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Investigating the Diversity of Radial Glia Fates in the Rat Neocortex

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Abstract

Radial Glia (RG) are a mitotically active population of cells which reside within the ventricular zone at the lateral ventricle and give rise to the pyramidal neurons and astrocytes of the neocortex. Through cellular divisions, RG produce two daughter cells, one which resides in the ventricular zone and becomes another RG while the other is an immature progenitor which migrates away from the ventricle and populates the growing cortex. RG have been found to be a heterogeneous population of cells which express different surface antigens and genetic promoters which may influence the cellular fate of their progeny. In this study we have investigated the progenitor profiles of two promoters, nestin (a neural intermediate filament) and GLAST (astrocyte specific glutamate transporter) within the RG. *In-utero* electroporation was used to transfect reporter plasmids under the control of promoter driven Cre-Recombinase into the RG lining the lateral ventricle during mid-neurogensesis (E14). It was found that there was a large amount of overlap between the nestin and GLAST expressing populations of RG, however, there was still a small subset of cells which exclusively expressed GLAST. This prompted us to investigate the lineage of these two promoters using the PiggyBac transposon system which uses promoter driven episomal plasmids to incorporate a reporter gene into the genome of the transfected cells, allowing use to trace their full progeny. Our data shows that nestin expressing RG generate mostly neurons and few astrocytes while the GLAST expressing RG generate a greater proportion of astrocytes to neurons.

Introduction

Radial glia are crucial in the development of the mammalian cerebral cortex. Derived from the cells of the neural epithelium, radial glia are a mitotically active population of cells which are located at the lateral ventricle and express distinct surface antigens such as *brain-lipid-binding protein* (Blbp), *glial-fibrillary-acidic protein* (GFAP) and *astrocyte specific glutamate transporter* (GLAST) which separate them from their neural epithelium precursors. Nestin expression, a neural intermediate filament protein, has been found to be expressed in the neural epithelium in addition to some radial glia (Pinto and Gotz, 2007). Sharing characteristics with the neural epithelium, radial glia have a bipolar morphology containing an end foot on the

ventricular surface, with an apical process that extends to the pia (Kriegstein and Gotz, 2003, Kriegstein and Buylla, 2009).

Radial glia are progenitors in the developing cortex, first producing neurons and then astrocytes (Noctor et. al 2002, 2004). Radial glia undergo asymmetrical cellular division yielding two daughter cells, one which inherits the apical process and the function of a radial glia, while the other is an immature progenitor which migrates up the apical process and populates the cortex (Noctor et. al 2001, Voigt, 1989). It has been shown that there is spatial heterogeneity within the radial glia pool (Marin and Rubenstein, 2001, Fogarty et. al 2007). Radial glia of the dorsal-lateral cortex produce radially migrating progenitors which produce cortical neurons and glia while radial glia of the ventral cortex, specifically of the lateral and medial ganglionic eminences, primarily produce tangentially migrating progenitors of cortical interneurons and oligodendroglia, respectively.

As eluded to earlier, radial glia at different time points produce different cell types. At the start of neurogenesis at embryonic day 9.5 (E9.5), the radial glia produce progenitors which populate the cortex and become deep layer (V-VI) cortical neurons. As neurogenesis continues, radial glia produce precursors to upper layer cortical neurons (II-III) and during the middle of cortical neurogenesis at E17.5 radial glia begin producing astrocytes and continue to do so well into the postnatal weeks (Kriegstein and Gotz, 2003).

McCarthy et al. 2001 has suggested that the entire pool of radial glia are not constantly dividing and producing progenitors, but rather, there are temporally activated populations which are responsible for populating specific cell types. This was shown by identifying cohorts of radial glia that are not mitotically active throughout the entire neurogenic period. This suggests that there are certain populations of radial glia which are active early in neurogenesis and produce neurogenic progenitors. Then at the end of neurogenesis and the beginning of gliogenisis, a new population begins to produce gliogenic progenitors. It was also found that some radial glia were bi-potent and can give rise to multiple cell types.

Studies have also suggested that expression of different promoters may influence the fate restrictions of radial glia as either bi-potent or uni-potent precursors (Hartfuss et. al 2001). This raises interest in whether certain promoters may be associated with different cell types and that

perhaps there may be neuronal or glial indicative progenitors; or at least promoters which are more strongly associated with one cell type over another. Fluorescence activated cell sorting (FACS) has been used to isolate radial glia during midneurogenesis in order to grow them *in-vitro*. These radial glia were shown to mainly produce neurons, while there were some clusters which had non-neuronal fates and a small cohort produced both neurons and non-neuronal cell types (Malatesta et. al 2000). Interestingly, Noctor et. al 2002, using retroviral labeling of radial glia was able to produce neuronal and non neuronal clones from a single cell, thus showing that radial glia have the ability to produce a wide array of cell types.

Transgenic mice using Cre/loxP fate mapping have shown radial glia throughout the entire central nervous system, at some point during development, pass through a neurogenic stage (Anthony et. al 2004). The Cre/loxP recombination system was later adapted as episomal plasmids to transfect and label cells of the postnatal rat retina and embryonic mouse brain using *in-utero electroporation* under the control of specific promoters (Matsuda and Cepko, 2006). The use of site specific recombination systems such as Cre/loxP, allows for specific control over the expression of reporter genes. Under the control of a specific promoter, Cre-recombinase recognizes a *neo* stop cassette bordered by loxP sites upstream of a reporter gene such as GFP. Cre-recombinase cleaves out the stop cassette and recombines the DNA, allowing expression of the downstream reporter gene. Using the Cre/loxP recombination system under control of radial glia promoters in transgenic mice (Anthony and Heintz, 2008) has been able to trace the lineage of radial glia in the ventral and dorsal telencephalon. The results show that there is heterogeneous promoter expression between radial glia from early neurogenic and later gliogenic time points.

The use of the episomal Cre/loxP recombination system is useful in labeling non-dividing cells because the plasmids are not integrated into the genome of the transfected cells and are therefore sequentially diluted through successive cell divisions. This allows us to preferentially label terminally dividing cells at the time of transfection. In order to investigate dividing cells and label their lineage, a reporter gene must be inserted into the transfected cells' genome inorder to pass it along to the progeny. The PiggyBac transposase system accomplishes this by inserting a vector including the reporter gene directly into the genome of the transfected cell at

TTAA repeats, thus allowing the observation of an entire cell's progeny, regardless of its mitotic activity (Wu, 2007).

In this study we want to investigate whether radial glia subpopulations distinguished by differences in promoter expression have different developmental fates within the same localized area within the ventricular zone. We have investigated the embryonic expression of the nestin and GLAST promoters using Cre/loxP recombination. Previously, nestin has been found to be co-localized with immature neuronal markers (Barry and McDermott, 2005) and GLAST has been shown to be expressed in radial glia and stay on in mature astrocytes (Barry and McDermott, 2005, Anthony and Heintz, 2008).

In order to isolate unique progenitor types, we compared promoter expression with one another and found that the majority of radial glia co-express both promoters while a small cohort of cells were found to be exclusive to GLAST promoter. Since the developmental fates of nestin and GLAST expressing radial glia have been suggested to have different fate potentials, we investigated the progeny of the promoter expressing radial glia using PiggyBac Transposase. We found that the progeny from the nestin condition generated a greater proportion of neurons to astrocytes while the GLAST condition generated a progeny with a greater proportion of astrocytes to neurons. Our data suggests that subpopulations of radial glia isolated based on promoter expression can have different fate potentials.

Materials and Methods

In this study, *in-utero* electroporation was used to transfect ventricular zone radial glia with either nestin, GLAST or CAG promoters that either directly or indirectly drove the expression of reporter genes.

Plasmid Constructs

Embryonic Experiments

For Cre/loxP recombination, two plasmid constructs were required: CAG-Cre, Nestin-Cre or GLAST-Cre and CALNL-tdTomato. CALNL-tdTomato was constructed via PCR amplification and insertion of tdTomato into CALNL-GFP vector following excision of GFP by restriction endonucleases. CAG-Cre and CALNL-GFP were gifted from Dr. Constance Cepko. Existing

nestin and GLAST promoter constructs were used to insert PCR amplified Cre-recombinase inserts following excision of reporter genes by restriction endonucleases. The two plasmids were also co-transfected with either CAG-mRFP or nestin second intron enhancer/hsp68 minimal promoter eGFP (Nestin/hsp68-eGFP) (gifted from Dr. Steven Goldman).

Long Term Survival Experiments

For genomic transposition of reporter genes into ventricular radial glia, two plasmid constructs were also required: Nestin or GLAST driving PiggyBac Transposase (Nestin-PBase or GLAST-PBase) and ZG-s (containing a β-actin promoter driving GFP cassette). Existing nestin and GLAST promoter constructs were used to insert PCR amplified PBase inserts following excision of reporter genes by restriction endonucleases. CAG-PBase and ZG-s were gifted from Dr. Mario Capecchi.

In-utero electroporation

In-utero electroporation was performed on embryonic rat pups to transfect ventricular radial glia with our plasmid constructs. Transfection took place at E14 or E15 for embryonic and lineage tracing experiments, respectively. Pregnant Charles River wistar rats at gestational day 14 or 15 were anesthetized with Ketamine/Xylazine (100/10 mixture, 0.1 mg per g body weight, intraperitoneally) after which a midline laparotomy was performed in order to expose the uterine horns. Approximately 1-2µL of 1.5µg/µL plasmid DNA solution (for CAG-mRFP, 0.5µg/µl was used) in 1X phosphate buffered saline (PBS) mixed with 2mg/ml fast green dye (Sigma) was microinjected by pressure into the lateral cerebral ventricles of embryos through the uterine muscle and amniotic sac via glass capillary tubes. Following injection, electroporation was accomplished by discharging a 500µF capacitor charged to 70-110V with a sequencing power supply using a BTX ECM830 pulse generator (Genetronics). The voltage pulse was discharged across a pair of BTX tweezertrodes that pinched the head of each embryo through the uterus with the anode placed outside the uterine wall over the ventral telencephalon of the embryo. After electroporation of all embryos was completed, the uterus was returned to the abdominal cavity which was then filled with warm, sterile, physiological saline. The abdominal muscle and skin incision sites were then closed using silk sutures. Transfected embryos used for the embryonic experiments were allowed to survive for two days (E14-16). The brains were then harvested and

placed in 4% paraformaldehyde solution. For lineage tracing experiments, the animals were allowed to survive until P15, at which time transcardial perfusion was performed with 4% paraformaldehyde and their brains were harvested.

Immunohistochemistry and microscopy

Embryonic and postnatal brains were fixed overnight in 4% paraformaldehyde and then sectioned on a VT100 Leica vibratome at 50-60µm sections. Free floating sections were obtained and placed in 1X PBS. Tissue was stained using standard immunohistochemistry protocols using the following antibodies: rabbit anti-GFP (Molecular Probes 1:1000, Cat. No. A11122), mouse anti-GFP (Molecular Probes 1:1000, Cat. No. 332600) and rabbit anti-dsRed (Clontech 1:1000, Cat. No. 632496).

Embryonic Experiments

Images for figures were taken using apotome confocal microscopy (Zeiss) with 7-10µm Z-stacks and adjusted in Adobe Photoshop CS2 (San Jose, CA). Images were taken at the ventricular surface to visualize the transfected radial glia. For data analysis, images were obtained via fluorescence confocal microscopy (Leica). One image per section, 3-5 sections per brain per condition (n=3-5 brains per condition). Z-stacks 7-10µm thick were obtained for each image using a 40x oil immersion lens.

Long Term Survival Experiments

Images for data analysis were obtained by a Nikon upright microscope with SPOT camera using a 40x oil immersion lens. Images spanning the neocortex were obtained by montaging single images from the pial surface to cortical layer VI. This resulted in producing a single neocortical column image per slice. Between 5 and 6 slices were examined per brain, per condition (n=5 brains per condition).

Data Analysis

Fluorescence microscopy images for both embryonic and long term survival experiments were processed and analyzed by Adobe Photoshop CS2 (San Jose, CA) and ImageJ software (NIH, Bethesda, MD), respectively.

Embryonic Experiments

Quantification of promoter expression in ventricular zone radial glia was accomplished by manual cell counting. Thin slices allowed for reliable quantification of single cells. All cells were quantified within the imaged field as only GFP+, only RFP+ or co-expressing both GFP and RFP. Quantifications were expressed as a percentage of the total transfected population. Statistical Analysis: Means and standard error quantifications were performed in Microsoft Excel (Redmond, Washington). Unpaired student t-test with unequal variance was performed in Kalidograph (Synergy Software, Reading, PA) to determine significance across conditions.

Long Term Survival Experiments

Quantifications of cell types in postnatal grey matter were accomplished by manual cell counting. For each image, GFP+ cells were analyzed within the grey matter based on morphological characterization as either neurons or astrocytes. ImageJ was used for cell type tallies. Cell type quantifications were expressed as a percentage of total GFP+ cells within grey matter. Statistical Analysis: Means and standard error quantifications were performed in Microsoft Excel (Redmond, Washington). Paired student t-test with equal variance was performed in Kalidograph (Synergy Software, Reading, PA) to determine significance within conditions.

Results

In-utero electroporation was used to transfect radial glia located at the wall of the lateral ventricle with our plasmid constructs. Promoters used to drive various reporter genes allowed us to identify and investigate subpopulations of ventricular zone radial glia.

Cre/LoxP Recombination System Reliably Labels Ventricular Zone Radial Glia

Figure 1A shows a schematic of the rat embryo *in-utero* electroporation transfection procedure. Negatively charged plasmids are pulled towards the positive electroporation paddle upon an electrical discharge across the paddles leading to the transfection of radial glia along the ventricular zone with our plasmid constructs.

Cre/loxP recombination requires two plasmids to express the desired reporter gene. Chicken β-actin with cytomegalovirus enhanced promoter (CAG) was used as the promoter to drive the expression of Cre-recombinase (CAG-Cre). In a separate construct, expression of green fluorescent protein (GFP) by CAG promoter was blocked by a neo stop cassette flanked by loxP (CALNL-GFP). Cre-recombinase recognizes loxP and recombines the plasmid to exclude the flanked segment (neo stop cassette) and allow the expression of the reporter gene (Figure 1B). Because the CALNL GFP plasmid requires recombination to express GFP, we first wanted to test the reliability of the Cre/loxP system for leaky GFP expression.

Cells were transfected at E14 with either CAG-mRFP + CALNL-GFP or CAG-mRFP + CAG-Cre + CALNL-GFP and harvested at E16. Because CAG is a ubiquitous promoter, CAG-mRFP (monomeric red fluorescent protein) was used as a control in order to label all the cells that were transfected (Figure 1C) In the upper panels, cells transfected in absence of Cre, showed strong expression of mRFP (Top Left Panel) and no expression of GFP (Top Middle). In the bottom panels, cells were transfected with Cre and strong expression of GFP was seen (Middle Bottom Panel). Upon merging the two images, it can be seen that there is high co-expression of GFP and mRFP. These data show that Cre/loxP recombination is reliable due to the fact that no GFP expression was observed in the absence of Cre. In addition, the high co-expression of both reporter genes under the control of the same ubiquitous promoter indicates that the three plasmid system works efficiently and that both promoters are equally recognized by the transfected population of cells.

Subpopulations of Radial Glia Recognized by Nesitn and GLAST Promoters

Nestin and GLAST are genes whose expression in radial glia may suggest certain developmental fates (Hartfuss et. al 2001, Barry and McDermott 2005, Malatesta et. at 2000). Therefore, we wanted to observe their expression profiles within the starting population of midneurogenic radial glia at E14 in order to detect if there are distinct subpopulations of radial glia that can be isolated based on promoter expression. This was accomplished by using the promoter driven Cre/loxP recombination system. Cells were transfected with Nestin/hsp68-eGFP, CALNL-tdTomato and either nestin-Cre or GLAST-Cre (Figure 2A).

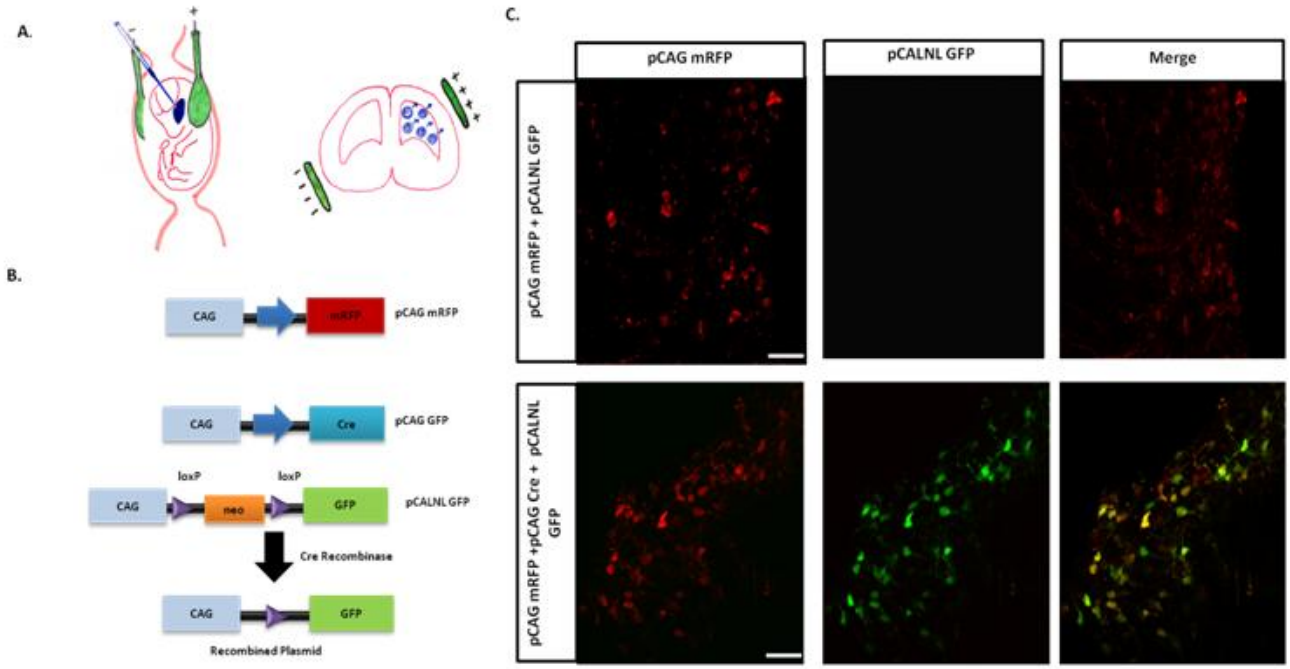

Figure 1. Cre/loxP Recombination System Shows no Leaky Reporter Gene Activity while Affectively Labeling Radial Glia Progenitors in the Ventricular Zone. A. Schematic of experimental procedure. *In-utero*, interventricular injection of plasmid system takes place at E14. Tweezertrodes hold the head of the embryo in place and are positioned on opposite sides of the brain. *In-utero* electroporation allows for transfection of cells along the ventricular wall **B**. Plasmid constructs used in Cre/LoxP recombination reliability experiments. Chicken β-actin with cytomegalovirus enhanced promoter (CAG) drives the expression of monomeric red fluorescent protein (mRFP) as internal control. Ubiquitous promoter CAG, upstream from GFP, is blocked by a neo stop cassette flanked by loxP. Following recombination by Cre-Recombinase, CAG drives the expression of GFP. Cre-Recombinase is driven by CAG. **C**. Reliability of the Cre/loxP recombination system. Confocal microscopy images taken of radial glia on the ventricular wall after two day survival transfection (E14-16). Cre-Recombinase is required to drive the expression of GFP (Top Panels). In the presence of Cre, GFP is strongly expressed (Bottom Panels). High co-localization of CAG driven mRFP and GFP demonstrates that both plasmid systems are equally recognized. Scale Bar: 50μm.

To assess the effectiveness of our Cre-recombination system, a control condition was used (Nestin/hsp68-eGFP + Nestin-Cre + CALNL-tdTomato) in order to determine the expression of two different reporter genes (eGFP and tdTomato, which is expressed as a red fluorescent protein) under the direct and indirect control of the nestin promoter, respectively. In the top panels of Figure 2B, we observed high expression of both eGFP and tdTomato. In the merge panel, very little cells can be seen which only express either GFP or tdTomato, suggesting that our system is effective. Upon quantification, it was found that an overwhelming majority of transfected cells (99%±1) were GFP+/tdTomato+ (Figure 2C), thus supporting our observations that our triple plasmid system is working effectively and that both the Nestin/hsp68 promoter and the Nestin prompter are equally recognized by the transfected population of cells.

In the experimental condition (nestin/hsp68-eGFP + GLAST-Cre + CALNL-tdTomato) we observed high expression of both GFP and tdTomato (Figure 2B, first two bottom panels). However, upon merging, our images show tdTomato+ cells which lack eGFP expression. Upon quantification it was found that although there is still a high percentage of overlap (87%±2), 10%±2 of cells were tdTomato+/GFP- (Figure 2C). These data show that there is a subpopulation of radial glia at E16 which are GLAST+/nestin-, while the majority of cells are GLAST+/nestin+. In order to test if these embryonic subpopulations had different fate potentials, we set out to determine their progeny.

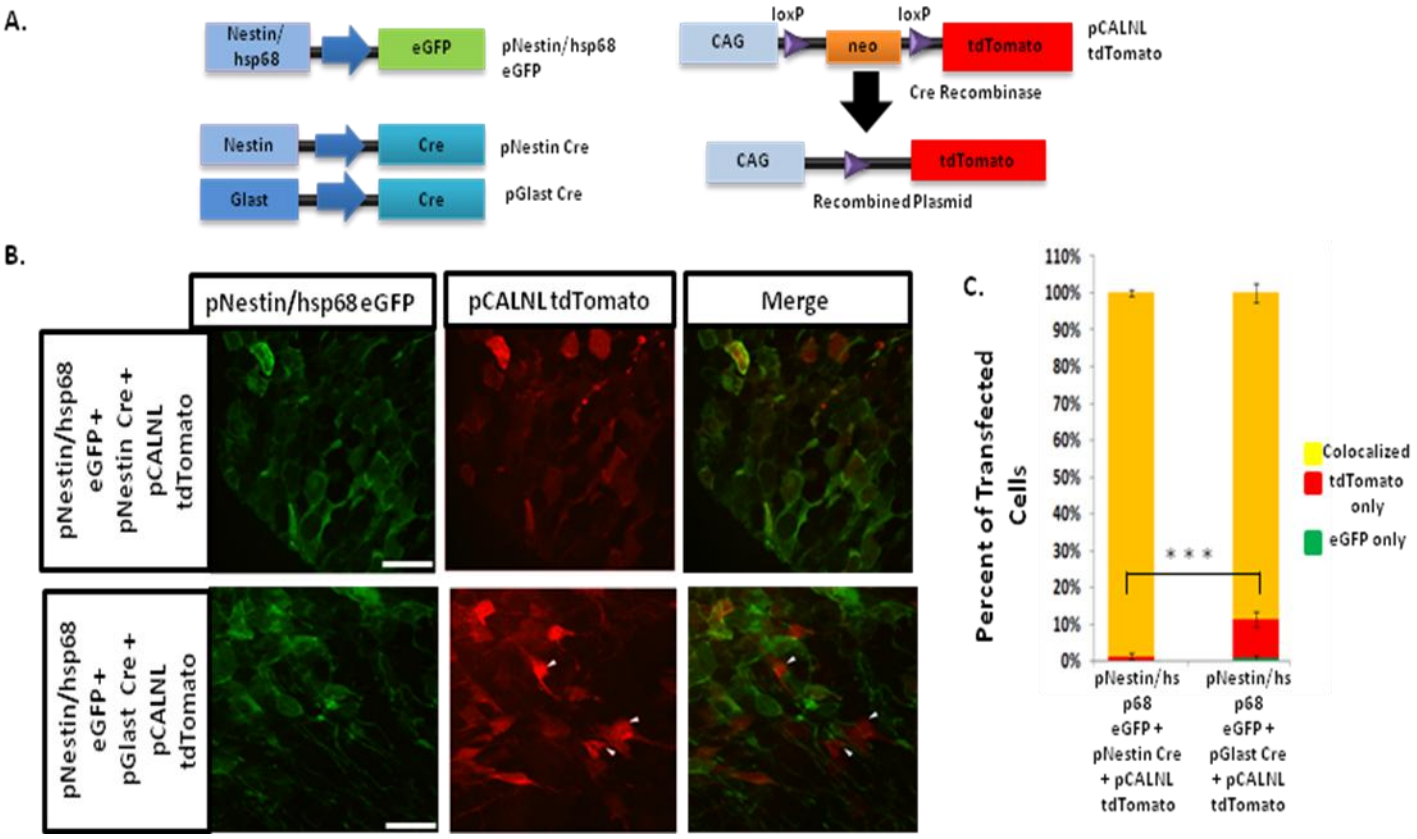

Figure 2. Subpopulations of Radial Glia are Identified Based on Expression of Nestin and GLAST Promoters. A. Plasmid constructs used in embryonic analysis of nestin and GLAST expression. Nestin/hsp68 driving enhanced green fluorescent protein (eGFP) used as internal control. Nestin and GLAST promoters separately drive Cre expression. Recombination drives the expression of tdTomato under the control of CAG. **B.** Confocal microscopy images of two day survival (E14-16), triple plasmid transfection of radial glia with Nestin/hsp68-eGFP and gene specific promoter driven Cre-Recombinase. Nestin/hsp68 is compared with Nestin-Cre in order to ensure that both promoters are equally recognized by the cell population. High overlap between both nestin plasmids can be seen in the merge (Top Panels). Nestin and GLAST promoter expression is compared; although many cells are nestin + and GLAST +, there are some cells which are nestin - and GLAST + (Bottom Panels). Scale Bar: 20µm. **C.** Bar Graph showing quantification of nestin + and GLAST + cells as a percentage of transfected cells. Nestin/hsp68-eGFP + Nestin-Cre condition shows high percentage of overlap (98.6%). 88.2% of transfected cells were both nestin + and GLAST +, leaving a subpopulation of GLAST +, nestin – cells (10.3%). (n=3-5 brains per condition) Unpaired Student t-test with unequal variance was performed. ($p<.0001$)

Nestin and GLAST show Different Lineages of Neurons and Astrocytes

Above, we investigated the immediately transfected starting population of ventricular zone radial glia. Due to the subpopulations that were distinguished based on their promoter expression, we wondered if this heterogeneity could lead to different fate potentials within the radial glia. In order to investigate the lineage of these cells, we employed a transposition system in order to insert a reporter gene into the transfected cells' genome, thus passing the reporter gene to all of its daughter cells and allowing us to track the full progeny.

In order to investigate the entire lineage generated by radial glia expressing these promoters, we used the PiggyBac Transposase system. PiggyBac (PBase), under the control of a promoter, recognizes the 3' and 5' translating regions (TR) on ZG-s and inserts it into the genome of the transfected cell at TTAA sequences (Wu, 2007). For these long term survival experiments, cells were transfected at E15 with either nestin-PBase or GLAST-PBase and ZG-s (containing a CAG-eGFP cassette) and harvested at P15. The cells were also co-transfected with CAG-mRFP to identify the direct progeny of the transfected cells (Figure 3A).

Because PBase dependent transposition was based upon nestin and GLAST promoter expression, we were able to track the lineages of specific subpopulations of radial glia (Figure 3B). Within the images for both conditions, two expression patterns can be seen; eGFP+ and mRFP+/eGFP+ (yellow cells). The yellow cells in both conditions show the immediate progeny of the originally transfected population. Since CAG-mRFP and ZG-s are episomal plasmids, their signal would have been lost by successive cell divisions. Therefore the yellow cells represent the progeny of radial glia which were terminally dividing at the time of transfection.

Cells which were only eGFP+ represent cells which had promoter driven PBase transposition of ZG-s. This is due to the fact that their loss of mRFP signal indicates that they continued to divide after transfection. Therefore, the only way to maintain their eGFP signal would have been through genomic insertion of ZG-s.

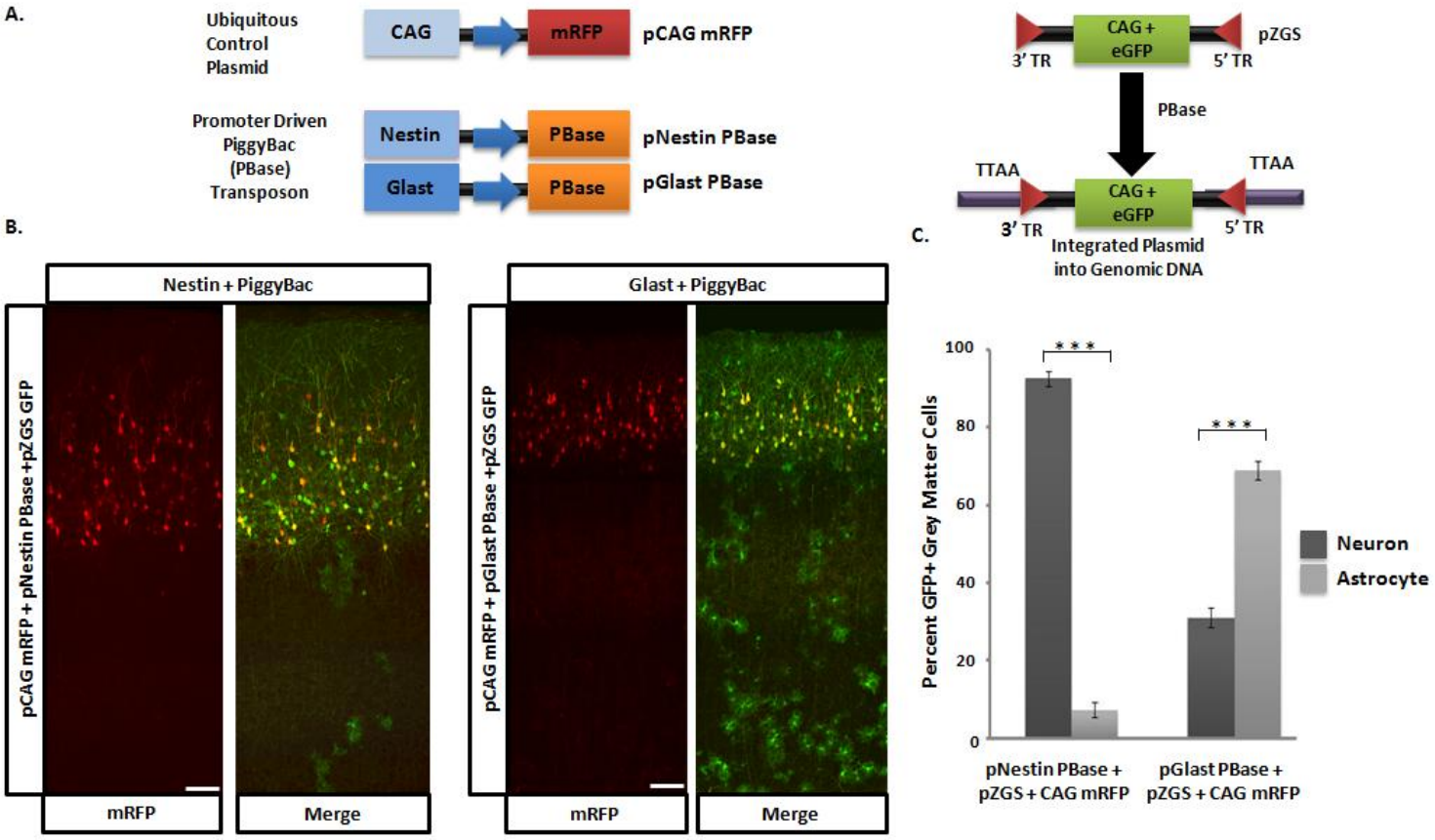

Figure 3. Nestin and GLAST driven PiggyBac Generates Different Lineages of Neurons and Astrocytes. A. PiggyBac Transposon labels promoter specific lineages **A.** Plasmid constructs used in lineage trace experiments. CAG-mRFP used to label the original cohort of transfected cells and indicates their direct progeny. Nestin and GLAST promoters separately drive the expression of PiggyBac (PBase). PBase inserts ZG-s, which contains CAG driving eGFP, into the genome of transfected cells at TTAA sequences. **B.** Confocal microscopy images of promoter specific lineages of radial glia at E15. Animals harvested at P15. Nestin driven PBase labels high amounts of neurons and few astrocytes. GLAST driven PBase labels mostly astrocytes, but also neurons. Scale Bar: 100µm **C.** Bar graph showing quantification of cell types associated with nestin and GLAST lineages. Nestin driven PBase labeled high amounts of neurons (92.7%) and few astrocytes (7.3%). GLAST driven PBase labeled mostly astrocytes (69.0%) and some neurons (31.0%) (n=3-5 brains per condition) Paired student t-test with equal variance was performed ($p<.0001$).

Two main cell types, pyramidal neurons and protoplasmic astrocytes were observed in both the nestin-PBase and GLAST-PBase conditions. The pyramidal neurons show large cell body with a long apical dendrite and many basal dendrites (Megias et. al 2001) while the protoplasmic astrocytes were identified by their short, thick, bushy processes (Levison and Goldman, 1993). The majority of pyramidal neurons in both conditions showed co-expression of eGFP and mRFP while the astrocytes were only GFP+ (Figure 3B). This shows that the neuronal generating radial glia stopped dividing shortly after transfection while the astrocytic generating radial glia where continually dividing.

The proportions of cell types generated differed greatly between both conditions. As observed in Figure 3B, the nestin-PBase condition generated primarily neurons with few astrocytes, while the GLAST-PBase condition generated both neurons and astrocytes with the majority of the progeny being astrocytic. Upon quantification, our observations were confirmed. 93%±2 of the GFP+ grey matter cells in the nestin PBase condition were neurons while in the GLAST PBase condition, 69%where astrocytes and 31% where neurons. These data show that the different subpopulations of radial glia characterized by their promoter expression can give rise to different mature cell types.

Discussion

Evidence for heterogeneity within Radial Glia

Previous studies have shown that the ventricular zone can be identified as a heterogeneous population of progenitors and that these differences may lead to the generation of different mature cell types. Morphological characterization has identified a non radial glia cell type within the ventricular zone which questions the radial glial role as the soul progenitor of the neocortex. These cells were characterized as lacking the long radial fiber distinct to radial glia and were also found to express a different promoter than radial glia. These cells were termed short neural precursors (SNPs) (Gal et. al 2006), although it was not determined if they may be dividing radial glia which lost their radial fiber. Regardless, this study shows that different promoter expression can identify different types of progenitors.

Molecular heterogeneity has been supported by the differential expression of certain cell surface markers. Throughout development, the reactivity of radial glia to these markers change, showing higher positivity in the post neurogenic population (Hartfuss et. al 2001). Subsets of radial glia showing heterogeneous positivity for the cell surface markers were also found to differ in transcription factor expression and mitotic activity. This group also showed that nestin and GLAST reactivity decreased throughout development; however, more radial glia expressed GLAST than nestin throughout. It has also been shown that spatial and temporal heterogeneity can contribute to the cell types generated. Anthony et. al, 2004 was able to demonstrate via lineage tracing that the cortex and ganglionic eminences generate different cell types at the same time. It was found that cells in the ganglionic eminence began producing astrocytes while the

cortical ventricular zone was still neurogenic. The cortical ventricular zone did eventually produce astrocytes, although it was at a later time period.

These previous data have demonstrated that the heterogeneity found within the ventricular zone can be attributed to many cellular characteristics. The ability to separate subsets of radial glia based on promoter expression may give clues towards the developmental fates of these subsets. Our study investigated the expression profiles of nestin and GLAST within midneurogenic ventricular radial glia within the same neocortical location. We were able to distinguish two subpopulations of radial glia based on promoter expression: GLAST+/nestin+ and GLAST+/nestin-. Most radial glia (87%±2) were found to be GLAST+/nestin+.

Embryonic Heterogeneity based on Nestin and GLAST

Previous work using transgenic mice containing nestin driven CreER has identified a radial glia progeny of granule neurons and Bergmann glia in the anterior cerebellum in addition to cortical neurons (Chen et. al 2008). GLAST driven recombination has also shown a neuronal progeny for radial glia early in development while switching to astrocytes and cerebellar glia latter (Anthony et. al 2008). In the rat spinal cord, nestin+ and GLAST+ radial glia populations were suggested to have different fate potentials. Nestin+ radial glia were found to be co-localized with the immature neuronal marker 2F7 while only GLAST+ radial glia were shown to give rise to astrocytes (Barry and McDermott, 2005). Because nestin and GLAST have been shown to be associated with different fate potentials, our lineage investigation was crucial in order to understand the significance of our embryonic radial glia subpopulations.

Subpopulations and Fate Potential

With the PiggyBac transposase system, we were able to investigate the lineage produced by the subpopulations of radial glia we previously identified. The nestin driven PiggyBac condition generated mainly neurons (93%) while the GLAST driven PiggyBac condition generated both neurons (31%) and astrocytes (69%). From our previous embryonic data we identified the majority of the nestin+ radial glia to also be GLAST+. Therefore it is expected that the GLAST driven PiggyBac condition produced neurons. In addition, since the nestin expressing radial glia generated few astrocytes in its lineage (7 %), we suggest that most of the astrocytes of the dorsal telencephalon are generated from the small subset of GLAST+/nestin-

radial glia. In this study we have identified progenitor pools within the same location of the ventricular zone. Previously, progenitor diversity was analyzed globally; our study has found that ventricular zone diversity can be found within a localized region of the ventricular zone.

Our data demonstrates that small subsets of radial glia, distinguished based on specific promoter expression are able to generate large populations of different cell types. This adds to the expanding list of heterogeneity within the ventricular zone and how it may lead to the generation of diversity within the brain. Our study analyzed the pyramidal neurons and astrocytes generated within the neocortex. Therefore, it may be interesting to investigate the neuroblasts within the olfactory bulb, which travel from the ventricular zone, in order to determine if they have contribution from either of our radial glia subpopulations. Also, it would be interesting to see if the GLAST subpopulations can further be sub-divided by analyzing the types of astrocytes and other glia, if any, that they generate. Further investigation into the diversity of ventricular zone radial glia and the heterogeneity of the ventricular zone in general will allow greater understanding of how cells from the same starting population can give rise to such a diverse structure as the brain.

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