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Characterization of Foxp2 and Foxp1 mRNA and Protein in the Developing and Mature Brain

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ABSTRACT

Foxp2 and *Foxp1* are recently identified members of the Fox family of winged-helix/forkhead transcription factor genes. A recent study has found that mutations in human *FOXP2* produce a severe language disorder. Since Foxp2 appears to be important in language, we wanted to explore the expression of this gene and a homologous gene, Foxp1, in the developing brain. In the present study, we investigated the time course and localization of Foxp2 and Foxp1 mRNA and protein expression in the developing and adult mouse using in situ hybridization and immunohistochemistry. Foxp2 and Foxp1 are expressed as early as E12.5 and persist into adulthood. Foxp2 and Foxp1 were most highly expressed in the developing and mature basal ganglia. Expression of Foxp2 was also observed in the cerebral cortex (layer 6), cerebellum (Purkinje neurons), and thalamus. Foxp1 expression was observed in the cerebral cortex (layers 3–5), hippocampus (CA1), and thalamus. Very little ventricular zone expression was observed for Foxp2 and Foxp1 and the expression of both of these genes occurred following neuronal migration, suggesting a role for these genes in postmigratory neuronal differentiation. Furthermore, we demonstrated the expression of *FOXP2* in human fetal brain by RT-PCR, in the perisylvian area of the left and right cerebral hemispheres, as well as in the frontal and occipital cortices. Overall, the widespread expression of Foxp2 in the developing brain makes it difficult to draw specific conclusions about which areas of Foxp2 expression are critical to human language function. J. Comp. Neurol. 460: 266–279, 2003.

Indexing terms: central nervous system; cerebrum; hippocampus; winged-helix; forkhead; transcription factor

Members of the winged-helix/forkhead family of transcription factors appear to be involved in the development of the central nervous system, especially in the development of the telencephalon. One of the winged-helix/forkhead family gene members, *Foxg1* (or BF-1), is localized in the progenitor cells of the telencephalic neuroepithelium, and Foxg1 null mutant mice have dramatically reduced cerebral hemispheres (Tao and Lai, 1992; Xuan et al., 1995). This reduction in the size of the cerebral hemispheres is thought to be a result of a premature differentiation of telencephalic neuroepithelial cells, leading to a decrease in the progenitor cell population of the ventricular zone (Xuan et al., 1995). These studies suggest that Foxg1 is a key regulator in the development of the cerebral cortex through its regulation of progenitor cell proliferation and differentiation.

Other members of the winged-helix/forkhead family of transcription factors were also shown to be critical in the

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development of nontelencephalic tissues. The winged-helix/forkhead gene, *Foxb1* (or Fkh5 or MF3), is expressed in the developing diencephalon, midbrain, hindbrain, and spinal cord (Kaestner et al., 1996; Labosky et al., 1997; Wehr et al., 1997; Alvarez-Bolado et al., 1999, 2000a,b). Moreover, mice with targeted deletions in the *Foxb1* gene exhibit gross abnormalities in the diencephalon and midbrain, suggesting a role of this gene in the development of these anatomical structures (Wehr et al., 1997).

Recently, two additional new members of the Fox family of winged-helix/forkhead DNA-binding proteins were identified (Shu et al., 2001). These proteins, called Foxp2 and Foxp1, were found to act as transcriptional repressors in lung epithelial gene transcription (Shu et al., 2001). Northern blot analyses of the Foxp2 and Foxp1 mRNA indicate that these gene products are expressed in the central nervous system of both rodents and humans (Lai et al., 2001; Shu et al., 2001). More specifically, Foxp2 and Foxp1 in situs verified this finding in the E16.5 embryonic mouse brain with robust expression of Foxp2 in the developing basal ganglia, thalamus, and inner cortical plate, with a low level of expression in the cortical ventricular zone and intense labeling of Foxp1 in the developing basal ganglia and outer cortical plate (Shu et al., 2001). Interestingly, mutations in the *FOXP2* gene were recently identified in patients with a severe speech and language disorder, mainly an expressive language deficit accompanied by an articulation disorder (Fisher et al., 1998; Vargha-Khadem et al., 1998; Lai et al., 2001). This finding strongly suggests that *FOXP2* is a critical gene involved not only in the production of language, but also in the development of the brain. However, the precise localization and time course of Foxp2 expression are currently unknown. The current study aimed to identify the time course of Foxp2 and Foxp1 expression as well as a detailed examination of the expression pattern of both of these genes in the brain, specifically in the cerebral cortex, basal ganglia, hippocampus, and cerebellum.

MATERIALS AND METHODS

Animals and histological procedures

All of the mice used in the present studies to characterize the normal expression patterns of Foxp2 and Foxp1 were from wildtype Swiss Webster mice acquired from Taconic (Germantown, NY). These mice were sacrificed at a range of developmental ages (E, embryonic; P, postnatal): E12.5, E14.5, E16.5, E17.5, P0.5, P1.5, P3.5, P6.5, P9.5, P15.5, and adult. In addition to the characterization of these genes in wildtype mice, we also examined their expression in adult *reeler* mutant mice (Caviness and Sidman, 1973). All mice were overdosed with sodium pentobarbital and prepared for either in situ hybridization (ISH) studies or immunohistochemistry (IHC) studies, as described below. For ISH, the heads of mice were frozen in isopentane on dry ice and stored at −80°C until processed. For IHC, mice were perfused transcardially with 0.01 M phosphate-buffered saline (PBS, pH 7.4) followed by cold 4%-paraformaldehyde made in PBS. Perfused brains were immediately removed and postfixed in 4%-paraformaldehyde for up to 7 days at 4°C, followed by cryoprotection in 30% sucrose in PBS. All animal use procedures were reviewed and approved by the Harvard Medical School Standing Committee on Animals and were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

All brains for ISH and IHC processing were sectioned either coronally or sagittally (10–20 μm) in a cryostat and mounted on Superfrost plus microscope slides (Fisher Scientific, Pittsburgh, PA). All slides containing cut brain sections were stored at −80°C (ISH) or −20°C (IHC) until processed for ISH or IHC using standard histological techniques as described below.

Foxp1 and Foxp2 in situ hybridization

Nonradioactive ISH was performed as previously described (Berger and Hediger, 2001) using digoxigenin (DIG)-labeled cRNA probes. The Foxp2 probes were constructed from the cloning of the mouse Foxp2 cDNA (NCBI: NM_053242; GI: 16716566) into the pCMV-Tag3B vector (Stratagene, La Jolla, CA) at the EcoRI and XhoI sites, producing a pCMV-Tag3B/Foxp2 plasmid (Shu et al., 2001). Similarly, the Foxp1 probes used for our ISH studies were derived from the cloning of the mouse Foxp1 cDNA (NCBI: NM_053202; GI: 16716508) into the EcoRI and XhoI sites of the pCMV-Tag2A vector (Stratagene) resulting in pCMV-Tag2A/Foxp1 (Shu et al., 2001).

Probes for Foxp2 were generated from the pCMV-Tag3B/Foxp2 plasmid at the following nucleotide (nt) sequence positions in the Foxp2 cDNA, Probes: Foxp2-1 (nt 99–601; 503 bp), Foxp2-2 (nt 1408–1904; 497 bp), Foxp2-3 (nt 463–1013; 551 bp), and Foxp2-4 (nt 1760–2057; 298 bp). All Foxp2 nucleotide probe sequences were from nucleotides downstream of the ATG start site. These probes were generated from PCR-amplified sequences using primers constructed to amplify the sequences described above (*Foxp2-1,* forward primer: 5′-TGGGAGATCAAGTGGTGACA-3′, reverse primer: 5′-GCTGCTCTTTTGCTTGCTTT-3′; Foxp2-2, forward primer: 5′-AATGTGGGAGCCATACGAAG-3′, reverse primer: 5′-CCACTGGAGGCGTTATTGAT-3′; *Foxp2-3,* forward primer: 5′-CAACAGCAGCAGCAGCAAC-3′, reverse primer: 5′-GAGGCCCCAGTCTCCTCA-3′; *Foxp2-4,* forward primer: 5′-TAACAGGAAGTCCAACTTTAGTAAA-3′, reverse primer: 5′-ATTGGACAGTCTTCATCCTCTG-3′).

Probes for Foxp1 were generated from Foxp1 sequences in the pCMVTag2A/Foxp1 vector at the following nucleotide (nt) sequence positions in the Foxp1 cDNA, Probes: Foxp1-1 (nt 768–1268; 501 bp), Foxp1-2 (nt 1806–2057; 252 bp), Foxp1-3 (nt 575–972; 398 bp), and Foxp1-4 (nt 1730–2030; 301 bp). All Foxp1 nucleotide probe sequences were from nucleotides downstream of the ATG start site. The probes were generated from PCR-amplified sequences using primers constructed to amplify the sequences described above (*Foxp1-1,* forward primer: 5′-GCAGCAGCTCTGGAAAGAAG-3′, reverse primer: 5′-GCAGACTTGGAGAGGGTGAC-3′; *Foxp1-2,* forward primer: 5′-GGCTTCCATGGCTGAGAATA-3′, reverse primer: 5′-CTGTGGTTGGCTGTTGTCAC-3′; *Foxp1-3,* forward primer: 5′-ATGCTGGAAAACAGCCGAAA-3′, reverse primer: 5′-GTGCTCCTCGTGGGACAAG-3′; *Foxp1-4,* forward primer: 5′-TCAGTGGTAACCCTTCCCTTATT-3′, reverse primer: 5′-AGAGGGCCTTCAGCTTCCT-3′).

All reverse primers contained T7 promoter sequences (5′-TAATACGACTCACTATAGGG-3′) on their 5′-ends in order to drive the transcription of the cRNA antisense probes. All forward primers contained T3 promoter sequences (5′-AATTAACCCTCACTAAAGGG-3′) on their 5′-

ends in order to drive the transcription of the cRNA sense probes. All primers were chosen using Primer3 (Rozen and Skaletsky, 2000).

All in situ hybridization studies were performed using digoxigenin (DIG)-labeled cRNA probes (Berger and Hediger, 2001). Frozen brain sections (10 μm) were cut in a cryostat and placed onto Superfrost plus microscope slides. Sections were then fixed and acetylated, followed by hybridization at 68°C over 3 nights to each probe (approximate concentration 100 ng/ml). Hybridized probes were visualized using alkaline phosphatase-conjugated anti-DIG Fab fragments (Roche, Indianapolis, IN) and 5-Bromo-4-chloro-3-indolyl-phosphate/Nitro blue tetrazolium (BCIP/NBT) substrate (Kierkegard and Perry Laboratories, Gaithersburg, MD). Sections were rinsed several times in 100 mM Tris, 150 mM NaCl, 20 mM EDTA pH 9.5, and coverslipped with glycerol gelatin (Sigma, St. Louis, MO). Control sections were incubated in either an identical concentration of the sense probe transcript or with another probe directed against a different sequence from the same gene in order to determine whether this second probe demonstrated an identical labeling pattern. All probes were sequenced to verify each probe's nucleotide composition.

Foxp1 and Foxp2 immunohistochemistry

Tissue sections (20 μm) were washed extensively in 0.01 M PBS (pH 7.4). Following these PBS rinses, all sections were permeabilized with 0.04% (v/v) Triton X-100 in PBS (PBS-TX) (pH 7.4) for 10 minutes. Immediately after PBS-TX treatment, the tissue sections were rinsed in 3% hydrogen peroxide in PBS for 30 minutes in order to inactivate endogenous peroxidases. Following peroxide treatment, tissue sections were washed three times for 10 minutes each in PBS-TX. Sections were incubated in 10% normal goat serum (Vector Laboratories, Burlingame, CA) made in PBS-TX for 1 hour, followed by an overnight incubation at 4°C with a rabbit polyclonal primary antibody directed against either Foxp2 (Lu et al., 2002) or Foxp1 (Lu et al., 2002) diluted 1:250, in PBS-TX containing 1% normal goat serum. Following incubation with the primary antibody, all tissue sections were washed three times in PBS-TX. For visualization of the Foxp2 and Foxp1 proteins by the peroxidase/diaminobenzidine IHC method, the sections were incubated for 1 hour in biotinylated goat antirabbit IgG (Vector Laboratories) diluted 1:500 in PBS-TX containing 1% normal goat serum. Following another three rinses in PBS-TX, sections were incubated in an avidin-biotin-horseradish peroxidase solution (Vector Laboratories) made in PBS-TX for 1 hour. Visualization of the antibody-antigen complex was accomplished using 3,3′-diaminobenzidine with nickel intensification (Vector Laboratories). Tissue was reacted for about 5 minutes until a black color developed. Lastly, the sections were rinsed in PBS, followed by water, and coverslipped with Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI). Sections were visualized using a Zeiss Axioskop light microscope (Carl Zeiss, Thornwood, NY) and photographed using a SPOT-RT Slider digital camera (Diagnostic Instruments, Sterling Heights, MI). For visualization of the Foxp2 and Foxp1 proteins by the fluorescent IHC method, sections were incubated for 1–2 hours in Cy3-conjugated goat antirabbit IgG (Jackson Laboratories, Bar Harbor, ME) diluted 1:500 in PBS-TX containing 1% normal goat serum. Sections were rinsed for 10 minutes, three times in PBS-TX, followed by a final wash in PBS. Lastly, the sections were coverslipped with Vectashield (Vector Laboratories). Tissue sections were visualized using an Olympus Provis AX-70 fluorescent microscope (Olympus America, Melville, NY) and photographed using a SPOT-RT Slider digital camera (Diagnostic Instruments). Controls for immunohistochemical procedures consisted of omitting the primary antibody as well as using irrelevant rabbit antibodies which bind in a nonoverlapping pattern with Foxp2 and Foxp1. No specific immunoreactivity (staining) was observed under the no primary control condition, in addition to the fact that the other rabbit antibodies (non-Foxp1 or 2) we tested did not demonstrate similar patterns of labeling. Additional verification of the specificity of the antibody staining for Foxp1 and Foxp2 comes from the fact that similar patterns of expression were observed in both the in situ hybridization and immunohistochemistry studies.

In order to determine whether these Foxp-positive cells were neuronal, we did double-labeling with both Foxp antibodies in combination with the neuronal marker, NeuN, in addition to a qualitative morphological analysis. NeuN immunohistochemistry was performed concomitantly with the Foxp staining. The NeuN antibody (mouse; Chemicon International, Temecula, CA) was added at a concentration of 1:1,000 with the Foxp antibody and incubated overnight. For visualization of NeuN by fluorescence, sections were incubated for 1–2 hours with both the Foxp secondary antibodies and a FITC-conjugated goat antimouse IgG (Jackson Laboratories) diluted 1:500.

For determining the laminar identity of the cortical layers as well as identifying various neuroanatomical structures, we used the DNA label Hoechst 33342 (Molecular Probes, Eugene, OR). This label allows for the identification of all nuclei in the developing and mature mouse brain.

RT-PCR

RNA was extracted from four cortical locations of frozen human fetal brain at 14 weeks of gestation (Brain and Tissue Bank for Developmental Disorders, University of Maryland, Baltimore, MD) using Trizol reagent (Invitrogen, Carlsbad, CA). Specifically, tissues were dissected from the right and left areas surrounding and including the perisylvian regions as well as from the rostral tip of the frontal lobe and the caudal tip of the occipital lobe. mRNA was then isolated from the total RNA pool of 10 μg and prepared for cDNA synthesis by Dynabeads mRNA DIRECT kit (Dynal Biotech, Lake Success, NY). First and second strand cDNA was synthesized using the SuperScript Choice System cDNA synthesis kit (Invitrogen). After second strand cDNA synthesis the oligo (dT) beadbound cDNA was washed once with 0.5 M EDTA, incubated at 75°C for 10 minutes in 5 mM Tris (pH 7.5), 0.5 mM EDTA, 1 M NaCl, 1% SDS, and 10 μg/ml glycogen and washed four more times in the same solution to inhibit DNA polymerase. The cDNA was then washed four times in 5 mM Tris (pH 7.5), 0.5 mM EDTA, 1 M NaCl, 200 μg/ml BSA. After the final wash, 1 μl of the oligo (dT) bead-bound cDNA was amplified by PCR using primers chosen from the 3′-end of the *FOXP1* sequence (NCBI: AF275309; GI: 14582215) (forward primer: 5′-TCAGTGGTAACCCTTCCCTTA-3′, reverse primer: 5′-GTACAGGATGCACGGCTTG-3′), the *FOXP2* sequence (NCBI: AF337817; GI: 15919271) (forward primer: 5′-

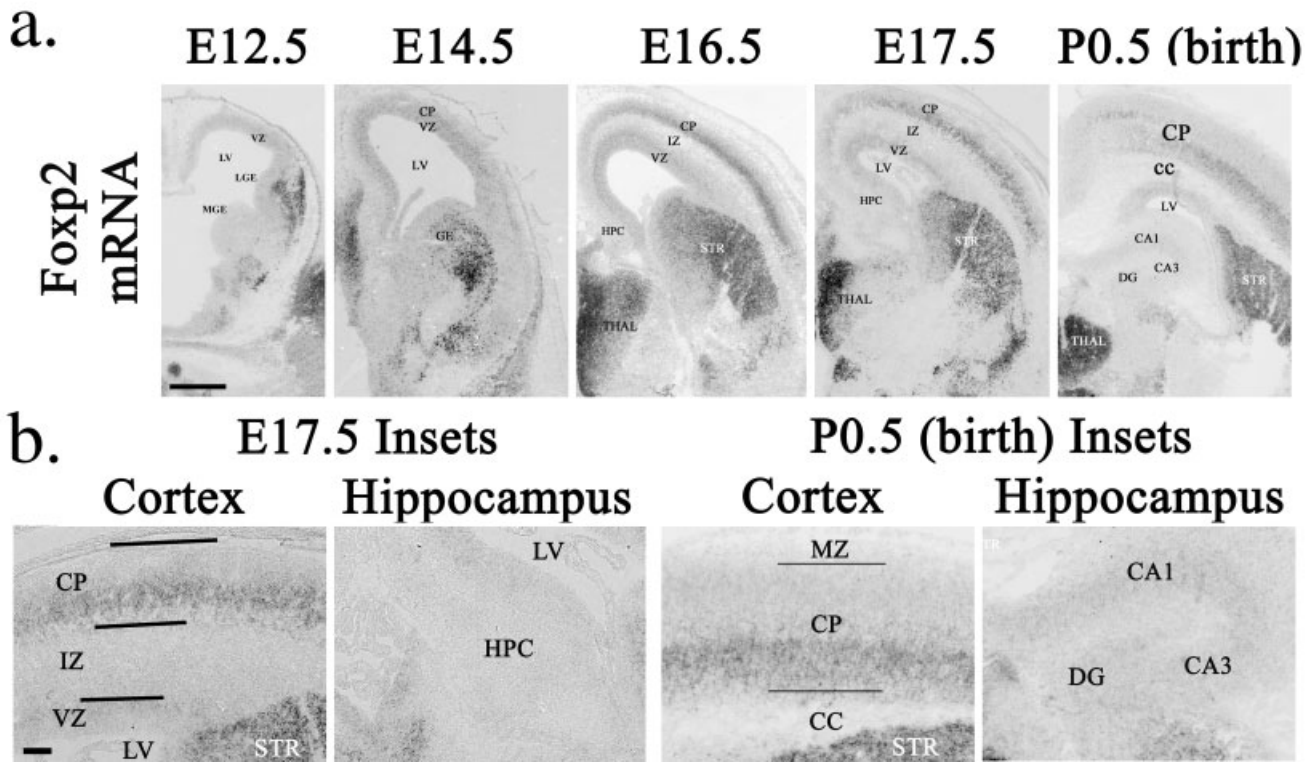

Fig. 1. **a:** Representative photomicrographs demonstrating the expression patterns of Foxp2 mRNAs in the developing mouse brain from embryonic day 12.5 (E12.5) to birth (postnatal day 0.5 (P0.5)). Scale bar = 500 μm. **b:** High-power magnifications demonstrating the expression patterns of Foxp2 mRNAs at E17.5 and P0.5 in the developing cortex and hippocampus. Scale bar = 100 μm. Note the expression of Foxp2 in the inner layers of the cortical plate, in the developing striatum, and in the striatal subventricular zone. CC: corpus callosum; CP: cortical plate; DG: dentate gyrus; GE: ganglionic eminences; HPC: hippocampus; IZ: intermediate zone; LGE: lateral ganglionic eminences; LV: lateral ventricle; MGE: medial ganglionic eminences; MZ: marginal zone; THAL: thalamus; STR: striatum; VZ: ventricular zone.

CCACGAAGACCTCAATGGTT-3′, reverse primer: 5′-TCACGCTGAGGTTTCACAAG-3′), the *doublecortin* sequence (NCBI: AF034634; GI: 2739175) (forward primer: 5′-TGCCTGTAACACCCCTCTTC-3′, reverse primer: 5′-TGAATGCTGCGAATCTTCAG-3′), and the *beta-tubulin* sequence (NCBI: NM001069; GI:4507728) (forward primer: 5′-GGTGTCCGAGTACCAGCAGT-3′, reverse primer: 5′-GAAGAGCACCAGAGACCCAG-3′). The amplification products (*FOXP1*: 255 bp; *FOXP2*: 253 bp; *doublecortin*: 151 bp; *beta-tubulin*: 233 bp) were analyzed by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and transluminated by UV light. All primers were chosen using Primer3 and screened against a human mispriming library by Primer3 (Rozen and Skaletsky, 2000).

RESULTS

Foxp2 expression in mouse cerebral cortex

Foxp2 mRNAs and proteins were found in various structures throughout the developing and mature mouse brain. Virtually all of the Foxp2 positive cells observed in the brain appeared to be neurons, based on nuclear morphology and colocalization with the neuronal marker NeuN (data not shown). Cortical expression of Foxp2 mRNA was observed as early as E14.5 in the lateral aspects of the cortical plate (Fig. 1A). One difference observed between the mRNA and protein expression of Foxp2 was that the protein expression sometimes lagged behind the mRNA expression, as would be expected. Additionally, Foxp2 protein was always observed in the nucleus of the neuron consistent with its role as a transcription factor. Foxp2 was found in the frontal, parietal, and occipital cortices, with very little expression in the cingulate cortex. In addition, there was no expression of Foxp2 in the piriform cortex, suggesting that Foxp2 is only expressed in neocortex (or six-layered cortices) and not in paleocortex (or three-layered cortices). Interestingly, Foxp2 mRNA and protein was restricted to the deeper layers of the cortical plate and subplate (Figs. 1–3). Moreover, upon formation of all six layers of the cortex, Foxp2 expression was restricted only to a subpopulation of neurons in layer 6 (Fig. 3). The only deviation from this pattern was a tendency to see Foxp2 positive cells in layer 5 of the very medial aspects of the cortex (Fig. 3, P3.5, P6.5) and in very posterior aspects of the cortex (data not shown). Furthermore, there was a possible gradient in Foxp2 expression in the cortex, with more expression occurring in the lateral aspects of the cortex and less expression in more medial aspects of the cortex (Fig. 1, E16.5, E17.5, P0.5). Lastly, very little expression of Foxp2 was observed in the ventricular zone at the mRNA level (Fig. 1). There was no

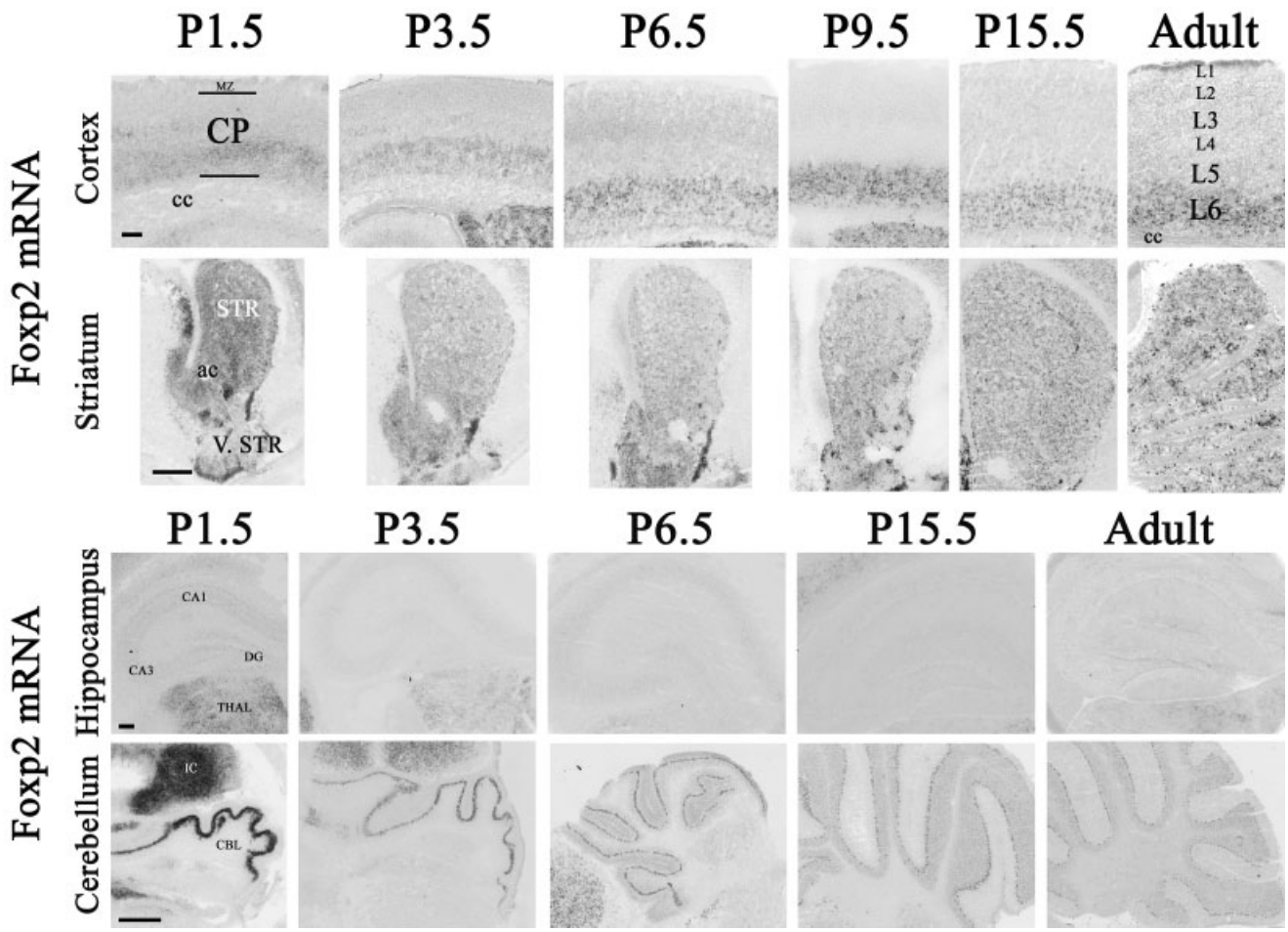

Fig. 2. Representative photomicrographs demonstrating the expression patterns of Foxp2 mRNAs in the postnatal brain from postnatal day 1.5 (P1.5) to adult in the cortex, striatum, hippocampus, and cerebellum. All sections are in the coronal plane except for the adult striatal sections, which are in the sagittal plane. Note the restricted expression of Foxp2 to layer 6 of the cortex. Both the striatum and cerebellum (Purkinje neurons) express Foxp2, but there is an absence of expression in the hippocampus. Scale bar = 100 μm for the cortical and hippocampal photomicrographs and 500 μm for the striatal and cerebellar photomicrographs. ac: anterior commissure; CBL: cerebellum; cc: corpus callosum; CP: cortical plate; DG: dentate gyrus; IC: inferior colliculus; L1: cortical layer 1; L2: cortical layer 2; L3: cortical layer 3; L4: cortical layer 4; L5: cortical layer 5; L6: cortical layer 6; MZ: marginal zone; STR: striatum; V. STR: ventral striatum; THAL: thalamus.

Foxp2 protein expression observed in the ventricular zone at any of the time points examined by immunohistochemistry (Fig. 3). The consistent absence of Foxp2 protein expression in the ventricular zone suggests that this protein becomes expressed either during late neuronal migration or during neuronal differentiation, based on the relative absence of Foxp2 expression in the intermediate zone (area of neuronal migration) and its abundance in the cortical plate.

Foxp2 expression was not observed in the hippocampus either at the mRNA or protein level at any of the time points examined (E12.5 to adult). No expression was noted in the dentate gyrus, CA1 fields, CA3 fields, or subiculum (Figs. 1–3).

Foxp2 expression in mouse basal ganglia

Foxp2 expression was also observed in the developing ganglionic eminences and mature basal ganglia. Specifically, Foxp2 mRNA was observed in the deep aspects of the ganglionic eminences (GE) in the developing brain as early as E12.5 (Fig. 1A). We observed very little Foxp2 mRNA and protein in the GE ventricular zone (Figs. 1A, 3). Furthermore, Foxp2 mRNA was observed in the subventricular zone of the GE (Fig. 1), with weaker protein staining in this same area (Fig. 3). In fact, the pattern of expression of Foxp2 suggests that these genes are activated in either migratory or postmigratory striatal neurons. Following these early time points, Foxp2 mRNA and protein was expressed continually in the striatum throughout development and into adulthood (Figs. 2, 3). However, there was a relative absence of Foxp2 expression in the pallidum. In the adult, Foxp2 mRNA and protein were localized throughout the basal ganglia, more specifically, in the striatum (caudate-putamen), substantia nigra, and the ventral striatum (i.e., nucleus accumbens).

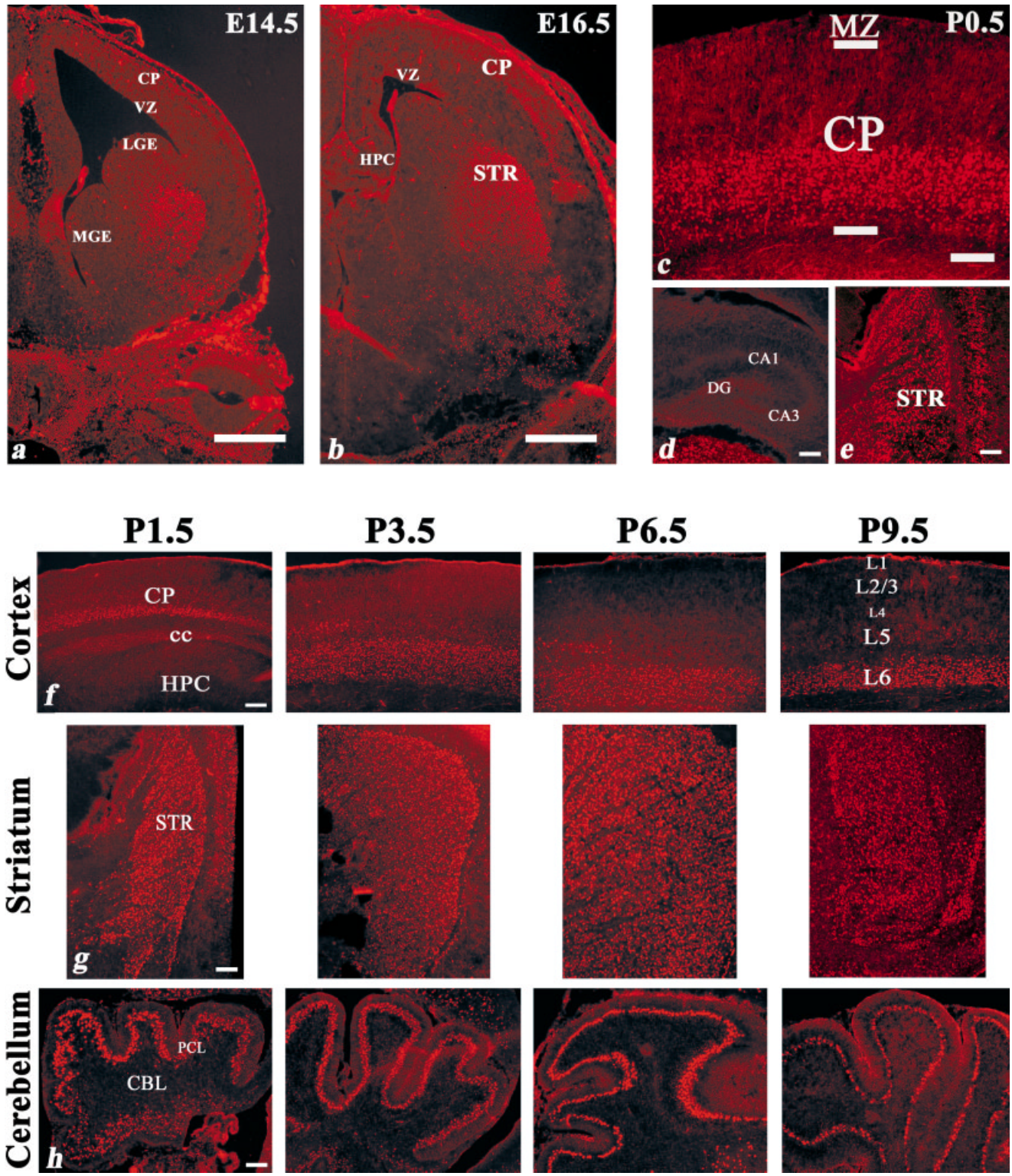

Fig. 3. Representative photomicrographs demonstrating the pattern of Foxp2 protein expression in the developing brain at ages (**a**) E14.5, (**b**) E16.5, (**c–e**) P0.5 (birth), and in the postnatal (**f**) cortex, (**g**) striatum, and (**h**) cerebellum (P1.5, P3.5, P6.5, P9.5). Foxp2 staining is in red. Individual hemispheres are shown for E14.5 and E16.5, with higher magnification images at P0.5 (birth) of the (**c**) cortex, (**d**) hippocampus, and (**e**) striatum. Foxp2 expression is observed in the deep ganglionic eminences at E14.5 and E16.5. Cortical expression appears in the lateral aspects of the cortex at E16.5. By P0.5 (birth), the expression of Foxp2 is in the deeper aspects of the cortical plate, with robust expression in the developing striatum. Very little expression of Foxp2 is observed in the ventricular zone. No Foxp2 expression is observed in the hippocampus at any of these time points (only E14.5, E16.5, and P0.5 shown). In the postnatal cortex, Foxp2 expression is observed mostly in the deep layers of the cortex during cortical layer formation and, upon completion of layer formation, Foxp2 expression is mostly observed in layer 6. However, there are occasional Foxp2 positive cells observed in layer 5, but mostly in more medial aspects (see (**f**) P3.5 and P6.5) and more posterior aspects of the cortex (data not shown). Cingulate cortex tends to lack Foxp2 expression. Robust expression of Foxp2 is observed throughout the striatum and ventral striatum; however, with relatively little expression in the pallidum. Additionally, cerebellar expression of Foxp2 is also intense, mostly in the Purkinje neurons. No Foxp2 expression is observed in the postnatal hippocampus at any of the time points tested (data not shown). Scale bar = 500 μm for **a,b**, 100 μm for **c–h**. Note: the photomicrograph of the striatum (**g**) at P9.5 is in the sagittal plane. CBL: cerebellum; cc: corpus callosum; CP: cortical plate; DG: dentate gyrus; HPC: hippocampus; L1: cortical layer 1; L2: cortical layer 2; L3: cortical layer 3; L4: cortical layer 4; L5: cortical layer 5; L6: cortical layer 6; LGE: lateral ganglionic eminences; MGE: medial ganglionic eminences; MZ: marginal zone; PCL: Purkinje cell layer; STR: striatum; VZ: ventricular zone.

TABLE 1. Expression patterns of Foxp2 and Foxp1 in the Mature Mouse Brain

Anatomical structure	*Foxp2*	*Foxp1*
Cortex		
Layer 1	−	−
Layer 2	−	−
Layer 3	−	+
Layer 4	−	+
Layer 5	−	+
Layer 6	+	−
Hippocampus		
Dentate gyrus	−	−
CA1	−	+
CA3	−	sp
Subiculum	−	+
Piriform cortex	−	−
Striatum	+	+
Ventral striatum	+	+
Cerebellum	+	−
Olfactory bulb	+	−
Amygdala	+	sp
Septum	sp	−
Thalamus	+	+
Hypothalamus	+	sp
Superior colliculus	+	+
Inferior colliculus	+	−
Substantia nigra	+	sp
Inferior olive	+	+

(+) expression; (−) no expression; (sp) sporadic cells.

Foxp2 expression in other structures of the mouse brain

Table 1 lists a variety of other structures that express Foxp2 in the mature mouse brain. Expression of Foxp2 was observed in the cerebellum as early as E14.5 (data not shown). In more mature cerebellum, most of the Foxp2 expression occurred in the Purkinje cells of the cerebellum (Figs. 2, 3). Foxp2 expression appeared localized in most of the cerebellar Purkinje cells, with no expression occurring in the granule cells (Figs. 2, 3). In addition, Foxp2 also was localized in the neuronal nuclei of the deep cerebellar nuclei.

Additional structures which demonstrated Foxp2 expression were the olfactory bulbs, the anterior olfactory nucleus, the olfactory tubercle, the amygdala, the septal nucleus, the thalamus, the hypothalamus, the inferior olive, and the superior and inferior colliculi. Although Foxp2 expression was found in these structures, not all of the neurons in these structures expressed Foxp2. For instance, Foxp2 was observed in only some of the thalamic nuclei, such as in the paraventricular thalamic nuclei, in the lateral posterior thalamic nuclei, the habenula, the medial and lateral geniculate, and a variety of dorsal thalamic nuclei. Also, the paraventricular nucleus of the hypothalamus also contained Foxp2-expressing neurons, while the ventromedial nucleus of the hypothalamus was absent of Foxp2-containing cells.

Foxp1 expression in mouse cerebral cortex

Expression of both the mRNA (ISH) and protein (IHC) of Foxp1 were virtually identical. A difference that was observed between the mRNA and protein of Foxp1 was that the expression of the protein lagged behind the mRNA. Moreover, nuclear localization of the Foxp1 protein in neurons was always observed, in accordance with its function as a transcription factor. Foxp1 mRNAs and proteins were found in a variety of structures throughout the embryonic, postnatal, and adult brain. Our analyses indicated that all of the cells that were Foxp1-positive demonstrated colocalization with NeuN (data not shown) and had nuclear sizes and morphology consistent with neurons. Foxp1 mRNA expression was observed in lateral aspects of the cortical plate as early as E14.5 (Fig. 4). Foxp1 expression was found in all of the cortical areas examined (cingulate, frontal, parietal, and occipital cortices). Similar to Foxp2 expression, Foxp1 was not observed in the piriform (paleocortex) cortex and seemed restricted only to neocortex (Figs. 4, 6). Ventricular zone expression of Foxp1 was very low at the mRNA level and was not detectable at the protein level (Figs. 4, 6). During corticogenesis, Foxp1 was observed mostly in the outer layers of the cortical plate (Figs. 4, 6). Upon formation of the cortical layers, Foxp1 expression was restricted to layers 3–5, with few Foxp1-positive cells in layers 2 and 6 (Figs. 5, 6). However, as the cortex develops late postnatally (>P6.5) and into adult, there is an increasing trend in observing more Foxp1-positive neurons in layer 6. From these patterns of expression, it appears that Foxp1 may also be involved in the differentiation of these cells into a neuronal phenotype since this gene is turned on either during late neuronal migration or after neuronal migration.

Hippocampal localization of Foxp1 mRNA and protein was observed as early as E17.5 and persisted into adulthood (Figs. 4–6). Foxp1 was strongly expressed in the subiculum and in the hippocampal CA1 fields (Figs. 4–6). Little or no expression of Foxp1 was observed in the hippocampal CA3 field, with no Foxp1 expression in the dentate gyrus.

Foxp1 expression in mouse basal ganglia

Foxp1 expression was strongest and very robust in the developing and mature basal ganglia. There was no apparent Foxp1 mRNA in the ganglionic eminence (GE) until E14.5 (Fig. 4A) and very little Foxp1 expression in the GE ventricular zone (Figs. 4A, 6). However, there was Foxp1 mRNA expression in the GE subventricular zone, which was much greater than that observed at the protein level (Figs. 4, 6). Most of the Foxp1 expression was in the deep aspects of the GE, suggesting that Foxp1 is expressed in either migratory or postmigratory striatal neurons (Figs. 4a, 6). Striatal expression of Foxp1 continued throughout late embryonic and postnatal development and persisted into adulthood (Figs. 5, 6). Foxp1 expression in the adult was found throughout other basal ganglia structures such as the substantia nigra and the ventral striatum; however, there was a virtual absence of pallidal Foxp1 expression.

Foxp1 expression in other structures of the mouse brain

Table 1 lists a variety of structures in the mature mouse brain that express Foxp1. Cerebellar expression of Foxp1 was not observed at any of the time points examined (Fig. 5). However, Foxp1 was observed in the neuronal nuclei of the deep cerebellar nuclei (data not shown). Additional structures which expressed Foxp1 were the anterior olfactory nucleus, the olfactory tubercle, sporadic cells in the amygdala, the thalamus, sporadic cells in the hypothalamus, the pontine nuclei, the inferior olive, and in the deep layers of the superior colliculus. Thalamic expression of Foxp1 was restricted to very few structures, such as in the paraventricular thalamic nuclei and a variety of posterior thalamic nuclei. In addition, Foxp1 expression appeared less robust and in sporadic neurons in structures such as

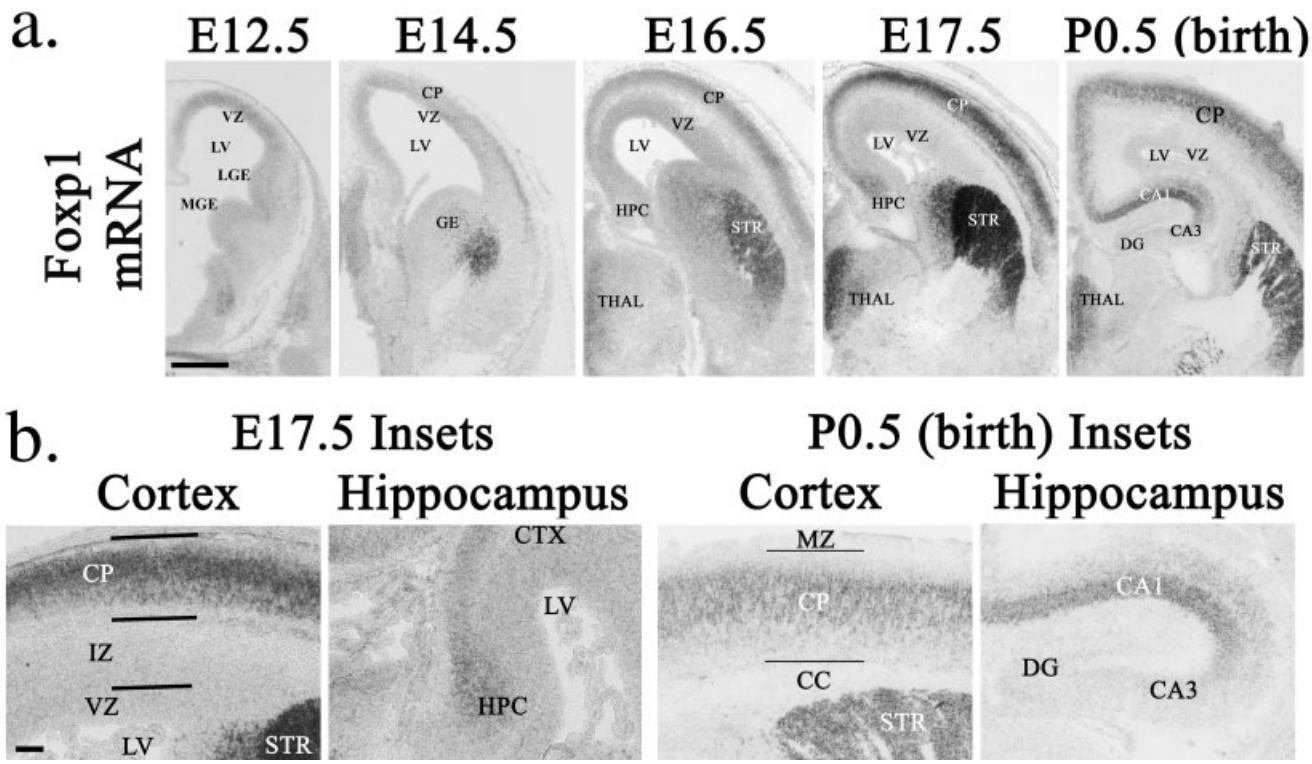

Fig. 4. **a:** Representative photomicrographs demonstrating the expression patterns of Foxp1 mRNAs in the developing mouse brain from E12.5 to birth (P0.5). Scale bar = 500 μm. **b:** High-power magnifications demonstrating the expression patterns of Foxp1 mRNAs at E17.5 and birth (P0.5) in the developing cortex and hippocampus. Scale bar = 100 μm. Note the expression of Foxp1 in the outer layers of the cortical plate and also in the developing striatum and hippocampus. CC: corpus callosum; CP: cortical plate; DG: dentate gyrus; GE: ganglionic eminences; HPC: hippocampus; IZ: intermediate zone; LGE: lateral ganglionic eminences; LV: lateral ventricle; MGE: medial ganglionic eminences; MZ: marginal zone; THAL: thalamus; STR: striatum; VZ: ventricular zone.

the substantia nigra, amygdala, hypothalamus, and septum.

Foxp2 and Foxp1 as layer-specific markers in the *reeler* mouse

Since Foxp2 and Foxp1 have distinctive expression patterns, we examined the expression patterns of these genes in the adult *reeler* mouse, which show disrupted and partially inverted cortical layering (Caviness and Sidman, 1973; D'Arcangelo et al., 1995). That is, instead of the normal layers 1–6, the *reeler* mouse shows very poor lamination, with a tendency for early-born neurons to be in the upper cortical plate and later-born neurons to be in the lower cortical plate. Interestingly, expression of Foxp2 in the adult *reeler* mouse was found in scattered neurons throughout the cortex, with predominate staining for Foxp2 in neurons that have migrated into the marginal zone (Fig. 7). Foxp2 was found in neurons abutting the pial surface of the brain (Fig. 7), consistent with the notion that the subplate and marginal zone fail to separate in *reeler* mice and that the laminar architecture is partially inverted in the *reeler* mouse (Caviness and Sidman, 1973; Sheppard and Pearlman, 1997), but also suggesting that many "overmigrated" neurons in *reeler* cortex represent layer 6 neurons. The wildtype littermate control had the normal expression of Foxp2 in layer 6 of the cortex, with no expression in the marginal zone (layer 1). In the adult *reeler* mouse, Foxp1 expression was found throughout the cortex. Scattered Foxp1 positive neurons were found in the marginal zone as well as throughout cortical layers 2–6 (Fig. 7). In the wildtype control, Foxp1 positive neurons were found mostly in cortical layers 3–5 (some layer 6 expression of Foxp1), with no Foxp1 cells in the marginal zone.

Expression of *FOXP2* and *FOXP1* mRNA in human fetal cortex

RT-PCR of RNA isolated from the cortex of a human brain at gestational week 14 of development demonstrated the presence of *FOXP2*. In fact, *FOXP2* expression was found in all cortical areas examined (Fig. 8). *FOXP2* was detected in the right and left perisylvian regions as well as in the frontal and occipital lobes. A similar pattern of expression for *FOXP1*, a homologous gene to *FOXP2*, was also observed (Fig. 8). In addition, *doublecortin*, a gene involved in neuronal migration, as well as the neuronal marker, *beta-tubulin*, were also present in all four areas of the developing cortex. These data indicate that *FOXP2* (and *FOXP1*) mRNAs are expressed in the human cortex at gestational week 14. However, we cannot determine from these data whether *FOXP2* is differentially expressed in the different areas of the cortex. Since *FOXP2* is implicated in human language expression, it will be interesting to determine whether the expression

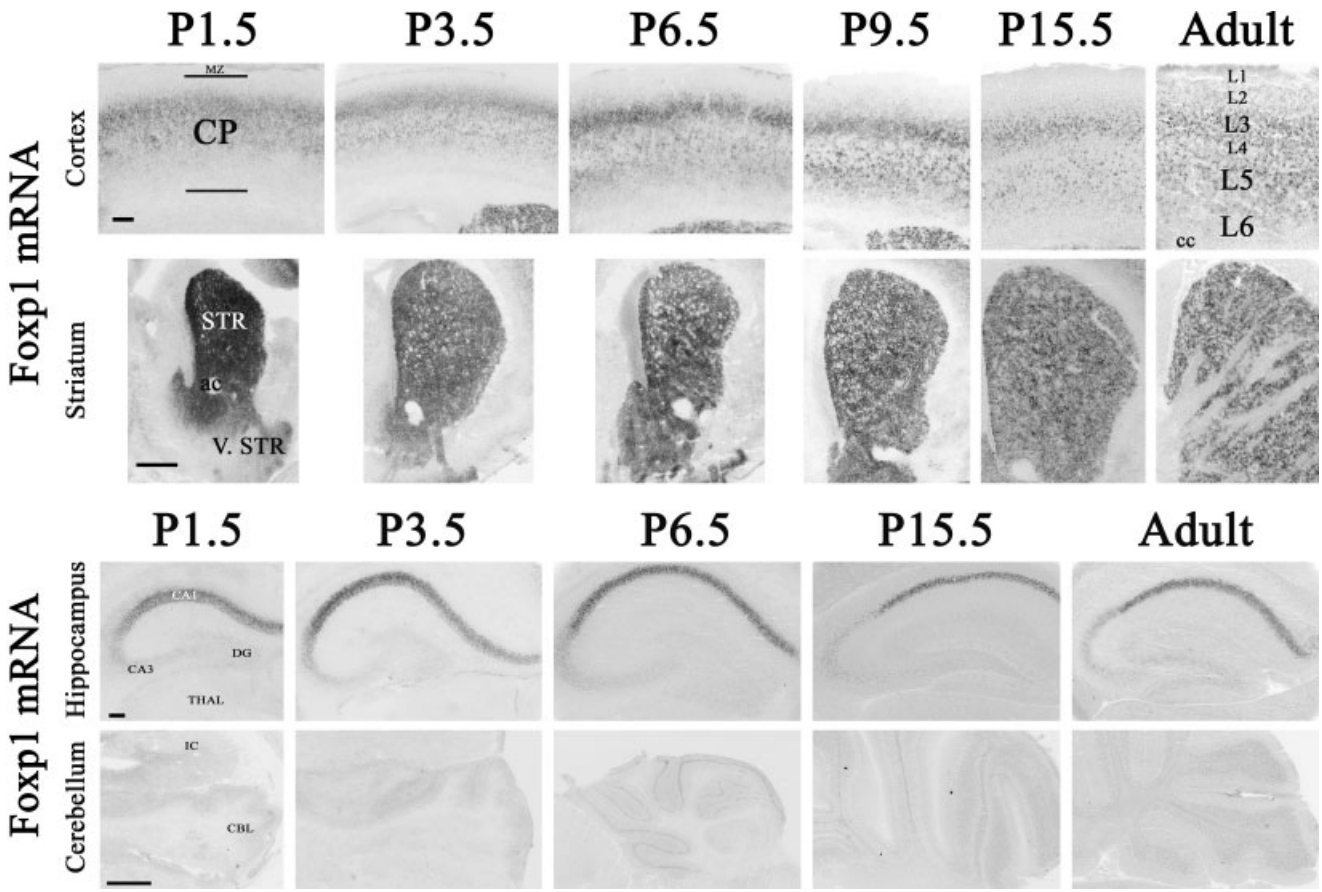

Fig. 5. Representative photomicrographs demonstrating the expression patterns of Foxp1 mRNAs in the postnatal brain from postnatal day 1.5 (P1.5) to adult in the cortex, striatum, hippocampus, and cerebellum. All sections are in the coronal plane except for the adult striatal section, which is in the sagittal plane. Note the restricted expression of Foxp1 to cortical layers 3–5. In addition, both the striatum and hippocampus (CA1 fields) express Foxp1, but there is an absence of expression in the cerebellum. Scale bar = 100 μm for the cortical and hippocampal photomicrographs, 500 μm for the striatal and cerebellar photomicrographs. ac: anterior commissure; CBL: cerebellum; cc: corpus callosum; CP: cortical plate; DG: dentate gyrus; IC: inferior colliculus; L1: cortical layer 1; L2: cortical layer 2; L3: cortical layer 3; L4: cortical layer 4; L5: cortical layer 5; L6: cortical layer 6; MZ: marginal zone; STR: striatum; V. STR: ventral striatum; THAL: thalamus.

pattern of *FOXP2* is differentially expressed in the left and right hemispheres, given the tendency for language function to localize to the left hemisphere.

DISCUSSION

In summary, Foxp2 and Foxp1 are found in various structures throughout the developing brain as well as in mature adult brain. Foxp2 expression was found throughout many telencephalic structures, most notably the cortex, striatum, and cerebellum. Cortical expression of Foxp2 was restricted mostly to layer 6 cortical neurons; however, not all layer 6 neurons. Foxp2 expression was also observed throughout many structures composing the basal ganglia (e.g., striatum, ventral striatum, substantia nigra) as well as in the cerebellum, specifically in the Purkinje cells. No hippocampal expression of Foxp2 was noted. The expression of Foxp1 was considerably different as compared to Foxp2, with the exception of the basal ganglia, since Foxp1 was highly expressed throughout the striatum, ventral striatum, and substantia nigra. However, the cortical expression of Foxp1 was quite different, in that labeling was found in layers 3–5. Moreover, Foxp1 expression was found throughout CA1 of the hippocampus, with no apparent Foxp1 expression in the cerebellum. It will be interesting to determine if similar patterns of expression are observed in the human brain and how these structures are involved in the expression of language.

Foxp2 expression in the developing and mature brain

Based on the recent data demonstrating that individuals with an expressive language disorder have mutations in the *FOXP2* gene (Lai et al., 2001), we wanted to define the anatomical structures that express Foxp2 throughout development in the mouse brain. Foxp2 expression was very robust in the basal ganglia. Striatal expression of Foxp2 appeared localized in the majority of striatal neurons. In addition, Foxp2 expression was also observed in other structures of the basal ganglia, such as the substantia nigra. However, there was very little Foxp2 expression

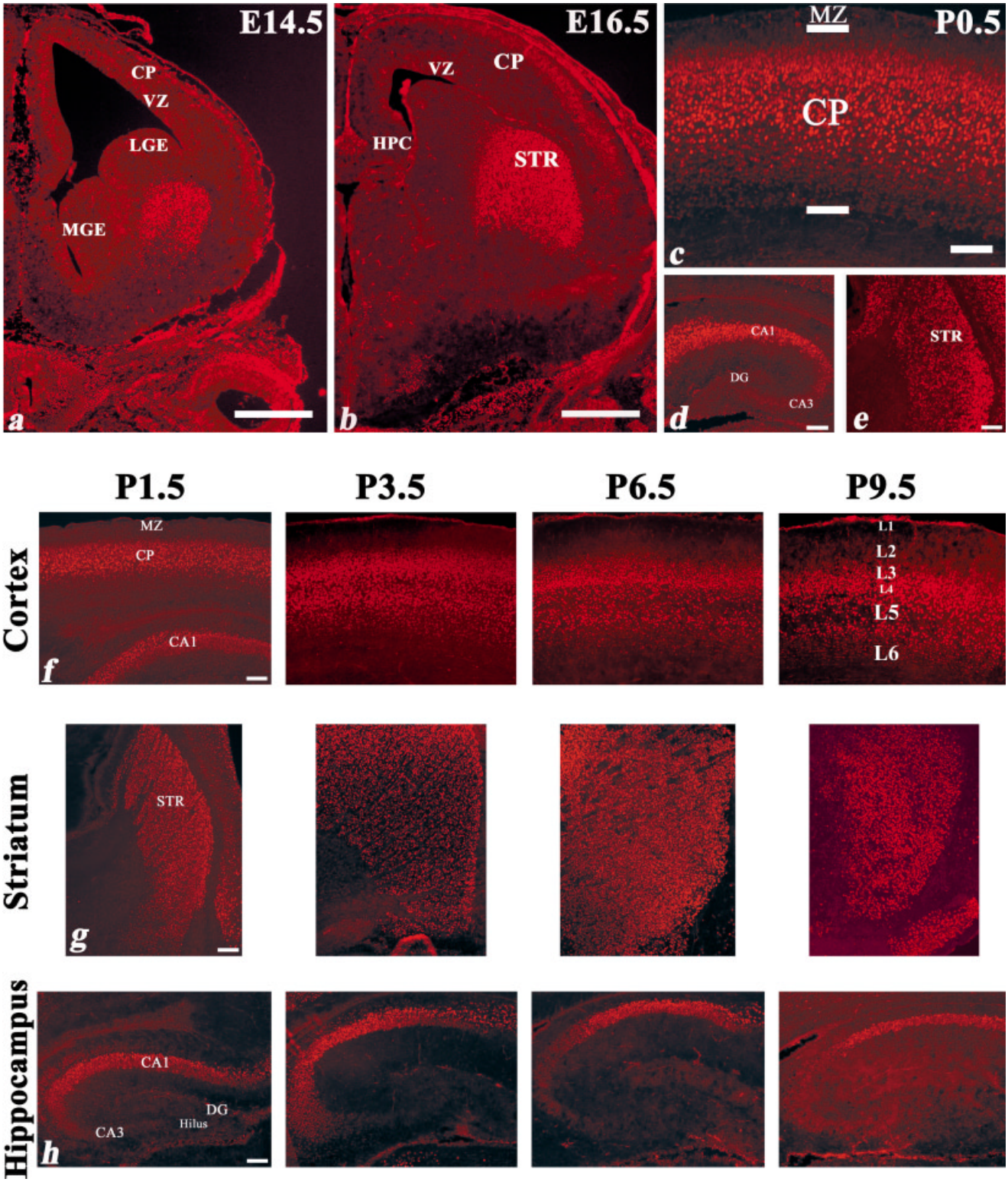

Fig. 6. Representative photomicrographs demonstrating the pattern of Foxp1 protein expression in the developing brain at ages (**a**) E14.5, (**b**) E16.5, (**c–e**) P0.5 (birth), and in the postnatal (**f**) cortex, (**g**) striatum, and (**h**) hippocampus (P1.5, P3.5, P6.5, P9.5). Foxp1 staining is in red. Individual hemispheres are shown for E14.5 and E16.5, with higher magnification images at P0.5 (birth) of the (**c**) cortex, (**d**) hippocampus, and (**e**) striatum. Similar to Foxp2, Foxp1 is expressed in the deep ganglionic eminences at E14.5 and E16.5. The expression of Foxp1 at P0.5 (birth) is in the upper aspects of the cortical plate, with intense expression in the developing striatum. In addition, hippocampal expression of Foxp1 is limited mostly to CA1 with very light staining in CA3 (no dentate gyrus staining is observed). In the postnatal cortex, Foxp1 expression is predominately in the outer layers of the cortical plate during cortical layer formation and, upon layer formation, Foxp1 expression is mostly observed in layers 3–5. However, occasionally Foxp1-positive cells are observed in layers 2 and 6. Like Foxp2, intense labeling of the Foxp1 protein is found in both the striatum and ventral striatum, with a relative absence of expression in the pallidum. In addition, Foxp1 expression is observed in CA1 of the hippocampus, with some expression in the hippocampal CA3 fields. Expression of Foxp1 is not observed in the developing or postnatal cerebellum except for some of the deep cerebellar nuclei cells (data not shown). Scale bar 500 μm for **a,b**, and 100 μm for **c–h**. Note: the photomicrograph of the striatum (**g**) at P9.5 is in the sagittal plane. CBL: cerebellum; cc: corpus callosum; CP: cortical plate; DG: dentate gyrus; HPC: hippocampus; L1: cortical layer 1; L2: cortical layer 2; L3: cortical layer 3; L4: cortical layer 4; L5: cortical layer 5; L6: cortical layer 6; LGE: lateral ganglionic eminences; MGE: medial ganglionic eminences; MZ: marginal zone; PCL: Purkinje cell layer; STR: striatum; VZ: ventricular zone.

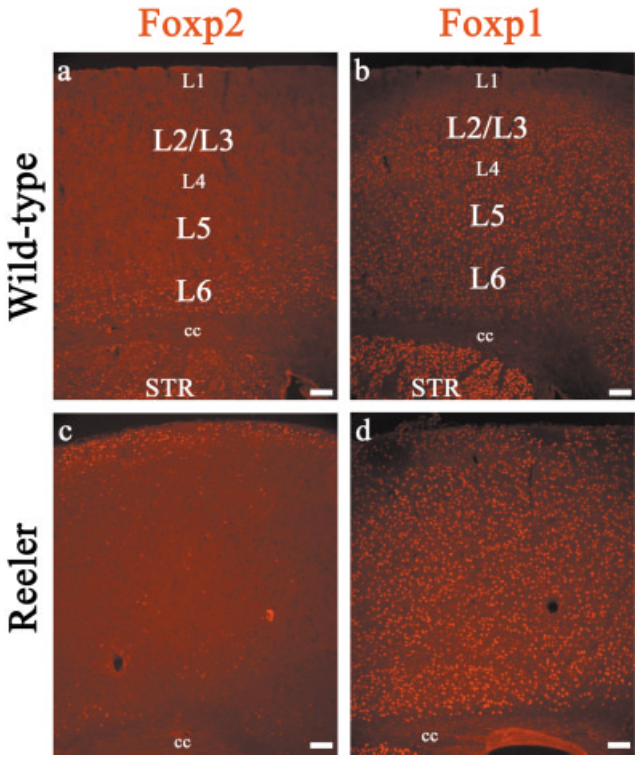

Fig. 7. Representative photomicrographs of Foxp2 staining in adult (**a**) wildtype and (**c**) *reeler* mice, in addition to Foxp1 staining in adult (**b**) wildtype and (**d**) *reeler* mice. One of the classic features of the *reeler* mouse is that it has an inversion in cortical layering. That is, instead of cortical layer 2 residing in the normal dorsal/top aspects of the cortex, layer 2 in the *reeler* mouse is in the deepest/ventral part of the cortex. Conversely, whereas a normal mouse has cortical layer 6 in the ventral/deep aspects of the cortex, layer 6 of the cortex in the *reeler* mouse is on the dorsal/top aspects of the cortex. Wildtype mice have the normal expression patterns of Foxp2 (cortical layer 6) and Foxp1 (cortical layers 3–5; some layer 6 positive neurons). However, in the *reeler* mouse these expression patterns are reversed. More specifically, Foxp2 staining is observed in the top layers of the cortex. In fact, these cells have migrated into the marginal zone. Foxp1 cells also appear in ectopic locations. Namely, Foxp1 expression is observed throughout the entire cortex and not in the restricted cortical layers that are normally observed. In addition, Foxp1-positive cells are observed in the marginal zone, but not nearly to the same extent as Foxp2. Scale bar = 100 μm. cc: corpus callosum; L1: cortical layer 1; L2: cortical layer 2; L3: cortical layer 3; L4: cortical layer 4; L5: cortical layer 5; L6: cortical layer 6; STR: striatum.

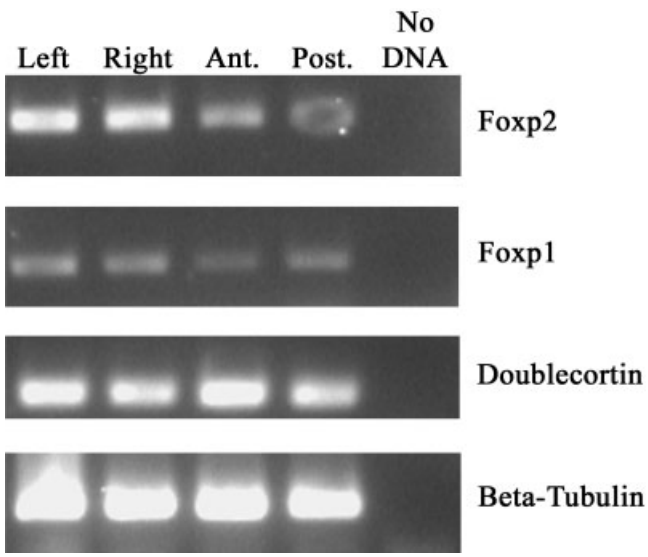

Fig. 8. Agarose gel stained with ethidium bromide demonstrating the RT-PCR product bands obtained for *Foxp2* (253 bp product), *Foxp1* (255 bp product), *doublecortin* (151 bp product), and *beta-tubulin* (233 bp product) from human cortical RNA. The cortical tissue was from the left perisylvian cortical region (Left), the right perisylvian cortical region (Right), the anterior tips of the frontal lobes (Ant.) or the posterior tips of the occipital lobes (Post.).

in the pallidum throughout development and into adulthood. Whether any of the Foxp2-positive neurons in the developing ganglionic eminences are migrating tangentially into the cortex remains to be determined (Anderson et al., 2001). Interestingly, *Dlx1* and *Dlx2* knockout mice have an abnormal subventricular zone, an area which expresses Foxp1 and Foxp2 (Anderson et al., 1997). Moreover, since *Dlx1* and *Dlx2*, as well as *Nkx2.1* and *Mash1*, genes are intricately involved in the development of the basal ganglia as well as in the differentiation of basal ganglionic neurons, it will be interesting to determine whether *Foxp1* and *Foxp2* are important either upstream regulators or downstream signaling partners of these genes (Anderson et al., 1997, 2001; Casarosa et al., 1999; Horton et al., 1999).

Foxp2 expression was also observed in the cerebellum. Interestingly, the cerebellar Foxp2 expression was only observed in the cerebellar Purkinje neurons in mouse, with additional expression in the deep cerebellar nuclei of the cerebellum. Since Foxp2 expression occurred early in cerebellar development and is expressed solely in the Purkinje cells, it will be important to determine whether Foxp2 is involved in the development, differentiation, or maintenance of Purkinje neurons.

One of the more interesting findings of this study was that Foxp2 expression was restricted mostly to layer 6 of the cortex. Layer 6 neurons are mostly large pyramidal neurons which mainly project back to the thalamus. What the role is for this Foxp2-positive subpopulation of layer 6 neurons in the cortex and whether they define neurons with specific projections to specific thalamic nuclei remains to be determined. Since patients with *FOXP2* mutations have a language disorder and language primarily localizes to the left hemisphere, studies examining the expression patterns of Foxp2 in both the normal human brain and in the brains of individuals with expressive language difficulties will be of significant interest in determining the role of this gene in language. Moreover, Tbr1, a member of the T box family of transcription factors, is also expressed in layer 6 cortical neurons (Hevner et al., 2001). How these two layer 6 markers are involved in determining the development and fate of layer 6 neurons as well as how these two transcription factors may be interacting in these processes remains largely unknown.

Since the predominant efferent projections of layer 6 pyramidal neurons are to the thalamus, it was interesting to observe Foxp2 expression was in the thalamus, most notably in the paraventricular thalamic nuclei, in the lateral posterior thalamic nuclei, the habenula, the medial and lateral geniculate, and a variety of dorsal thalamic

nuclei. It will be important to determine whether Foxp2-expressing layer 6 cortical neurons project to Foxp2-expressing neurons in the various thalamic nuclei described. Interestingly, Tbr1 mutants do not display appropriate axon pathfinding from cortical layer 6 to the dorsal thalamus, both areas of high Foxp2 expression (Hevner et al., 2002). It is interesting to speculate as to whether the absence of Tbr1 disrupts Foxp2 expression in layer 6 of the cortex, thereby altering the projections of layer 6 neurons to the dorsal thalamus. Whether Foxp2 is specifically involved in axon pathfinding remains to be determined.

Foxp1 expression in the developing human and mouse brain

Our results demonstrate that *FOXP1* is expressed in the developing human cortex (14 weeks gestation age) in the left and right perisylvian areas and the frontal and occipital lobes. In mouse brain, Foxp1 expression was observed as early as E12.5 and persisted into adulthood in a variety of neuroanatomical structures. Foxp1 was expressed most highly in the developing ganglionic eminences as well as in the mature striatum, with a relative absence of expression in the globus pallidum. This pattern of striatal Foxp1 expression was similar to that of Foxp2. These findings suggest that the Foxp genes are critical modulators of striatal development and adult function, since their expression continues in the mature striatum.

In addition to the striatal expression, Foxp1 was also observed in the developing and mature brain in patterns that do not tend to complement Foxp2 expression. Unlike Foxp2, which was found in layer 6 of the cortex, Foxp1 expression was observed mostly in layers 3–5, with scattered cells in layers 2 and 6, except in more mature cortex, where there is a tendency to see more Foxp1-expressing cells in layer 6. Similarly, Foxp2 was not observed in the hippocampus, but Foxp1 had robust expression in the hippocampal CA1 fields. In addition, there was no expression of Foxp1 in the cerebellum, whereas Foxp2 was found throughout the Purkinje cell layers of the cerebellum. Future studies are needed to determine whether the Foxp genes' functions are similar, but the two genes function in different cell types, or whether the different Foxp genes are performing different functions in these nonoverlapping structures.

Foxp2 and Foxp1 expression occurs in differentiated neurons

The majority, if not all, of the Foxp1- and Foxp2-positive cells are neurons based on nuclear morphology as well as colabeling with the neuronal marker, NeuN. Almost all of the Foxp2-positive cells colocalized with the neuronal marker, NeuN, indicating that these cells are neurons. It is important to note that not all of the neurons in layer 6 of the cortex were Foxp2-expressing neurons, but only a subpopulation of layer 6 neurons. Interestingly, Tbr1 is also expressed in layer 6 cortical neurons (Hevner et al., 2001). It will be interesting to see if Tbr1 and Foxp2 label different populations of layer 6 cortical neurons or whether there is a specific overlap between these two transcription factors. Overlapping expression patterns between Tbr1 and Foxp2 would suggest that Tbr1 might possibly regulate the expression of Foxp2, since Tbr1 expression occurs earlier than Foxp2. Future studies are needed that specifically address how Foxp2 is regulated and whether Tbr1 may be one of the regulators.

Qualitatively, very little, if any, expression of either Foxp1 or Foxp2 was observed in the cortical or striatal ventricular zone; however, subventricular zone expression was observed for both of these genes. Expression of both Foxp1 and Foxp2 began mostly in cells outside of the ventricular zone, presumably in either late migrating or postmigratory neurons. Therefore, our expression and developmental data favor the view that these genes are expressed in postmigratory neurons, since their expression begins when most cells should have already reached their postmigratory position. For instance, Foxp2 expression begins around E16.5 in the cortical plate, which is a time when most of the layer 6 neurons have finished their migration. In addition, very little Foxp staining is observed in the intermediate zone, the area through which postmitotic neurons migrate. Therefore, this interpretation would suggest that the Foxp genes are involved in the differentiation of neurons; however, further experiments are needed in order to definitively prove this point.

Foxp2 and Foxp1 as markers of cell identity

The overall pattern of Foxp2 and Foxp1 expression in the cortex demonstrates that these transcription factors can identify different cortical layers. Foxp2 expression mostly occurs in layer 6 of the cortex, whereas Foxp1 is found mostly in cortical layers 3–5. Due to the cortical layer disruption that is observed in the *reeler* mouse (Caviness and Sidman, 1973), expression of Foxp2 was found mostly in the marginal zone, and not in the deep layers of the cortex that is typically observed in normal cortex. This finding suggests that Foxp2 is involved in regulating the identity or functioning of these neurons and not involved in determining the neurons' cortical layer position. Furthermore, most of the Foxp2-positive layer 6 neurons are found in the marginal zone and in fact lie right up against the pial surface.

FOXP2 expression in human fetal brain

Mutations in the human *FOXP2* gene were recently identified in individuals with a severe language disorder (Lai et al., 2001). Since language function is clearly associated with the left perisylvian area, we were interested in determining whether *FOXP2* would be differentially expressed in the left and right hemispheres. Our RT-PCR experiments, using fetal brains, suggest that *FOXP2* is expressed in both the left and right hemispheres. However, our data are not quantitative and we cannot rule out that *FOXP2* could be more highly expressed in the left perisylvian area as compared to the right. Future work using in situ hybridization in human fetal brain might be able to address this issue.

Foxp2 expression and its potential relationship to language function

The widespread expression of Foxp2 in the developing nervous system makes it difficult to determine which of its loci of expression are essential to human speech production. Major regions of Foxp2 expression, including the caudate, the deep cerebral cortex, and the cerebellum, have all been previously recognized as being abnormal in size in patients with heterozygous *FOXP2* mutations (Vargha-Khadem et al., 1998; Watkins et al., 2002b).

Moreover, there are other data implicating not only the cerebral cortex (Rueckert et al., 1994; Wise et al., 1999; Blank et al., 2002; Holowka and Petitto, 2002; Gernsbacher and Kaschak, 2003), but also the caudate (Damasio et al., 1982; Aram et al., 1983; Lieberman et al., 1992; Mega and Alexander, 1994) and posterior lobes of the cerebellum (Fiez and Raichle, 1997), in various aspects of language production. Interestingly, the cerebral cortex, the caudate, and the cerebellum have also been implicated in stuttering (Fox et al., 1996). It will be important to determine the expression patterns of Foxp2 in both the normal human brain and in the brains of individuals with expressive language difficulties.

Lastly, since the medial geniculate nucleus/inferior colliculus/inferior olive, and the lateral geniculate nucleus/superior colliculus, are involved in hearing and vision, respectively, and highly express Foxp2, it will be interesting to determine if there are any subtle deficits in either the perception or coding of these sensory modalities in individuals with *FOXP2* mutations, since no obvious hearing or visual defects were reported in these patients (Fisher et al., 1998; Vargha-Khadem et al., 1998; Lai et al., 2001; Watkins et al., 2002a,b). In summary, the widespread expression of Foxp2 in developing brain makes it difficult to draw specific conclusions about which loci of expression are essential to language function in humans.

CONCLUSION

The expression pattern of Foxp2 in the mouse brain nicely parallels the structures that were demonstrated to be abnormal in patients with *FOXP2* mutations (Fisher et al., 1998; Vargha-Khadem et al., 1998; Lai et al., 2001; Watkins et al., 2002b). Future studies are needed to examine whether similar expression of *FOXP2* occurs in human brain throughout development. In addition, since language is unique to humans, it will also be of further interest to examine the expression of Foxp2 in nonhuman primates. How these neuroanatomical deficits in individuals with *FOXP2* mutations manifest in language dysfunction will be of intense interest in parceling out the roles of different anatomical structures in the production of language.

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