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Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon

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Abstract

Our understanding of the cellular and molecular mechanisms underlying the adult neural stem cell state remains fragmentary. To provide new models on this issue, we searched for stem cells in the adult brain of the zebrafish. Using BrdU tracing and immunodetection of cell-type-specific markers, we demonstrate that the adult zebrafish telencephalon contains self-renewing progenitors, which show features of adult mammalian neural stem cells but distribute along the entire dorso-ventral extent of the telencephalic ventricular zone. These progenitors give rise to newborn neurons settling close to the ventricular zone within the telencephalon proper. They have no equivalent in mammals and therefore constitute a new model of adult telencephalic neural stem cells. In addition, progenitors from the ventral subpallium generate rapidly dividing progenitors and neuroblasts that reach the olfactory bulb (OB) via a rostral migratory stream and differentiate into GABAergic and TH-positive neurons. These ventral progenitors are comparable to the mammalian neural stem cells of the subependymal zone. Interestingly, dorsal and ventral progenitors in the adult telencephalon express a different combination of transcription factors than their embryonic counterparts. In the case of *neurogenin1*, this is due to the usage of different enhancer elements. Together, our results highlight the conserved and unique phylogenic and ontogenic features of adult neurogenesis in the zebrafish telencephalon and open the way to the identification of adult neural stem cell characters in cross-species comparative studies.

Keywords: Zebrafish; Adult neurogenesis; Neural stem cell; Telencephalon; Olfactory bulb; Radial glia

Introduction

The continued production of neurons at adulthood has been observed in most vertebrates. However, our knowledge is mostly derived from two mammalian neural stem cell models, the subependymal zone (SEZ) of the lateral wall of the lateral ventricle and the subgranular zone (SGZ) of the hippocampus, where slow-dividing long-lasting progenitors with astrocytic characteristics give rise to newborn neurons (Alvarez-Buylla and Garcia-Verdugo, 2002; Doetsch and Hen, 2005; Garcia et al., 2004; Goldman, 2003; Kempermann, 2002; Lledo and Saghatelyan, 2005; Merkle et al., 2004; Taupin and Gage, 2002). Neuronal differentiation from the SEZ is preceded by a transient amplification phase and by the migration of committed neuronal progenitors towards the olfactory bulb (OB) along the rostral migratory stream (RMS). Newborn OB neurons, mostly GABAergic interneurons, might help discriminate odorant stimuli (Lledo and Saghatelyan, 2005).

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Many open questions remain on the biology of adult neural stem cells, for example, the characteristics of their "niche" of origin (Alvarez-Buylla and Lim, 2004) or the cell-intrinsic factors maintaining their undifferentiated character or orienting their commitment. Among other transcription factors (Molofsky et al., 2003, 2005; Shi et al., 2004), Sox2 was implicated in attributing stem cell properties, both in the SEZ and SGZ (Episkopou, 2005; Ferri et al., 2004). Also, Olig2 promotes the transient-amplifying precursor state in the SEZ and RMS, and Pax6 the commitment towards a TH-positive neuronal fate in the OB (Hack et al., 2005; Kohwi et al., 2005).

Adult neurogenesis is tightly restricted to the SEZ and SGZ in mammals but appears more widespread in other vertebrates like reptiles (Garcia-Verdugo et al., 2002), birds (Goldman, 1998), and fish (Zupanc, 2001). These models may yield insight into the mechanisms generating other neuronal subtypes than OB or hippocampal granule interneurons in the adult brain. Moreover, by comparison with mammals, they should highlight general versus derived properties of adult neurogenesis, thus crucial elements of neural stem cell maintenance. In teleost fish, proliferation and neurogenesis occur throughout life, correlating with long-lasting brain growth and a high capacity for regeneration (Zupanc, 2001). Proliferation sites are restricted to discrete foci, suggesting the existence of niches. In the zebrafish adult brain, newborn neurons form in the OB, dorsal telencephalon, hypothalamus, preoptic area, optic tectum, and cerebellum (Byrd and Brunjes, 2001; Cameron, 2000; Goldman et al., 2001; Zupanc et al., 2005). However, the location, identity and molecular characteristics of the progenitors involved remain unresolved. Here, we characterize self-renewing progenitors in the adult zebrafish telencephalon. We show that these progenitors are located both in dorsal and ventral telencephalic domains and express neural stem cell-like characteristics and a unique combination of molecular markers. We demonstrate that OB neurogenesis in zebrafish shares many features with that in mammals, validating the zebrafish model to study vertebrate adult neurogenesis. Finally, we identify a novel stem cell population in the dorsal telencephalon, providing a new niche to examine. Our findings establish new neural stem cell models in a genetically tractable animal, setting the stage for detailed comparative studies and forward genetics approaches on vertebrate adult neurogenesis.

Materials and methods

Fish strains

5- to 9-month-old wild-type zebrafish (*Danio rerio*) of the AB strain, or transgenic zebrafish (*-3.4ngn1:gfp*, *-3.1ngn1:gfp*) (Blader et al., 2003; Shin et al., 2003) in AB or ABO background were used.

BrdU labeling

To label cells in S phase, the fish were injected intraperitoneally with 50 µl/g body weight of the thymidine analog bromo-deoxy-uridine (BrdU) diluted in 110 mM NaCl pH 7.0 twice within 2 h. BrdU is taken up by cells in the S phase of mitosis and detectable by immunocytochemistry (Gratzner, 1982). The clearance time of BrdU has been estimated around 4 h in adult fish (Zupanc and Ott, 1999); thus, we injected BrdU twice with a 2-h interval followed by a survival time of 2 h after the last injection, to map cells undergoing division at the time of BrdU exposure. Other survival times ranged between 4 h and 8 weeks after the first pulse. Then the fish were anesthetized with Tricaine before they were killed in ice water. The brains were dissected out and fixed in 4% paraformaldehyde solution at 4°C for 4 h, then progressively dehydrated in MeOH and stored in 100% MeOH at −20°C.

Immunohistochemistry

Immunostainings were performed on 100-µm sections. The whole brains were embedded in 3% agarose in PBS and cut serially using a vibrating microtome (HM 650 V, Microm). The sections were blocked with 0.5%Triton X-100 and 10% normal goat serum in PBS for 1 h at RT, then were incubated in the primary antibodies diluted in the block buffer at 4°C overnight. Primary antibodies used in this study were rabbit anti-BLBP (1:1500; Feng et al., 1994), mouse anti-BrdU (1:100; Roche), rat anti-BrdU (1:200; abcam), rabbit anti-GFAP (1:100; DAKO), rabbit anti-GFP TP401 (1:500; ams), mouse anti-Hu C/D (1: 300; Molecular Probes), rabbit anti-Pax6 (1:300; Babco) mouse anti-PCNA (1:250; DAKO), mouse anti-PSA-NCAM (1:600; Chemicon). Primary antibodies were detected by subclass-specific secondary antibodies labeled with Cy2, Cy3, or Cy5 or enhanced with the tyramide amplification kit (PerkinElmer). The sections were then embedded in Aqua Polymount (Polyscience). Immunodetection of BrdU required a pretreatment with 2 M HCl followed by washes with borate buffer and PBS before the sections were incubated in anti-BrdU antibody. Double immunodetection with rabbit anti-Pax6 and rabbit anti-TH antibodies was performed following the protocol of the tyramide amplification kit (PerkinElmer), as follows. The primary antibody against Pax6 was diluted in blocking buffer to 1:5000 and amplified with the secondary antibody solution (anti-rabbit biotinylated 1:250 in block buffer). Following incubation in streptavidin–HRP (1:100 in PBS), the sections were incubated in tyramide solution coupled with either FITC or TRITC (1:30) for 15 min. Staining for TH was then performed following the standard protocol.

In situ hybridization

In situ hybridization was performed as for whole-mount embryos (Hauptmann and Gerster, 1994), starting with whole-mount brains. Following hybridization and rinses, the brains were then embedded in 3% agarose in PBS and cut serially using a vibrating microtome (HM 650 V, Microm) at 100 µm before blocking and incubation in anti-digoxigenin antibody, as described for embryos. For double ISH/immunodetections, the brains were first processed for ISH, then for immunocytochemistry. The following ISH probes were used: *dlx2a* (former *dlx2*) (Akimenko et al., 1994), *emx3* (former *emx1*) (Morita et al., 1995), *fabp7a* (Liu et al., 2003a), *gad67* (Martin et al., 1998), *ngn1* (Blader et al., 1997), *olig2* (Park et al., 2002), *pax6a* and *pax6b* (Nornes et al., 1998), *ash1a* (Allende and Weinberg, 1994), and *sox2* (PCR-amplified from GeneBank CF416982). All sections were photographed and analyzed under a Zeiss Axioplan microscope or a Zeiss confocal microscope (LSM 510 META).

Results

The zebrafish adult telencephalon contains distinct subpopulations of proliferating cells

Distinct proliferation sites have been described in the teleost adult brain (Ekstrom et al., 2001; Zupanc, 1999; Zupanc and Horschke, 1995), such as, in zebrafish, the retina (Cameron, 2000; Goldman et al., 2001), olfactory bulb (Byrd and Brunjes, 1998), telencephalon and cerebellum (Zupanc et al., 2005), hindbrain or spinal cord (Goldman et al., 2001). However, with the exception of the cerebellum (Zupanc et al., 2005), it has not been determined which of these sites give rise to neurons and/or glia. Here, we used BrdU-birth-dating to map the sites of proliferating cells and the fate of their progeny.

Sacrifice of animals 2 h after two times BrdU injections (see Materials and methods) allows detection of actively proliferating cells. Consistent with previous data (Zupanc et al., 2005), we observed that cell division was abundant in the adult zebrafish brain, with several prominent sites (Fig. 1A). The zebrafish telencephalon is commonly divided into the dorsally located pallium and the basally located subpallium; the pallium is itself subdivided into posterior, lateral, dorsal and medial domains, and the subpallium into dorsal and ventral components (Wullimann et al., 1996) (see Fig. 2F). In the telencephalon, the ventral subpallium was intensely labeled, appearing as a longitudinal stripe (indicated as (1) on Fig. 1) of BrdU-positive cells joining the posterior telencephalon with the olfactory bulb (OB). No staining was observed inside the OB itself, but a few positive cells, interpreted as glia (Byrd and Brunjes, 2001), were encountered in the superficial OB olfactory nerve layer (see Fig. 1B). On cross and horizontal sections (Figs. 1B–E), we observed that most proliferating cells were adjacent to the telencephalic ventricle at all dorsoventral (DV) levels (Figs. 1B–D). Dorsally, they line the outer surface of the telencephalon, in agreement with the interpretation that most of the ventricular surface of the pallium in teleosts is everted (red arrows in Figs. 1B–D) (Wullimann and Rink, 2002). Additionally, a prominent site of cell proliferation was detectable on posterior sections at the junction between the pallium and ventral telencephalic domains overlying the optic tract (Fig. 1D). Finally, a few positive cells could also consistently be observed in nonventricular locations, within the parenchyma (white arrowheads, Figs. 1B, C).

We next addressed whether all these cells displayed similar proliferation characteristics. Although BrdU-positive cells were found at all DV levels, we found that the growth fraction varied between these areas. Strikingly, at anterior levels, the highest density of BrdU-positive cells was observed in the ventral subpallium (numbered (1) on Figs. 1B, C), while it was decreased to a fourth in the dorsal subpallium and to a tenth in the medial or dorsal pallium (Fig. 1F). These observations suggest that the ventral subpallium contains either more or faster dividing proliferating cells than further dorsally located ventricular areas. To resolve this issue, we assessed the labeling index of each domain by calculating the number of BrdU-incorporating cells (number of cells in S phase) within the actively dividing cell population expressing the proliferating cell nuclear antigen PCNA (Figs. 2A–E) (Bravo et al., 1987; Mathews et al., 1984). In addition to a higher density of PCNA-positive cells along the ventricle in the ventral subpallium (Fig. 2B), we observed that the proportion of BrdU/PCNA double-labeled cells was consistently increased (two times) in this domain compared other DV levels (Figs. 1D–E). A high density of BrdU-positive cells and a high labeling index also characterized the ventricular surface of the posterior pallium (numbered 1p on Fig. 1D, and see Figs. 2E, F). Thus, most precursors in the ventral subpallium and posterior pallium are fast proliferating, while other ventricular telencephalic precursors proliferate more slowly. In agreement with a slower cell cycle at the ventricle of the dorsal subpallium, medial, dorsal and lateral pallium, we observed that the number of BrdU-incorporating cells in the latter domains continuously increased over 10 days in cumulative BrdU labeling experiments (not shown). We conclude that proliferating telencephalic cells contain several subpopulations that differ in their speed of division (summarized in Fig. 2F).

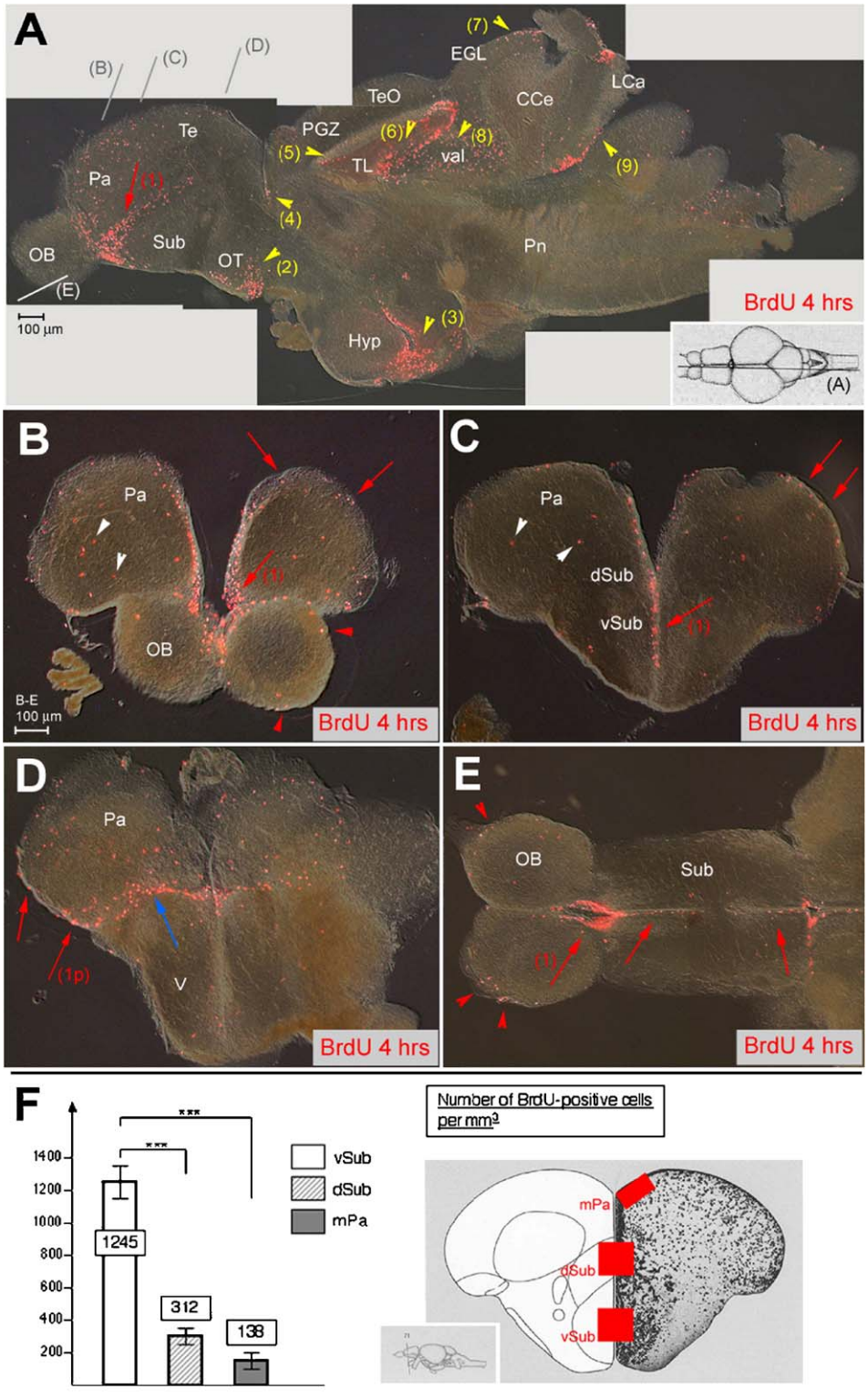
Migration of the progeny of adult telencephalic precursors

We next studied the progeny of ventricular proliferating cells by following BrdU-labeled cells 3 days, and 2, 3, 4, and 8 weeks after injection. In contrast to previous studies (Zupanc et al., 2005), this close spacing of time points allows monitoring cell migration as well as following fast proliferating cells.

Three days after BrdU injection, no major differences were observed in the telencephalon proper (Figs. 3A–C), but a few BrdU-labeled cells could now be detected within the OB (white arrowheads on Figs. 3A, B). A more obvious alteration in the location of BrdU-positive cells was then detectable 2 weeks after the BrdU pulse in the telencephalon and OB (Figs. 3D–F). For example, after 2 weeks, numerous positive cells were found inside the OB (white arrowheads in Figs. 3D, E). The zebrafish OB is organized in three layers (from external to internal: the olfactory nerve layer—ON, the glomerular layer—GL, and the internal layer—IL) (Byrd and Brunjes, 1998). BrdU-positive cells were predominantly found within the IL after 2 weeks, but they also populated the GL 4 to 8 weeks after injection (see below). Thus, cells generated outside the OB appear to migrate into the OB within 2 weeks. In parallel, the total number of positive cells in the ventral subpallial stripe had

Fig. 1. Proliferation sites in the zebrafish adult telencephalon. (A–E) BrdU immunocytochemistry (red staining) on brain sections of 6-month-old adult zebrafish sacrificed 4 h after 2 BrdU injections. Vibratome sections are observed under conventional fluorescence microscopy, (A) parasagittal section, anterior left, (B–D) cross-sections at increasingly posterior levels (indicated in panel A), dorsal up, (E) horizontal section (level indicated in panel A), anterior left. In panel A, intensely labeled sites are numbered (red arrows: telencephalon, yellow arrowheads: other domains). Note the stripe of intense staining within the ventral subpallium (numbered 1 in all sections). Other sites: (2) optic tracts, (3) ventricular zone of the hypothalamus, (4) anterior thalamus, (5) periventricular gray zone, (6) torus longitudinalis, (7) cerebellar external granular layer, (8) valvula cerebelli, (9) lobus caudalis cerebelli. Nonventricular proliferation is also visible in all cross-sections and indicated by white arrowheads. The only staining visible in the OB (E, red arrowheads) likely corresponds to glia in the superficial olfactory nerve layer (Byrd and Brunjes, 2001). In the posterior telencephalon, prominent proliferation is observed at the junction between the dorsal and ventral domains (blue arrow in panel D) and in the posterior pallium (red arrow numbered 1p in panel D). (F) Number of BrdU-positive cells in the telencephalon along the DV axis, counted in the areas boxed in red in the scheme on the right panel (Wullimann et al., 1996). Ventral subpallium: 1245 ± 245 BrdU-positive cells/mm^3, dorsal subpallium: 312 ± 136/mm^3, medial pallium: 138 ± 37/mm^3 (on a total of 9 sections from 3 different fish at anterior telencephalic levels, all areas containing a comparable total number of cells, statistical analysis performed using independent samples Student's *t* test). Abbreviations, after Wullimann et al. (1996): CCe: corpus cerebellaris, EGL: external granular layer, Hyp: hypothalamus, IGL: internal granular layer, LCa: lobus caudalis cerebelli, Pa: pallium, PGZ: periventricular gray zone, OB: olfactory bulb, OT: optic tract, Pn: pons, Sub: subpallium, Te: telencephalon, TeO: tectum opticum, TL: torus longitudinalis, V: posterior domain of the ventral telencephalon, val: valvula cerebelli.

decreased (white arrow in Figs. 3D, F, compare to red arrow in panels A, C), and most cells that had retained the BrdU label in this stripe were now clustered anteriorly, still along the ventricle (yellow arrow in Figs. 3D, E). In addition, BrdU-positive cells located along the ventricle in the dorsal subpallium and pallium shortly after BrdU injection had migrated into the parenchyma to a slightly more lateral position 2 weeks after BrdU labeling (Fig. 3F arrowheads, compare the position of labeled cells in the insets in panels C and F, yellow arrows to the ventricular surface). This redistribution continued over a longer time after BrdU injections, as visible after 19 days (Figs. 7A, B).



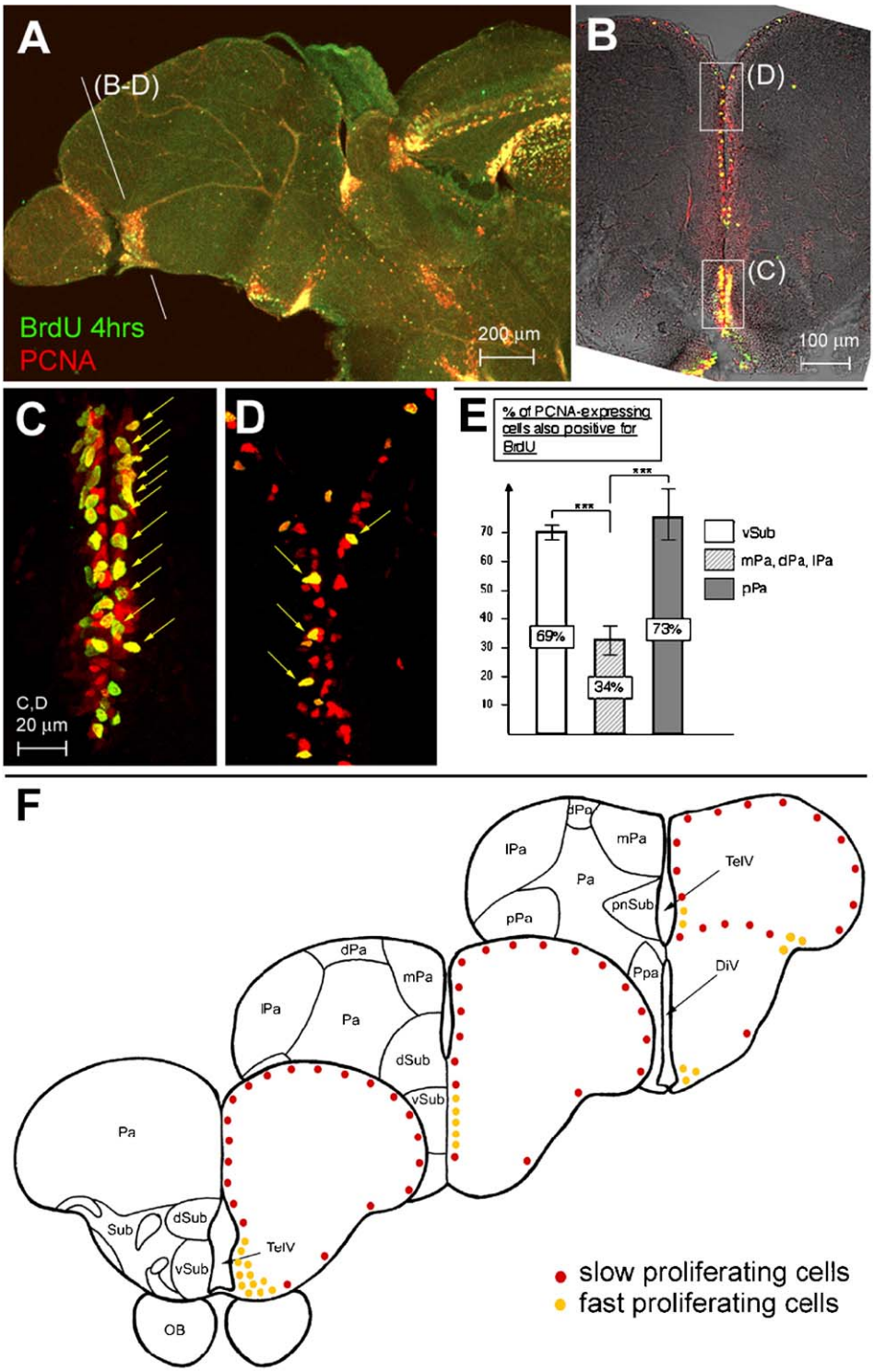


Fig. 2. Different cell cycle characteristics between proliferation zones in the adult zebrafish telencephalon. (A–D) Double immunolabeling for PCNA (red) and BrdU (green) on sagital (A, anterior left) and cross (panels B–D, dorsal up) vibratome sections observed under confocal microscopy; panels C, D are the areas boxed in panel B observed at higher magnification and on an optic section of 1.5 µm. PCNA expression and BrdU-positive cells are concentrated ventrally (compare the density of PCNA-only (red) and double-labeled (yellow arrows) cells in B–D). (E) Labeling index (number of PCNA-positive cells that are also BrdU-positive) in the telencephalon along the DV axis. In the anterior telencephalon: 69% in the ventral subpallium, $n = 115$ PCNA-positive cells counted, against 33.7% in the dorsal subpallium, medial, dorsal, and lateral pallium, $n = 327$; at the surface of the posterior pallium: 73%, $n = 26$. (F) Schematic representation of the zones of fast (yellow dots) and slow (red dots) proliferation in the zebrafish adult telencephalon, as identified in panels A–E. Cross-sections are oriented along the AP axis. Abbreviations: see Fig. 1, and DiV: diencephalic ventricle, dPa: dorsal pallium, dSub: dorsal subpallium, lPa: lateral pallium, mPa: medial pallium, pnSub: postcommisural nucleus of the ventral subpallium, Ppa: preoptic nucleus, pPa: posterior pallium, TelV: telencephalic ventricle, vSub: ventral subpallium.

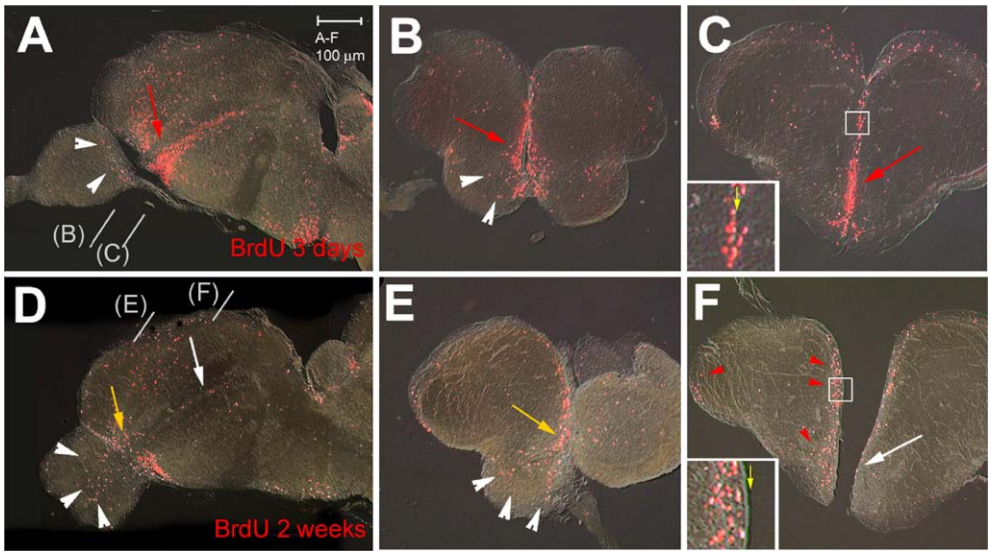


Fig. 3. BrdU-labeled cells (red staining) in the zebrafish adult telencephalon 3 days (A–C) or 2 weeks (D–F) after BrdU injection. All views are vibratome sections observed under conventional fluorescence microscopy (A,D: parasagittal sections, anterior left; B, C, E, F: cross-sections at the levels indicated in panels A, D, dorsal up). Note major changes after 2 weeks: (i) the ventral subpallial stripe is diminished caudally (white arrows in panels D, F) and mostly maintained anteriorly (yellow arrows in panels D, E), (ii) labeled cells are observed in the OB (white arrowheads in panels D, E), and (iii) labeled cells have left the ventricle in the pallium and dorsal subpallium (red arrowheads in panel F, compare insets in panels panels C, F, yellow arrow to the ventricle).

Together, these results indicate that within 2 weeks, most progeny arising from ventricular BrdU-positive cells relocalizes to populate the anterior domain of the ventral subpallial ventricular stripe and the OB, or the telencephalic parenchyma.

Adult telencephalic precursors generate newborn neurons

These results prompted us to address whether the different subpopulations of ventricular telencephalic dividing cells were neurogenic progenitors. We first studied expression of Hu proteins, markers for young postmitotic neurons (Mueller and Wullimann, 2002). We observed that Hu-positive cells were detectable in domains immediately adjacent to the proliferation zones along the telencephalic ventricle and were also abundant in the OB (Fig. 4 and not shown). Together with the results above, this is compatible with a model where ventricular telencephalic proliferating cells give rise to neurons throughout the telencephalon including the OB.

We next examined whether and when ventricular BrdU labeled cells acquire expression of Hu, and we obtained similar results at all telencephalic AP levels. Within the telencephalon proper 4 h after BrdU injection, none of the BrdU-positive cells co-expressed Hu (Fig. 4A), while double-labeled cells appeared starting 3 days after BrdU injection (e.g., Figs. 4B, D), and their proportion increased over 2 weeks to 74% ($n = 406$ BrdU-positive cells) (Figs. 4C, E). Thus, three quarters of all proliferating cells in the telencephalon had acquired a neuronal identity 2 weeks after BrdU incorporation (Fig. 4G). Contrasting with these populations within proximity of the ventricle, proliferating cells detected in the parenchyma (Figs. 1B, C) never expressed Hu (not shown). Interestingly, neuronal differentiation proceeded at a slower rate in the OB where only 41% of all BrdU-positive cells were Hu-positive 2 weeks after BrdU injection (Figs. 4F, H).

Together, these results demonstrate that de novo neurogenesis is taking place throughout the adult telencephalon, from precursors initially located along the ventricle that give rise to Hu-positive newborn neurons close to the ventricular surface and in the OB.

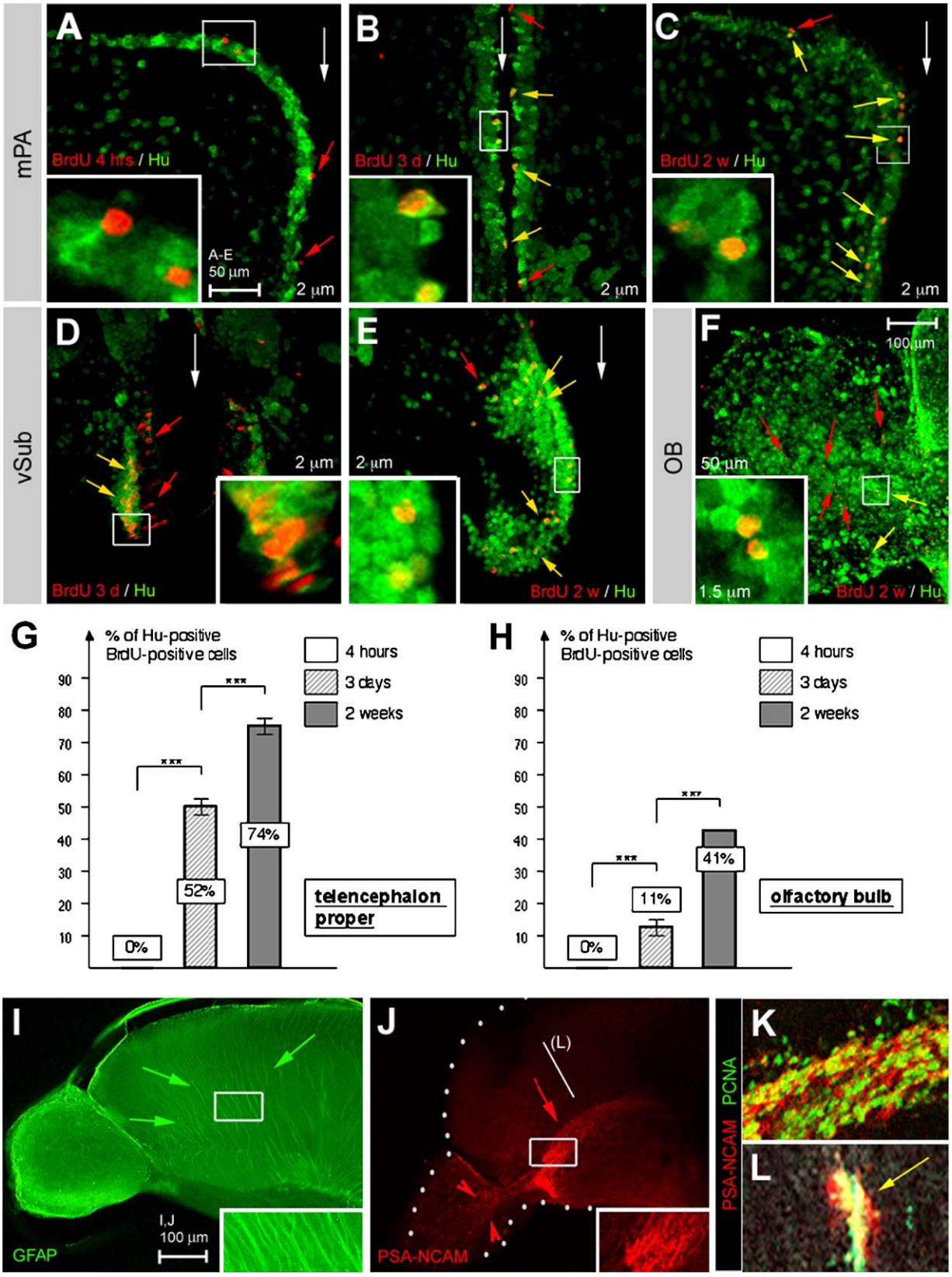
Ventral telencephalic precursors form a RMS-like migration route of neuronal progenitors towards the olfactory bulb

The progressive colonization of the OB by the progeny of dividing cells has been previously documented, but the origin of these cells was not determined (Byrd and Brunjes, 1998; Zupanc et al., 2005). We showed above that no BrdU-positive cells could be detected within the OB shortly after BrdU labeling, suggesting that newly generated neurons must originate somewhere else. Moreover, the number of BrdU-labeled cells in the ventral subpallial stripe decreases over time, paralleled by an increase in the number of BrdU-positive cells in the OB. These observations suggest that OB neurons might originate from dividing cells located at the ventricle of the ventral subpallium. To confirm this interpretation, we aimed at identifying the migratory path and characteristics of the cells en route to the OB. Unlike in mammals, we found no evidence for organized GFAP-positive tubes in the adult zebrafish telencephalon; GFAP-positive cells were present but rather loosely oriented tangentially (Fig. 4I), in agreement with a recent report mapping radial cells in the adult zebrafish brain using the marker AroB (Menuet et al., 2005). However, we observed intense PSA-NCAM staining along the entire subpallial stripe (Figs. 4J–L), reaching into the OB (Fig. 4J, red arrowheads), comparable to the organization of young migrating neuroblasts labeled by PSA-NCAM in the mammalian brain (Doetsch et al., 1997; Jankovski and Sotelo,

1996; Peretto et al., 1997). These results strongly suggest that the ventral subpallial stripe is a migration pathway for telencephalic neuronal progenitors and neurons fated to the OB, thus a possible functional equivalent to the mammalian RMS.

Ventricular telencephalic progenitors give rise to GABAergic and TH-positive neurons in the olfactory bulb

To determine whether adult neurogenesis contributed to the GABAergic population in the OB (Fig. 5A) (Kim et al., 2004;



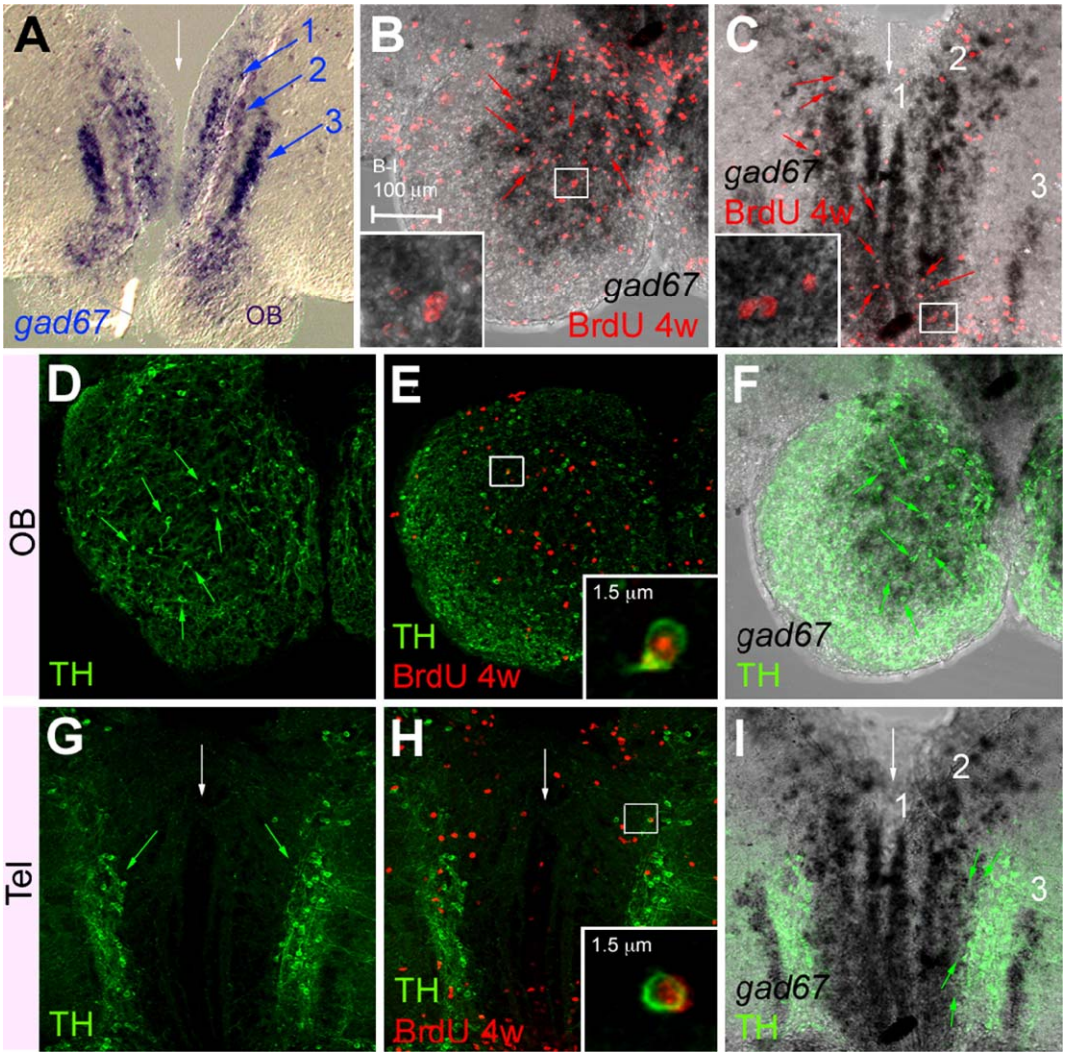


Fig. 5. Telencephalic progenitors give rise to *gad67*- and/or TH-positive neurons in the OB and in the anterior subpallium and medial pallium. Localization of GABA-ergic neurons, revealed by *gad67* (A–C, F, I) (ISH, blue or black) expression and compared with the expression of TH (D–I) (immunodetection, green) and/or the localization of BrdU-positive cells after 4 weeks of tracing (B, C, E, H) (red). Panels B and D–F are focussed on the OB, panels C and G–I on the subpallium and medial pallium, white arrows to the midline. All views are cross-sections, anterior up, panels B–I are confocal photomicrographs. In the OB, newborn neurons in the IL largely express *gad67* (B, red arrows to examples of double-positive cells, one example is magnified in inset). Most TH-positive neurons in the GL (D) are *gad67*-positive (F, green arrows to examples of double-positive cells) and a subset of these neurons are newborn (E). In the subpallium and medial pallium, *gad67* expression is organized in three bilateral columns (numbered 1–3 in A, C, I), which largely contain newborn neurons (C red arrows to examples of double-positive cells, one example is magnified in inset). Some newborn neurons also express TH (H), but these are mostly not GABAergic (I, green arrow to the restricted subpopulation of *gad67*/TH double-positive cells).

Martin et al., 1998), as is the case in mammals, we analyzed whether BrdU-positive cells turned on expression of the GABA synthesizing enzyme Gad67 (now Gad1, zebrafish nomenclature committee) after long-term tracing. After 4 weeks, a large proportion of the BrdU-positive population in the OB expressed *gad67* (Fig. 5B), demonstrating de novo GABAergic

Fig. 4. Adult neurogenesis and neuroblast migration in the zebrafish telencephalon. (A–F) Double immunodetection of Hu proteins (green) and BrdU (red) after increasing tracing times (4 h, 3 days—3d, 2 weeks—2w, as indicated). Panels A–E are cross-sections, dorsal up, and panel F is a sagittal section, anterior left, all observed under confocal microscopy with focus on the medial pallium (mPa) (A–C), ventral subpallium (vSub) (D, E) or OB (F). The position of the midline is indicated by white arrows. Examples of cells positive for BrdU but negative for Hu are indicated by red arrowheads, and examples of double positive cells by yellow arrowheads. All insets are high magnifications of the boxed areas. BrdU/Hu double positive cells can be found along the entire DV extent of the telencephalic ventricle, and to a lesser extent in the OB, after 3 days of tracing. G,H: Percentage of BrdU-positive cells also expressing Hu in the anterior telencephalon in the vicinity of the ventricular zone (G) and in the OB (H). Percentage of double-labeled cells for *n* BrdU-positive cells counted in the telencephalon proper: 0%, $n > 500$ at 4 h, 52%, $n = 942$ at 3 days, 74%, $n = 406$ at 2 weeks. Percentage in the OB: 0%, $n > 200$ at 4 h, 11.2%, $n = 269$ at 3 days, 41.5%, $n = 458$ at 2 weeks. Statistical analysis performed using independent samples Student's *t* test. I: Immunodetection of glia using an anti-GFAP antibody (green staining) (sagittal view, anterior left). Unlike in the rodent telencephalon, no longitudinally oriented fibers are visible. Rather, the projections of the detected glial cells (green arrows, see also inset) are arranged radially. (J–L) Immunodetection of PSA-NCAM (red staining) and PCNA (in panels K–L, green staining) (J: sagittal view, anterior left, OB and telencephalon delimited by the white dots; (K) sagittal view, anterior left, high magnification of a double-labeled preparation at the same level as boxed in panel J; (L) cross-section, dorsal up, at the level indicated in panel J). Note the prominent stripe of PSA-NCAM (red arrow in panel J) in the location of proliferating cells in the ventral subpallium (yellow staining in the overlay with PCNA expression in panels K–L). PSA-NCAM-positive cells reach into the olfactory bulb (red arrowheads in panel J).

neurogenesis in the adult zebrafish OB. Further consistent with adult neurogenesis of TH-positive neurons in the mammalian OB (Kosaka et al., 1995), we observed a low proportion of BrdU-positive cells expressing TH 4 or 8 weeks following BrdU injection (Figs. 5D, E and not shown), demonstrating that some TH-positive neurons in the adult OB (Byrd and Brunjes, 1995; Edwards and Michel, 2002) are newborn. Within 4 weeks, these account for less than 5% of the total TH-positive population, consistent with the lower proportion of newly generated TH-positive neurons in the mammalian OB (Hack et al., 2005). It is likely that these neurons also belong to the newborn *gad67*-positive population since, both in rodents and zebrafish, TH-positive neurons were reported to produce GABA in the OB (Edwards and Michel, 2002; Hack et al., 2005; Kohwi et al., 2005) (see also Fig. 5F). Together, these results demonstrate that de novo neurogenesis in the adult zebrafish contributes to the generation of several types of OB neurons, which include GABAergic interneurons in the IL and TH-positive interneurons in the GL (see Fig. 6H), comparable to the situation in mammals.

Recent results in the mouse highlight a bias for adult telencephalic Pax6-positive progenitors to give rise to TH-positive neurons of the OB GL (Hack et al., 2005; Kohwi et al., 2005). Thus, to further compare the zebrafish model to mammalian models of adult OB neurogenesis, we tested the relation between newborn TH neurons and the expression of *pax6* genes. Two *pax6* genes have been identified in zebrafish, with largely overlapping expression in embryos (Nornes et al., 1998). Most unexpectedly, we observed that the situation in adults was different: while *pax6b* expression was restricted to

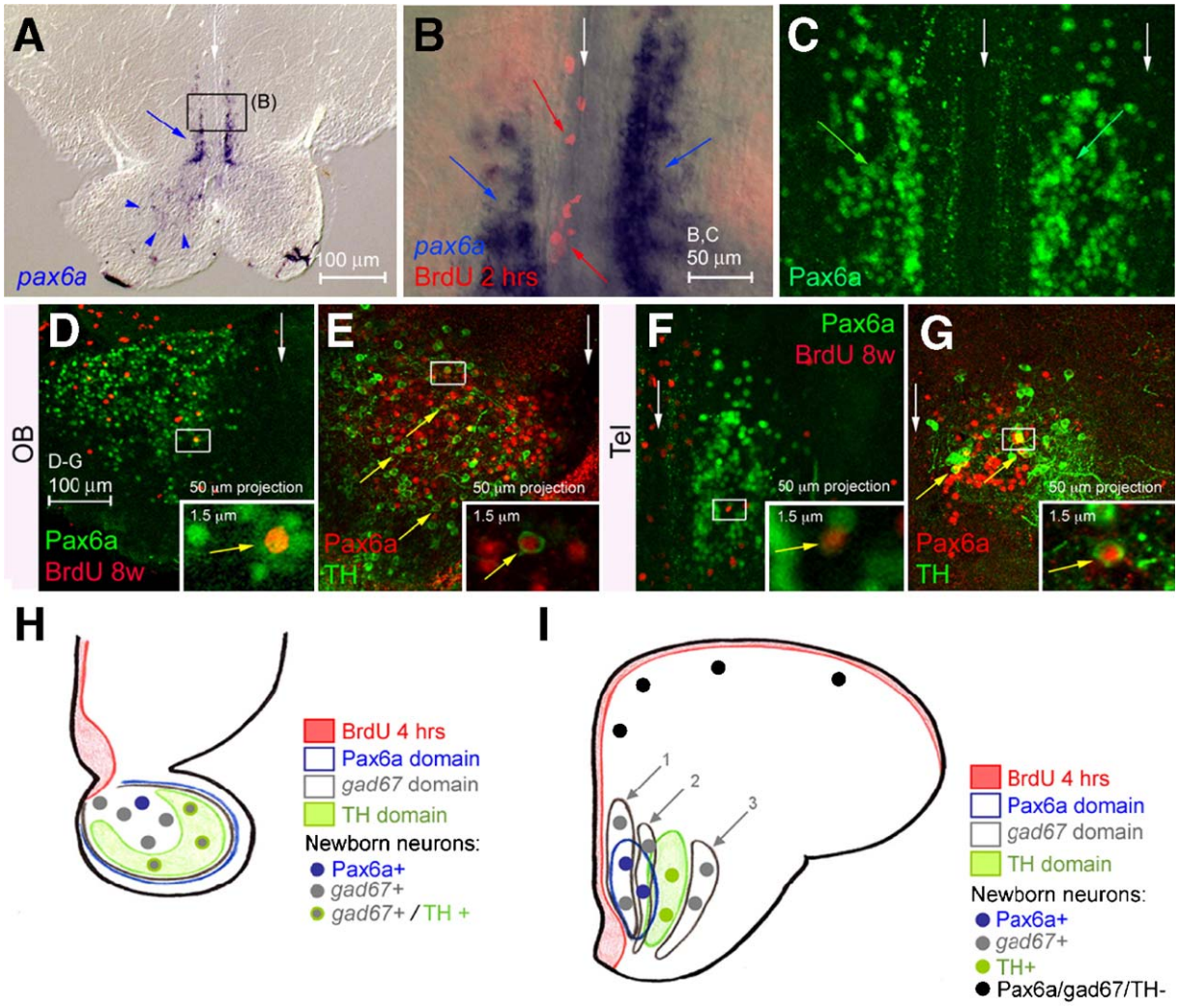


Fig. 6. Pax6a is expressed in neurons of the OB and anterior telencephalon. (A–G) Comparison of *pax6a* expression (A, B: in situ hybridization, blue; C–G: immunodetection, green or red) with BrdU incorporation (red) after 4 h (B) or 8 weeks (D, F) or with TH (E, G, green). All views are cross-sections, dorsal up, with white arrows to the midline; insets are high magnifications of the boxed areas; panel B is an overlay between bright field and fluorescence. Pax6a is expressed in the OB (A, arrowheads, D,E) and in bilateral telencephalic longitudinal stripes (A, B blue arrows, C green arrows, F, G), but not in proliferating cells (red arrows in panel B, see also C). After 8 weeks of tracing, cells expressing *pax6a* RNA (not shown) or protein and doubly positive for BrdU can be observed in both the Ob and telencephalon (D, F) (double labeled cells in insets). Coexpression of Pax6a and TH can also be detected in these domains (E, G) (examples of double positive cells are indicated by arrows and some are magnified in insets). (H, I) Schematics summarizing *gad67*, TH and Pax6a expression in the OB (H) and in the anterior subpallium and pallium (I) as well as the identity of newborn neurons 2 months after BrdU incorporation (color-coded).

telencephalic progenitors and was absent from the OB (see Figs. 9D, D′), *pax6a* expression was complementary. *pax6a* transcripts were found in cells adjacent to but nonoverlapping with ventricular progenitors in the telencephalon (Figs. 6A, B), as well as in numerous cells in the OB (Fig. 6A). None of the cells expressing *pax6a* was PCNA-positive (not shown) or had incorporated BrdU after a 4-h pulse (Fig. 6B). These findings were confirmed using a polyclonal antibody directed against mouse Pax6 that we found selectively labeling regions of zebrafish Pax6a (but not Pax6b) expression (Fig. 6C): positive cell bodies were strikingly absent from the ventricular zone. In the OB, BrdU/Pax6a-double positive cells were observed after 8 weeks of tracing (Fig. 6D), and few TH/Pax6a-double positive cells were also found (Fig. 6E). Thus, the Pax6a- and TH-positive populations overlap, and newborn OB neurons express Pax6a (see Fig. 6H).

Ventricular telencephalic progenitors give rise to GABAergic and TH-positive neurons in the telencephalon proper

As described above (Figs. 4, 5A), ventricular progenitors in the adult zebrafish telencephalon also contribute post-mitotic neurons to the telencephalon proper. In the subpallium and medial pallium, after a few weeks of tracing, these neurons appear aligned as bilateral dorsoventral columns adjacent to the ventricular zone. Strikingly, we observed that *gad67* (Figs. 5A, C), TH (Figs. 5G, I), and Pax6a (Figs. 6A–C) displayed similarly organized expression domains in this location. *gad67* is transcribed in three parallel columns (numbered 1 to 3 in order from medial to lateral, Fig. 5A). TH-positive cell bodies were mostly distributed between stripes 2 and 3, with a small overlap into stripe 2 (Fig. 5I), while Pax6a expression was medial, covering stripes 1 and 2 (compare Figs. 5A and 6A) and partially overlapping with TH expression laterally (Fig. 6G) (see schematic in Fig. 6I). The latter observation contrasts with the situation in juveniles, where TH and Pax6a expression in the telencephalon proper are exclusive (Wullimann and Rink, 2001). All domains are located adjacent to, but not within, the PSA-NCAM stripe (not shown), suggesting that these cells are not fated to the OB but rather overlap with the population described above of newborn neurons fated to the telencephalon proper. Together, these observations suggest the exciting possibility that newborn telencephalic neurons might share subtype identity and/or molecular attributes with newborn OB neurons.

To address this issue, we next determined the identity of newborn telencephalic neurons in the anterior dorsal subpallium and medial pallium by comparing BrdU-positivity in long-term tracings with the expression of the above markers. We found that, within 4 weeks, newborn neurons distribute over the three *gad67* stripes and that a large proportion of these neurons express *gad67* (see Fig. 5C after 8 weeks, and not shown). Newborn neurons positive for Pax6a (Fig. 6F) or TH (Fig. 5H) were also found. Within the time-frame of our experiments, none of the TH-positive newborn neurons in the telencephalon proper coexpressed Pax6a or *gad67* (not shown), in contrast to the OB. Together, these findings demonstrate that the ventricular zone of the subpallium and ventral pallium contribute newborn GABA or TH-positive neurons to the telencephalon proper, but that, unlike in the GL of the OB, these populations are mostly nonoverlapping.

Self-renewing progenitors are located in the dorsal and ventral ventricular zone of the zebrafish adult telencephalon

We demonstrated above that the zebrafish adult telencephalic ventricular zone serves as a source of progenitors for de novo neuronal differentiation in the adult OB and telencephalon proper. This prompted us to analyze the location, cellular, and molecular characteristics of these progenitors. To locate long-lasting progenitors, we analyzed after long-term BrdU tracing which cells that had retained the label were still cycling. To this aim, we traced ventricular BrdU-positive cells for 19 days and double-stained for PCNA expression. While, as described above, many BrdU cells during this time exited the proliferation zones, a few BrdU/PCNA double-labeled cells could still be found along the telencephalic ventricle (including its everted dorsal part) (2.2% of the PCNA-positive cells, $n = 180$) (Figs. 7A–C). Such cells are also observable anteriorly in the ventral subpallium, in a higher proportion (12.5% of the PCNA-positive cells, $n = 112$) (Figs. 7A, D, D′). These results were confirmed after 2 months of tracing (not shown). Thus, self-renewing, label-retaining progenitors are located both within the dorsal and ventral telencephalic ventricular zone. Their proportion among the proliferating cells is however five times higher ventrally than dorsally.

Ventricular telencephalic progenitors have molecular characteristics of glia

Neural stem cells in the adult mammalian brain are also slow dividing and label-retaining and are further characterized by their astroglia-like identity (Alvarez-Buylla and Garcia-Verdugo, 2002; Campbell and Gotz, 2002; Goldman, 2003; Gotz et al., 2002). To determine if this was the case in the adult fish telencephalon, we studied expression of the brain–lipid-binding protein BLBP that labels glial cells in mammals and birds (Anthony et al., 2004; Rousselot et al., 1997). Of the two zebrafish *blbp* genes, *fabp7a* and *b* (Denovan-Wright et al., 2000; Liu et al., 2003b), we found that *fabp7a* was strongly expressed in the telencephalic ventricular zone (Fig. 8B), in a manner identical to *sox2* (Figs. 8A–A″), *sox9a* and *sox10* (not shown), other common markers of adult neural precursors in mouse and fish (Ellis et al., 2004; Episkopou, 2005; Ferri et al., 2004; Komitova and Eriksson, 2004; Pevny and Placzek, 2005). FABP7a-positive cell bodies were observed to cover the ventricular zone, extending radial processes towards the pial surface of the brain (Figs. 8C–E). Two hours after BrdU incorporation, 42% of the BrdU-labeled progenitors were also positive for FABP7a (from 224 counted cells) (Figs. 8C, F). Importantly, however, the percentage of FABP7a-positive BrdU-positive cells decreased over time, as BrdU-labeled cells exited the ventricular zone (27% of co-labeling after 3 days, $n > 500$ counted cells, and 14% after 2 weeks, $n > 500$

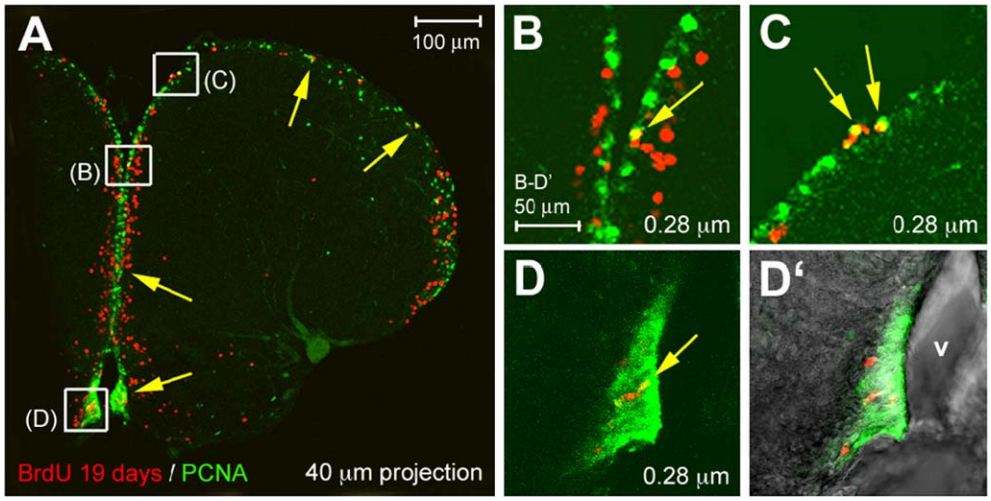


Fig. 7. Long-lasting neuronal progenitors in the zebrafish adult telencephalic ventricular zone. Co-immunodetection of PCNA (green) and BrdU (red) after long-term tracing (19 days), observed under confocal microscopy on cross-sections (thickness indicated, dorsal up). Panels B–D are high magnifications of the areas boxed in panel A, panel D′ is a brightfield and fluorescence view of panel D to locate the ventricle (v). While most labeled cells have exited the ventricular zone, a few BrdU-positive cells are retained in cycle (yellow arrows); these cells are located throughout the DV extent of the telencephalic ventricular zone.

counted cells) (Figs. 6D–F), consistent with the acquisition of a neuronal fate by most of the progeny. We conclude that the ventricular progenitors of the adult zebrafish telencephalon share classical characteristics of mammalian neural stem cells, in particular the expression of cellular and molecular glial characters.

Molecular code characterizing zebrafish adult telencephalic neuronal progenitors

Besides a few cases (Doetsch et al., 2002; Hack et al., 2005; Kohwi et al., 2005; Parras et al., 2004), the transcription factors attributing progenitor properties and fate of SEZ neuronal precursors in the rodent adult brain remain largely unknown.

We found that *emx3* (previously *emx1*, zebrafish nomenclature committee) (Morita et al., 1995), *dlx2a* (previously *dlx2*) (Akimenko et al., 1994), *olig2* (Park et al., 2002), *pax6b* (Nornes et al., 1998), *ash1a* (Allende and Weinberg, 1994) and *ngn1* (now *neurog1*) (Blader et al., 1997) were expressed in BrdU-positive ventricular progenitors of the zebrafish adult telencephalon (Figs. 9A–F′ and not shown). Unexpectedly, in striking contrast to the embryonic situation, the expression domains of these markers were largely overlapping and encompassed the entire ventricular progenitor zone in the subpallium and medial pallium (blue arrows in Figs. 9A–F′). However, the everted, dorsal, and lateral parts of the pallium progenitor zone expressed *pax6b* and *ash1a* (blue arrowheads in Figs. 9D, E) but not *emx3*, *dlx2*, *olig2*, and *ngn1* (white arrowheads in Figs. 9A–C, F). Interestingly, these observations suggest that progenitors located along the ventricular zone of the subpallium and medial pallium in the adult express a similar combination of transcription factors, unlike embryonic neuronal progenitors, and this combination differs with that expressed by adult progenitors in the dorsal and lateral pallium. Thus, the combination of factors expressed in zebrafish adult telencephalic progenitors might not be inherited from embryonic stages but might be recruited de novo to mediate adult neurogenesis.

To examine this idea, we tested whether the telencephalic expression of *ngn1* was controlled by distinct regulatory elements in the adult and embryo. The elements driving *ngn1* expression in the embryonic telencephalon consist of 212-bp-long regulatory region named “lateral stripe element” (LSE) in position −6702 to −6490 of the *ngn1* 5′ noncoding sequence. When this element is deleted, e.g. in the transgenic line *-3.4ngn1:gfp*, which contains only 3.4 kb upstream sequence, no telencephalic expression is observed in the embryo (Blader et al., 2003, 2004). In striking contrast, we found that, in the telencephalon of adult *-3.4ngn1:gfp* transgenic fish, GFP was a faithful reporter of endogenous *ngn1* expression: GFP expression was prominent in cell bodies along the ventricle of the subpallium (Figs. 9G, G′) and medial pallium (Figs. 9H, H′) and partially overlapped with BrdU-incorporating cells (Figs. 9G–H′, yellow arrows). Only one out of three transgenes containing the 3.4 kb upstream sequence of *ngn1* reproduced this pattern, suggesting that additional elements are required for robust expression (not shown). We conclude that *ngn1* expression in zebrafish adult telencephalic progenitors is not simply inherited from embryonic precursors but corresponds to a de novo recruitment of *ngn1* via new enhancer elements. Together, these results argue for a specific combination of factors mediating adult versus embryonic telencephalic neurogenesis in zebrafish and suggest that Pax6b (but not Pax6a), Ash1a, Olig2, Emx3, Dlx2, and Ngn1 might be instructive components of this combination.

Discussion

Our analysis of neurogenesis and neural precursor proliferation in the adult zebrafish telencephalon revealed several key insights. First, we identified, in the dorsal zebrafish telencephalon, a novel zone of adult neurogenesis that has apparently no

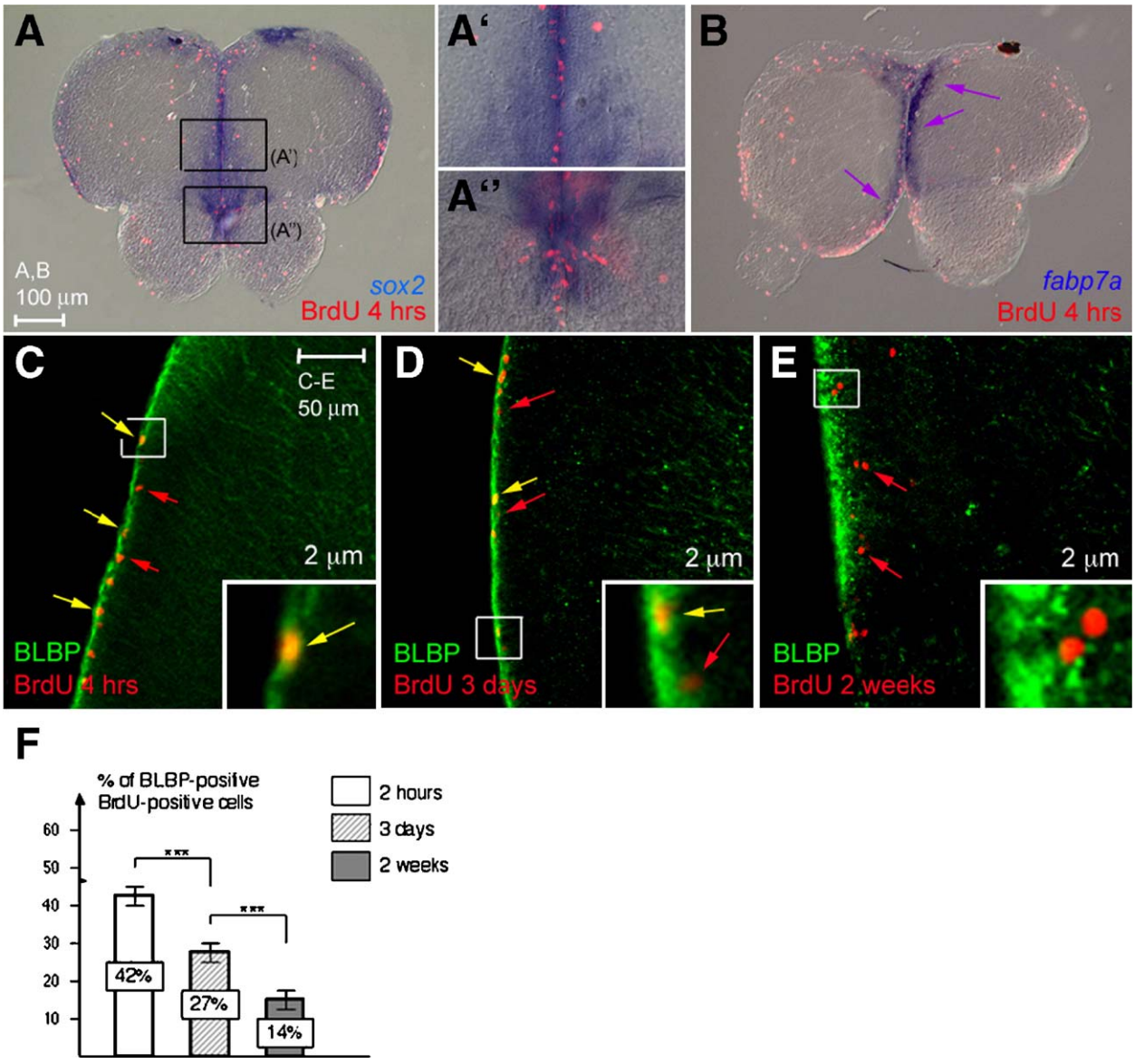


Fig. 8. Telencephalic progenitors express *sox2* and have molecular characteristics of glia. (A–B) Comparison of BrdU incorporation (red) after 4 h tracing with the expression of *sox2* (A–A″) or *fabp7a* (B) (blue, in situ hybridization). Cross-sections, dorsal up. Telencephalic progenitors are found in the expression domain of both markers. (C–E) Analysis in confocal microscopy (thickness 2 µm) of BLBP protein (green) and BrdU incoporation (red) after 4 h (C), 3 days (D) or 2 weeks (E). Examples of cells positive only for BrdU are indicated by red arrows, examples of cells doubly positive by yellow arrows. (F) Percentage of BLBP-positive cells that are also BrdU-positive after progressively longer tracing times. Note that this number strikingly decreases over time.

equivalent in the mouse telencephalon. As this domain also contains long-term label-retaining precursors, it may represent a novel zone of adult neural stem cells. Second, we describe for the first time that adult neurogenesis of olfactory bulb neurons in the fish closely resembles the mammalian counterpart: it also originates in the ventral telencephalon with PSA-NCAM-positive neuroblasts migrating towards the OB where GABAergic and dopaminergic neurons are generated life-long. However, in contrast to mammals, precursors in the ventral telencephalon also generate new neurons destined to the ventral telencephalon proper, notably including TH-positive neurons. Thirdly, we demonstrate that adult neural precursors in the fish telencephalon occur at all dorso-ventral levels but are distinct in their transcriptional code from those present at these positions during embryonic development. Thus, adult and embryonic neurogenesis differ in their molecular terms. Taken together, these data therefore provide not only insights into the phylogenetic but also the ontogenetic differences of neurogenesis in vertebrates.

Novel zones of adult neurogenesis in the dorsal and ventral telencephalon

BrdU-labeling in the adult fish revealed not only proliferation all along the ventricle in the dorsal telencephalon as previously shown (Zupanc et al., 2005) but also that the progeny of these cells differentiates into neurons. These neurons settle at just a short distance from the ventricular zone within the parenchyma.

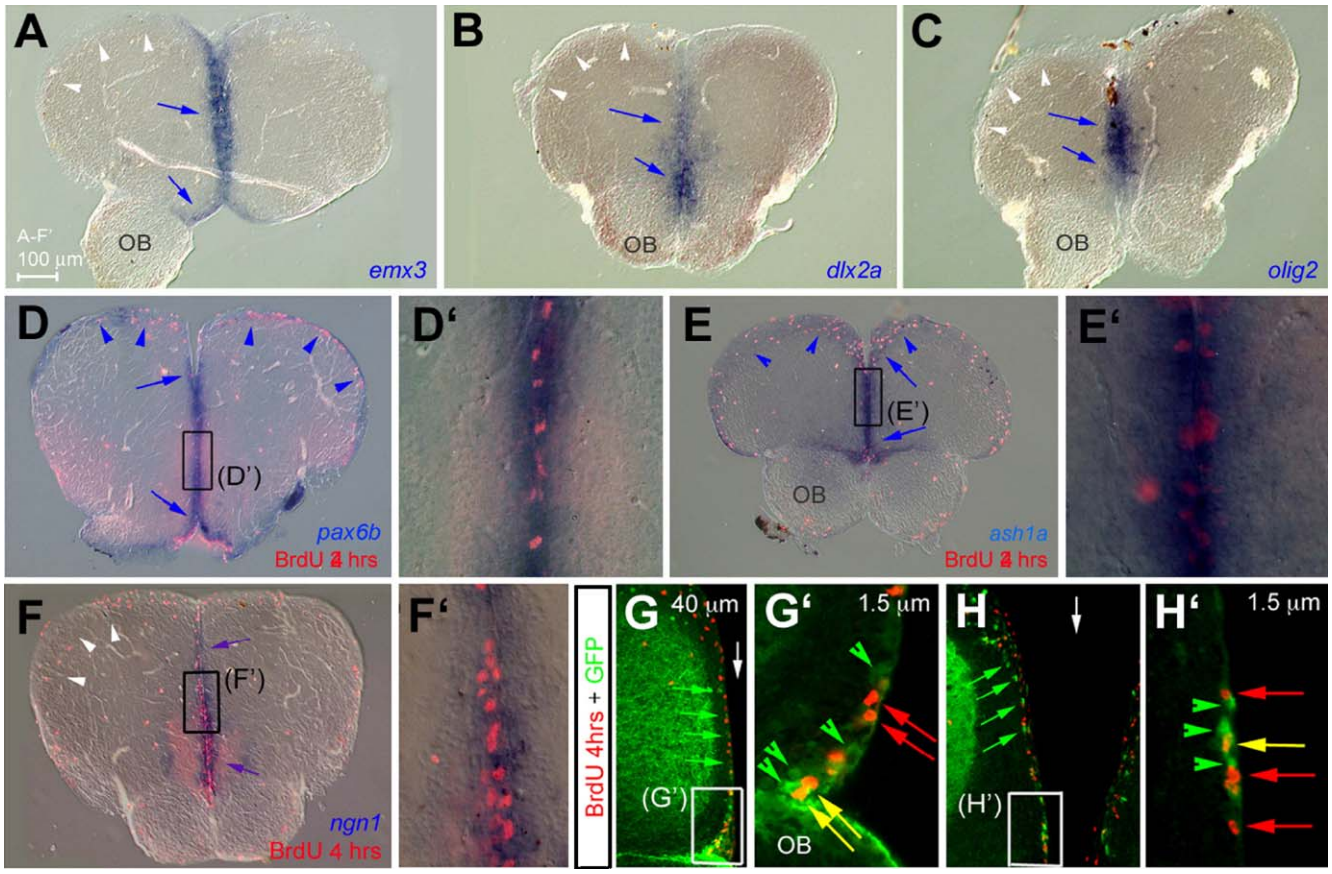


Fig. 9. Unique transcription factors code and promoter usage in adult zebrafish telencephalic progenitors. (A–F′) Compared expression of *emx3* (A), *dlx2a* (B), *olig2* (C), *pax6b* (D, D′), *ash1a* (E, E′), and *ngn1* (F, F′), together with BrdU incorporation after 4 h survival (red staining in panels D–F′), on anterior cross-sections (dorsal up). All markers are expressed in progenitors along the telencephalic ventricle at the midline (blue arrows, note the co-labeling with BrdU in panels D–F′). In addition, *pax6b* and *ash1a* are expressed in progenitors along the dorsally everted telencephalic ventricle (blue arrowheads in panels D, E), while other markers are not (white arrowheads in panels A–C, F). (G–H′) Determination of the enhancer fragment driving *ngn1* expression in adult ventricular telencephalic progenitors by comparing BrdU incorporation (red) and GFP protein (green) in the *-3.4ngn1:gfp* transgenic line (Blader et al., 2004; Blader et al., 2003). Cross-sections observed in confocal microscopy, dorsal up, green arrowheads and arrows point to cells expressing GFP only, red arrows to cells positive for BrdU only, and yellow arrows to double-labeled cells. Panels G and G′ focus on the ventral subpallium, panels H and H′ on the medial pallium, white arrows to the midline. Note that GFP distribution in *-3.4ngn1:gfp* is in agreement with endogenous *ngn1* expression (compare with F). OB: olfactory bulb.

Since the posterior pallium in zebrafish has been suggested as a functional equivalent of the hippocampus in mammals (Wullimann and Rink, 2002) (see Fig. 1D blue arrow), the fast proliferating cells in this region (see Fig. 1D and Zupanc et al., 2005) may correspond to adult neurogenesis in the hippocampal region in mammals (Gage, 2000). However, neurogenesis in the mammalian hippocampus is restricted to the dentate gyrus by nonventricular precursors (Gage, 2000, Taupin and Gage, 2002), an obvious difference to the location of adult precursors all along the telencephalic ventricle in fish.

In the dorsal telencephalon of mammals, adult neurogenesis is restricted to the dentate gyrus and does not occur in the cerebral cortex, while it is widespread throughout the dorsal telencephalon in zebrafish. Interestingly, also in reptiles newborn neurons populate all major subdivisions of the cortex (Font et al., 2001; Garcia-Verdugo et al., 2002) by a short migration to the parenchyma close to the VZ of the dorsal telencephalon (Perez-Canellas and Garcia-Verdugo, 1996). The identity and functional significance of these neurons have not been determined, and it will be of great interest to understand whether dorsally born neurons in reptiles and zebrafish are functionally equivalent.

Adult newborn neurons populate also the ventral domains of the adult fish telencephalon proper. As in the dorsal telencephalon, proliferation occurs along the ventricle, and newborn neurons that acquire GAD and TH settle very close to their site of origin. This pattern of neuron generation in the dorsal and ventral telencephalon suggests that the telencephalon of fish grows by addition of new neurons at the outside, similar to the retina and the midbrain (Marcus et al., 1999; Nguyen et al., 1999). While adult neurogenesis in the ventral telencephalon was also observed in reptiles and birds (Alvarez-Buylla and Kirn, 1997; Font et al., 2001; Garcia-Verdugo et al., 2002; Goldman, 1998; Solis, 2000), in mammals, all neurons generated along the ventro-lateral wall of the telencephalon migrate towards the olfactory bulb. New striatal neurons can be added from the underlying precursor zone in the mouse only after lesion in the overlying striatum (Arvidsson et al., 2002; Parent et al., 2002). As the teleostean subpallium has been proposed to be homologous to the mammalian striatum and septum (Wullimann and Rink,

2002), the molecular cues governing adult neurogenesis of TH-positive neurons in this region will be of particular interest.

Adult generation of olfactory bulb neurons in the zebrafish resembles mammalian olfactory bulb neurogenesis

The addition of newborn neurons to the zebrafish adult OB has been demonstrated previously, but the origin and fate of these neurons was not determined (Byrd and Brunjes, 1998; Zupanc et al., 2005). Following progenitor cell fate over closely spaced time points revealed that BrdU-labelled cells leave the ventral subpallial ventricle and enter the OB. We further discovered the likely migration route of newborn neurons as a stripe of PSA-NCAM-immunoreactive cells reaching into the OB. Indeed, PSA-NCAM regulates neuronal migration to the bulb in the mouse (Chazal et al., 2000) and chains of migrating PSA-NCAM-positive neuroblasts have been observed throughout vertebrates (Doetsch and Scharff, 2001). Moreover, the heterogeneity among the precursors in the subpallium comprising long-term label-retaining progenitors, fast dividing precursors and PSA-NCAM-expressing neuroblasts closely resembles the precursor types observed in the mammalian SEZ (Alvarez-Buylla and Garcia-Verdugo, 2002). Finally, the identity of the newly generated neuronal subtypes in the OB shows close similarities between mouse and zebrafish with *gad67*+/TH− neurons added to the IL and *gad67*+/TH+ neurons settling in the outer part of the zebrafish OB. Interestingly, we also observed Pax6 expression in the adult OB in both the GL and IL (for comparison see, Hack et al., 2005; Kohwi et al., 2005). Pax6 is necessary and sufficient for adult neurogenesis in the mouse OB, and instructs a dopaminergic phenotype when maintained in postmitotic neurons (Hack et al., 2005; Kohwi et al., 2005). Taken together, adult neurogenesis of GABAergic and dopaminergic neurons in the OB may be a conserved feature among most vertebrates, while neurogenesis present in other telencephalic regions of the zebrafish has come to an end in some vertebrate classes.

Identification of neural stem and progenitor cells in the adult zebrafish telencephalon

Our results further provide evidence for the existence of label-retaining precursors, a feature of stem cells, within the ventricular zones of the adult zebrafish telencephalon. BrdU-labeling was retained for weeks by a subset of precursors lining the ventricle and that remained proliferating. Since these cells were still detectable in animals over 2 years of age (data not shown), they likely exist throughout adulthood, a further feature of adult tissue stem cells. These data demonstrate that a subset of adult telencephalic precursors in the zebrafish is slow-dividing and self-renewing. Notably, these label-retaining cells express glial characteristics similar to neural stem cells in the two unique sites of adult neurogenesis in mammals (Doetsch et al., 1999; Seri et al., 2001). Taken together with some molecular expression characteristics (e.g. *sox2*) these findings strongly suggest that the telencephalic ventricular zone of the zebrafish contains long-lasting neural progenitors with stem cell-like properties. Importantly, these label-retaining progenitors are present throughout the dorso-ventral extent of the telencephalic ventricle, in contrast to mammals.

Interestingly, adult neural precursors in the fish telencephalon differed significantly from their embryonic counterparts. For example, one main distinguishing feature between adult and embryonic precursors is the acquisition of glial characteristics that appear only late in zebrafish embryos (Marcus and Easter, 1995). Along the same line, *sox2* that is highly expressed in adult telencephalic precursors is not expressed ubiquitously in the embryonic neural plate of zebrafish (www.zfin.org). Moreover, we discovered that the elements required for *ngn1* expression in adult telencephalic progenitors are distinct from those regulating expression in the telencephalon at embryonic stages (Blader et al., 2004). This provides the first evidence that a molecular mechanism specifies adult neural precursors differently from those during development. In fact, the gene expression domains that we examined here in the adult zebrafish telencephalon differ considerably from those at embryonic ages. For example, *emx3* and *ngn1* are expressed in the dorsal telencephalon of zebrafish embryos, and *dlx*, *ash1* and *olig2* are expressed ventrally (Akimenko et al., 1994; Allende and Weinberg, 1994; Blader et al., 2004; Kawahara and Dawid, 2002; Nornes et al., 1998; Park et al., 2002). In addition, zebrafish *pax6a* transcripts are restricted to the ventral telencephalon and *pax6b* transcripts are absent from the telencephalon at the 24 h post-fertilization (hpf) stage (see Blader et al., 2004 and our unpublished observations). This is notably different from their expression pattern in the ventricular zone of the adult fish, where most of these genes (including *pax6b*, but not *pax6a*) were expressed all along the dorso-ventral axis. Similarly, Pax6 and Emx2 are expressed dorsally while Dlx1,2,5, Mash1 and Olig2 are expressed ventrally in the telencephalon of mouse embryos (Campbell, 2005, and references therein), but these transcription factors are expressed within the same region in the adult ventral telencephalon, the zone of OB neurogenesis (Galli et al., 2002; Hack et al., 2005; Kohwi et al., 2005; Parras et al., 2004). While it is not clear whether this change is due to cell migration or transcriptional regulation, it points to the unique composition of precursors in adult neurogenic zones, both in zebrafish and in mice. Live imaging analyses in zebrafish as well as genetic screens will help to better understand how the distinguishing features between embryonic and adult precursors come about. This should provide direct insight into the mechanisms regulating adult stem cell hallmarks, such as those characterizing the slow-dividing self-renewing neural stem cells that are not present during development.

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