Genet 2001;10:699–703. [PubMed: 11257102]

Niehrs C, Pollet N. Synexpression groups in eukaryotes. Nature 1999;402:483–487. [PubMed: 10591207]

Nomura T, Osumi N. Misrouting of mitral cell progenitors in the Pax6/small eye rat telencephalon. Development 2004;131:787–796. [PubMed: 14736749]

Otaegi G, Yusta-Boyo MJ, Vergano-Vera E, Mendez-Gomez HR, Carrera AC, Abad JL, Gonzalez M, de la Rosa EJ, Vicario-Abejon C, de Pablo F. Modulation of the PI 3-kinase-Akt signalling pathway by IGF-I and PTEN regulates the differentiation of neural stem/precursor cells. J Cell Sci 2006;119:2739–2748. [PubMed: 16787946]

Parise P, Finocchiaro G, Masciadri B, Quarto M, Francois S, Mancuso F, Muller H. Lap2alpha expression is controlled by E2F and deregulated in various human tumors. Cell Cycle 2006;5:1331–1341. [PubMed: 16760672]

Pollard K, van der Lann M. A new algorithm for hybrid hierarchical clustering with visualization and the bootstrap. Journal of Statistical Planning and Inference 2003;117:275–303.

Rizo J, Rosenmund C. Synaptic vesicle fusion. Nat Struct Mol Biol 2008;15:665–674. [PubMed: 18618940]

Ross SE, Greenberg ME, Stiles CD. Basic helix-loop-helix factors in cortical development. Neuron 2003;39:13–25. [PubMed: 12848929]

Ruzhynsky VA, McClellan KA, Vanderluit JL, Jeong Y, Furimsky M, Park DS, Epstein DJ, Wallace VA, Slack RS. Cell cycle regulator E2F4 is essential for the development of the ventral telencephalon. J Neurosci 2007;27:5926–5935. [PubMed: 17537963]

Sephton CF, Good SK, Atkin S, Dewey CM, Mayer P 3rd, Herz J, Yu G. TDP-43 is a developmentally regulated protein essential for early embryonic development. J Biol Chem 2010;285:6826–6834. [PubMed: 20040602]

Sherr CJ. Cancer cell cycles. Science 1996;274:1672–1677. [PubMed: 8939849]

Stevens CF. Neuronal diversity: too many cell types for comfort? Curr Biol 1998;8:R708–R710. [PubMed: 9778523]

Swiss VA, Casaccia P. Cell-context specific role of the E2F/Rb pathway in development and disease. Glia 2010;58:377–390. [PubMed: 19795505]

Vergano-Vera E, Yusta-Boyo MJ, de Castro F, Bernad A, de Pablo F, Vicario-Abejon C. Generation of GABAergic and dopaminergic interneurons from endogenous embryonic olfactory bulb precursor cells. Development 2006;133:4367–4379. [PubMed: 17038521]

Vicario-Abejon C, Yusta-Boyo MJ, Fernandez-Moreno C, de Pablo F. Locally born olfactory bulb stem cells proliferate in response to insulin-related factors and require endogenous insulin-like growth factor-I for differentiation into neurons and glia. J Neurosci 2003;23:895–906. [PubMed: 12574418]

von Lindern M, Fornerod M, van Baal S, Jaegle M, de Wit T, Buijs A, Grosveld G. The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemiaspecific dek-can mRNA. Mol Cell Biol 1992;12:1687–1697. [PubMed: 1549122]

Wagner N, Krohne G. LEM-Domain proteins: new insights into lamin-interacting proteins. Int Rev Cytol 2007;261:1–46. [PubMed: 17560279]

Whitman MC, Greer CA. Synaptic integration of adult-generated olfactory bulb granule cells: basal axodendritic centrifugal input precedes apical dendrodendritic local circuits. J Neurosci 2007;27:9951–9961. [PubMed: 17855609]

Whitman MC, Greer CA. Adult neurogenesis and the olfactory system. Prog Neurobiol 2009;89:162–175. [PubMed: 19615423]

Wu LS, Cheng WC, Hou SC, Yan YT, Jiang ST, Shen CK. TDP-43, a neuropathosignature factor, is essential for early mouse embryogenesis. Genesis 2010;48:56–62. [PubMed: 20014337]

Xu X, Bieda M, Jin VX, Rabinovich A, Oberley MJ, Green R, Farnham PJ. A comprehensive ChIP-chip analysis of E2F1, E2F4, and E2F6 in normal and tumor cells reveals interchangeable roles of E2F family members. Genome Res 2007;17:1550–1561. [PubMed: 17908821]

Yang YH, Speed T. Design issues for cDNA microarray experiments. Nat Rev Genet 2002;3:579–588. [PubMed: 12154381]

Yun K, Mantani A, Garel S, Rubenstein J, Israel MA. Id4 regulates neural progenitor proliferation and differentiation in vivo. Development 2004;131:5441–5448. [PubMed: 15469968]

Yuste R. Origin and classification of neocortical interneurons. Neuron 2005;48:524–527. [PubMed: 16301166]

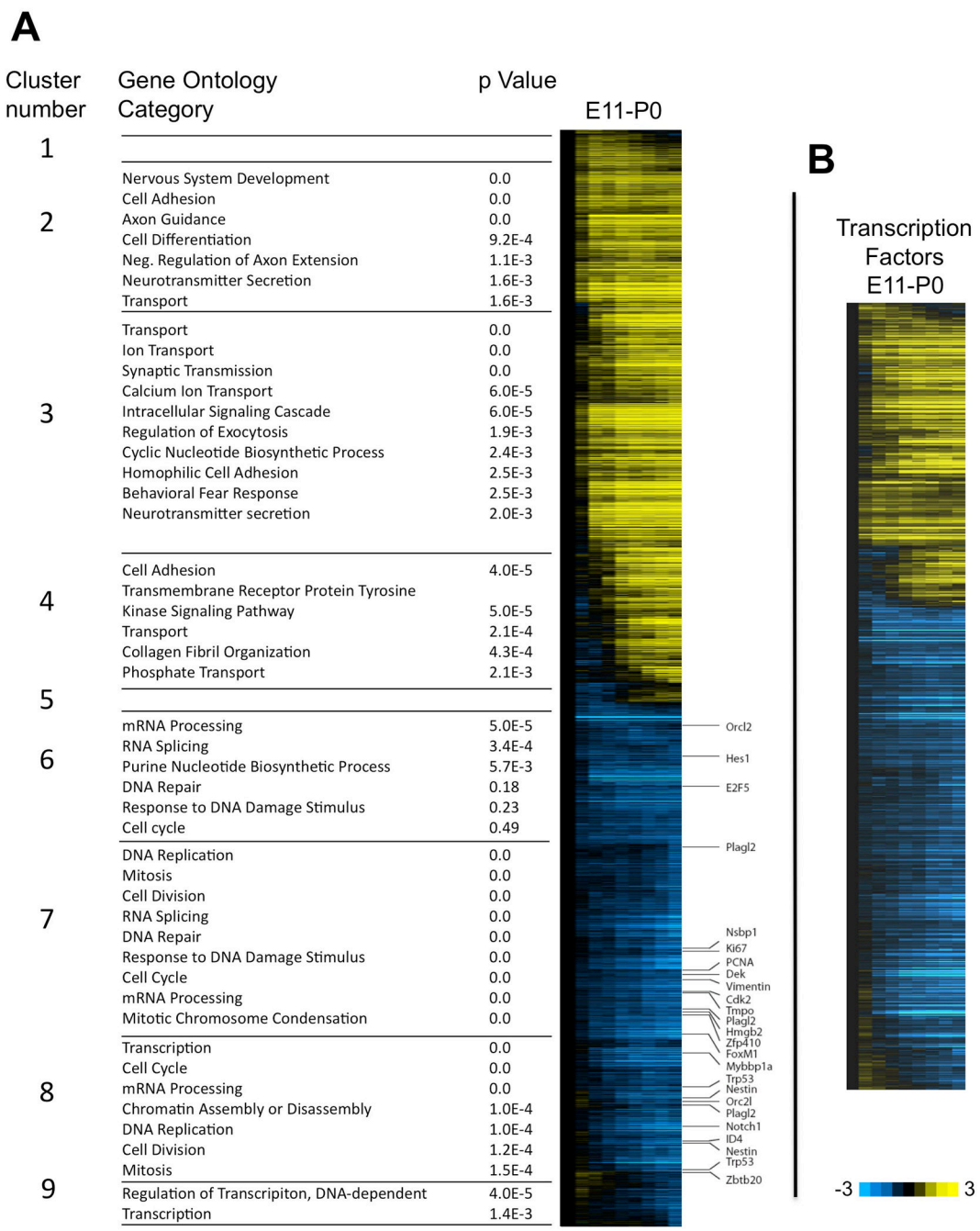

Figure 1. Time course of RNA expression in the embryonic olfactory bulb

(A) Microarray data from embryonic days E11 to P0 normalized to E11. Differentially expressed genes were clustered using the HOPACH algorithm and displayed as a heat map. Blue time points represent decreases from E11 values and yellow represent increases. Table on left lists overrepresented GO terms for clusters 1–9, along with their corresponding p values. P values indicated as 0.0 are less than 10^{-5}. The locations of selected NPC markers are indicated. **(B)** Heat map of differentially expressed TFs.

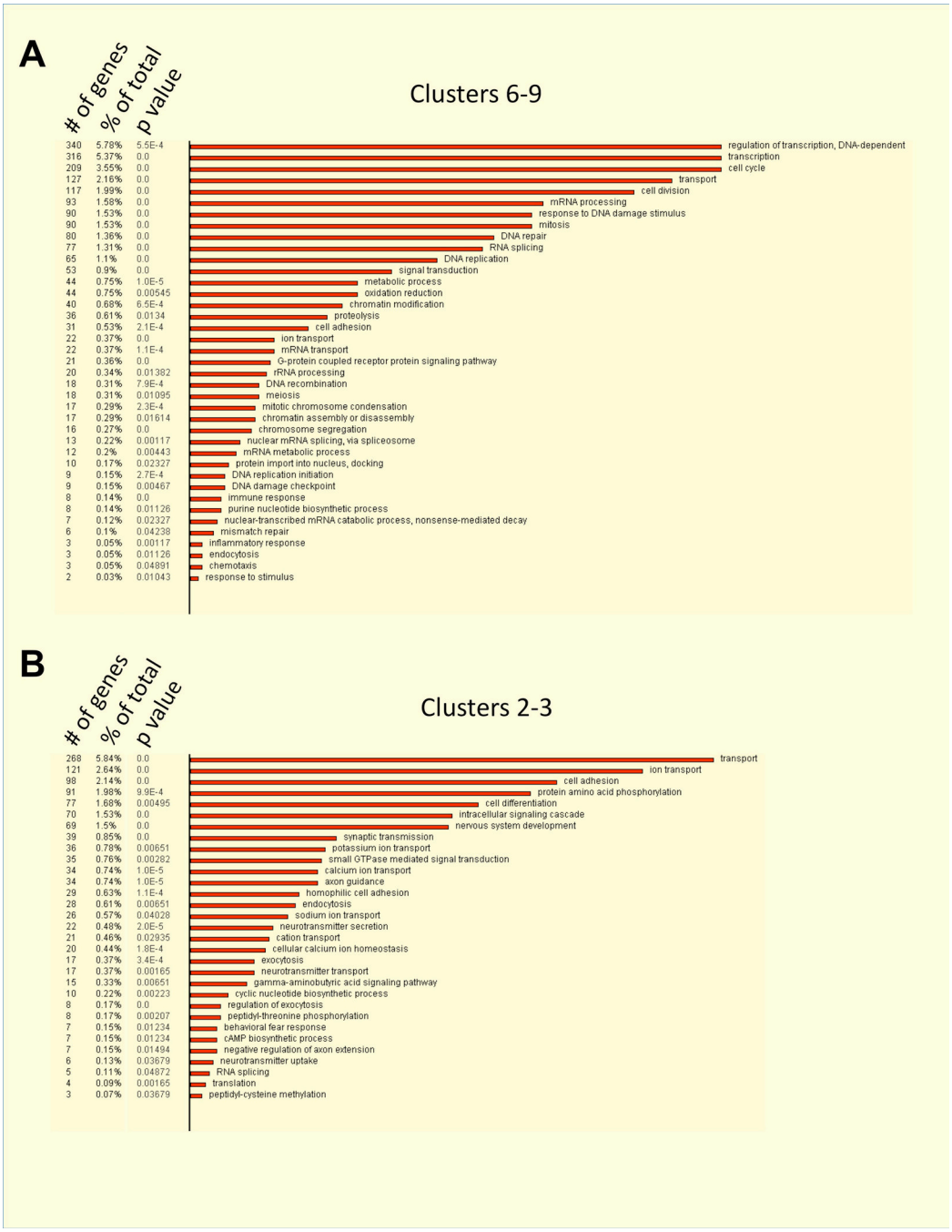

Figure 2. Graphical representation of GO ontology terms enriched in clusters 6–9 and clusters 2–3

The number of genes with a particular GO ontology term in each cluster set is shown in absolute numbers and as a percentage of all of the differentially expressed genes. Clusters 6–9 are enriched for terms associated with proliferating cells, notably cell cycle, cell division, mitosis, DNA repair and DNA replication. Terms enriched in clusters 2–3 include differentiation, nervous system development and synaptic transmission. P values indicated as 0.0 are less than 10^{-5}.

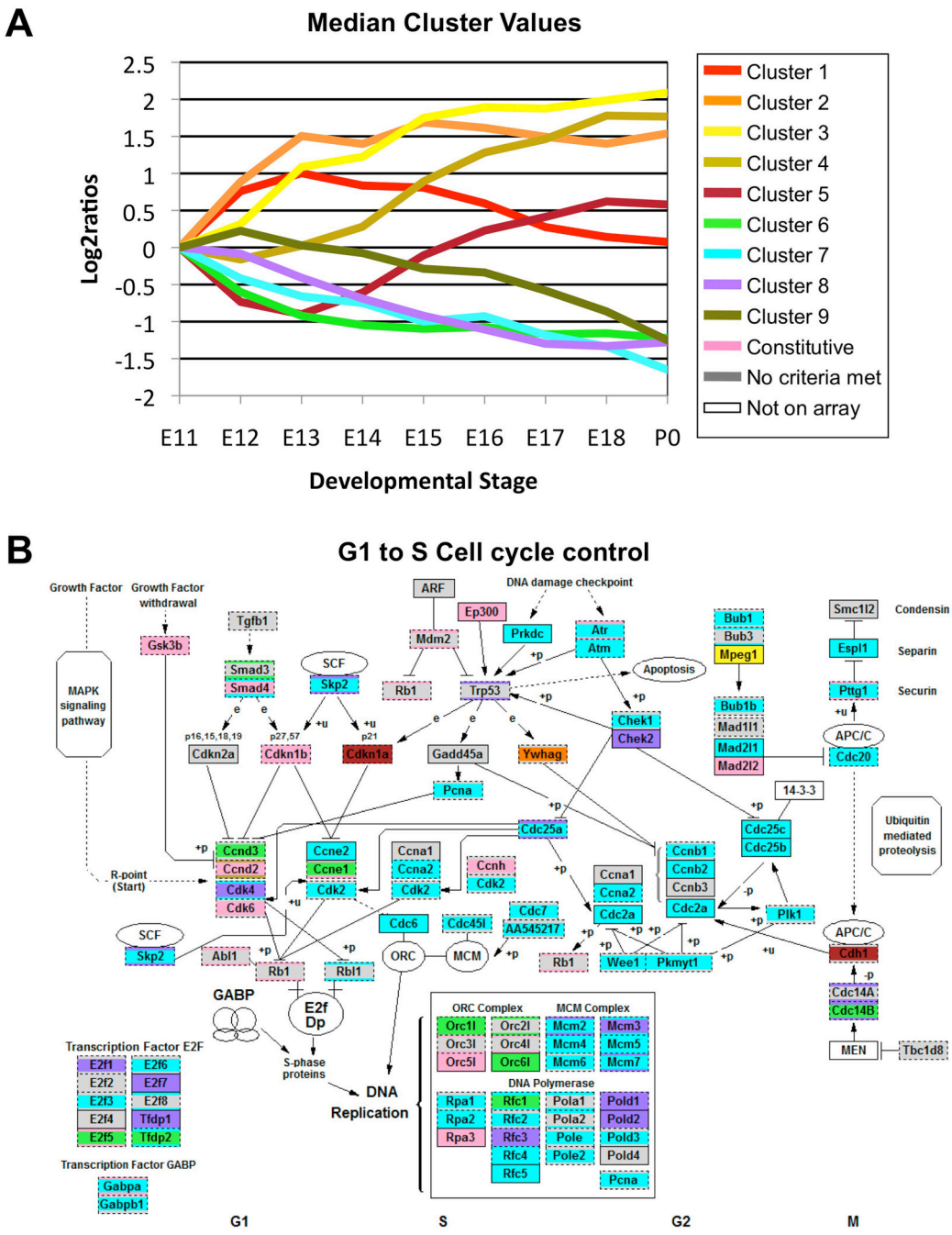

Figure 3. Cell proliferation markers are predominantly located in clusters 6–9

(A) Median log2ratio for expression values between time point and E11. Color key applies for both panels. **(B)** HOPACH cluster information superimposed on G1 to S cell cycle and DNA replication pathways. The cluster membership for each gene in the pathway is indicated by color according to the key in panel A. Genes shaded in different colors for foreground and border had two or more probes that localized to different clusters. Most of the genes in these pathways are located in clusters 6–9, or are constitutively expressed.

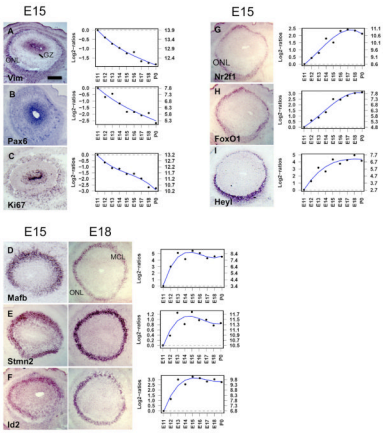

Figure 4. Expression patterns of genes with different profiles are expressed in distinct cell types

RNA *in situ* hybridizations of coronal sections of the OB at E15 and E18 along with time course expression data for selected genes. **(A–C)** E15 OB *in situs* using probes to the neural progenitor markers *Vim*, and *pax6*; and the proliferation marker *Ki67*. All three markers label the germinal region of the OB. *Vim* and *Ki67* also label the nerve layer which is a common pattern for probes that label the germinal region. **(D–F)** E15 OB *in situ* hybridizations for the TF genes *Nr2f1*, *Fox01*, and *Heyl*. These probes specifically label the nerve layer. **(G–I)** OB *in situ* hybridizations for the TF genes *Mafb*, *Stmn2* and *Id2* performed at E15 and E18. At E15 the genes exhibited a diffuse ring of expression in the OB; at E18, labeled cells could be identified as mitral cells. ONL: olfactory nerve layer. GZ: germinal zone. MCL: mitral cell layer. Bar = 200 μm. For microarray expression plots, units for the X-axis are time, log2ratio values are plotted on the left Y axis, A values on the right. (see Materials and Methods).

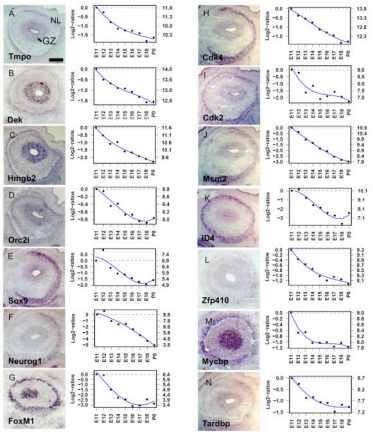

Figure 5. Localization of cluster 6–9 genes to the olfactory bulb germinal zone

RNA *in situ* hybridizations were performed on OBs from E15.5 embryos for the following genes from clusters 6–9: **(A)** *Tmpo*, **(B)** *Dek*, **(C)** *Hmgb2*, **(D)** *Orc2l*, **(E)** *Sox9*, **(F)** *Neurog1*, **(G)** *FoxM1*, **(H)** *Cdk4*, **(I)** *Cdk2*, **(J)** *Mcm2*, **(K)** *Id4*, **(L)** *Zfp410*, **(M)** *Mycbp*, and **(N)** *Tardbp*. All probes hybridize to the germinal region (GZ in panel A) and to a greater or lesser extent to the nerve layer (NL in panel A). Bar = 200 μm.

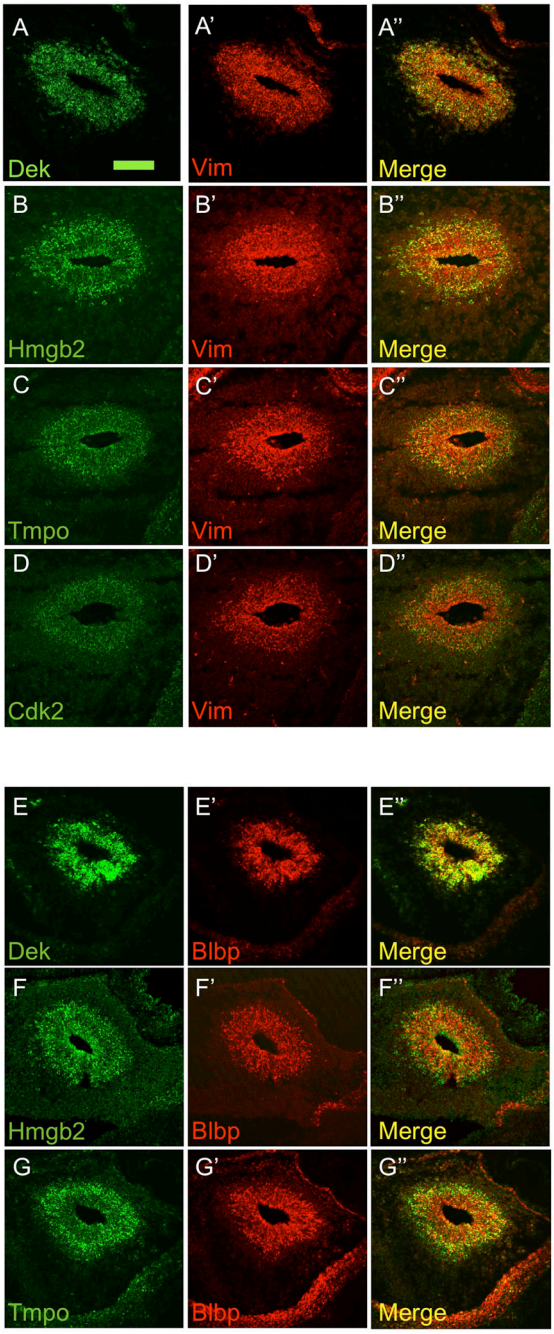

Figure 6. Double-label fluorescent RNA *in situ* hybridization analysis of neural progenitor cell candidate genes at E15.5

Representative genes were selected for co-localization with the NPC markers *Vim* (A–D) and *Blbp* (E–G). Micrographs show the region of the OB including and immediately surrounding the germinal zone. *Dek* (A, E), *Hmgb2* (B, F), *Tmpo* (C, G) and *Cdk2* (D) co-localize with *Vim* and/or *Blbp*, indicating that they are expressed in the population of OB NPCs. Bar = 200 μm.

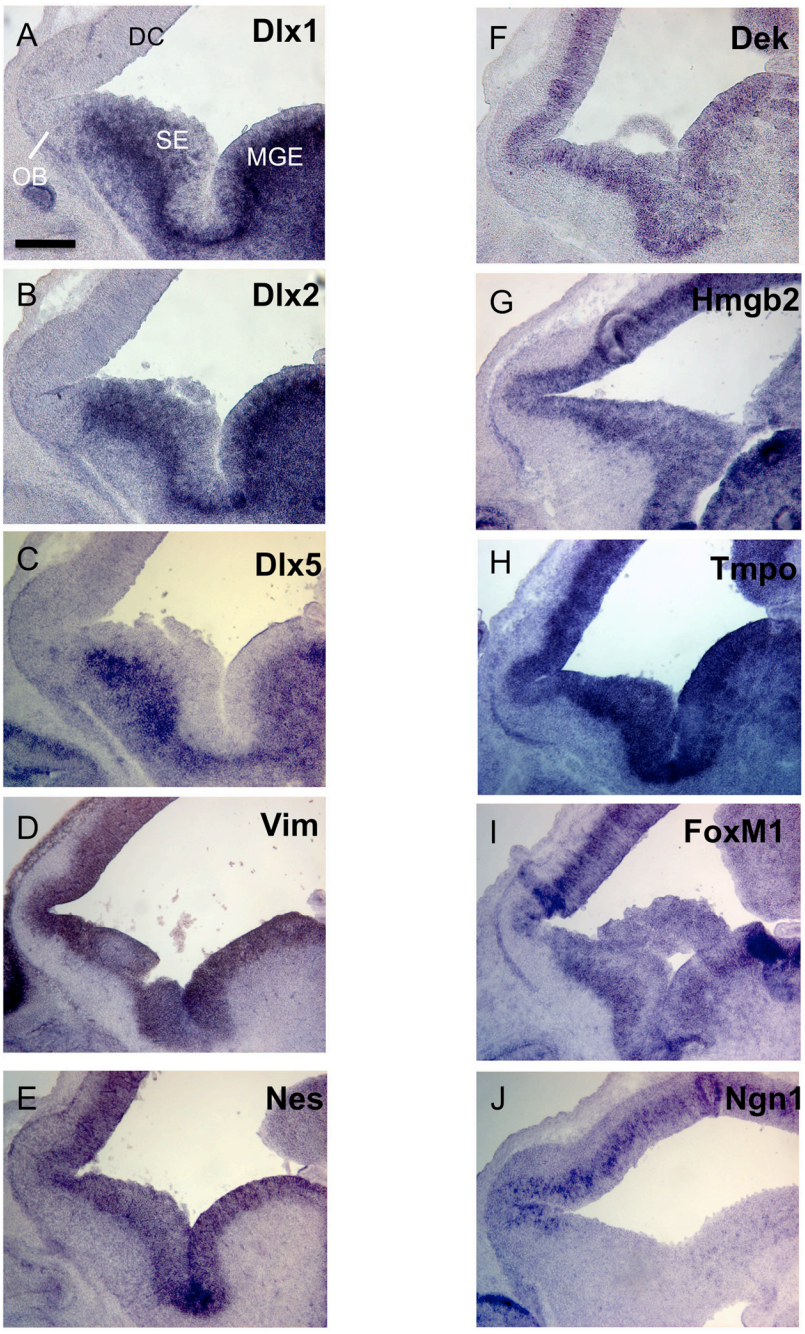

Figure 7. Expression patterns of genes from clusters 6–9 are distinct from those of *Dlx1*, *Dlx2*, and *Dlx5* in the early telencephalon

(A–C) By RNA in situ hybridizations were performed on parasagittal sections of OBs from E13.5 embryos. *Dlx1*, *Dlx2*, and *Dlx5* exhibit strong expression in the septum and medial ganglionic eminence, but very little staining in the nascent OB. **(D–E)** In contrast, *Nes* and *Vim* staining is present throughout the germinal region of the telencephalon including the developing OB. **(F–J)** Candidate genes from clusters 6–9 including *FoxM1*, *Dek*, *Hmgb2*, *Tmpo*, and *Ngn1* (*Neurog1*) exhibit similar staining patterns to *Nes* and *Vim*, with Ngn1 expression excluded from the septum and ganglionic eminence. OB, olfactory bulb; DC, dorsal cortex; SE, septum; MGE, medial ganglionic eminence. Bar = 200 μm.

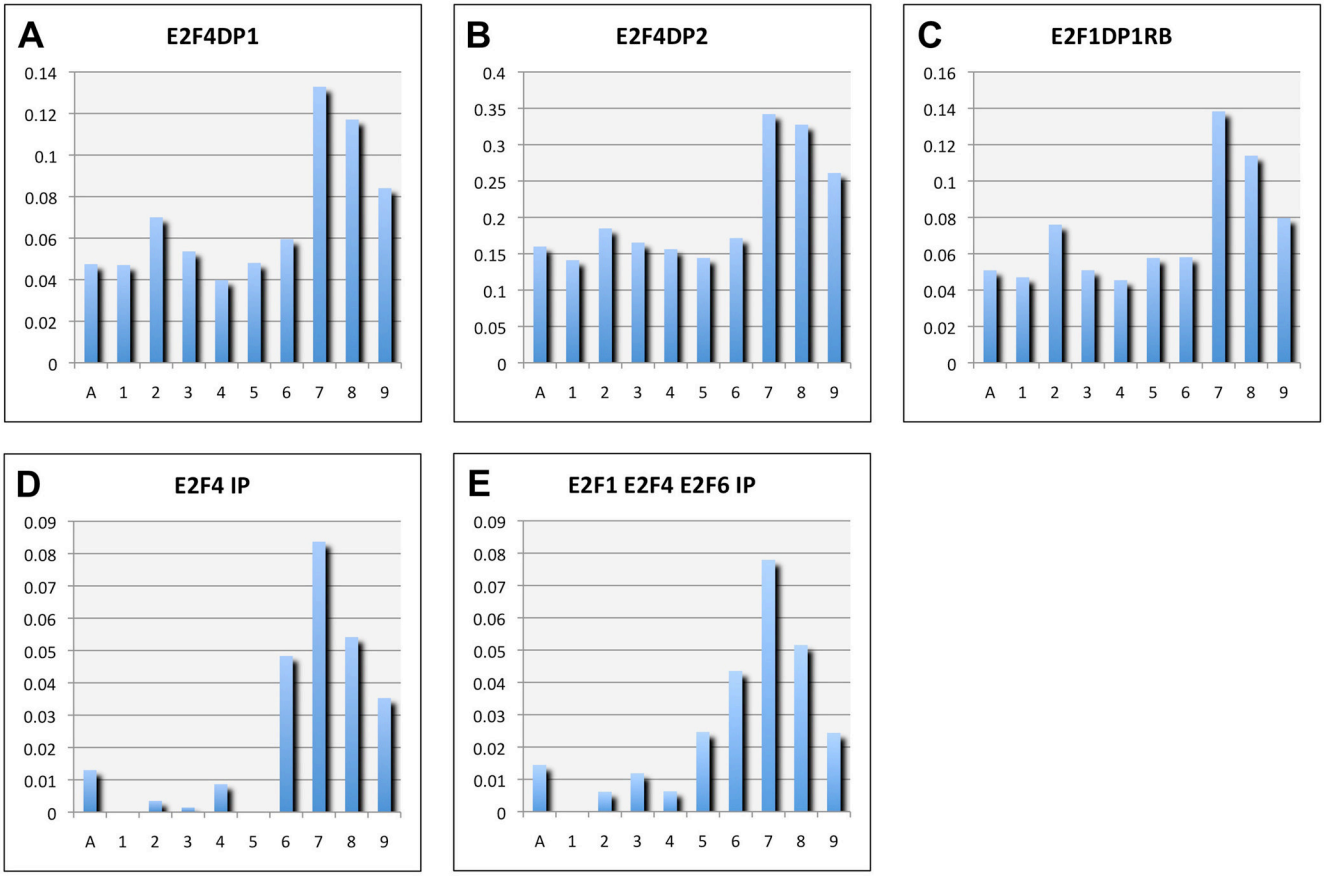

Figure 8. Genes with E2F binding sites are enriched in NPC clusters

(A–C) Three E2F binding sites in the rVista database were over-represented in NPC clusters 6–9: E2F1/DP1, E2F4/DP1, and E2F4/DP2. Cluster numbers are indicated below the X of each plot. The Y axis indicates the mean number of binding sites per gene in each cluster. The first column (column A) is the calculation for all genes on Affymetrix chip. **(D, E)** Similar plots were generated for genes with binding sites for E2F4 (Conboy et al., 2007) and E2F1/E2F4/E2F6 (Xu et al., 2007) as detected by chromatin immunoprecipitation (ChIP). Note the enrichment of genes with E2F family binding sites in the NPC clusters using both informatics predictions (A–C) and direct ChIP analyses (D, E).

Table 1
Classes of TFs encoded by differentially expressed genes

TFs encoded by genes in differentially expressed genes matched to Interpro domain descriptions. Domains are tabulated by the number of genes in clusters 6–9, number in clusters 2–3, and overall number in the dataset. Many transcription factors contain more than one domain and therefore are included in more than one class.

Transcription Factor Class	Number Overall	Clusters 6–9	Clusters 2–3
Total TFs	507	320	135
Znf	120	73	34
Pro rich	60	28	21
Homeobox	41	28	7
HLH	33	16	14
HMG	22	19	3
ANK	21	11	8
Antifreeze	18	13	3
AAA ATPase	13	13	0
bZip	12	2	5
BTB/POZ	12	5	4
POU	12	9	1
Nuclear receptor	10	1	5
RNA Pol	9	7	2
WD40	7	5	2
MCM	7	7	0
p53_like_TF_DNA-bd	7	4	1
Forkhead	6	1	1
PAS	6	0	6
E2F	5	5	0
Ets	5	2	1
Chromodomain	5	4	1
EF Hand	4	1	1
No Match	102		

Table 2

Genes confirmed by RNA in situ hybridization to be expressed in the olfactory bulb germinal zone

Genes with more than one cluster number reflect the behavior of different probe sets for the same gene and may result the recognition of different splice forms.

Cluster Number	Gene Symbol	Gene Name	Gene Function
6	E2f5	E2F transcription factor 5	Transcription Factor/DNA Binding Protein
6	Hes1	hairy and enhancer of split 1	Transcription Factor/DNA Binding Protein
6	Limd1	LIM domains containing 1	Transcription Factor/DNA Binding Protein
6	Mycbp	c-myc binding protein	Transcription Factor/DNA Binding Protein
6	Neurog1	neurogenin 1	Transcription Factor/DNA Binding Protein
6	Bmpr1a	bone morphogenetic protein receptor, type 1A	Receptor
6	Bmpr1b	bone morphogenetic protein receptor, type 1B	Receptor
6, 7	Tardbp	TAR DNA binding protein	Transcription Factor/DNA Binding Protein
6, 7	Tfdp2	transcription factor Dp 2	Transcription Factor/DNA Binding Protein
6, 8	Orc2l	origin recognition complex, subunit 2-like	Transcription Factor/DNA Binding Protein
6, 9	Zbtb20	zinc finger and BTB domain containing 20	Transcription Factor/DNA Binding Protein
7	Cdk2	cyclin-dependent kinase 2	Transcription Factor/DNA Binding Protein
7	Dek	DEK oncogene	Transcription Factor/DNA Binding Protein
7	E2f6	E2F transcription factor 6	Transcription Factor/DNA Binding Protein
7	Gabpa	GA repeat binding protein, alpha	Transcription Factor/DNA Binding Protein
7	Hmgb2	high mobility group box 2	Transcription Factor/DNA Binding Protein
7	Hmgb3	high mobility group box 3	Transcription Factor/DNA Binding Protein
7	mKi67	antigen identified by monoclonal antibody Ki 67	Proliferation Marker
7	Pcna	proliferating cell nuclear antigen	DNA Replication Factor
7	Plagl1	pleiomorphic adenoma gene-like 1	Transcription Factor/DNA Binding Protein
7	Rpo1-1	RNA polymerase 1-1	Transcription Factor/DNA Binding Protein
7	Tmpo	thymopoietin	Transcription Factor/DNA Binding Protein
7	Ttrap	Traf and Tnf receptor associated protein	Transcription Factor/DNA Binding Protein
7	Vim	vimentin	Intermediate filament
7	Whsc1	Wolf-Hirschhorn syndrome candidate 1	Transcription Factor/DNA Binding Protein
7	Zfp410	zinc finger protein 410	Transcription Factor/DNA Binding Protein
7, 8	Cdk4	cyclin-dependent kinase 4	Transcription Factor/DNA Binding Protein
7, 8	MCM2	minichromosome maintenance deficient 2 mitotin	Transcription Factor/DNA Binding Protein
7, 8	Plagl2	Pleiomorphic adenoma gene-like 2	Transcription Factor/DNA Binding Protein
7, 8	Ssbp3	single-stranded DNA binding protein 3	Transcription Factor/DNA Binding Protein
7, 8	Zfp521	zinc finger protein 521	Transcription Factor/DNA Binding Protein
7, 8	Hes6	hairy and enhancer of split 6	Transcription Factor/DNA Binding Protein
8	Ankhd1	ankyrin repeat and KH domain containing 1	Transcription Factor/DNA Binding Protein
8	E2F1	E2F transcription factor 1	Transcription Factor/DNA Binding Protein
8	E2F7	E2F transcription factor 7	Transcription Factor/DNA Binding Protein
8	FoxM1	forkhead box M1	Transcription Factor/DNA Binding Protein

Cluster Number	Gene Symbol	Gene Name	Gene Function
8	Gli2	GLI-Kruppel family member GLI2	Transcription Factor/DNA Binding Protein
8	Mybbp1a	MYB binding protein (P160) 1a	Transcription Factor/DNA Binding Protein
8	Nes	nestin	Intermediate filament
8	Nr2e1	nuclear receptor subfamily 2, group E, member 1	Transcription Factor/DNA Binding Protein
8	Pax6	paired box gene 6	Transcription Factor/DNA Binding Protein
8	Rbl1	retinoblastoma-like 1 (p107)	Transcription Factor/DNA Binding Protein
8	Sox9	SRY-box containing gene 9	Transcription Factor/DNA Binding Protein
8	Tcf3	transcription factor 3	Transcription Factor/DNA Binding Protein
8	Notch1	Notch gene homolog 1	Transcription Factor/DNA Binding Protein
8	Id4	inhibitor of DNA binding 4	Transcription Factor/DNA Binding Protein
8	Atf7ip	activating transcription factor 7 interacting protein	Transcription Factor/DNA Binding Protein
3, 8	Dnmt3a	DNA methyltransferase 3A	Transcription Factor/DNA Binding Protein
8, 9	Trp53	transformation related protein 53	Transcription Factor/DNA Binding Protein
8,9	Trim28	tripartite motif protein 28	Transcription Factor/DNA Binding Protein