Comprehensive Expression Map of Transcription Regulators in the Adult Zebrafish Telencephalon Reveals Distinct Neurogenic Niches

Nicolas Diotel, Rebecca Rodriguez Viales, Olivier Armant, Martin März, Marco Ferg, Sepand Rastegar,* and Uwe Strähle*

[1]Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Campus Nord, Karlsruhe, Germany

ABSTRACT

The zebrafish has become a model to study adult vertebrate neurogenesis. In particular, the adult telencephalon has been an intensely studied structure in the zebrafish brain. Differential expression of transcriptional regulators (TRs) is a key feature of development and tissue homeostasis. Here we report an expression map of 1,202 TR genes in the telencephalon of adult zebrafish. Our results are summarized in a database with search and clustering functions to identify genes expressed in particular regions of the telencephalon. We classified 562 genes into 13 distinct patterns, including genes expressed in the proliferative zone. The remaining 640 genes displayed unique and complex patterns of expression and could thus not be grouped into distinct classes. The neurogenic ventricular regions express overlapping but distinct sets of TR genes, suggesting regional differences in the neurogenic niches in the telencephalon. In summary, the small telencephalon of the zebrafish shows a remarkable complexity in TR gene expression.

The adult zebrafish telencephalon has become a model to study neurogenesis. We established the expression pattern of more than 1200 transcription regulators (TR) in the adult telencephalon. The neurogenic regions express overlapping but distinct sets of TR genes suggesting regional differences in the neurogenic potential. J. Comp. Neurol. 523:1202–1221, 2015.

INDEXING TERMS: zebrafish; forebrain; adult neurogenesis; transcription regulator; expression pattern; database

Differential transcription of genes is a mechanism underlying a wide variety of cellular processes including cell proliferation, differentiation, and survival (Norton, 2000; Ferg et al., 2014). The transcription of genes is controlled by transcriptional regulators (TRs). Neurogenesis is based on regulatory cascades of TRs, eventually leading to differentiation of the many distinct neuronal cell types. Differential expression of TR genes is a key feature not only during development but also in the maintenance and function of the complex structures of the central nervous system (CNS) in the adult.

The zebrafish genome encodes 3,302 putative TR genes representing ~12.7% of total protein coding genes (Armant et al., 2013). Of these, about 2,600 genes are detectably expressed in the 1-day-old embryo (Armant et al., 2013). The large representation of this gene ontology group in the genome underscores the importance of precise control of gene transcription during various processes such as development and body homeostasis. TRs affect transcription of genes at different levels from influencing the state of chromatin, to interaction with DNA regulatory elements, to the modulation of the general transcription machinery by cell-

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The first two authors contributed equally to this work.

Current address: Inserm U1188, Université de La Réunion, Plateforme CYROI, Saint-Denis de La Réunion, France

Current address: Centre for Pulmonary Hypertension Thoraxclinic and Institute of Human Genetics, University of Heidelberg, Germany

Current address: Department of Pediatrics, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

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*CORRESPONDENCE TO: Sepand Rastegar or Uwe Strähle, Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Campus Nord, PO box, Karlsruhe, Germany. E-mail: sepand.rastegar@kit.edu or uwe.straehle@kit.edu.

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specific employment of different components. Very frequently, TR genes are subject to differential transcription themselves, thereby serving specific regulatory roles in the regionally restricted hierarchical steps of organ development. In the developing CNS, for example, TRs are expressed in distinct territories such as the prosomeres of the forebrain or the rhombomeres of the hindbrain (Narita and Rijli, 2009; Lauter et al., 2013).

TRs can be subdivided roughly into three different functional classes: transcription factors, chromatin remodeling factors, and factors of the general transcription machinery. One characteristic of transcription factors (TFs) is that they contain one or more DNA-binding domains (Ptashne and Gann, 1997). Upon binding to a specific DNA sequence TFs activate or repress transcription of target genes. Frequently, multiple TFs act in a combinatorial fashion and the interaction of factors determines the specific regulatory outcome (Pabo and Sauer, 1992; Latchman, 1997; Lee and Young, 2000). Forced expression of single TFs or TFs in specific combinations can change cell identity from one cell type to another and can induce pluripotency in differentiated cells (Davis et al., 1987; Schafer et al., 1990; Stühmer et al., 2002; Takahashi and Yamanaka, 2006; Young et al., 2007a,b). TFs interact with factors of the general transcription machinery and other TRs including coactivators and corepressors. Furthermore, some of the TRs can act as chromatin remodelers such as histone acetylases, deacetylases, or methylases and play crucial functions controlling access to the DNA via regulation of chromatin structure (Luo and Dean, 1999; Clapier and Cairns, 2009).

In recent years, the zebrafish has become a well-recognized model for studying adult neurogenesis (Kizil et al., 2012b; Grandel and Brand, 2013; Schmidt et al., 2013). In mammals, predominantly two regions of the brain exhibit neurogenic properties during adulthood, i.e., the subventricular zone of the telencephalon and the subgranular zone in the dentate gyrus, respectively (Ming and Song, 2011; Grandel and Brand, 2013; Lacar et al., 2014). In comparison, the brain of the adult zebrafish exhibits an enormous capacity to generate new neurons (Grandel et al., 2006; Pellegrini et al., 2007; Ayari et al., 2010; März et al., 2010a, 2011; Baumgart et al., 2012; Kishimoto et al., 2012; Diotel et al., 2013). It harbors 16 proliferative zones distributed in many brain regions (Zupanc et al., 2005; Lindsey and Tropepe, 2006; Adolf et al., 2006; Grandel et al., 2006; Kaslin et al., 2008; Grandel and Brand, 2013). Moreover, injury increases this baseline of constitutive neurogenesis even further, leading to effective production of neurons and repair of the injured tissue (Zupanc, 2006; Ayari et al., 2010; Kroehne et al., 2011; März et al., 2011; Baumgart et al., 2012; Kishimoto et al., 2012; Kizil et al., 2012a,c; Diotel et al., 2013; Edelmann et al., 2013; Kyritsis et al., 2013). The proliferative activity observed in the adult zebrafish brain is due to the persistence of neurogenic progenitors, such as radial glial cells (RGCs) and neuroblasts (Adolf et al., 2006; Pellegrini et al., 2007; Lam et al., 2009; März et al., 2010a; Lindsey et al., 2012). The adult zebrafish telencephalon contains RGCs that express the markers glial fibrillary acidic protein (GFAP), S100b, and nestin. The cell bodies of RGCs reside along the entire ventricular surface of the everted telencephalon. Zebrafish, as all ray-finned fishes, are characterized by a T-shaped ventricle between the two everted lobes of the telencephalon. As a consequence, proliferative cells in the periventricular regions occupy also the areas immediately below the tela choroidea. Previously, it was suggested that this eversion is the result of outward folding and growth of the pallial lobes laterally (see Folgueira et al., 2012, and references therein). More recently, a two-step model of formation of the zebrafish telencephalon was proposed (Folgueira et al., 2012). This entails first formation of the anterior intraencephalic sulcus followed by posterior growth of the telencephalon (Folgueira et al., 2012).

In the adult telencephalon, RGCs can be distinguished by their cell cycle kinetics. Type I cells are RGCs that are quiescent, while type II cells are slowly proliferating (PCNA-positive) RGCs that produce committed progenitors such as neuroblasts. Neuroblasts correspond to type III cells. The ventral subpallium contains faster proliferating cells than ventricular zones further dorsal (Adolf et al., 2006). The different progenitor populations show a distinctive distribution in the telencephalon. Type I and II cells are mainly found in the pallial ventricular zone and are absent from the rostral migratory stream (RMS), located at the subpallial medial ventricular zone. The RMS encompasses adjacent parts of the ventricular/periventricular zones of the ventral nucleus of the telencephalic area (Vv) and the dorsal nucleus of the ventral telencephalic area (Vd). The RMS is mostly composed of fast proliferating type III cells that express polysialylated neuronal cell adhesion molecule (PSA-NCAM). Regional differences not only appear to exist in the proliferation rate and type of progenitors in neurogenic regions (Adolf et al., 2006; März et al., 2010a,b), but limited expression studies also indicated that there are differences in gene expression. For example, the mRNA of the bHLH TF Olig2 is expressed in two subregions of the proliferative zone of the telencephalon, one of which is located in the RMS and expresses PCNA. The other region is located ventral to the RMS and is PCNA-negative (März et al., 2010b), suggesting regional differences in the programming of the stem cell niches in the telencephalon.

To understand the sequence of regulatory events that lead to the generation of new neurons, knowledge of the regionally restricted regulatory programs in progenitors, differentiated neurons, and glia is necessary. Based on deep sequencing data, 1,202 TR genes were chosen and subjected to a detailed *in situ* expression analysis in the adult zebrafish telencephalon. This comprehensive expression map provides insights into a potential regulatory role of specific TRs in adult neurogenesis and maintenance of differentiation and function of the telencephalon.

MATERIALS AND METHODS

Zebrafish strains

Experiments were performed on 6–12-month-old adult AB wildtype or transgenic zebrafish *Tg(−3.9nestin:GFP)*, (RRID: ZFIN_ZDB-GENO-100308-4; zf168Tg) (Lam et al., 2009) and *Tg(olig2:EGFP)*, (RRID: ZFIN_ZDB-GENO-041129-1; vu13Tg) (Shin et al., 2003) which were maintained on a 14/10-hour light-dark cycle at 28.5°C in recirculation systems (Schwarz, Germany; Müller and Pfleger, Germany) and fed with commercial food and in-house hatched brine shrimp as described (Westerfield, 2007).

Dissection and fixation

Fish were anesthetized in 0.02% tricaine methanesulfonate (MS-222, pH 7) before being killed in ice water (Westerfield, 2007). Brains were carefully removed and fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS, pH 7.4) overnight at 4°C. They were then stepwise dehydrated in a methanol/PBS concentration series and stored at −20°C (Adolf et al., 2006; Schmidt et al., 2014). Each *in situ* hybridization (ISH) and immunohistochemical staining was repeated at least 3 times.

Immunohistochemistry

Immunostainings were performed on free-floating transverse vibratome-sections as described in Adolf et al. (2006). Sections were cut on a vibratome (Vibratome 1500) to a thickness of 50 μm. Primary polyclonal chicken anti-GFP antibody (1:1,000, Aves Labs, Tigard, OR; Cat# GFP-1020, RRID: AB_10000240) was labeled with secondary antibodies (of the Alexa Fluor series; Alexa 488, Invitrogen, La Jolla, CA; Cat# A11039, RRID: AB_142924). The sections were mounted on glass slides in Aqua Polymount (Polysciences, Warrington, PA) (Adolf et al., 2006; Schmidt et al., 2014). Immunohistochemistry using GFP antibody on wildtype zebrafish results in no labeling.

In situ hybridization

Digoxigenin (DIG) labeled antisense riboprobes were used from the previously described collection (Armant et al., 2013). ISH on whole adult brains were performed as described (Adolf et al., 2006; Schmidt et al., 2014). Briefly, brains were rehydrated through MetOH/PBS gradient series and washed several times in 0.1% Tween, PBS buffer (PTw; pH 7.4). They were next incubated for 30 minutes in PTw containing proteinase K (10 μg/ml) at room temperature (20°C). After postfixation in 4% PFA for 30 minutes and washes in PTw, brains were then prehybridized for 3 hours before overnight incubation at 65°C in hybridization buffer (pH 6) containing the DIG-labeled probes. The second day, after several washing steps, brains were incubated briefly in blocking buffer (pH 7.4) before embedding in 2% agarose. They were sectioned using the Leica vibrating blade microtome VT1000 S at 50 μm thickness and blocked again for 1 hour at room temperature. Incubation with anti-digoxigenin-AP, Fab fragments (1:4,000; Roche, Nutley, NJ; Cat# 11093274910, RRID: AB_514497) was performed overnight at 4°C. The next day the brain sections were washed with PTw before staining with NBT/BCIP buffer (pH 9.5). For fluorescent ISH on adult brains, signal amplification was performed using a tyramide amplification kit according to the manufacturer's instructions

Abbreviations

Candt	commissura anterior, pars dorsalis
Dc	central zone of the dorsal telencephalic area
Dd	dorsal zone of the dorsal telencephalic area
Dl	lateral zone of the dorsal telencephalic area
Dm	medial zone of the dorsal telencephalic area
Dp	posterior zone of the dorsal telencephalic area
dV Dm	dorsal ventricular zone of Dm
ENd	entopeduncular nucleus
GFAP	glial fibrillary acidic protein
Lot	lateral olfactory tract
Mot	medial olfactory tract
mV Dm	medial ventricular zone of Dm
Par	parenchyma (brain tissue with the exception of the ventricular zone)
PSA-NCAM	polysialylated neuronal cell adhesion molecule
PTw	0.1% Tween, PBS buffer (PTw)
RGCs	radial glial cells
RMS	rostral migratory stream
Sup	rest of the subpallium corresponding to the subpallial region without specific annotations in the zebrafish brain atlas (region of the subpallium surrounded by the Lot, Dc, Vd, Vv, Va, Vl and ENd, excluding Vc and Mot)
SY	sulcus ypsiloniformis,
TF	transcription factor
TR	transcriptional regulator
Va	area below Vv
Vc	central nucleus of the ventral telencephalic area
Vd	dorsal nucleus of the ventral telencephalic area
VDd	ventricular zone of Dd (VDd)
VDl	ventricular zone of Dl
VDp	ventricular zone of Dp
Vl	lateral nucleus of the ventral telencephalic area
Vv	ventral nucleus of the ventral telencephalic area
VVd	ventricular zone of Vd
VVv	ventricular zone of Vv

TABLE 1.

Antibodies, Model Organisms and Software Tools Used in This Work

Antibody	Immunogen	Manufacturer, host species, mono/polyclonal, catalog number	Dilution used	Research Resource Identifiers (RRID)
Sheep anti-digoxigenin Fab fragments antibody, AP conjugated	DIG	Roche, sheep polyclonal, Fab fragments conjugated to AP, #11093274910	1:4,000	AB_514497
Sheep anti-digoxigenin Fab fragments antibody, POD conjugated	DIG	Roche, sheep polyclonal, Fab fragments conjugated to POD, # 11207733910	1:1,000	AB_514500
Chicken anti-GFP	GFP	Aves Labs, chicken, polyclonal, GFP-1020	1:1,000	AB_10000240
Alexa Fluor 488 goat antichicken IgG (H+L)	Chicken IgG (H+L)	Molecular Probes (Invitrogen), goat, # A11039	1:1,000	AB_142924

Model organisms	Interest	Research Resource Identifiers (RRID)
Tg(−3.9nestin:GFP) zebrafish	GFP expression in nestin-positive cells corresponding to neural progenitors: RGCs and further committed progenitors	ZFIN_ZDB-GENO-100308-4
Tg(olig2:EGF) zebrafish	GFP expression in oligodendrocytes and oligodendrocyte progenitor cells	ZFIN_ZDB-GENO-041129-1

Tools	Interest	Research Resource Identifiers (RRID)
TreeView 3.0	Graphically browse results of clustering	OMICS_01574

(TSA Plus Cyanine 3 System, Perkin Elmer, Boston, MA). Briefly, during the first day brains were processed as previously described with an additional step that corresponds to the quenching of endogenous peroxidase in 1% (v/v) H_2O_2 prior to proteinase K treatment. On the second day, brains were transversely sectioned, blocked, and incubated overnight with an anti-digoxigenin-poly-POD antibody (1:1,000; Roche; Cat# 11207733910, RRID: AB_ 514500). The next day sections were stained with tyramide Cy3 solution (1:100) in 0.002% (v/v) H_2O_2 in PTw. The sections were then washed in PTw and processed for immunohistochemistry using an anti-GFP antibody (1:1000; Aves Labs, polyclonal antibody, chicken anti-GFP, Cat# GFP-1020; RRID: AB_10000240) and a secondary antibody coupled to Alexa 488 (1:1000; Molecular Probes (Invitrogen); Cat# A11039; RRID AB_142924). The patterns obtained were analyzed using the anatomical landmarks of the zebrafish brain atlas as reference to annotate gene expression patterns (Wullimann et al., 1996). In order to classify TR gene expression with respect to cell proliferation at the ventricular zone, we scored in addition gene expression in ventricular domains adjacent to the anatomical landmarks of Wullimann et al. (1996). This was the case for the dorsal nucleus of the ventral telencephalic area (Vd), the ventral nucleus of the ventral telencephalic area (Vv), the lateral zone of the dorsal telencephalic area (Dl), the medial zone of the dorsal telencephalic area (Dl), and the posterior zone of the dorsal telencephalic area (Dp). Consequently, several anatomical annotations were added such as ventricular zone of Vv (VVv), ventricular zone of Vd (VVd), medial ventricular zone of Dm (mV Dm), dorsal ventricular zone of Dm (dV Dm), ventricular zone of Dl (VDl), and ventricular zone of Dp (VDp). In addition, as our expression analyses revealed the existence of a distinct region below the Vv, we named this zone Va.

Research Resource Identifiers (RRIDs) of antibodies and strains as far as the identifiers were available are summarized in Table 1.

Clustering analysis

To cover all areas of the telencephalon, gene expression patterns were scored and annotated to the standard anatomical landmarks using on average 15 (±2) sections per telencephalon. Levels of expression were estimated by visual observations relative to regions within a section or between sections treated in parallel in the same staining experiment. Tissue annotations of *in situ* expression data were transformed into a matrix of gene expression (0 no expression, 0.5 moderate expression, 1 high expression) and subjected to hierarchical clustering using the uncentered correlation metric and pairwise average linkage method (de Hoon et al., 2004). The resulting heatmaps were visualized with Treeview 3.0 (RRID: OMICS_01574) (Saldanha, 2004) (white: no expression, pink: moderate expression, red: high expression). Expression patterns of 769 TR genes expressed in a restricted manner in the entire telencephalon and of 574 TR genes expressed in the VZ were clustered.

Disclosures

Zebrafish were maintained in the fish facility of the Institute of Toxicology and Genetics (ITG) at Karlsruhe Institute of Technology (KIT). Experiments on animals were performed in accordance with the German animal

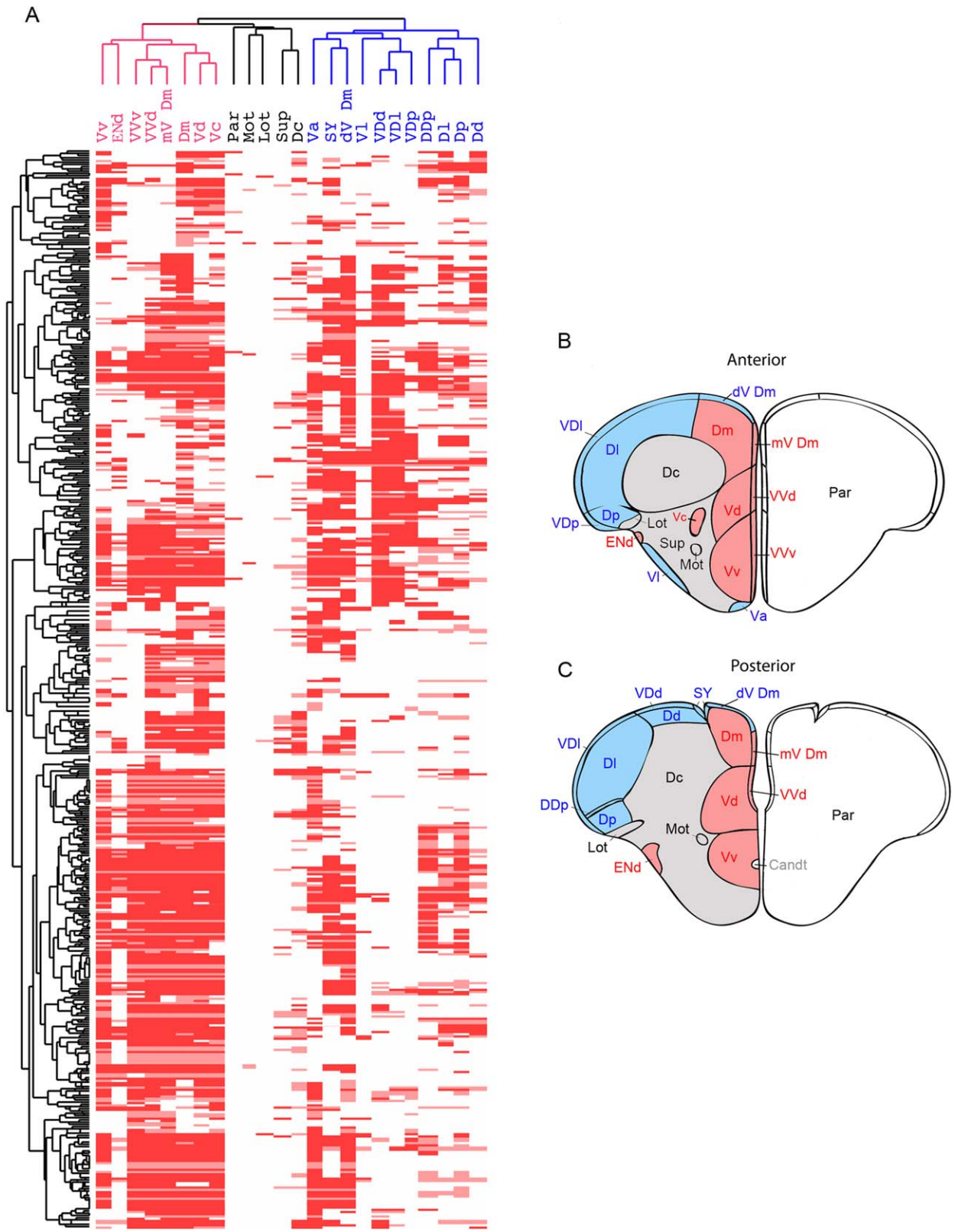

Figure 1. Hierarchical clustering of expression patterns of TR genes ($n = 769$) in the telencephalon of adult zebrafish. **A:** Clustering of TR gene expression patterns deposited in the AGETAZ database. Note ubiquitously expressed TR genes were excluded from the analysis. Anatomical terms used are indicated on top. With respect to anatomical areas, expression patterns fall into three major classes comprising dorsolateral structures (blue), ventromedial structures (red), and a third class in black comprising distinct scattered domains (gray). White: no expression; pink = moderate expression; red = strong expression. **B,C:** Scheme of adult telencephalon transverse sections at two different rostrocaudal levels (B: anterior, C: posterior) and the associated anatomical terms. Dorsolateral structures are marked in blue, ventromedial structures are marked in red, and areas comprising distinct scattered patterns are highlighted in gray. B: A scheme of a section at an intermediate anteroposterior level of the telencephalon and (C) a more caudal section.

protection standards and were approved by the Government of Baden-Württemberg, Regierungspräsidium Karlsruhe, Germany (Aktenzeichen 35–9185.81/G-272/12 "Adulte Neurogenese").

RESULTS

Systematic analysis of TR gene expression in the telencephalon of adult zebrafish

To establish a comprehensive expression atlas of TR genes, we took advantage of a recently published cDNA collection of 2,149 zebrafish TR cDNAs, encoding DNA binding proteins, chromatin remodeling proteins, and factors of the general transcriptional machinery (Armant et al., 2013). We wished to limit the analysis to clones with a high likelihood of spatially restricted expression in the telencephalon. We used two prescreening criteria for selecting genes for *in situ* expression analysis. First, we compared the expression pattern of 196 genes in the 1-day-old embryo and the adult telencephalon. Genes with a nonrestricted expression pattern in the 1-day-old embryo (Armant et al., 2013) also showed a ubiquitous expression in the adult brain (data not shown). Thus, we excluded from the analysis genes that are expressed ubiquitously in the embryo. Second, we referred to RNA sequencing data generated from the adult zebrafish telencephalon (Rodriguez Viales et al., 2014) and selected TR genes whose transcripts were detected at a minimum of 10 reads per kilobase per million reads (rpkm $\geq$10). In total, by excluding the genes that exhibited a ubiquitous expression in the embryo, we finally analyzed 1,202 genes by ISH with DIG-labeled antisense probes on transverse sections through the adult telencephalon.

The expression patterns were essentially annotated according to the anatomical atlas of the zebrafish brain (Wullimann et al., 1996). Moreover, for detailed analysis of gene expression in the neurogenic regions, we also scored expression in the ventricular areas immediately adjacent to the subdivisions described by Wullimann et al. (1996). In addition, we introduced a new anatomical region Va in the subpallium (see schemes in Fig. 1). Sections at three distinct anteroposterior levels of the telencephalon were analyzed and expression patterns were scored relative to anatomical landmarks of the telencephalon. The expression data and anatomical annotations together with supplemental information such as gene expression levels and chromosome location were compiled in a publicly available database named AGETAZ for Atlas of Gene Expression in the Telencephalon of Adult Zebrafish that is accessible at http://cory.itg.kit.edu/agetaz/index.php.

Hierarchical clustering of expression data

Hierarchical clustering of expression patterns of 769 regionally restricted genes was employed to identify TR genes coexpressed in specific anatomical structures. Ubiquitously expressed genes were excluded from this analysis. At a global level, expression domains of TR genes segregated into three distinct clusters reflecting the overall organization of the adult telencephalon (Fig. 1). The TR gene expression patterns of the ventromedial domains (cluster 1) are more closely related to the medial zone of the dorsal telencephalic area (cluster 2), while the expression patterns of the dorsolateral domains (cluster 3) take up a more distant relation. Cluster 1 contains the subpallial domains ventral nucleus of the ventral telencephalic area (Vv), ventricular zone of Vv (VVv), dorsal nucleus of the ventral telencephalic area (Vd), ventricular zone of Vd (VVd), central nucleus of the ventral telencephalic area (Vc), but also the pallial medial zone of the dorsal telencephalic area (Dm) and medial ventricular zone of Dm (mV Dm), and the entopeduncular nucleus (ENd) (Fig. 1A–C, red areas in the schemes). Interestingly, shared gene expression in this group is conserved throughout the rostrocaudal axis of the telencephalon (Fig. 1B,C, red). Cluster 2 is composed of the central zone of the dorsal telencephalic area (Dc), the lateral olfactory tract (Lot), the medial olfactory tract (Mot), and the rest of the subpallial parenchyma without specific annotations in the zebrafish brain atlas. We refer to this latter region as Sup in our scoring of expression patterns. In cluster 2, most of the TR genes are detected in the Dc, which contains more cells in comparison to the Lot, Mot, and the Sup. Finally, cluster 3 is composed of dorsolateral brain regions of the pallium (dorsal zone of the dorsal telencephalic area (Dd), ventricular zone of Dd (VDd), the sulcus ypsiloniformis (SY); the dorsal ventricular zone of Dm (dV Dm), the lateral zone of the dorsal telencephalic area (Dl), the posterior zone of the dorsal telencephalic area, (Dp), the ventricular zone of Dl (VDl), the ventricular zone of Dp (VDp) but also of two subpallial nuclei (Va and Vl).

While this overall clustering approach suggested shared TR gene expression programs in the three clusters of anatomical regions, most TR genes are expressed in multiple ventral and dorsal domains (Fig. 1), suggesting that these factors may be involved in more general functions or act in a combinatorial fashion together with other factors to generate region-specific regulatory control.

Patterns of coexpressed genes

TR genes act frequently in a combinatorial fashion. Coexpression of specific genes may thus reflect a potential functional interaction. Furthermore, synexpression of TR genes in different regions of the telencephalon may indicate common regulatory mechanisms. We

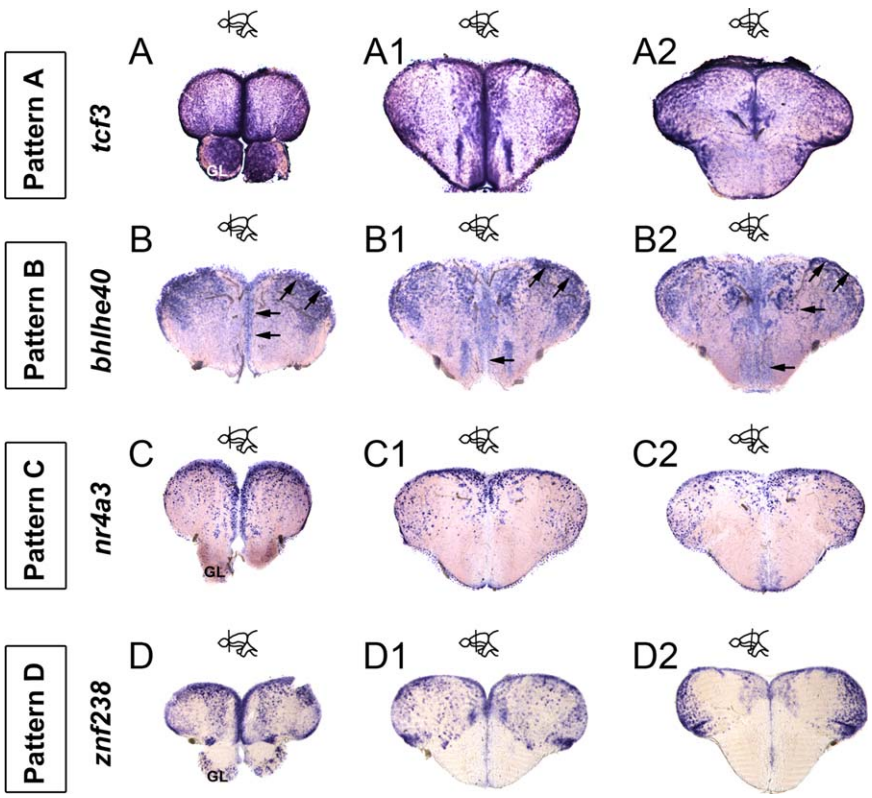

Figure 2. Mapping of distinct expression patterns exhibited by TRs at three different levels of the telencephalon (pattern A–M). **A–M:** Schematic distribution of TR gene expressing cells in three representative telencephalic sections taken from the zebrafish brain atlas (adapted from Wullimann et al., 1996). In all, 562 TR genes were classified into 13 different restricted expression pattern categories annotated from A to M. The 640 remaining TRs that did not fit to these 13 categories each show a unique combination of these different patterns. A–H: Examples of ISH of TR genes in the telencephalon of adult zebrafish exhibiting the expression patterns A (*tcf3*), B (*bhlhe40*), C (*nr4a3*), D (*znf238*), E (*vax1*), F (*prdm12*), G (*zfp36l1a*), and H (*six6a*). The *E-protein* gene *tcf3* is ubiquitously expressed in the telencephalon; the *bhlhe40* gene displays a widespread expression, whereas the zinc finger *znf238* is mostly detected in the pallium. In contrast the ventral homeobox 1 (*vax1*) and the *PR domain containing 12* genes (*prdm12*) are mainly expressed in the subpallium, in the ventral and central telencephalic nuclei. The *zinc finger protein 36, C3H type-like 1a* (*zfp36l1a*) exhibits a pattern reminiscent of oligodendrocytes and the *sine oculis-related homeobox 6a* (*six6*a) is mainly detected in the dorsal nucleus of the ventral telencephalic area. Arrows in B–B2 show differences of *bhlhe40* staining between different regions of the same section such as the ventricular zone of the ventral nucleus of the ventral telencephalic area (VVv) and the lateral zone of the dorsal telencephalic area (Dl) in B1. I–M: Examples of ISH of TR genes in the telencephalon of adult zebrafish exhibiting the expression patterns I (*sox4a*), J (*pou3f3b*), K (*foxj1a*), L (*meis1*), and M (*dmrta2*). *sox4a* is detected along the ventricular/periventricular layer according to the rostrocaudal axis, *pou3f3b* in a large ventricular/periventricular stripe notably near the RMS. In contrast, *dmrta2* is expressed only in ventricular cells, and *foxj1a* appears to be expressed in the RMS. The *meis1* gene is expressed around the RMS. Arrows highlight the strong *sox4a* and of *foxj1a* expression in the RMS (I1–II2 and K–K2), the *dmrta2* expression in the ventricular zone (M1–M2). Scale bar = 200 μm.

therefore investigated TR genes according to shared patterns of expression (Fig. 2; Supplemental Table 1) using our annotation of expression domains to anatomical landmarks (Wullimann et al., 1996). We identified 13 specific patterns (A–M, Fig. 2) that are shared by at least two TR genes. These include the categories "ubiquitous" (pattern A) and "widespread" (pattern B) expression as well as 11 other categories with different more region-restricted patterns of expression. The genes classified in this way correspond to a total of 562 genes. The remaining 640 genes represented unique patterns that were not shared entirely with another gene and were therefore left unclassified. The E-zone tool was installed as a search option in the AGETAZ database (http://cory.itg.kit.edu/agetaz/index.php) for detection of genes coexpressed in a particular region, allowing further rapid evaluation of coexpression of these 640 genes with those classified in specific patterns.

Pattern A: ubiquitous expression.

Among the 1,202 genes, we identified 354 genes that were ubiquitously expressed, corresponding to 29.5% of the total investigated genes. For example, the basic helix-loop-helix *transcription factor 3* (*tcf3*, also termed E12/E47) is expressed in the entire telencephalon from the olfactory bulbs to caudal regions of the

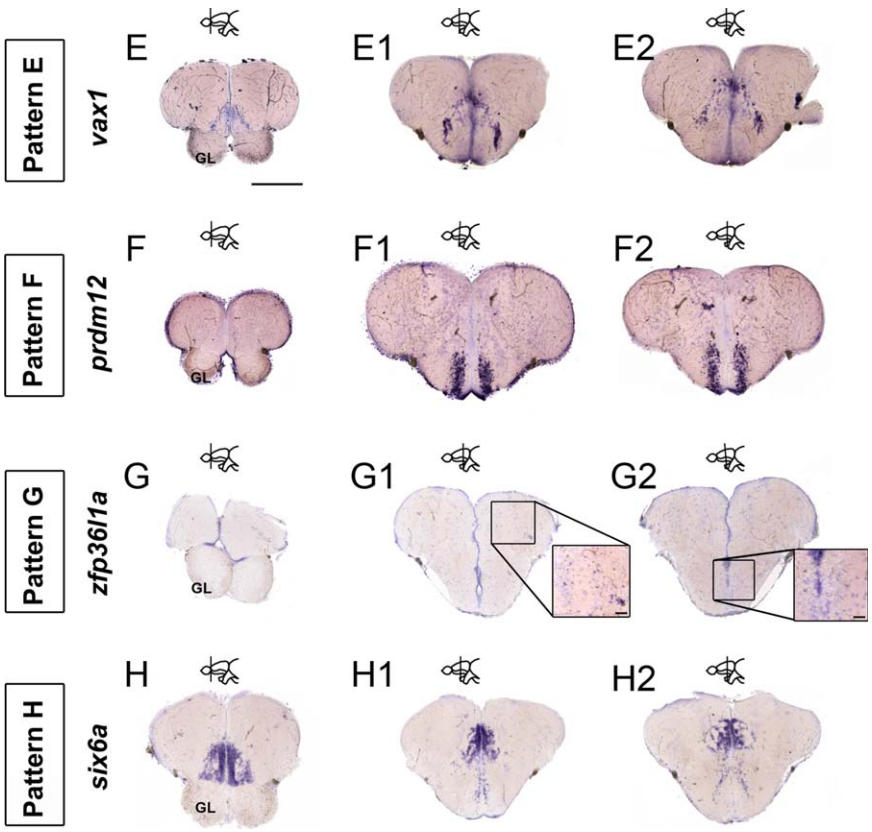

Figure 2. (continued).

telencephalon (Fig. 2A–A2). Expression of factors which we classified in pattern A as ubiquitously expressed are characterized by uniform staining intensities in all telencephalic nuclei.

Pattern B: widespread expression with variable intensity in subregions.

In contrast to the homogenous staining of ubiquitously expressed TR genes, the genes of this category as, for example, the *basic helix-loop-helix family, member e40* (*bhlhe40*, Fig. 2B–B2) are expressed at different levels in some brain nuclei and regions. *bhlhe40* shows a stronger staining in the lateral zone of the dorsal telencephalic area (Dl) compared to the medial ventricular zone. We identified 143 genes (~11.9% of total genes analyzed) which were detected in all telencephalic regions, but showed differences in expression intensity that varied in different regions from gene to gene.

Pattern C: expression in single cells with strong perinuclear staining.

We noticed genes whose expression is characterized by the appearance of single cells with strong perinuclear staining (Figs. 2C–C2, 3A–F). The genes subsumed under pattern C are thus grouped according to the subcellular distribution of their mRNA rather than the overall expression pattern in the telencephalon. For example, the nuclear receptor *nr4a3* is expressed in a limited number of cells in close vicinity of the periventricular stripe in the dorsal telencephalic area in the anterior part of the telencephalon, and in the parenchyma (Fig. 2, pattern C–C2). The medial ventricular layer does not express detectable levels of *nr4a3* transcripts. We also observed positive cells in the dorsal, lateral, medial, and posterior zones of the dorsal telencephalic area, as well as in a central zone. However, in these regions there were less expressing cells compared to the other dorsal telencephalic regions. Only few positive cells were detected in the subpallium. Perinuclear staining pattern of expression was observed for 10 TR genes corresponding to 0.8% of the total investigated genes. Interestingly, *jun B proto-oncogene* (*junb*), *jun B proto-oncogene b* (*junbb*), and *FBJ murine osteosarcoma viral oncogene homolog B* (*fosb*), corresponding to factors that form the activator protein 1 complex (AP1), belong also to this class of patterns (Fig. 3A–F).

Pattern D: pallial expression.

Pattern D comprises TR genes that were almost exclusively detected in cells of the pallial region. These genes

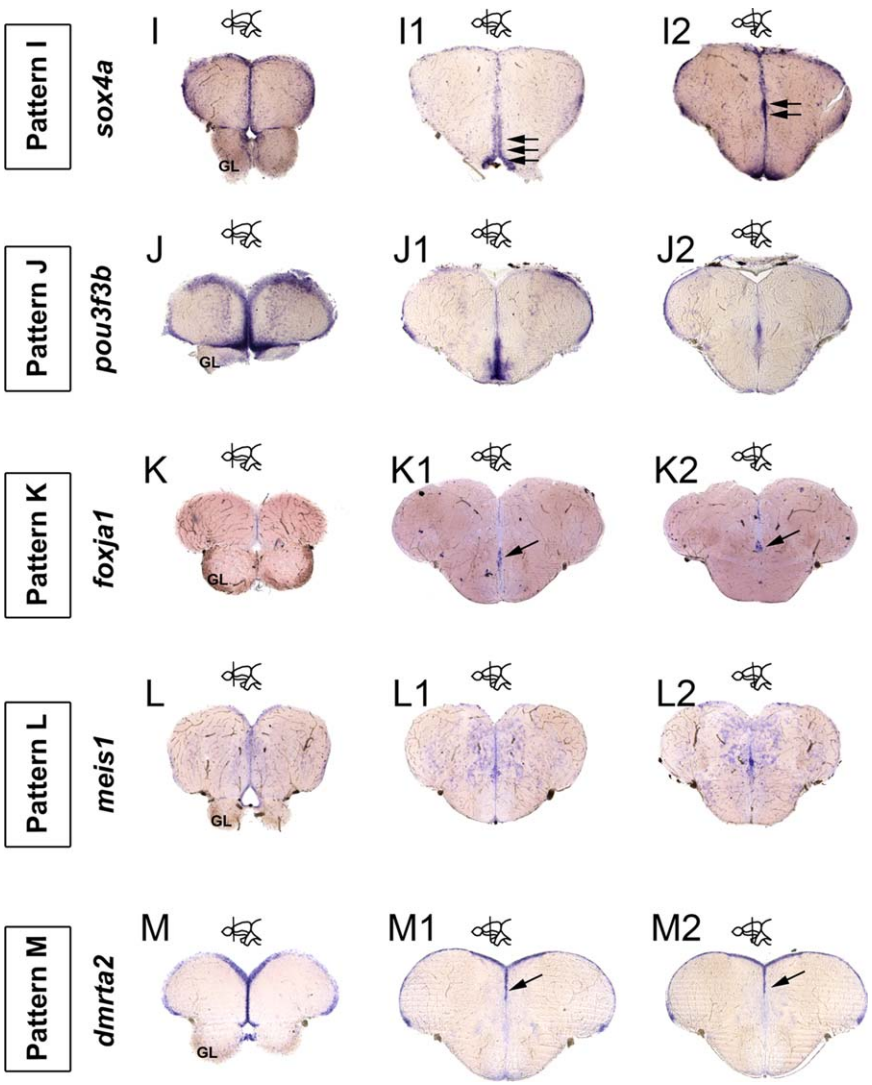

Figure 2. (continued).

are not or only weakly expressed in the subpallium. One example of a pattern D gene is the C2H2-type zinc finger transcription factor *znf238* (or *rp58*). Its transcripts were detected in the anterior part of the telencephalon in the dorsal telencephalic area (D), in the parenchyma, and the periventricular region at the junction between the olfactory bulbs and the telencephalon. Expression was also observed in the glomerular layer of the olfactory bulbs (GL). More posteriorly, *znf238* transcripts were scored in the central (Dc), dorsal (Dd), lateral (Dl), medial (Dm), and posterior (Dp) zones, in the parenchyma and in the periventricular region of the dorsomedial and dorsolateral telencephalon (Fig. 2D–D2). We identified 13 genes with such a pattern of expression, corresponding to ~1.1% of the total investigated genes. This pattern of expression includes also *neurod*, as previously shown (Ganz et al., 2012).

Pattern E: strong expression in the central nucleus of the ventral telencephalic area (Vc).

The expression of five genes (~0.4% of the total investigated genes) was high in the central nucleus (Vc) of the ventral telencephalic area (Fig. 2E–E2). In addition to their prominent expression in Vc, expression at a lower level was detected in other regions of the brain. For example, the *ventral anterior homeobox 1* gene (*vax1*) is strongly expressed in the Vc (Fig. 2E–E2) and weaker expression was detected along the medial and dorsomedial ventricular/periventricular layer of the telencephalon and in ventral and dorsal nuclei of the ventral telencephalon. We only observed a low expression in a few cells in the central, lateral, and posterior zones of the dorsal telencephalic area. We did not identify any gene among the

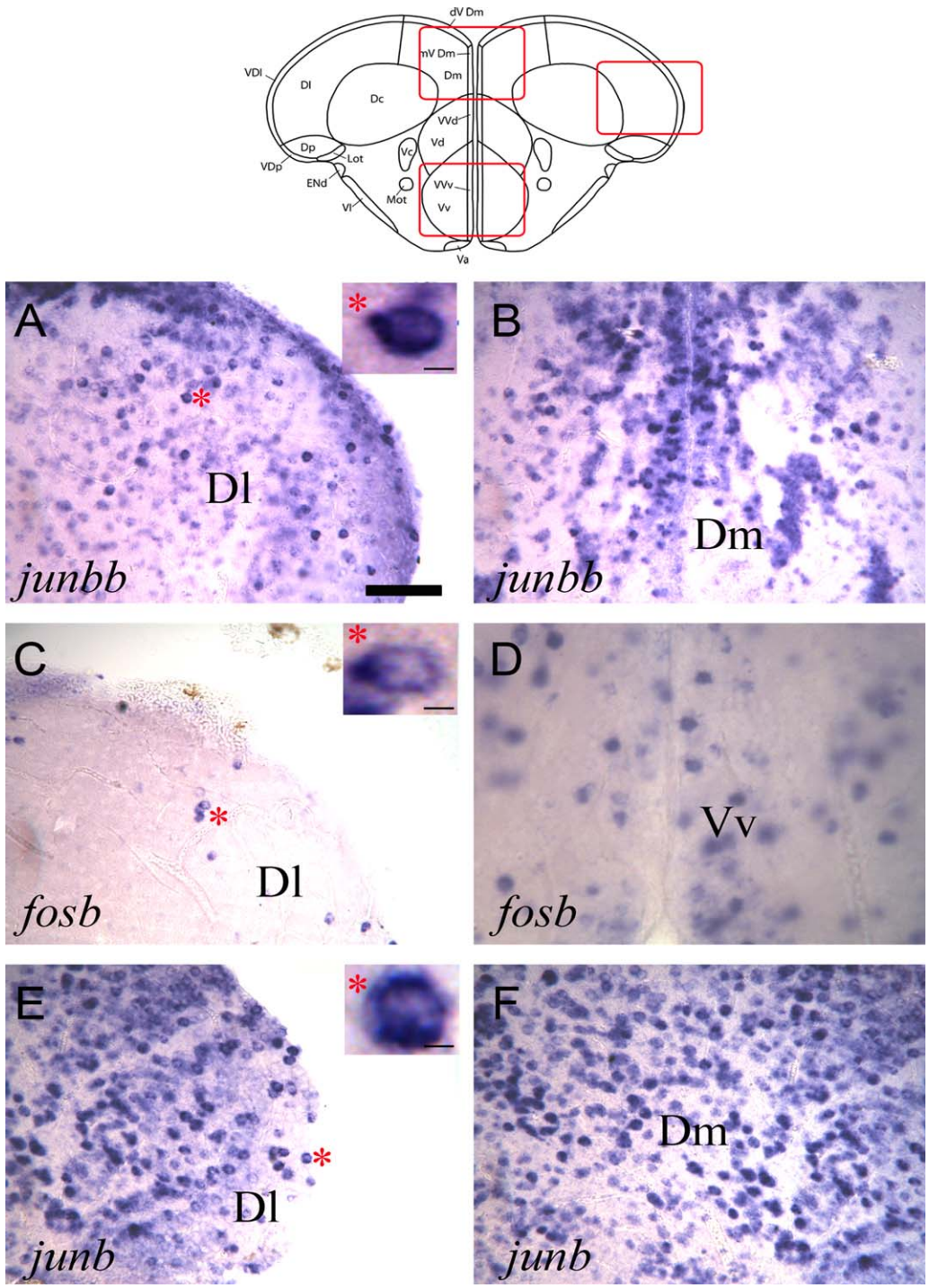

Figure 3. Pattern C genes exhibit an intense staining around the cell nucleus. *Transcripts of jun B proto-oncogene b* (*junbb*, **A,B**), *FBJ murine osteosarcoma viral oncogene homolog B* (*fosb*, **C,D**) and *jun B proto-oncogene* (*junb*, **E,F**) transcripts are enriched in the perinuclear space of expressing cells. A,C,E: Inserts provide high-magnification views of perinuclear staining (red stars) showing a more intense labeling around the cell nucleus. The schematic cross-section through the telencephalon indicates the regions of interest which have been studied. A,C,F: Regions from dorsolateral telencephalon. B,D,F: Ventral region of the telencephalon and medial zone of the pallium. Scale bar = 70 μm; 5 μm for inserts.

1,202 genes analyzed that was exclusively expressed in the central nucleus of the ventral telencephalic area. The pattern E genes are the *ventral anterior homeobox 1* gene (*vax1*), the *sp9 transcription factor* (*sp9*), the *human immunodeficiency virus type I enhancer binding protein 1* (*hivep1*), the *SRY-box containing gene 2* (*sox2*), and the *SUB1 homolog S. cerevisiae* (*SUB1*).

Pattern F: strong expression in the ventral nucleus of the ventral telencephalic area (Vv).

An example for expression pattern F is the *positive regulatory (PR)-domain zinc-finger 12* transcripts (*prdm12*). *prdm12* expression was strongly detected in the ventral nucleus of the ventral telencephalic area (Vv), in a very distinct stripe of cells (Fig. 2F–F2). In addition, a very weak expression was observed in the whole telencephalon. We identified seven factors exhibiting the pattern E (∼0.6 % of the total investigated genes). Among these genes, we also identified *isl1* that was previously reported to be expressed specifically in the subpallium of zebrafish (Mueller and Wullimann, 2009; Ganz et al., 2012).

Pattern G: expression in oligodendrocytes.

Transcripts expressed in pattern G (∼0.2% the total investigated genes) exhibit a distribution in the parenchyma reminiscent of oligodendrocytes (März et al., 2010b). Cells of the oligodendrocyte lineage are characterized by the expression of the TF *olig2* whose expression has been described in detail previously (März et al., 2010b). In addition to *olig2*, there is only one other gene that shares this restricted expression pattern: the putative transcription factor *zfp36l1a* (Fig. 2G–G2). It is expressed in single cells in the parenchyma and exhibits a distribution reminiscent of oligodendrocyte precursor cells (OPCs).

Pattern H: strong expression in the dorsal nucleus of the ventral telencephalic area (Vd).

The TF *sine oculis-related homeobox 6a* (six6a), is strongly expressed in the dorsal telencephalic area (Fig. 2H) and more posteriorly in the dorsal nucleus of the ventral telencephalic area (Fig. 2H1,H2). Some weaker expression can also be observed along the periventricular layer at the level of the ventral nuclei of the subpallium (Fig. 2H–H2). No expression was detected in the ventricular cells. We scored six TR genes expressing this pattern: *dachshund a* (*dacha*), *sine oculis-related homeobox 6a and 3b* (*six6a, six3b*), v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived avian b (*mycl1b*), POU class 3 homeobox 1 (*pou3f1*), and *KIAA1107*. These correspond to ∼0.5% of the investigated genes.

Pattern I: strong expression in the periventricular layer and fast proliferative region (RMS).

SRY-box containing gene 4a (sox4a) is one of the genes exhibiting pattern I. Its expression was detected along the periventricular layer in the anterior part of the telencephalon at the olfactory bulb/telencephalon junction and more posteriorly in the dorsomedial and dorsolateral periventricular zones (Fig. 2I–I2). However, expression was more prominent at the level of the RMS-like region composed of fast dividing progenitors (Fig. 2I–I2, arrows). In addition to *sox4a*, the *zinc finger homeobox 4* (*zfhx4*), the *gastrulation brain homeobox 1* (*gbx1*), the *SRY-box containing gene* (*six3a*), and the *LIM homeobox 8a* (*lhx8a*) belong to this pattern I (∼0.4% of total investigated genes).

Pattern J: strong expression in the RMS and some parts of the ventricular zone.

TR genes exhibiting the pattern J such as the *POU class 3 homeobox 3b* (*pou3f3b*) in many aspects show a distribution quite similar to pattern I (Fig. 2J–J2). However, at the level of the olfactory bulbs/telencephalon junction, expression is detected in a larger ventricular/periventricular stripe and some staining is also observed in the parenchyma of the dorsal telencephalic area. More caudally, we also detected a weak expression in the parenchyma, mostly in the central nucleus of the pallium. We also recorded *pou3f3b* expression in the ventricular zone of the medial zone (Dm), lateral (Dl), and posterior (Dp) zones of the dorsal telencephalic area. Strong expression was observed along a large ventricular/periventricular layer in the ventral nucleus of the ventral telencephalon and more posteriorly in the region localized at the junction between the Vv and the Vd corresponding to the RMS-like region. Apart from *pou3f3b*, there are only two other transcripts exhibiting pattern J: *v-myc myelocytomatosis viral related oncogene, neuroblastoma derived* (*mycn*) and the *SRY-box containing gene 19b* (*sox19b*) (corresponding to ∼0.2% of the 1,202 investigated genes).

Pattern K: strong expression in the RMS.

In total, five TR transcripts were only or mainly detected in the RMS, corresponding to ∼0.4% of the 1,202 investigated genes. *Forkhead box J1a* (*foxj1a*) is one member of this group. In the anterior region, we detected only weak expression along the ventricular layer, notably in the dorsomedial telencephalon. More caudally, we observed strong expression in a small region localized between the ventral and dorsal nuclei of the ventral telencephalic area, corresponding to the RMS-like stripe (Fig. 2K–K2) composed of type III progenitors.

Pattern L: strong expression around the RMS.

The *androgen receptor (ar)*, the *myeloid ecotropic viral integration 1 (meis1)*, and the *scratch homolog 2, zinc finger (scrt2)* are the three TR genes that exhibit the pattern L characterized by a strong expression around

the RMS (Fig. 2L–L2) representing ~0.2% of the total number of investigated genes. The expression of these genes is also detected at lower levels in other brain regions such as the Dm or the Dl.

Pattern M: ventricular expression.

We identified six TR genes (almost 0.5% of the investigated genes) expressed specifically at the ventricular zone of the telencephalon, where the different progenitor types reside (Fig. 2M–M2). The *doublesex and mab-3 related transcription factor like family a2* (*dmrta2)* gene that is expressed in the ventricular zone is one example. The expression of these genes appears to be excluded from the RMS.

Expression of selected TR genes in neurogenic zones

The pattern of expression of genes of patterns I to M identifies these genes as potential regulators of neurogenesis (Fig 2I–M2). In addition, seven other genes were identified with high expression in the ventricular zone but with additional weaker expression in subventricular regions, for example, *zinc finger protein 36, C3H type-like 2* (*zfp36l2*), *hairy-related 6* (*her6*), and *SRY-box containing gene 9a* (*sox9a*) (Fig. 4D–F). To verify that these genes are indeed expressed in neural progenitors, fine mapping of the expression of selected TR genes (*her4.1; id1; sox9a; nf1a; sox4a; pou3f3b)* was performed (Fig. 4I–N3). To this end, we carried out fluorescent ISH on *Tg(nestin:gfp)* brains. This *nestin*-based transgene marks type I, II and III progenitors in the adult zebrafish telencephalon (Lam et al., 2009; März et al., 2010a). *Id1* and *her4.1* are strongly expressed in many *nestin:GFP*-positive cells (Fig. 4I–J3). *Sox9a* showed a high expression level in neural progenitor cells (Fig. 4K–K3), but is also detected in parenchymal cells (arrow in Fig. 4K). *nfia* and *sox4a* showed a stronger expression in cells located next to the ventricular stem cell layer (Fig. 4L–M3), but were also detected in some *nestin:GFP*-positive cells (arrows in Fig. 4L1–L3 and M1–M3). In contrast to *nfia*, *sox4a* expression was restricted to the fast proliferative zone corresponding to the RMS (Fig. 4M), most likely corresponding to type III cells. *Pou3f3b* expression was exclusively detected in cells adjacent to the neural stem cells in Vv (Fig. 4N–N3) and was not detectable in *nestin:*GFP-positive progenitors.

Regional differences in TR gene expression in the ventricular zones

There appear to be local differences in the ventricular zones of the telencephalon, indicated by distinct proliferative rates (März et al., 2010a) as well as by the existence of distinct patterns of gene expression (patterns I to M, Fig. 2I–M2). Thus, we next analyzed these regional differences in TR gene expression more systematically. We subjected 22 TR genes expressed predominantly in the ventricular zone to hierarchical clustering. To this end, we subdivided the ventricular zone according to adjacent anatomical landmarks (scheme Fig. 5). We noted significant differences in the expression of individual TR genes in the ventricular zone in various regions (Fig. 5). The first cluster contained two genes expressed only in the RMS. The second cluster of ventricular subdomains is more complex and includes the ventricular zones of the RMS Vv (VVv), Vd (VVd), Dd (VDd), Dl (VDl) the medial ventricular zone of Dm (mV Dm), and the dorsal ventricular zone of Dm (dV Dm). The different proliferative regions have overlapping but not identical patterns of TR gene expression. *dmrta2*, *her4.1, npas3b, id1, her4.4, sox9a, nfia*, and *zfp36l1b* are mainly expressed at the ventricular zone except the RMS (Fig. 5). In posterior regions, *hif1al, foxj1a, mych, CR774186.3*, and *tcf7l2* are expressed in the ventricular zone of Vd (VVd) marking the RMS (Fig. 5), whereas *mycn, sox19b, pou3f3b, sox4a*, and *sox2* are expressed in the ventricular zone of Vd (VVd) in anterior sections marking the anterior RMS (Fig. 5).

This analysis of gene expression in the ventricular zone did not take into account that TR genes may have multiple functions. By having excluded those genes which are expressed in the ventricular zone but also in other non-neurogenic domains, we introduced a bias into the analysis. To overcome this, we clustered the ventricular expression domains of all TR genes that are expressed in the ventricular zone including those TR genes that are expressed also in other domains of the telencephalon in a restricted fashion. We excluded "ubiquitous" (pattern A) and "broadly expressed" (pattern B) genes. Clearly, the ventricular regions of the different brain areas express overlapping but distinct sets of genes, strongly suggesting that neurogenic regions differ regionally (Fig. 6A,B). Expression of *smad5*, *junb*, and *gata3* is detected in the ventricular zones of VVd, mV Dm, SY (except for *junb)*, and also in Dm, Vd, and Vc. Moreover, TR genes specifying ventral identity during embryonic telencephalon development such as *isl1*, *nkx2.2a*, and *lhx8a* are also expressed in the adult ventricular zone of the dorsal nucleus of the ventral telencephalic area (VVd), in the medial ventricular zone of the dorsal telencephalic area (mV Dm), as well as in the medial zone of the dorsal telencephalic area (Dm), dorsal zone of the ventral telencephalic area (Vd), and central nucleus of the ventral telencephalic area (Vc)

(Fig. 6A,B). Novel candidate regulators coexpressed in ventral VZ include the zinc finger TF encoding gene *si:ch211–222k6.2*, the PHD and RING finger domains chromatin remodeling gene *uhrf1*, and the *vestigial like homolog 2bA* (*vgll2b*). A small subset of eight TR genes (*six3a*, *six6a*, *lhx8a, e2f7, otpb, encl1, tbx2a*, and *mycl1b*) is expressed only in Vd and nine TR genes (*foxj1a, foxk2, tcf7l2, hif1a1, mych, npas3b, sal11a, lima1*, and *invnslabpb*) only in the ventricular zone of Vd (VVd). For further details on the TR genes expressed in particular ventricular regions, consult the AGETAZ database (http://cory.itg.kit.edu/agetaz/index.php) using the search options to group coexpressed genes. Taken together, these clustering results indicate that the ventricular zones, in addition to expressing genes that control cell proliferation and maintenance of stem cells, express neuronal differentiation markers, suggesting that the different neurogenic niches in the telencephalon have region-specific identities.

DISCUSSION

Transcription regulators are well-known key players in development and maintenance of the CNS by controlling the formation of neurons and glia from progenitor and stem cells (Shirasaki and Pfaff, 2002; Gray et al., 2004). We report here the expression pattern of 1,202 TR genes in the telencephalon of the adult zebrafish, summarized in the searchable expression database AGETAZ (http://cory.itg.kit.edu/agetaz/index.php). Annotation of the pattern of expression of each gene to morphological landmarks at three anteroposterior levels of the telencephalon allows identification of genes that are coexpressed and may thus potentially interact functionally. In a previous study, we showed that 3,302 TR genes with at least one protein domain related to transcriptional regulation are present in the zebrafish genome. Of those, 2,580 TR genes are detectably expressed in the 24-hour postfertilization zebrafish embryo (Armant et al., 2013). Since we attempted to exclude genes exhibiting ubiquitous expression in the telencephalon, the number of expressed TR genes in the adult telencephalon is likely to be even higher than 1,202 TR genes. This indicates an amazing regulatory complexity in the supposedly simple telencephalon of the teleost zebrafish.

Patterns of gene expression identify regions with potentially shared regulatory mechanisms

Expression of TR genes is one fundamental mechanism that controls cell type. By using hierarchical clustering algorithms, we identified anatomical areas that share TR gene expression and thus potentially underlying regulatory mechanisms. Overall, three major anatomical clusters with similar gene expression were identified. Cluster 1 comprises the nucleus ENd, the ventromedial domains including Vv, VVv, Vd, VVd, Vc, but also the pallial zones Dm and mV Dm spanning the entire rostrocaudal axis of the telencephalon (Fig. 1). The second cluster is composed of the Dc, the Lot, the Mot, the Sup, and the parenchyma (Par). Cluster 3 is mainly composed of dorsolateral brain regions of the pallium (dV Dm, Dl, Dp, VDl, and VDp) but also of two subpallial nuclei (Va and Vl) (Fig. 1). The fact that the Va and Vl have expression features similar to pallial structures suggests distinct and specific functions of these brain areas compared to the other subpallial regions. Interestingly, these clusters of anatomical landmarks do not strictly obey the pallial and subpallial divisions, suggesting that regulatory mechanisms are shared between these broad anatomical subdivisions of the telencephalon.

Overall, this clustering, which was focused on anatomical annotations, gives a tendency and points at a global similarity of brain regions. However, many TR genes are expressed in a much more fine-grained pattern. We thus decided to focus on the expression of individual TR genes and to identify the genes that are expressed in the same pattern, hence forming synexpression groups (Niehrs and Pollet, 1999). In total, 562 of the 1,202 genes analyzed exhibited expression patterns that were shared with at least one other TR gene among this set of 562 genes. We scored 13 such expression groups forming patterns A to M. Among the 562 genes, a large proportion was expressed in the entire telencephalon (354 genes, pattern A) or broadly with varying intensities of expression in different parts of the telencephalon (143 genes, pattern B). Other gene patterns comprised more specific areas such as pallial regions or subpallial nuclei, oligodendrocytes, or regions of proliferation such as the ventricular zones including the RMS. We also noted genes that are expressed in varying patterns but whose transcripts were enriched at the perinuclear space subcellularly (10 genes, pattern C). Interestingly, a subset of these genes (*fosb, junba, jun*, and *junbb*) encodes the subunits of the transcription factor AP1 (Halazonetis et al., 1988; Shaulian and Karin, 2002). The 640 remaining genes show unique expression patterns and thus could not be categorized into a group. This finding underscores the importance of the free combinatorial action of TR genes that will drive different regulatory outcomes depending on the specific cellular context.

A crucial question is, of course: Would the genes that are coexpressed indeed point at potential

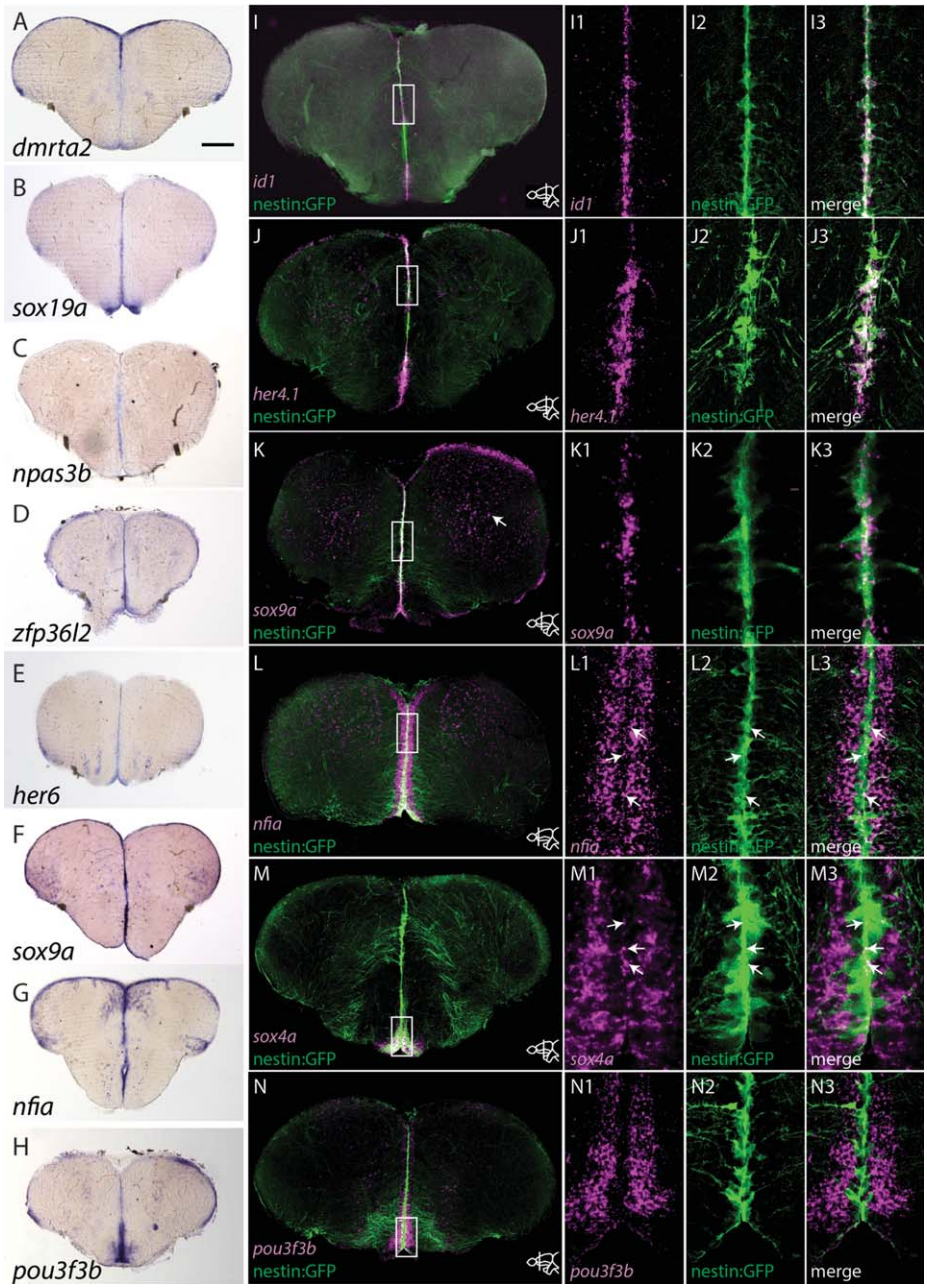

Figure 4. TR gene expression at the ventricular zone. **A–I:** The reported transcripts are mainly or exclusively expressed in the ventricular zones. **I–M3:** Fluorescent ISH of TR gene mRNA (magenta) followed by immunohistochemistry against GFP on *Tg(−3.9nestin:GFP)* brains (green). I–K3: *Id1*, *her4.1*, and *sox9a* are coexpressed in numerous *nestin:*GFP-positive cells. *Sox9a* is also detected in parenchymal cells (arrow in K). **L–N3:** *Nfia* and *sox4a* are expressed in cells next to the *nestin:*GFP-positive cells and very rarely in *nestin:*GFP-positive cells (arrows in L1–L3 and M1–M3). In contrast to *nfia*, *sox4a* expression is restricted to the fast proliferative zone Vv (M–M3). N–N3: *Pou3f3b* expression is exclusively detected in cells adjacent to the RGCs in Vv. Scale bar = 100 μm for A–H; 130 μm for I–N; 35 μm for I1–I3, L1–L3; 20 μm for J1–J3, K1–K3; 22.5 μm for M1–M3, N1–N3.

functional interactions? Drawing from the mammalian literature, for example, Rp58 regulates Neurod1 expression in mouse (Xiang et al., 2012) and zebrafish *rp58* and *neurod* share a common distribution in the zebrafish pallium (pattern D). Similarly, the two transcription factors *vax1* and *sox2*, known to be required for ependymal cell development and neural stem cell proliferation in mammals (Ferri et al., 2004; Soria et al., 2004), are strongly expressed in Vc. In mouse, Vax1 deficiency results in a severe depletion of GABAergic neurons in the neocortex (Taglialatela et al., 2004) and the zebrafish Vc is known to contain GABAergic neurons (Kim

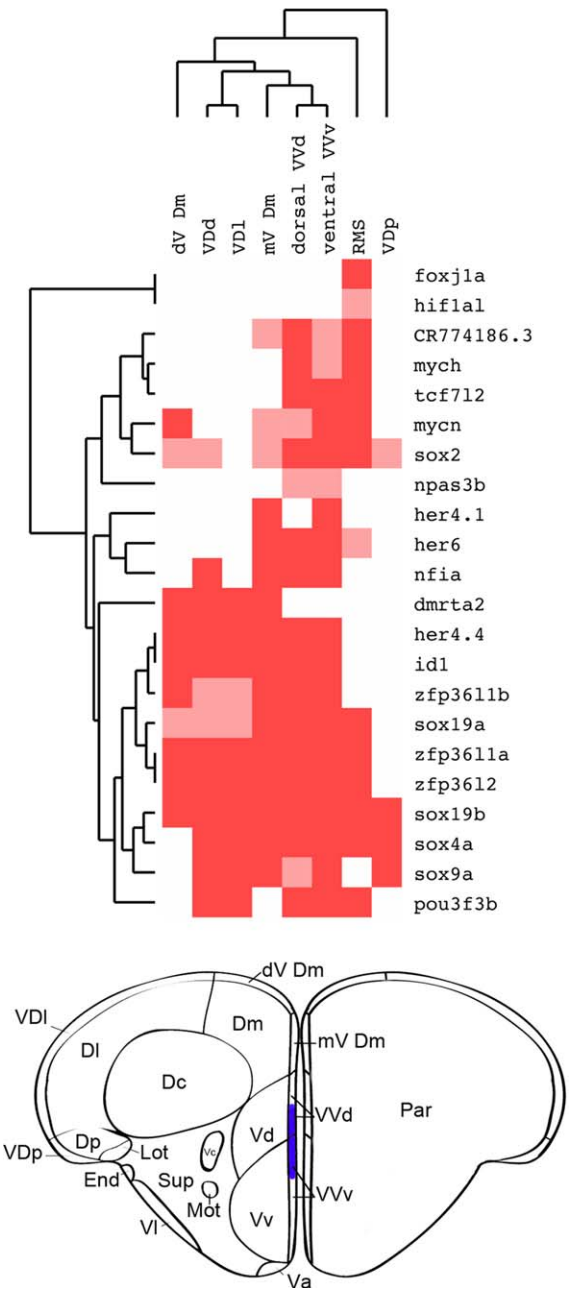

Figure 5. Hierarchical clustering of TR genes with expression along the ventricular zone. The TR genes of this cluster are only or mainly expressed in the ventricular zone including the RMS. The scheme illustrates the different brain areas. The region of the VVv and VVd in blue corresponds to the RMS.

et al., 2004; Adolf et al., 2006). It is tempting to hypothesize that *vax1* and *sox2* also play important roles in the differentiation and/or maintenance of GABAergic neurons in the zebrafish Vc. Molecular anatomy and expression patterns of genes involved in the establishment of neurotransmitter systems such as GABAergic neurons have been shown to be highly conserved between zebrafish and rodent models (Mueller and Wullimann, 2009). For instance, the zebrafish orthologs of mammalian *Mash1* and *dlx2* are known for their involvement in specification of GABAergic neurons (Mueller and Wullimann, 2009, and references therein). Similarly, our data on expression domains of *lhx6* in the ventral subdivision of the precommissural dorsal subpallium is in agreement with a previous report published by Mueller et al. (2008). This further reinforces the conclusions of Mueller et al. (2008) showing that *lhx6* and *lhx7* display a similar distribution during forebrain development in tetrapods and in the zebrafish telencephalon. However, conclusions should be tempered, given that the large number of adult cortical GABAergic neurons originated in the subpallium during mouse development and that all the subpallium of zebrafish is primarily GABAergic (Xu et al., 2004; Mueller et al., 2006). The expression atlas of the zebrafish telencephalon will help to elucidate the functional mechanisms operating in the zebrafish telencephalon. Moreover, these data will provide a bridge to the vast mammalian literature to compare the telencephalon of the zebrafish with that of mammals as previously documented for some genes, notably *tbr1*, *eom* (*tbr2*), *lhx6* and *lhx7, and dlx2* (Mione et al., 2001; Mueller et al., 2008; Mueller and Wullimann, 2009; Ganz et al., 2012).

Gene expression in neurogenic domains

The adult zebrafish telencephalon abundantly generates new neurons and also regenerates injured brain tissue highly effectively. Patterns I to M are linked to proliferative regions and thus may contain regulators of adult neurogenesis. We found 13 TR genes with a strong and specific ventricular expression, including some genes whose expression was predominantly detected at the ventricular zone, but also in other regions of the telencephalon, such as *znf36l2, her6*, and *sox9a*. Only six of the screened genes exhibited an expression restricted exclusively to the ventricular zone. Our results are in accordance with previous results showing *her4* and *her6* expression along the ventricular layer (Chapouton et al., 2010, 2011). We also noted a strong *her9* expression along the ventricle. But in contrast to Chapouton et al. (2011), we observed a wider *her9* expression including also cells in the brain parenchyma. *Dmrta2* initially described for its implication in sexual development is exclusively expressed in cells at the ventricular surface, suggesting a role in adult neurogenesis in addition to its previously reported involvement in embryonic neurogenesis (Yoshizawa et al., 2011). The expression of the basic helix-loop-helix PAS (Per, Arnt, Sim) domain transcription factor gene *npas3b* is restricted to the ventricular region. In mice, NPAS3 was noted for its implication in the modulation of neurogenesis in the hippocampus (Pieper et al., 2005).

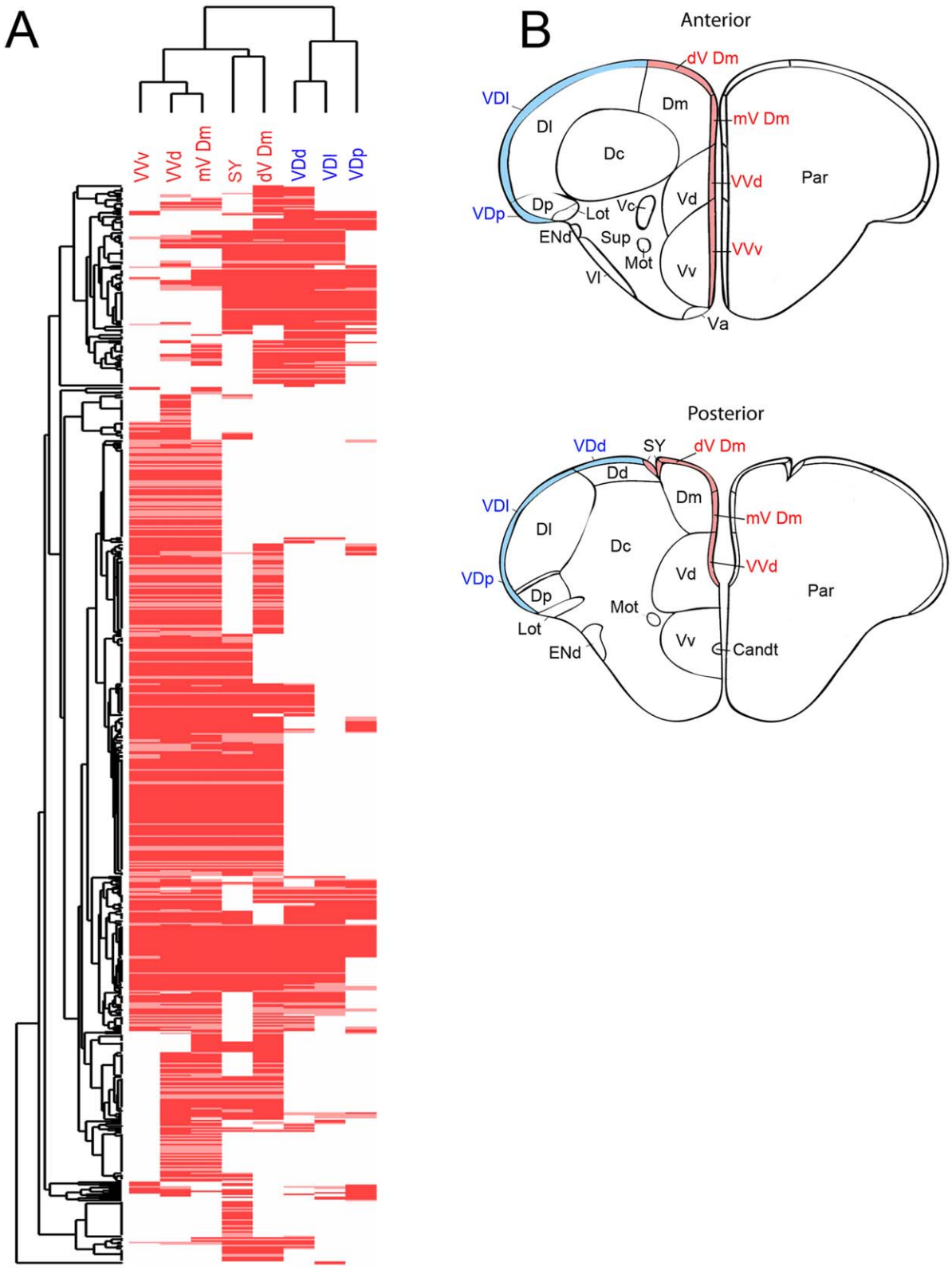

Figure 6. Hierarchical clustering of TR genes expressed in specific regions of the VZ. **A:** Clustering of 547 VZ expressed genes focusing on anatomical terms corresponding to ventricular domains (indicated on top). **B:** Scheme of adult telencephalon transverse sections at two different rostrocaudal levels (top, anterior; bottom, posterior) and the associated anatomical terms. Dorsolateral VZ are marked in blue and ventromedial VZ in red.

In the zebrafish telencephalon, the hippocampus-like region was proposed to be the posterolateral domain of the dorsal telencephalon at the border between the Dl and Dp regions (also referred to as the dorsolateral proliferating zone, DPZ) (Wullimann and Rink, 2002; Rodríguez et al., 2002; Salas et al., 2003; Broglio et al., 2005; Adolf et al., 2006; Grandel et al., 2006). In zebrafish, the *tfap2c* gene (coding for AP-2) was detected in the DPZ. In the adult mouse brain, different AP-2 subtypes were also detected in the hippocampus (Shimada et al., 1999). Furthermore, *sox9* transcripts appeared to be expressed in the DPZ in zebrafish, and in the hippocampus in mouse (Heng et al., 2014). We also noted a strong expression of *smad1* in the zebrafish equivalent of the mammalian hippocampus. In rat, *Smad* expression is also detected in the hippocampus and SMAD proteins are activated following ischemia (Nakajima et al., 2014). This further underscores the proposition that the DLZ in the zebrafish telencephalon is indeed homologous to the mammalian hippocampus.

In zebrafish, the RMS-like region is composed of type III progenitor cells (März et al., 2010a). We identified 14 genes expressed in the RMS, five transcripts of which are restricted to the posterior RMS (*CR774186.3, foxj1a, hif1al, mych, tcf7l2*) and five (*mycn, sox19b, sox2, pou3f3b, sox4a*) are specific to the anterior RMS. Some of these genes are also expressed in the mammalian RMS. In the mouse and rat brain, FoxJ1 is involved in postnatal neurogenesis (Jacquet et al., 2011; Devaraju et al., 2013; Muthusamy et al., 2014). Foxj1-expressing cells give rise to neuroblasts in the adult rat brain (Devaraju et al., 2013) and knockin experiments in mice showed that Foxj1 expressing cells contribute to the formation of new neurons in the olfactory bulb (Muthusamy et al., 2014). In addition, the HMG box factor *sox2* is strongly expressed in the RMS-like region in zebrafish (Lam et al., 2009). Its expression was also observed in the telencephalic neurogenic niches in adult mice, including the subventricular zone, the RMS, and the subgranular zone (Kang and Hebert, 2012). Similarly, the mouse POU-homeodomain transcription factor Pou3f3 has been shown to influence neurogenesis in the ventricular zone and to modulate neuronal migration (Dominguez et al., 2013). The hypoxia inducible factor 1 (alpha subunit, like) expressed in the RMS in the zebrafish telencephalon is implicated in neural stem cell function and the regenerative response to stroke in mammals (Cunningham et al., 2012). Thus, taken together, TR gene expression in the neurogenic niches in the zebrafish telencephalon bears significant resemblance to the equivalent regions in the mammalian telencephalon. This suggests conserved mechanisms and further underscores the relevance of the dataset provided here.

In contrast to the restricted neurogenic potential of the mammalian telencephalon, however, neurogenesis occurs along the entire ventricular surface of the zebrafish telencephalon. As outlined by the distinct cell division rates (März et al., 2010a) and the different expression of TR genes (Adolf et al., 2006; März et al., 2010a), there are differences between the RMS and the other proliferative regions. This was further supported by the systematic cluster analysis. The patterns of expression of genes in the ventricular zone were clearly not uniform and even in regions outside of the RMS significant differences were noted. This suggests that the neurogenic niches differ in various regions and may reflect a commitment of the neural stem cells to differentiate in one or the other direction. For example, the Vv expresses genes that may control differentiation towards GABAergic neurons, while regions located more dorsally generate neurons of the glutamatergic lineage (Mueller et al., 2008; Mueller and Wullimann, 2009). Interestingly, genes that are expressed in a region-specific manner in the embryonic telencephalon such as nkx2.2, *nuclear factor I/X* (*CCAAT-binding transcription factor*), *prdm12* (*PR domain containing 12*) in the embryonic subpallium suggest that mechanisms have been retained from embryo ontogenesis to adult maintenance of the CNS. It is tempting to speculate that a code as employed in the embryonic spinal cord may exist also in the telencephalon that is maintained into adulthood in the zebrafish. However, this notion remains to be supported by functional experiments, for which this study has laid the foundation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ROLE OF AUTHORS

SR and US designed the experiments and supervised the work. RRV and MM conducted the ISH experiments. RRV, ND, SR and US analyzed the ISH data. OA provided the RNA sequencing data and performed the bioinformatic analysis. MF programmed the AGETAZ database. ND, RRV, SR, and US wrote the article.

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