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# Characterization of Foxp Expression in the Embryonic and Neonatal Mouse Brain

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## Introduction

The developing mammalian neural tube is composed of three distinct regions along the medial-lateral axis. The medial-most ventricular zone (VZ) is composed of neural stem cells (NSCs), which are able to self-renew and differentiate into a variety of mature neural cell types: neurons, astrocytes and oligodendrocytes. As NSCs differentiate, they migrate laterally into the subventricular zone (SVZ). Differentiated neural cell types are located in the lateral-most mantle zone (MZ). The process by which NSCs differentiate in embryonic tissue is very precisely controlled over a protracted period of time. However, this process is not well understood.

The neural tube is divided along the anterior-posterior axis, the posterior-most region becoming spinal cord. The anterior neural tube is subdivided along the anterior-posterior axis into three brain regions: the forebrain, midbrain, and hindbrain. Within each region there is further subdivision into specific brain structures in which a wide variety of neurons are generated. Despite this complexity, studies have shown that many of the signaling pathways and transcription factor families responsible for the control of progenitor maintenance/differentiation in the spinal cord are also employed in the developing brain.

Past research carried out in the Novitch lab suggests that members of the Forkhead domain family of transcription factors (Foxp) are important in the control of NSC maintenance/differentiation within the developing spinal cord. FOXP2, FOXP4, and FOXP1 are progressively expressed in the motor neuron domain of the ventral spinal cord. FOXP2 is expressed in neural stem cells in the ventricular zone, FOXP4 is expressed in late-stage progenitors and immature motor neurons, and FOXP1 is expressed in mature motor neurons.

The aim of the current study is to identify regions that express FOXP2, FOXP4, and FOXP1 in the mouse brain at embryonic and post-natal stages. Given the expression of FOXP2, FOXP4, and FOXP1 in the spinal cord, it is hypothesized that these genes will show a similar pattern of expression in the embryonic brain and play a role in progenitor maintenance/differentiation.

## Materials and Methods

Embryonic day (E) 11.5 brains were fixed for approximately one hour in 4% paraformaldehyde and the postnatal (P) 0.5 brains were fixed overnight, and subsequently transferred into 30% sucrose. The brains were mounted and frozen in O.C.T. compound and sectioned to 16-25 microns. All embryonic brains were sectioned coronally. The postnatal brains were sectioned both coronally and sagittally. The tissue was stained using a variety of primary antibodies (Table 1).

Subsequently, the appropriate FITC-, Cy3-, and Cy5- conjugated secondary antibodies were used to fluorescently label primary antibodies. Expression of the target proteins was visualized using a confocal microscope.

**Table 1. Primary antibodies**

| Name | Host | Dilution | Cell Types Marked |
|---|---|---|---|
| Foxp2 | Rabbit | 1:8000 | |
| Foxp4 | Goat | 1:250 | |
| Foxp1 | Guinea Pig | 1:1600 | |
| Sox2 | Goat | 1:100 | Neural stem/progenitor cells |
| Olig2 | Guinea Pig | 1:20000 | Oligodenrocyte precursors, motor neuron progenitors (in the spinal cord) |
| Ki67 | Rabbit | 1:1000 | Proliferating stem/progenitor cells |
| TuJ1 | Rabbit | 1:100 | Immature neurons |
| NeuN | Mouse | 1:20000 | Neurons |

## Results and Discussion

In the E11.5 forebrain, the expression of FOXP2 and FOXP4 is restricted to specific regions. FOXP1 expression, however, is very low in the forebrain, suggesting that the gene has not yet been turned on in this area at this stage of development. Further, FOXP2 and FOXP4 are both expressed in the cells within E 11.5 prethalamus that are SOX2 positive, suggesting that these cells are NSCs. In the cortex, FOXP4 positive cells are also SOX2 positive, indicating that they are NSCs. The FOXP2 positive cortical regions are TUJ1 positive, suggesting that these cells are immature neurons.

In the embryonic lateral ganglionic eminence (LGE) and medial ganglionic eminence (MGE), the cells that express FOXP2 are also TUJ1 positive, but do not express SOX2 or OLIG2. This suggests that these cells are immature differentiated neurons. Because it has been shown that cytoskeletal changes, cell specification, migration, cell cycle exit, and cell adhesion properties are all functionally required in young differentiating neurons, it is possible that the Foxp2 serves one or more of these functions. The cells in the VZ of the LGE and MGE, which express low levels of FOXP4, are SOX2 positive and/or OLIG2 positive, suggesting that these cells are NSCs. It has been shown that cell maintenance, differentiation, proliferation, adhesion, and migration occur within progenitor cells. Therefore, Foxp4 may have an impact on some of these processes.

Based on the expression profiles seen in the E 11.5 mouse brain, Foxp2 and Foxp4 may be employed in different, yet region specific ways in the embryonic brain. However, these results show that the progressive expression of FOXP2, FOXP4, and FOXP1 seen in the spinal cord is not mirrored in the embryonic mouse brain.

In the P0.5 brain, FOXP2, FOXP4, and FOXP1 are all expressed in several functionally important regions. Because the cerebellum is a brain region that continues to develop postnatally, the cells in this structure are still differentiating at P 0.5. In the postnatal cerebellum, FOXP2 and FOXP4 are expressed in the external granular layer, a known location of granule cell precursors that are beginning to exit the cell cycle and differentiate. FOXP1 expression is highly concentrated within the area known as the destination for differentiated granule cell neurons after they migrate through the Purkinje cell layer.

The dentate gyrus of the hippocampus is a known region of NSCs in the post natal and adult brain. FOXP2 and FOXP4 are not expressed in this region, suggesting that they do not play a role in postnatal NSC development in the hippocampus. However, FOXP1 is strongly expressed in CA1, CA2, and CA3, regions of the hippocampus known to be locations of differentiated neurons.

Another brain region where NSCs have been observed postnatally and into adulthood is the SVZ. FOXP2 is expressed in this proliferative area, which also shows expression of KI67. This suggests that the cells in this region are proliferating NSCs. Importantly, cortical and thalamic expression of FOXP4 and FOXP2 can be seen in both the embryonic and postnatal brain, suggesting that the expression in these regions is maintained throughout development.

In conclusion, while FOXP2, FOXP4, and FOXP1 are not progressively expressed in the developing brain as they are in the spinal cord, the characterization of their expression in the brain is a beneficial explorative approach to determining their functions in brain development and ultimately their purposes in the adult brain.

## Future Directions

Given the data obtained regarding the expression of FOXP2, FOXP4, and FOXP1 in the mouse brain, the next step would be to determine the function of these genes in the various regions that they are expressed. This research could be done by identifying the types of cells that express these genes and analyzing their known functions.

Additionally, it would be helpful to look at Foxp2, Foxp4, and Foxp1 knock out (KO) mutant mice to see how neurogenesis is affected in the absence of these genes. Because Foxp2 KO mice die slightly before weaning age (21 days) and Foxp1 and Foxp4 KO mice are embryonic lethal, it would be best to create a variety of conditional knockout mice using Cre drivers that target specific brain regions allowing for the investigation of the roles of Foxps in neurogenesis. For example, the Novitch lab currently has Foxp4 conditional knockout mice under the control of Olig2 Cre recombinase, which removes Foxp4 expression in all regions expressing Olig2, such as the MGE. The behavior of adult conditional knockout mice could also be investigated for abnormalities that could be caused by irregular brain development. Given the functional redundancy seen in Foxps in other regions, it may be necessary to create mice lacking all three Foxp genes in order to study their functions.

Furthermore, it would be possible to create Foxp GFP mice in which GFP is used to tag each of the Foxps in order to track the fate of Foxp cells. This type of test would allow for tracking of migrating neuronal populations as was done in the well-characterized migration of LGE/MGE neurons to the cortex.

## References

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