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Developmental Origins of Astrocyte Heterogeneity: The final frontier of CNS development

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Abstract

Astrocytes are the most abundant cell type in the CNS, have diverse physiological roles in both health and disease, and exhibit phenotypic heterogeneity. In spite of the overwhelming evidence that astrocytes are a diverse population, there has been relatively little consideration of their molecular heterogeneity. In this review we will summarize what is known about the heterogeneity of astrocytes and outline challenges that have limited studies understanding their molecular diversity. Approaches that have sought to overcome these limitations will be discussed, with an emphasis on recent progress in the field of developmental gliogenesis, which has revealed that positional identity during embryogenesis is an organizing feature of astrocyte diversity. These recent findings, coupled with emerging technologies that allow for direct isolation of astrocyte populations, have led us to propose that approaches rooted in astrocyte development may be the key to unlocking this immense, untapped diversity.

Keywords

Heterogeneity; Astrocyte; Embryonic development; Patterning

Introduction

Astrocytes were first described by Virchow in 1846 and were originally thought to be a homogenous population of cells which function to support neurons [1]. Subsequently, Camillo Golgi and Ramon y Cajal observed diverse astrocyte morphologies in the human cerebellum, laying the foundation for the dissection and study of this potentially immense cellular diversity [2,3]. Since the time of Golgi and Cajal, numerous astrocyte morphologies across various regions of the CNS have been described [4,5], hinting that an extensive reservoir of molecular heterogeneity lies within these diverse astrocyte populations. Supporting this idea of molecularly diverse sub-populations of astrocytes are the observations that they are functionally diverse and directly contribute to a myriad of cellular processes essential for normal CNS physiology, including: synaptogenesis, neurotransmission, trophic regulation and blood brain barrier formation, to name a few [6,7]. Moreover, astrocyte-like cells in the adult SVZ or SGZ can function as multi-potent stem cells in vivo and are the major source of adult neurogenesis in the olfactory bulb and

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hippocampus [8,9]. This extensive functional diversity, when coupled with morphological heterogeneity strongly suggests the existence of molecularly distinct sub-types of astrocytes. However, whether these diverse functions can be performed by all astrocytes, or are distributed across a spectrum of distinct sub-populations remains an open question. Therefore, linking molecular and functional heterogeneity of astrocytes has either become the “elephant in room” or the “holy grail”, depending upon ones perspective (neuron-centric v. glial-centric, respectively).

The goal of this review is to summarize what we know about astrocytes and their heterogeneity, highlighting recent experimental approaches that have been used to dissect their molecular diversity *in vivo*. Indeed, several reviews have focused on the functional diversity of astrocytes [6,7], and on the general knowledge so far gained [4,10]. However, here we will discuss what is known about the molecular heterogeneity of astrocytes and the inherent limitations of characterizing their diversity. Furthermore, we will discuss what is known about the developmental mechanisms that control astrocyte heterogeneity and provide perspective on how these paradigms may guide the use of existing technologies to study these cells and understand their contributions to normal CNS physiology and disease.

Challenges in the study of astrocyte development

If astrocytes are the most abundant cell type of the CNS, why do we know so little about them? Studies on astrocyte development and function have been hindered over the years for two key reasons: 1) Differences across species in astrocyte/glial biology and 2) the lack of a clearly defined developmental endpoint. A common strategy to understand a developmental process is to study it in lower organisms and determine whether the regulatory paradigms are conserved in higher organisms. In the late 80's/early 90's several groups employed this strategy to the study of neurogenesis and found that bHLH transcription factors controlling Drosophila neurogenesis also control neurogenesis in mammals [11-13], paving the way for our current understanding of neurogenesis in the CNS. When this same approach was applied to the study of astrocytes/glial cells it was not met with the same success. In Drosophila the generation of glial cells is controlled by the transcription factor gcm (glial cells missing) [14,15], however manipulation of the mammalian homologue of gcm in mouse models did not affect the generation of glial cells (astrocytes or oligodendrocytes) [16-19]. This key finding indicates that the developmental origins of astrocytes/glial cells are fundamentally different across species, suggesting that astrocyte/glial contribution to the functioning CNS also differ across species. Indeed, both the number and complexity of astrocytes has increased over evolution [20,21], further complicating the use of lower organisms for the study of astrocyte development, diversity, and function. Nevertheless, in spite of these key differences, some aspects of astrocyte function are likely to be conserved in invertebrate models, warranting use of these models in the study of astrocyte/glia function.

Another important limitation is the lack of a clearly defined developmental endpoint for astrocytes, which is crucial for the identification of terminally differentiated cells and has aided the study of other cell types in the CNS. Terminally differentiated neurons are post-mitotic, have distinct morphologies (including axons and dendrites) and activities [22]. Likewise terminally differentiated oligodendrocytes are also post-mitotic, have a distinct morphology, and express a set of myelin genes that are essential for their singular role in myelination [23]. These properties can be used to measure progress through a developmental lineage and as criteria for functional studies both *in vitro* and *in vivo*. Conversely, astrocytes do not play by the same set of rules. GFAP-expressing astrocyte populations retain mitotic potential, as evidenced by their robust local proliferation in the post-natal cortex and BrdU-labeling studies in the brain and spinal cord [24,25]. These properties suggest that mature

astrocytes are a “moving target” and thus, unlike differentiated neurons and oligodendrocytes, may not be developmentally or functionally static, especially *in vitro*. Astrocytes demonstrate a variety of morphologies and when coupled with their functional diversity [6], results in a situation where it can be difficult to know what population you are studying and which properties to test for, hindering functional studies that might serve to decode morphological/molecular relationships. Collectively, these properties have conspired to make the study of astrocyte development and function enigmatic and reinforce the importance of assigning unique molecular signatures to functional sub-types of astrocytes.

Morphological heterogeneity of astrocytes

Since a major limitation in our understanding of astrocytes stems from their immense diversity, we will begin with the most recognizable form of heterogeneity, morphology. Since the time of Cajal, astrocytes have been categorized into 2 broad morphologies; protoplasmic with long unbranched processes and fibrous astrocytes with short and highly branched extensions [3,4]. Protoplasmic astrocytes are found in the gray matter and generally express S100β, while fibrous astrocytes are located within the white matter and express GFAP [26,27]. Functionally, protoplasmic astrocytes associate with both synapses and endothelial cells, thus directly participating in the “neurovascular unit” [10,28-30]. Less is known about the function of fibrous astrocytes, but their location in the white matter suggests they may participate in myelination [27]. Collectively, these observations suggest a link between astrocyte morphology and function, supporting the possibility that molecularly distinct astrocytes perform unique functions.

Another way of identifying morphologically distinct sub-populations of astrocytes is to compare their morphologies across brain regions. Indeed, the presence and/or proportion of protoplasmic and fibrous astrocytes varies across brain regions, indicating regional heterogeneity of these populations. Similarly, a recent study identified nine morphologically distinct GFAP-or S100β- expressing astrocytes [5], distributed in varying proportions across different brain regions, supporting the notion that different brain regions, which perform different motor and/or cognitive functions, are likely to harbor astrocytes with distinct molecular and functional properties. While gross morphology can be used as an entry point into the dissection of astrocyte heterogeneity, it does not always correlate with function. For example, Bergmann and Muller glia are specialized “radial” astroglia found in the cerebellum and retina, respectively, that are morphologically similar to radial glial, possessing long processes that extend to the pia surface [31,32]. Like radial glia, during development Bergmann glia provide a substrate upon which granule cell precursors migrate [33], however Muller glia have not been linked to precursor migration and are thought to have a role in regeneration [34]. While their role in development may differ, in the adult both Bergmann and Muller glia are thought to modulate synaptic activity and thus function similar to protoplasmic astrocytes [35,36]. That Bergmann and Muller glial are morphologically distinct from protoplasmic astrocytes, yet have similar functions, further supports the notion that astrocyte morphology can be dissociated from function. Together, these observations indicate that categorizing functional groups of astrocytes based on gross morphology does not provide sufficient enough resolution to make predictions about the function of a given morphological class across the CNS.

Approaches to dissecting astrocyte heterogeneity

The coupling of morphological and functional properties of regionally distinct astrocytes has provided a limited understanding of their heterogeneity and highlights the importance of uncovering the molecular underpinning of these morphologically and functionally diverse populations across different regions of the CNS. While cataloging molecular signatures of

morphologically distinct astrocyte subpopulations across a variety of brain regions is a worthwhile endeavor, the reality is that the field of astrocyte biology has been hindered for years by a paucity of reliable markers. While developmental and functional studies on neurons and oligodendrocytes have been facilitated by the existence of sub-type and stage specific markers, analogous markers for astrocyte lineages have not existed. Traditionally, a vast majority of studies on astrocytes have relied on the expression of GFAP, a marker of terminally differentiated astrocyte that poorly labels protoplasmic astrocytes (see figure 1) [4,27] and is expressed in adult Type B multipotent cells of the rodent SVZ [8,33], indicating that it cannot be used as a stage-specific marker. Moreover, GFAP becomes upregulated in reactive and cultured astrocytes reflecting a state quite different from that of normal, resting astrocyte populations [10,37,38]. Given the limited number of astrocyte markers and their importance in dissecting heterogeneity, a major directive in the field of astrocyte development is the identification of new markers, both general and sub-type specific. Below is an overview of recent approaches that have been implemented in the identification of new astrocyte markers.

Approach 1: Identify region specific differences

Given that different regions of the adult brain contain astrocytes with vastly different morphologies [3,5], it follows that these populations likely have unique molecular signatures. In one such study, Bachoo, et al, compared the gene expression profiles of astrocytes from various developmental stages and regions, isolated using both *in vitro* culture and complementary *in vivo* samples [39]. Here the goal was to identify common gene signatures across samples that delineated a unified astrocyte profile. Instead, they found that each brain region contained unique molecular profiles and that significant differences existed between cultured astrocytes and primary astrocytes. In spite of these limitations, several new markers of astrocytes were validated *in vivo*, including Id3 and AldoC (Figure 1). More recently, Yeh, et al. cultured astrocytes isolated from cortex, cerebellum, optic nerve and brainstem and compared their gene expression profiles [40]. These studies found that each population contained both common and unique cohorts of genes, with astrocytes from the optic nerve having the most divergent gene expression profile. While the data generated from this study contains a wealth of information, limited *in vivo* confirmation of candidates was performed, as the broader goal of this study was to identify region specific expression of tumor suppressor genes. That different brain regions contain molecularly distinct astrocytes *in vivo* was confirmed when Doyle, et. al examined the heterogeneity of directly isolated astrocytes from different brain regions using translating ribosome affinity purification (TRAP) coupled with BAC transgenics [41]. Their data indicated that astrocytes isolated from the cortex and the cerebellum have different gene clustering data sets. While the use of state-of-the-art mouse tools to directly examine molecular heterogeneity of region-specific astrocytes represents a significant advance in the field, these studies did not, however, validate any candidate genes that could be used to mark astrocytes from different regions.

Approach 2: Comparison of astrocytes with other neural cell types

Another approach to identify novel markers of astrocytes is to compare their expression profiles with mature neurons and oligodendrocytes. Up to this point a major limitation in the use of *in vitro* systems to study astrocytes is that they are mostly derived from a small, proliferating glial precursor population that demonstrates immature phenotypes, but expresses several mature markers [42]. Thus it is likely that the derivates of these cells propagated in culture do not fully recapitulate the *in vivo* astrocyte constituency. In a landmark paper by the Barres group [43], a novel approach to the purification and culture of glial precursors was developed that allowed for characterization and unambiguous comparison of the gene expression profiles of astrocytes, neurons, and oligodendrocytes.

Using an S100β-EGFP transgenic mouse line, classic immunopanning techniques were coupled with FACS analysis and applied to acute cultures derived from forebrain. This resulted in highly purified astrocyte and oligodendrocyte cultures that could be used as a substrate for gene expression profiling studies and resulted in the generation of the astrocyte transcriptome database. In addition to providing an extensive cataloging of the molecular profile of astrocytes, several markers were validated *in vivo*, including Aldh1l1, which is rapidly becoming the "new" standard marker of astrocytes, marking both fibrous and protoplasmic astrocytes (See below and figure 1 and ref. [44]). While delineating the transcriptome of astrocytes represents a major advance in our understanding of their biology these studies do not provide any additional insight into their heterogeneity.

Approach 3: Functional properties as markers of heterogeneity

Given the diverse roles of astrocytes, another source of heterogeneity are the molecules associated with these functions [6]. In particular several studies have found that astrocytes from different regions demonstrate heterogeneity in their expression of ion channels and coupling molecules. For example, the K+ channel, Kir4.1, is enriched in astrocytes of the ventral horn of spinal cord compared to those of the dorsal horn and corresponds with the rates of K+ uptake by astrocytes in local dorsal and ventral populations [45]. Glutamate receptors provide an example of regional heterogeneity, where functional NMDA receptors have been reported in cortical and spinal cord astrocytes and Bergmann glia of the cerebellum [46-48], but not in the hippocampus [49]. Along these same lines, it is interesting to note that hippocampal astrocytes were found to be sub-divided into at least three electrophysiologically distinct types [50], further suggesting ion channel heterogeneity amongst astrocytes. Coupling via GAP junctions is another feature of astrocytes that demonstrates regional heterogeneity, where the expression of connexin 43 and 30 is more pronounced in the barrel field compared to the septal region, or other regions of the cortex [51]. In each of these examples, especially for the channel proteins, expression of the given molecule is not restricted to astrocytes, as they are also expressed on neurons and therefore unlikely to represent astrocyte specific markers.

An alternative approach that also employs a functional perspective on molecular heterogeneity is to use molecules that are specifically associated with astrocyte function. One astrocyte specific function is to remove extracellular glutamate from synapses, which is crucial for preventing excitotoxicity and modulating neurotransmission [52-54]. This function is performed by the glutamate transporters, GLT-1 and GLAST, which developmental studies indicated are specifically expressed on astrocytes and astrocyte precursors [55-57]. In a study by the Rothstein group, GLT-1 and GLAST transgenic BAC reporters were generated and found to have region specific activity, where in the adult GLT-1 demonstrated expression in the cortex, grey matter areas of the hippocampus, and spinal cord, while GLAST was expressed in the cerebellum, dentate gyrus of the hippocampus, and spinal cord [58]. Interestingly, intercrossing these mouse lines revealed heterogeneity within the regions that expressed both reporters, identifying distinct combinations of single-GLAST/DsRED+, single-GLT-1/EGFP+, or double-GLAST/DsREd +;GLT1/EGFP+ sub-populations within the spinal cord, cortex, hippocampus, and cerebellum. More recently, similar approaches were implemented using GLT-1 and Aldh1l1 BAC reporter mice and combinations of single- and double- reporter positive populations were identified in the spinal cord and cortex, suggesting that GLT-1 and Aldh1l1 expression also marks sub-populations of astrocytes [59]. These studies clearly indicate the presence of both regional and local heterogeneity in astrocyte populations in the CNS and suggest the use of combinations of astrocyte specific molecules as tools for identifying sub-populations of astrocytes.

Developmental markers of astrocytes

The use of GLAST and GLT-1 to identify local subpopulations of astrocytes, is based on the observation that they are expressed in developing astrocyte precursors during embryogenesis and early post-natal stages. This highlights the importance of using developmental studies as a guide in the identification of general and sub-type specific markers of astrocytes. While our understanding of the molecular controls of early astrocytogenesis remains rudimentary, several developmental studies have identified new markers of astrocyte precursors, albeit with some limitations. For example, the first indication of glial specification is marked by the induction of NFIA/B [60] and GLAST [57] at E11.5. These markers continue to be expressed in astrocytes during precursor migration (E13.5-E16.5) and thus do not become truly astrocyte-specific until several days post-specification in the ventricular zone (VZ). One caveat is that both NFIA/B and GLAST are also expressed in oligodendrocyte precursors. Other reported markers of astrocytes and/or their precursors include FGFR3 [61], FABP7 [62]/BLBP [63], and Sox9 [64]. However, many of these markers are also expressed during neurogenic stages, thus do not exclusively mark VZ cells committed to the astrocyte lineage and therefore do not specifically mark astrocyte precursors. Nevertheless, in the future, intersectional approaches like those taken with GLAST and GLT-1 [58], with these (or other) markers may reveal new layers of astrocyte and astrocyte precursor heterogeneity.

Patterning and Astrocytes: A way to diversify

A conserved developmental strategy used to generate cellular diversity within a given lineage is the patterning of embryonic tissue. In the developing CNS patterning has been shown to control the generation of various sub-populations of projection neurons and interneurons in the spinal cord and forebrain [65]. Neural tube patterning is the process by which extrinsic morphogenic signals (BMP, Wnt, Shh) are integrated into neuroepithelial populations lining the VZ and converted to cell intrinsic transcription factor expression. In the spinal cord, this results in a set of domains along of the dorsal ventral axis, each expressing a unique combination of these HD transcription factors, which ultimately gives rise to distinct sub-populations of interneurons or motor neurons. After the initial wave of neurogenesis (~E9-E11), the VZ populations begin generating glia around E12.5, a developmental transition regulated by Sox9 and NFIA [60,64,66]. While NFIA and Sox9 are key regulators of gliogenesis and eventual markers of astrocytes, they do not mark distinct sub-populations. This raises the question of whether the patterning mechanisms that control the generation of neuronal diversity are re-utilized during gliogenesis and serve to organize local astrocyte heterogeneity. Previous studies have linked morphogens associated with patterning to astrocyte heterogeneity without making a direct connection to the “transcriptional codes” that control pattern formation. For example, *in vitro* studies revealed that astrocytes cultured in the presence of BMPs have distinct morphologies from those cultured in LIF [67]. While the presence of these sub-populations has not been rigorously validated *in vivo*, deletion of BMPR's in astrocytes influences astrocyte morphology and response during spinal cord injury [68]. More recent studies indicate that local subsets of astrocytes in the forebrain receive Shh signaling, as marked by Gli1 reporter mice [69]. Moreover, deletion of the Shh co-receptor smoothened (Smo) results in reactive gliosis specifically in the Gli1-expressing astrocytes, implicating Shh-signaling in the regulation of a distinct astrocyte sub-population *in vivo* [69]. These studies implicate BMP and Shh in the diversification of astrocytes and suggest that they may do so by using patterning independent mechanisms.

Embryonic origins of astrocyte heterogeneity

The first evidence that glial cell diversity is the result of patterning came from the observation that a vast majority (>85%) of spinal cord oligodendrocytes are derived from the pMN domain, a ventral domain that specifically expresses the bHLH transcription factor Olig2, while the remaining domains (p1-p3) generate astrocytes [70,71]. The concept that patterning is directly linked to the generation of astrocytes arose indirectly from analysis of the Olig2-null mice, where in the absence of Olig2, the pMN domain is converted to a supernumerary copy of the adjacent domain (p2), and the cells that were originally fated to become oligodendrocytes are converted to astrocytes [72,73]. Subsequently it was found that cross-repressive interactions between Olig2 and Scl operate at the interface of the pMN and p2 domains to control astrocyte versus oligodendrocyte fate decisions [74]. Together, these observations indicate that astrocytes are generated from a restricted region of the neural tube, and further support the notion of separate domains for oligodendrocyte and astrocyte production along the dorsal/ventral axis of the embryonic spinal cord.

While these initial studies provided the first evidence that progenitor patterning is an organizing principle controlling the generation of glial diversity (ie. oligodendrocyte versus astrocyte), they did not decode the link between patterning, positional identity, and molecularly distinct sub-populations of astrocytes. Subsequent studies in the Anderson lab made the direct link between patterning and astrocyte diversity in the spinal cord by analyzing the gene expression profiles of astrocytes generated in the absence of Olig2 [75]. These studies identified the patterning transcription factors Pax6 and Nkx6.1, as well as Reelin and Slit as highly expressed in these converted astrocyte populations. Analysis of their temporal and spatial expression patterns during gliogenesis demonstrated that the combinatorial expression of Pax6 and Nkx6.1 marks three subtypes of ventral white matter astrocytes (termed VA1, VA2, and VA3), which can be identified, based on their patterns of Slit1 and Reelin expression. These astrocyte subtypes exhibit positional identity and are organized into domains of the ventral white matter along the dorsal-ventral axis, which mirror the dorsal-ventral arrangement of their progenitors in the p1, p2 and p3 VZ domains at earlier stages (Figure 2). VA1 astrocytes (Pax6+/Reelin+) are the most dorsal and located in the lateral spinal cord white matter, while VA3 astrocytes (Nkx6.1+, Slit1+) are the most ventral and VA2 astrocytes (Pax6+, Nkx6.1+, Reelin+, Slit1+) are located in an in-between ventral-lateral white matter domain. Functional studies revealed that Pax6 is essential for VA1 and VA2 astrocyte identity by promoting Reelin and repressing Slit1 expression, indicating that cross-repressive interactions controlling VZ patterning also control astrocyte sub-type identity. Specification of VA3 astrocytes appears to be dependent on multiple factors including Nkx6.1 and Nkx2.2, suggesting the involvement of other patterning genes in the specification of astrocyte sub-types. In sum, these studies indicate that patterning and positional identity control the diversification of glial sub-types and, importantly, white matter astrocytes in the spinal cord.

Recently, the concept that astrocytes are patterned in the spinal cord was elegantly confirmed using conditional Cre-lines that are specifically active in VZ domains along the dorsal/ventral axis of the spinal cord to trace the fate of astrocyte progenitors derived from these domains [44]. Importantly, these approaches were extended to the developing forebrain and met with similar results, suggesting that patterning may be a generalized principle of astrocyte diversity throughout the CNS. Moreover, this study found that both white matter (fibrous) and grey matter (protoplasmic) astrocytes are derived from these domains, indicating common VZ origins of these populations. Interestingly, the lineage tracing approach allowed for careful analysis of astrocyte precursor migratory patterns and revealed a stereotypical radial migration pattern that is congruent with their VZ sites of origin and stable throughout the life of the animal and after injury. Taken together, these

patterning studies indicate astrocyte diversity in the spinal cord and forebrain is pre-specified into positionally distinct subtypes by a homeodomain transcription factor code and these spatial relationships are maintained during migration, differentiation, and throughout adulthood.

Conclusions/Perspective

That astrocytes comprise approximately 50% of the cellular constituency of the CNS [76], coupled with the regional complexities of the adult brain, make dissecting their heterogeneity across the adult brain a daunting task. Several groups have investigated astrocyte heterogeneity using “lateral” approaches that involve comparison of gene signatures of astrocytes derived from different brain regions [39,41,43]. Given recent insights from developmental studies on astrocytes in the spinal cord and forebrain we propose a targeted, “top-down” approach that interrogates local heterogeneity within a given brain region (Figure 3). This approach will ideally make use of newly developed intersectional BAC transgenic and TRAP technologies [41,58], and established lineage tracing methods to directly isolate bulk astrocyte populations and delineate their gene expression profiles, ultimately uncovering their molecular heterogeneity. These studies will be complemented by *in vitro* studies that employ recent advances in glial precursor and astrocyte culturing techniques that can be used for functional studies [77]. The key to successful implementation of this model is a thorough understanding of astrocyte development throughout the CNS, as many of the tools required to implement these technologies requires prior knowledge of astrocytes or astrocyte precursor development. There is increasing evidence that astrocytes, like neurons, are patterned during early embryogenesis [44,75], therefore existing mouse tools that make use of morphogens and transcription factors associated with patterning could serve as a starting point for these studies.

The use of paradigms associated with patterning or glial development to dissect astrocyte heterogeneity is convenient, however it raises the question of whether astrocytes from other CNS regions are similarly patterned. This is a key question, as different regions of the CNS use different molecular and cellular developmental programs, thus what works in the spinal cord or forebrain may not be applicable to the hippocampus. Because the developing CNS is not necessarily a “unified field”, a comprehensive understanding of the molecules controlling patterning and early gliogenesis of a brain region to be studied is essential. Moreover, recent studies indicate that astrocytes from the spinal cord are relatively static throughout adulthood and seemingly tethered to their sites of origin in the VZ [44]. Whether this is true in other regions of the CNS will have a profound effect on how these patterning approaches are implemented. The ultimate goal of understanding astrocyte heterogeneity is to determine if sub-populations of astrocytes exist and whether they perform specific functions. Given recent insights into their developmental and functional diversity, this seems likely, but nevertheless remains an open question and one of the final frontiers of developmental neuroscience.

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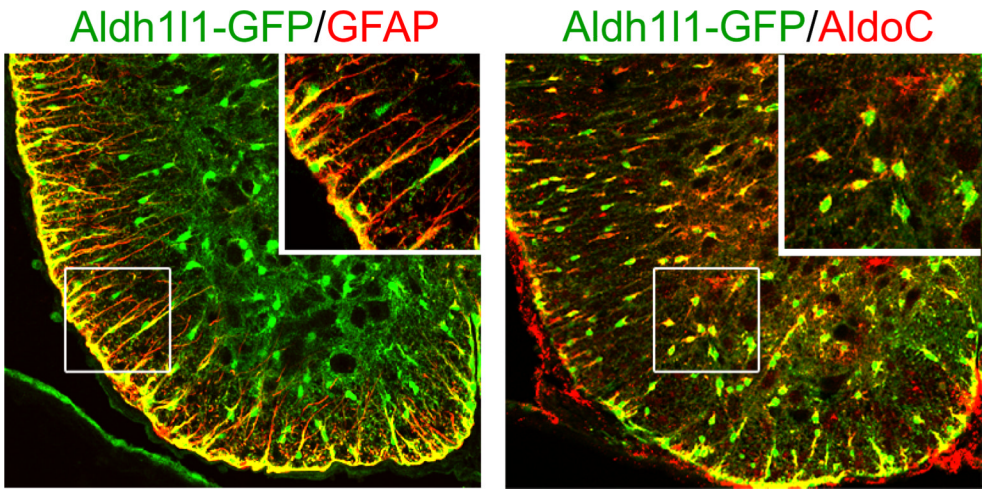

Figure 1. Expression of Aldh1l1 in spinal cord astrocytes

Spinal cord from P2 mice from the Aldh1l1-GFP mouse line stained with GFAP and AldoC. Alhd1l1 is expressed in both fibrous (white matter) and protoplasmic (grey matter) astrocytes. Note that GFAP-expressing astrocytes are generally restricted to the white matter and have fibrous morphologies. Reproduced from [44] with permission.

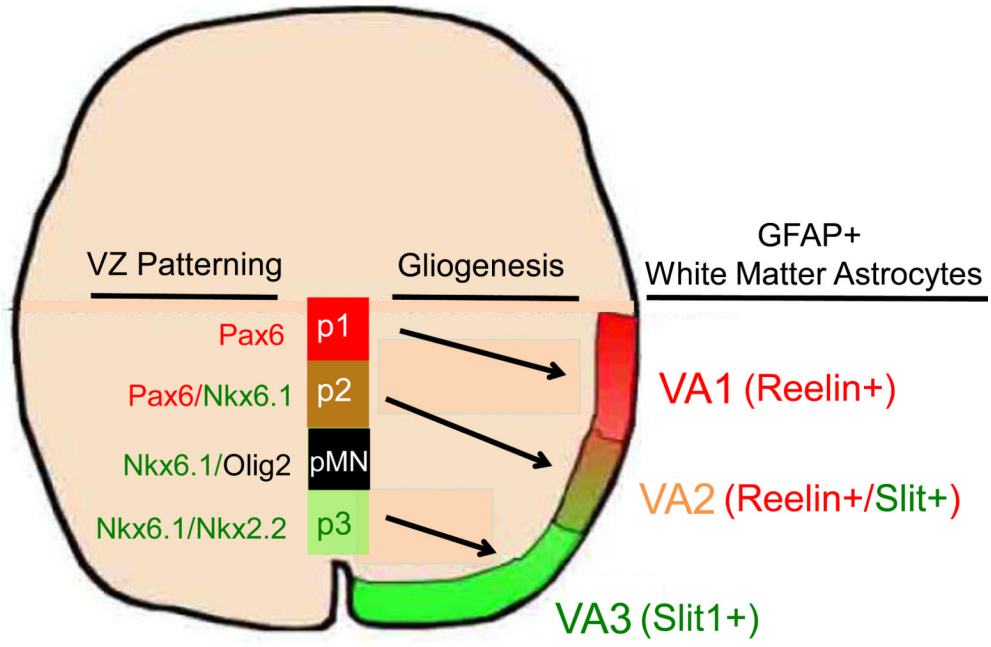

Figure 2. Schematic illustrating the VZ origins and the white matter destinations of VA1-VA3 astrocyte populations in the spinal cord

Note that the positions of VA1-VA3 in the white matter mirror their positions along the dorsal/ventral axis of the VZ during specification. Reelin and Slit are also expressed in the VZ populations during specification and Pax6 and Nkx6.1 expression is maintained in white matter astrocyte populations after migration and differentiation.

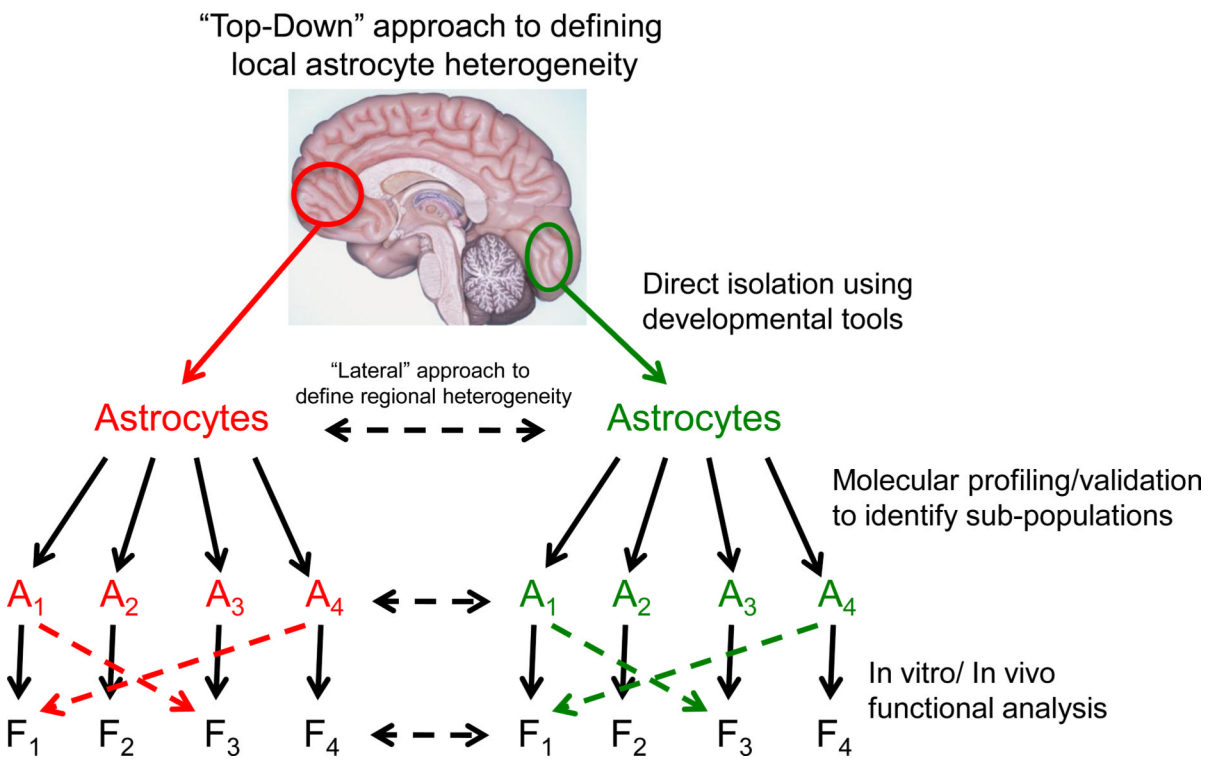

Figure 3. Top-Down approach to defining local astrocyte heterogeneity

Colored circles represent different brain regions. "A" represents a given astrocyte sub-population and "F" represents a given function of astrocytes. The colored dashed lines between "A" and "F" represent the possibility that a given astrocyte sub-population may perform more than one function. The black dashed lines between examples represent comparison between different regions at different levels of the "top-down" paradigm, and represent the "lateral approach" to interrogating astrocyte heterogeneity.