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# Ascl1 (Mash1) lineage cells contribute to discrete cell populations in CNS architecture

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ABSTRACT

Ascl1 (previously Mash1) is a bHLH transcription factor essential for neuronal differentiation and specification in the nervous system. Although it has been studied for its role in several neural lineages, the full complement of lineages arising from Ascl1 progenitor cells remains unknown. Using an inducible Cre-flox genetic fate-mapping strategy, Ascl1 lineages were determined throughout the brain. Ascl1 is present in proliferating progenitor cells but these cells are actively differentiating as evidenced by rapid migration out of germinal zones. Ascl1 lineage cells contribute to distinct cell types in each major brain division: the forebrain including the cerebral cortex, olfactory bulb, hippocampus, striatum, hypothalamus, and thalamic nuclei, the midbrain including superior and inferior colliculi, and the hindbrain including Purkinje and deep cerebellar nuclei cells and cells in the trigeminal sensory system. Ascl1 progenitor cells at early stages in each CNS region preferentially become neurons, and at late stages they become oligodendrocytes. In conclusion, Ascl1-expressing progenitor cells in the brain give rise to multiple, but not all, neuronal subtypes and oligodendrocytes depending on the temporal and spatial context, consistent with a broad role in neural differentiation with some subtype specification.



## Introduction

In the mammalian central nervous system (CNS), distinct types of neurons are assembled into elaborately interconnected circuits that process complex neural functions. To obtain this refined CNS architecture, diverse populations of neurons must be generated that migrate to specific positions in precise temporal order during embryogenesis. The molecular mechanisms regulating each developmental step in this process are not completely understood. Basic helix–loop–helix (bHLH) transcription factors play a central role in generating neuronal diversity by regulating subtype specification as well as differentiation (Bertrand et al., 2002). Ascl1 (previously Mash1) is a neural bHLH transcription factor restricted to proliferative zones in the developing brain and spinal cord in a spatially specific manner. Multiple studies of mouse embryos lacking Ascl1 suggest that Ascl1 is a neuronal differentiation factor required in diverse but specific neuronal subtypes in the developing central and peripheral nervous systems (Fode et al., 2000; Helms et al., 2005; Horton et al., 1999; Nakada et al., 2004; Pattyn et al., 2004; Perez et al., 1999). Neuronal lineages disrupted in the Ascl1 mutant include interneurons in dorsal spinal cord and telencephalon, both glutamatergic and GABAergic neurons in the mesencephalon, olfactory sensory epithelium, and neurons in the autonomic nervous system in the periphery.

Within a specific neural region, Ascl1 can also influence neuronal subtype specification (Fode et al., 2000; Helms et al., 2005; Nakada et al., 2004; Parras et al., 2002). In dorsal spinal cord, mouse embryos null for Ascl1 lose dI3 and dI5 neurons, whereas overexpression of Ascl1 in the chick neural tube leads to an increase in these specific neuronal populations (Nakada et al., 2004). In neural crest derivatives, Ascl1 is required for autonomic neurons but not sensory neurons (Perez et al., 1999). In the ventral spinal cord, Ascl1 functions in balance with Notch signaling at the choice point where the V2 interneuron population is split into two subpopulations, V2a and V2b (Del Barrio et al., 2007; Peng et al., 2007). Furthermore, Ascl1 is not restricted to neuronal lineages but is also present in progenitors to oligodendrocytes, but not astrocytes, in the spinal cord and adult brain (Battiste et al., 2007; Kim et al., 2007).

A complete fate map of Ascl1 derived lineages, particularly in the brain, has not been described largely due to the transient nature of Ascl1 expression that disappears as the cells exit the cell cycle and migrate extensively during development. This hurdle has been overcome by utilizing Cre recombinase in an in vivo genetic fate-mapping strategy to define mature CNS regions receiving contribution from progenitor cells that have transiently expressed Ascl1. This strategy was used to demonstrate that in spinal cord development, Ascl1 progenitors from embryonic day 10.5 (E10.5)–E12.5 give rise to dorsal horn interneurons whereas after E15.5 Ascl1 progenitors give rise to oligodendrocytes (Battiste et al., 2007). In the adult brain, Ascl1 was also found to be present in neuronal progenitors in the dentate gyrus of the hippocampus and the rostral migratory stream in the forebrain, and in oligodendrocyte precursors in white and gray matter (Kim

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et al., 2007). Consistent with this lineage analysis, forced expression of Ascl1 in neural progenitor cultures biased differentiation of the cells to neurons and oligodendrocytes at the expense of astrocytes (Gokhan et al., 2005; Sugimori et al., 2007).

Here we use the in vivo genetic fate-mapping strategy to identify brain regions containing neurons and oligodendrocytes originating from Ascl1 expressing progenitor cells from different embryonic stages. We find that Ascl1-expressing cells are generated continuously throughout embryogenesis from as early as E9.5. As in the spinal cord, the Ascl1 lineage includes both neurons and oligodendrocytes, but not astrocytes. We demonstrate that Ascl1 is present in neural progenitor cells in each major brain division. In the cerebellum, depending on the embryonic stage, Ascl1 defined lineages give rise to GABAergic neurons in deep cerebellar nuclei and Purkinje cells. Ascl1 lineages contribute to the trigeminal sensory system from the midbrain to the caudal medulla. Multiple telencephalic regions contain Ascl1 lineage cells including subsets of neurons in the striatum, olfactory bulb, amygdala and piriform cortex. Neurons in the neocortex arise from Ascl1 progenitors present at late embryonic stages. This study correlates the temporal and spatial origin of Ascl1 expressing progenitor cells and their final phenotypes throughout the brain. The diverse identity of the neurons is consistent with Ascl1 requiring additional molecular components for its specification function.

## Results and discussion

### *Ascl1 lineage cells identify discrete populations of neurons as well as oligodendrocytes throughout the brain*

In order to trace the fate of the transiently expressing Ascl1 progenitor cells into the mature brain, we used two BAC (bacterial artificial chromosome) transgenic mouse strains that have been previously described (Battiste et al., 2007; Helms et al., 2005) (Fig. 1). One strain, *Ascl1-GIC*, expresses constitutively active Cre recombinase in an Ascl1 pattern (Helms et al., 2005). We crossed *Ascl1-GIC* with *R26R-stop-lacZ* or *R26R-stop-YFP* Cre reporter mice to permanently label Ascl1 lineages, since Cre recombinase will excise the stop sequence upstream of LacZ or YFP (Soriano, 1999; Srinivas et al., 2001). Thus, at any given stage, β-gal or YFP positive cells are a cumulative representation of Ascl1 lineages up to that stage. In contrast, the second transgenic strain, *Ascl1-CreER™*, expresses an inducible Cre recombinase in the Ascl1 pattern, providing temporal control in labeling the Ascl1 lineages. Cre recombination is detectable within 6 h following tamoxifen treatment, and it persists for approximately 24 h (Hayashi and McMahon, 2002), thus, only the progenitor cells expressing Ascl1 in a restricted time window will be labeled. Together, these mouse strains, combined with neuroanatomical analyses and immunofluorescence with cell-type specific markers, have allowed us to characterize the fate of Ascl1-expressing cells as they progress through development and settle in the mature brain (Fig. 1).

An *Ascl1-GIC;R26R-stop-lacZ* mouse brain was harvested at postnatal day 30 (P30). An X-gal stained parasagittal section illustrates that Ascl1 lineage cells make extensive but specific contributions to each major subregion in the brain (Fig. 2A). Higher magnification images reveal this specificity (Figs. 2B–H). X-gal stained cells were detected primarily in the granule cell layer in the olfactory bulb (Fig. 2B), in cells dispersed throughout the cortex (Fig. 2C), in the striatum, septum and corpus callosum (Fig. 2D), and in presumptive noradrenergic cells in the locus coerulus (Fig. 2G). These results are consistent with previous studies on Ascl1 function in these lineages (Casarosa et al., 1999; Hirsch et al., 1998; Horton et al., 1999; Kim et al., 2007; Long et al., 2007; Marin et al., 2000). Furthermore, there were X-gal stained cells enriched in other brain regions including the preoptic area of the hypothalamus (Fig. 2E), the superior colliculus in the midbrain (Fig. 2F), and Purkinje cells in the cerebellum (Fig. 2H). Many X-gal stained cells were also found in white matter tracts, regions containing a high percentage of oligodendrocytes (Figs. 2D,H). The Ascl1 lineage cells were identified as neurons and oligodendrocytes since they co-label with the neuronal marker NeuN (Fig. 2I) and the oligodendrocyte marker Olig2 but not the astrocyte marker GFAP (Fig. 2J). This latter finding is consistent with the previous studies defining Ascl1 lineages in neurons and oligodendrocytes of the spinal cord and adult brain (Battiste et al., 2007; Kim et al., 2007; Parras et al., 2007).

Neurogenesis and gliogenesis are temporally dynamic. To reveal the fate of Ascl1 expressing cells from different stages of embryonic development, we utilized the tamoxifen-inducible Cre line, *Ascl1-CreER™*. *Ascl1-CreER™;R26R-stop-lacZ* embryos received tamoxifen by administering the drug to pregnant mice twice with a 6 h interval on a given embryonic day. The CreER™ recombinase is transiently

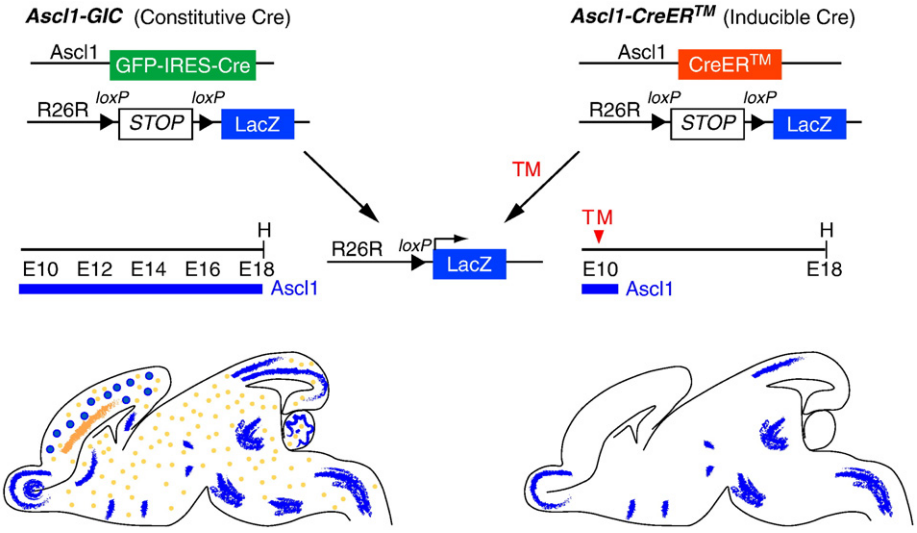


**Fig. 1.** Diagram of Ascl1 transgenic mice and the fate-mapping strategy. Two transgenic mouse models were generated with a BAC containing Ascl1 >200 kb flanking non-coding sequence. *Ascl1-GIC* replaces the Ascl1 coding sequence with GFP-IRES-Cre (Helms et al., 2005). This strain will reveal an accumulation of Ascl1 lineage cells when crossed with a Cre reporter mouse strain such as *R26R-stop-lacZ* (Soriano, 1999). *Ascl1-CreER™* replaces the Ascl1 coding sequence with an inducible Cre (Battiste et al., 2007). Only Ascl1 lineage cells originating at the time of tamoxifen (TM) treatment will be detected when crossed with *R26R-stop-lacZ*. Sagittal views of mouse brains from each paradigm are diagrammed to highlight that the inducible Cre will reveal only a subset of the Ascl1 lineage. Blue represents neurons and orange represents oligodendrocytes E, embryonic stage; H, harvest age.