Development/Plasticity/Repair

Repression of Fgf Signaling by Sprouty1-2 Regulates Cortical Patterning in Two Distinct Regions and Times

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A fundamental question in developmental biology is how signaling pathways establish a transcription factor code that controls cell proliferation, regional fate and cell fate. Morphogenesis of the rostral telencephalon is controlled in part by Fgf signaling from the rostral patterning center. How Fgf signaling is regulated in the telencephalon is critical for understanding cerebral cortex formation. Here we show that mouse *Sprouty1* and *Sprouty2* (*Spry1-2*), which encode negative feedback regulators of Fgf signaling, are affecting cortical proliferation, differentiation, and the expression of genes regulating progenitor identity in the ventricular zone. In addition, *Spry2* has a later function in regulating the MAPK pathway, proliferation, and gene expression in the cortex at mid-neurogenesis. Finally, we provide evidence that *Coup-TFI*, a transcription factor that promotes caudal fate, does so through repressing Fgf signaling, in part by promoting *Spry* expression.

Introduction

Fibroblast growth factor (Fgf) signaling during embryogenesis has a central role in regulating regional specification and morphogenesis of the forebrain (Shimamura and Rubenstein, 1997; Ye et al., 1998; Crossley et al., 2001; Fukuchi-Shimogori and Grove, 2001; Korada et al., 2002; Garel et al., 2003; Mason, 2007). At least five Fgf ligands (*Fgf3*, *Fgf8*, *Fgf15*, *Fgf17*, and *Fgf18*) are expressed in a nested pattern in the rostral patterning center (RPC) (Cholfin and Rubenstein, 2007), a neuroepithelial region of the telencephalon that is derived from the anterior-most neural plate. In addition, some Fgf ligands, such as *Fgf10*, are more broadly expressed, and regulate neuroepithelial differentiation (Sahara and O'Leary, 2009).

Alterations in Fgf signaling (in Fgf receptor mutants) (Hébert et al., 2003; Sansom et al., 2005; Gutin et al., 2006; Smith et al., 2006; Thomson et al., 2009) or Fgf ligand dosage alter telencephalic regional patterning and growth. Whereas reduced *Fgf8* and *Fgf17* caudalize the cortex (Garel et al., 2003; Storm et al., 2006; Cholfin and Rubenstein, 2007, 2008), reduced *Fgf15* has the opposite phenotype (Borello et al., 2008). Some of these phenotypes are controlled by alterations in the expression of *Coup-TFI*, a transcription factor with caudoventralizing, antiproliferative, and neurogenic properties (Armentano et al., 2007; Faedo et al., 2008).

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Fgfs signal through four Fgf receptors (FgfRs), which activate several transduction pathways, including the Ras-Erk, the PI3 kinase-Akt, and the PLC-calcium-PKC pathways (Mason, 2007). We presented evidence that *Coup-TFI* is a negative regulator of Ras-Erk and PI3 kinase-Akt signaling (Faedo et al., 2008), suggesting that *Coup-TFI* regulates cortical patterning, proliferation, and neurogenesis, at least in part through repression of Fgf signaling.

The four *Spry* genes are induced by Fgf signaling and serve as negative feedback regulators. Spry proteins function intracellularly to inhibit the Ras-MAPK pathway, although their precise biochemical mechanism(s) remains controversial (Kim and Bar-Sagi, 2004; Mason, 2007). Loss-of-function analyses in mice have shown that *Spry* genes are required for development of the midbrain/hindbrain, kidney (Basson et al., 2005), auditory epithelium (Shim et al., 2005), and tooth (Klein et al., 2006). Their roles in telencephalic development are unknown.

Fgf8 is required for *Spry1* expression in the RPC (Storm et al., 2006), while $Fgf17^{-/-}$ mutants do not show an obvious change in *Spry1* or *Spry2* expression (Cholfin and Rubenstein, 2008). *Spry2* expression in ventral cortical progenitors is positively regulated by *Fgf15*, which is expressed at low levels at the pallial–subpallial boundary (Borello et al., 2008). *Fgf15* also promotes the expression of *Coup-TFI* (Borello et al., 2008), unlike *Fgf8* and *Fgf17*, which repress *Coup-TFI* (Garel et al., 2003; Cholfin and Rubenstein, 2008). Moreover, Coup-TFI represses Erk phosphorylation when overexpressed (Faedo et al., 2008). Thus, here we investigated how *Coup-TFI* regulates Fgf signaling.

We demonstrate different functions of *Spry* during cortical development. First, we show that *Spry1* and *Spry2* expression in the RPC function together as negative regulators of Fgf signaling from the RPC to control early patterning, proliferation, differentiation, and progenitor identity of the rostral dorsal/medial pallium. Second, we show that *Spry2* has a later function in

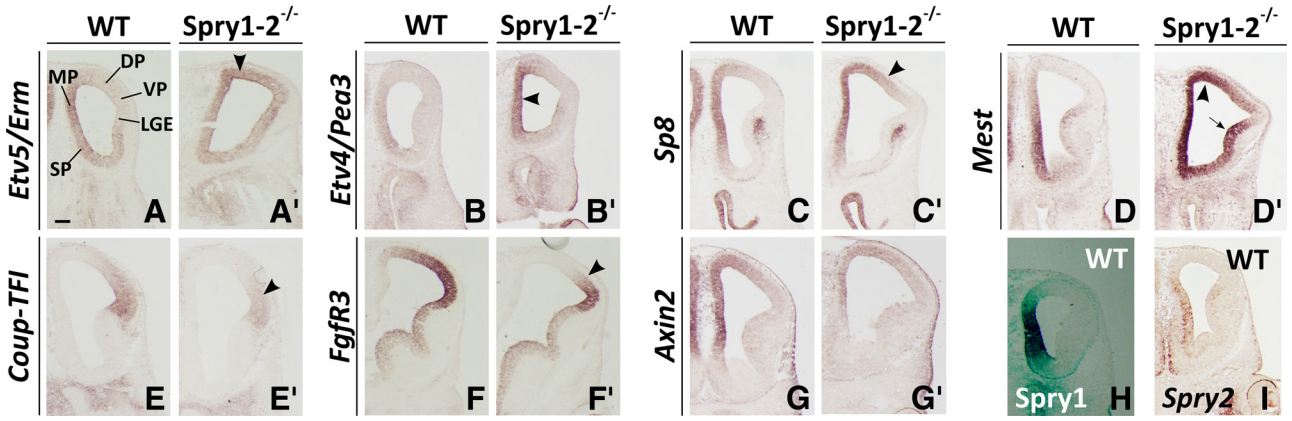

Figure 1. *Spry1-2* loss of function upregulates Fgf-regulated genes in the E12.5 RPC and dorsal telencephalon. ***A–D′***, RNA *in situ* hybridization for *Etv5* (***A***, ***A′***), *Etv4* (***B***, ***B′***), *Sp8* (***C***, ***C′***), and *Mest* (***D***, ***D′***) showing the upregulation of Fgf-regulated genes in the medial/dorsal pallium (arrowheads in ***A′***, ***B′***, ***C′***, ***D′***). ***E***, ***E′***, *Coup-TFI*, a gene with a complementary expression pattern to *Spry1-2*, and repressed by Fgf signaling, is downregulated. ***F***, ***F′***, *Fgfr3* expression, similar to *Coup-TFI*, and repressed by Fgf signaling, is shifted ventrally (***F′*** arrowhead). ***G***, ***G′***, *Axin2* RNA expression can be used as WNT signaling readout: in *Spry1-2*$^{-/-}$ its reduced expression suggests that Fgf signaling represses WNT signaling. ***H***, ***I***, Spry1 (β-galactosidase staining) and *Spry2* expression in WT sections. DP, dorsal pallium; VP, ventral pallium; MP, medial pallium; SP, septum; LGE, lateral ganglionic eminence. Scale bar, 200 μm.

regulating patterning and proliferation of the dorsal/ventral pallium through its expression in pallial progenitors. Finally, we show that *Coup-TFI* overexpression induces *Spry2*, which mediates some of *Coup-TFI*'s repressive properties on Fgf signaling (inhibition of Erk phosphorylation, proliferation, and *Etv* gene transcription).

Materials and Methods

Mice. D6/Coup-TFI mice were described by Faedo et al. (2008). Briefly, to generate D6/COUP-TFI transgenic mice, a 5.7Kb promoter region was cloned upstream of COUP-TFI open reading frame. The D6/COUP-TFI expression cassette was purified and injected into the pronucleus of fertilized (C57BL/6 × BALB/c) F1 mouse oocytes. Mice were genotyped by PCR using genomic DNA from the tail of postnatal and late embryonic stages or yolk sac from earlier embryos. The mouse mutant strain with null allele of Coup-TFI (Qiu et al., 1997) was used. The Spry1^{lacz} strain was used (Thum et al., 2008).

The production of *Spry1* and *Spry2* flox alleles have been described by Basson et al. (2005) and Shim et al. (2005). Mice homozygous for *Spry1-2*^{flox} were mated to β-actin-Cre transgenic mice to generate animals carrying the *Spry1-2*-null allele. To generate the D6/Coup-TFI;*Spry1-2*$^{-/-}$ line, *Spry1-2*$^{flox/flox}$ line was mated to β-actin-Cre transgenic mice to generate *Spry1-2* heterozygous mice. *Spry1-2* heterozygous mice were mated to D6/Coup-TFI mice to generate *Spry1-2*$^{+/-}$;D6/Coup-TFI mice. *Spry1-2*$^{+/-}$ mice were mated to *Spry1-2*$^{+/-}$;D6/Coup-TFI mice to produce *Spry1-2*$^{-/-}$;D6/Coup-TFI embryos. Mice heterozygous or homozygous for *Spry1-2* were identified by PCR assays described by Basson et al., 2005, and Shim et al., 2005. For *in situ* hybridization or immunofluorescence, either *Spry1-2*$^{+/-}$ or *Spry1-2*$^{+/+}$ were used as controls, since heterozygous mice did not show any phenotype.

For staging of embryos, midday of the day of vaginal plug formation was considered as embryonic day 0.5 (E0.5).

In situ *RNA hybridization. In situ* RNA hybridization was performed on frozen sections (20 μm thick) mounted on Fisher Superfrost/Plus slides. *In situ* RNA hybridization using digoxigenin (DIG)-labeled RNA probes was performed according to methods described at the Rubenstein laboratory website (http://physio.ucsf.edu/rubenstein/protocols/index.asp). Sections from the different genotypes shown in the figures have been processed simultaneously. In the D6/COUP-TFI, we used basal ganglia expression as an internal control to compare results between the different experiments and between experimental and WT samples.

The probes used and their sources were as follows: *COUP-TFI* (M. Tsai, Baylor College of Medicine, Houston, TX), *Fgfr1* (P. Lonai, Weizmann Institute of Medicine, Rehovot, Israel), Etv1/*Er81* (T. Jessell, Columbia University, New York, NY), *Sprouty1* (G. Martin, University of California, San Francisco, CA), *Sprouty2* (G. Martin), *Etv5/Erm* (A. Chotteau-Lelievre, Institut de Biologie de Lille, Lille, France), *Etv4/Pea3* (A. Chotteau-Lelievre), *Blbp* (*Fabp7*) (N. Osumi, Tokyo University School of Medicine, Sendai, Japan), *Sp8* (K. Campbell, University of Cincinnati College of Medicine, Cincinnati, OH), *Axin2* (B. Cheyette, University of California, San Franscisco, CA). *Mest* probe was produced in John Rubenstein's lab.

Immunohistochemistry. Immunohistochemistry was performed on frozen sections (10 or 20 μm thick) mounted on Fisher Superfrost/Plus slides. The slices were washed in PBS solution (PBS 0.1 M, pH 7.4), incubated in blocking solution (0.2% Triton X-100, 10% normal goat serum, 2% nonfat milk, 0.2% gelatin in PBS) for 1 h, and incubated 1 d at 4°C in the primary antibody diluted in 0.2% Triton X-100, 3% normal goat serum, 0.2% gelatin in PBS. For p44/42 Map kinase staining TBS was used instead of PBS. For Coup-TFI antibody, antigen unmasking procedure was performed by briefly boiling the section in sodium citrate 10 mM, pH 6. The antibodies used were as follows: monoclonal anti-βIII-tubulin antibody (clone TUJ1; Covance), 1:1000; monoclonal anti-BrdU antibody (clone B44; Becton Dickinson), 1:500; anti-phospho-p44/42 Map kinase (Thr202/Tyr204) antibody (Cell Signaling Technology) 1:100; anti-phospho-histone H3 (Ser10) (Millipore) 1:200; mouse anti-human Coup-TFI (clone H8132, Invitrogen) 1:1000; rabbit anti-Blbp (Millipore Bioscience Research Reagents) 1:1000.

For fluorescent immunohistochemistry, goat anti-rabbit Alexa-488, goat anti-mouse Alexa-594, or goat anti-rat Alexa-594 antibodies (Invitrogen), diluted at 1:300, were used. Images were acquired using a Nikon Eclipse 80i fluorescent microscope using Nikon Elements Software.

BrdU pulse analysis. Single injections of BrdU (40 mg/kg i.p.) were done following standard procedures. Animals were killed 1 h after BrdU injection. To quantify the number of cells, sections through the rostral cortex were divided in three vertical bins (200 μm^2) for counting.

Results

Spry1-2 regulate patterning in the RPC and the pallium

We began our investigation into the function of *Spry1-2* regulation of telencephalic development by analyzing their expression patterns in the embryonic stage (E) 12.5 telencephalon. As previously shown, *Spry1* and *Spry2* RNAs are expressed in the RPC along with Fgf ligands (Fig. 1*H*,*H′*; Fig. S1*A–F*, available at www.jneurosci.org as supplemental material) (Cholfin and Rubenstein, 2008). *Spry1* and *Spry2* have overlapping but distinct expression domains: *Spry2* is expressed in the core of the RPC with *Fgf8* (Fig. S1*C*,*D*, available at www.jneurosci.org as supplemental material), whereas *Spry1* expression extends rostrally and dorsally, similar

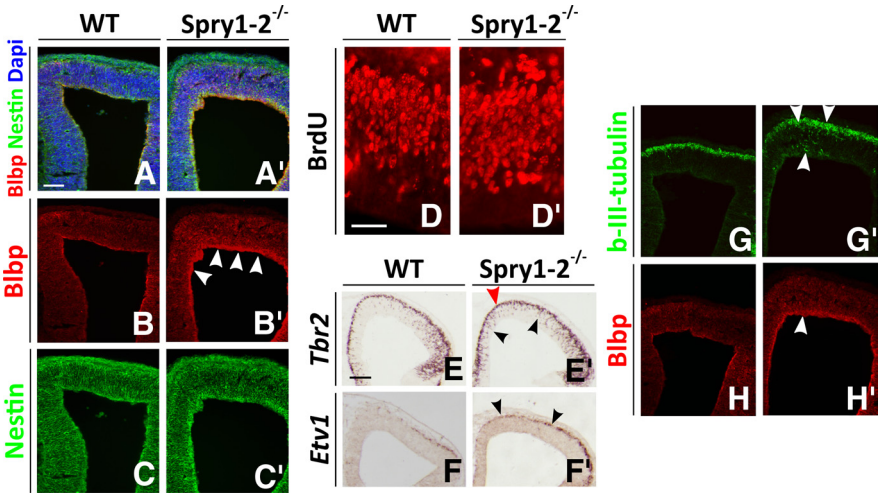

Figure 2. *Spry1-2*$^{-/-}$ shows increased proliferation, precocious progenitor cell maturation, and neurogenesis at E12.5. ***A–C′***, Immunofluorescence analysis for Blbp (***A–B′***, red), and Nestin (***A***, ***A′*** and ***C***, ***C′***, green) in the WT and *Spry1-2*$^{-/-}$ brains. Blbp, a marker for radial glia, is strongly upregulated in the dorsal/medial pallium (arrowheads in B′). ***D***, ***D′***, Short-pulse BrdU labeling of WT (***D***) and *Spry1-2*$^{-/-}$ (***D′***). BrdU was injected to pregnant females and E12.5 embryos were collected after 30′, and coronal sections were stained with anti-BrdU antibody. More BrdU^{+} cells are present in the dorsal pallium in *Spry1-2*$^{-/-}$ cortex (***D′***) than in WT (***D***). ***E***, ***E′***, RNA *in situ* hybridization for *Tbr2* in WT and *Spry1-2*$^{-/-}$ brains, showing the increase of *Tbr2* staining in preplate (red arrowhead) and in VZ/SVZ region (black arrowhead). ***F***, ***F′***, RNA *in situ* hybridization for *Etv1* in WT and *Spry1-2*$^{-/-}$ brains, showing the increase of *Etv1* staining in the preplate (arrowheads). ***G–H′***, β-Tubulin (green) and Blbp (red) immunodetection in WT and *Spry1-2*$^{-/-}$ brains. Arrowheads in ***G′*** point to increased preplate thickness in *Spry1-2*$^{-/-}$-null mice, in a region of high Blbp expression (***H′***). Scale bars: ***A–C′***, ***E–F′***, ***G–H′***, 200 μm; ***D***, ***D′***, 250 μm.

to *Fgf17* (Fig. S1*A*,*B*,*F*, available at www.jneurosci.org as supplemental material). Moreover, we detected Spry1 expression in the dorsal pallium using the Spry1^{lacz} line (Thum et al., 2008) (arrowheads in Fig. S1*A*, available at www.jneurosci.org as supplemental material).

To elucidate Spry1-2 function in telencephalic development we used *Spry1*, *Spry2*, and *Spry1-2*-null mutants. We studied the expression of genes that are reporters of Fgf signaling and are important for telencephalic development. Here we focused on the *Spry1-2* mutant because the phenotype was strong, although the single mutants (Spry1>Spry2) showed similar but less pronounced defects (Fig. S2, available at www.jneurosci.org as supplemental material).

We began by examining *Fgf8*, *Fgf15*, and *Fgf10* expression in the *Spry1-2* mutants at E12.5, and did not find obvious changes (Fig. S3, available at www.jneurosci.org as supplemental material). Next, we studied the expression of two Etv transcription factors which are positively regulated by Fgf signaling: *Etv5* (*Erm*) and *Etv4* (*Pea3*) (Münchberg et al., 1999; Raible and Brand, 2001; Roehl and Nüsslein-Volhard, 2001; Fukuchi-Shimogori and Grove, 2003; Cholfin and Rubenstein, 2008). *Etv5* and *Etv4* are expressed in the RPC and adjacent cortex similar to *Fgf* ligands and *Spry1-2* (Fig. 1*A*,*B*). In the *Spry1-2*-null mutant, *Etv5* and *Etv4* were upregulated in medial pallium and ectopically expanded in dorsal pallium (Fig. 1*A′*,*B′*, arrowheads), suggesting that Spry1-2 negatively regulate transcriptional responses to Fgf signaling in the RPC. The transcription factor *Sp8* is expressed in the RPC and the mediodorsal pallium (Fig. 1*C*). Consistent with its positive regulation by Fgf8 (Storm et al., 2006; Sahara et al., 2007; Cholfin and Rubenstein, 2008), its expression is expanded in the *Spry1-2* mutant (Fig. 1*C′*). *Mest/Peg1* expression is increased by Fgf8 signaling (Sansom et al., 2005); in agreement with this, we found upregulation of *Mest/Peg1* in the *Spry1-2* mutants (Fig. 1*D*,*D′*).

Next we examined the effect of loss of *Spry1-2* function on the expression of genes that are repressed by Fgf signaling. Both *Coup-TFI* (Fig. 1*E*,*E′*) and *FgfR3* (Fig. 1*F*,*F′*) were expressed less in the absence of *Spry1-2*. Therefore, the increase of *Evt4*, *Etv5*, and *Sp8*, along with the repression of *Coup-TFI* and *FgfR3*, strongly support the model that *Spry1-2* in the RPC negatively regulates Fgf signaling.

Finally, we investigated whether loss of *Spry1-2* function affects other signaling pathways in addition to Fgf signaling. Fgf and Wnt signaling show a reciprocal regulation during forebrain development (Shimogori et al., 2004). *Axin2* RNA expression can be used as WNT signaling readout (Jho et al., 2002). *Axin2* expression decreased in *Spry1-2*$^{-/-}$ mutants (Fig. 1*G*,*G′*), providing evidence that Spry1-2 mediate cross-regulation between Fgf and Wnt signaling.

In sum, at E12.5 *Spry1-2* have critical roles in regulating patterning in the rostral telencephalon by negatively regulating the expression of Fgf-responsive genes.

Spry1-2 regulate proliferation, differentiation, and progenitor identity in the telencephalon

Given the roles of Spry1-2 in patterning the rostral telencephalon, and the observed changes in gene expression in the VZ, we analyzed their functions in regulating proliferation and differentiation at E12.5. First, we asked whether Spry1-2 regulate maturation of the VZ. Blbp expression begins as immature neuroepithelial cells differentiate into neurogenic radial glia (Feng et al., 1994), concomitant with the initiation of neurogenesis (Anthony et al., 2004). In *Spry1-2* mutants, Blbp was strongly upregulated in the dorsal/medial pallium (Fig. 2*A–B′*, red, arrowheads), suggesting that the increased Fgf signaling promoted the transformation of neuroepithelial cells into radial glia cells (Sahara and O'Leary, 2009). On the other hand, expression of nestin (an intermediate filament present in all CNS precursors cells) did not show a marked change (Fig. 1*C*,*C′*, green), showing that *Spry1-2* negatively regulate the onset of Blbp expression in radial glia cells, suggesting that they repress their maturation.

We next evaluated whether loss of Spry1-2 function affected proliferation. We used a 30 min pulse of BrdU to label S phase progenitors in the VZ and SVZ at E12.5 (Fig. 2*D*,*D′*). Counts of BrdU labeled cells showed a statistically significant ~20% increase in the number of S-phase progenitors in the rostrodorsal pallium of *Spry1-2*$^{-/-}$ mice ($n = 3$, 76 ± 10 SD for WT, 93 ± 8 SD for *Spry1-2*$^{-/-}$, $p < 0.02$).

To test whether *Spry1-2* affected differentiation, we examined the expression of three early neuronal markers: *Tbr2*, *Etv1*, and βIII-tubulin. *Tbr2* is a T-box transcription factor expressed in the dorsal pallium beginning with the onset of neurogenesis and the appearance of intermediate (basal) progenitor cells (IPCs) (Bulfone et al., 1999; Englund et al., 2005). Conditional ablation of *Tbr2* in the developing forebrain results in the loss of IPCs and their differentiated progeny (Arnold et al., 2008; Sessa et al., 2008). We found that Tbr2's expression in *Spry1-2*$^{-/-}$ brains was increased in the VZ/SVZ (Fig. 2*E*,*E′*, black arrowheads), and the preplate (red arrowhead), suggesting that Spry1-2 negatively regulate differentiation of early neurons and basal progenitors. *Etv1* (*Er81*) is a transcription factor of the ETS family positively regu-

lated by Fgf; it has a different expression pattern from *Etv4* and *Etv5*, as it is expressed in the preplate of the ventrolateral pallium (Fig. 2*F*). In *Spry1-2*$^{-/-}$ mutant brains, *Etv1* was upregulated in the dorsal pallium (Fig. 2*F*,*F*′, arrowheads), providing evidence that Spry1-2 repress early neurogenesis. To further explore this hypothesis, we examined βIII-tubulin expression. Indeed, there was a statistically significant increase ($p < 0.02$) in the thickness of the βIII-tubulin^{+} preplate in the same region in which *Etv1* was upregulated and scattered ectopic βIII-tubulin^{+} cells in the progenitor zone (Fig. 2*G*,*G*′, arrowheads). Interestingly, this region of increased βIII-tubulin expression (Fig. 2*G*′, arrowheads) coincided with the increased Blbp expression (Fig. 2*H*,*H*′). We also analyzed Tbr1 expression in the same region, and found a similar increase, with a higher number of Tbr1^{+} cells in the Spry1-2$^{-/-}$ preplate compared to WT (Fig. S4, available at www.jneurosci.org as supplemental material).

Finally, we compared cell cycle exit in WT and Spry1-2$^{-/-}$ brains by using a 24 h BrdU pulse. We found that Spry1-2$^{-/-}$ progenitors have a higher tendency of exiting the cell cycle compared to WT (Fig. S4*A*,*A*′, quantification in *A*″, available at www.jneurosci.org as supplemental material).

In summary, our loss-of-function analysis indicates that Spry1-2 inhibit progenitor cell maturation (repressing onset of Blbp expression), proliferation, and early neurogenesis.

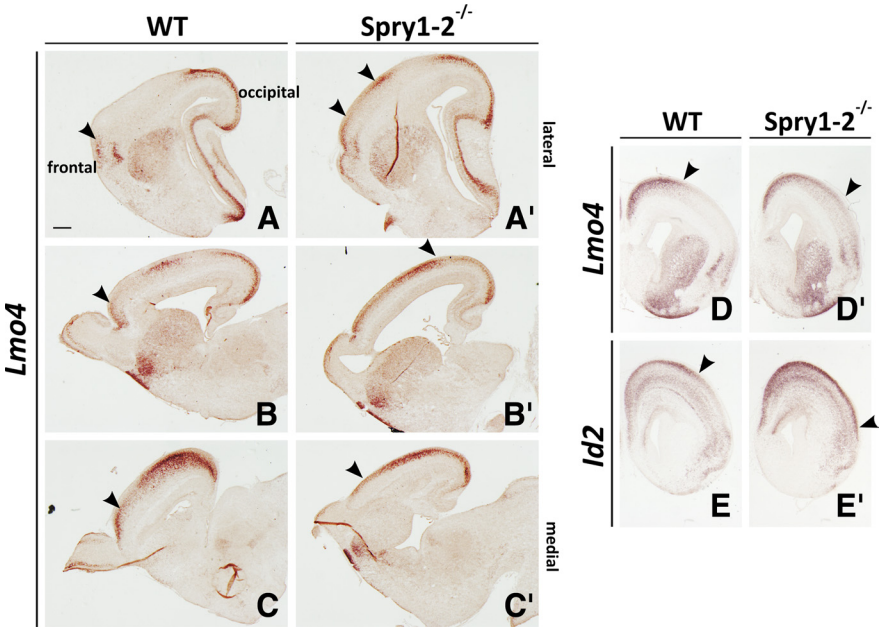

Figure 3. Frontal cortex areas are expanded in *Spry1/2*$^{-/-}$ cortex at E18.5. ***A–C′***, RNA *in situ* hybridization analysis of sagittal sections of E18.5 WT (***A–C***) and *Spry1-2*$^{-/-}$ (***A′–C′***) with *Lmo4*. ***A***, ***A′***, Lateral sections. ***B–C′***, Medial sections. Black arrowheads in ***A*** to ***C*** mark the caudal border of the frontal *Lmo4* expression domain, shifted more caudally in *Spry1-2*$^{-/-}$ brains (***A′–C′***, arrowheads). ***D–E′***, RNA *in situ* hybridization analysis of coronal sections of E18.5 WT (***D***, ***E***) and *Spry1-2*$^{-/-}$ (***D′***, ***E′***) with *Lmo4* (***D***, ***D′***) and *Id2* (***E***, ***E′***), showing the expansion of the frontal/dorsal expression domain border (arrowheads). Scale bar: 200 μm.

Frontal area expansion in Spry1/2$^{-/-}$ cortex at E18.5

Consistent with an upregulation and ectopic expression of molecular markers of the dorsomedial pallium and its progenitor pool, we found an increase in the size of frontal cortical areas. The LIM domain transcription factor *Lmo4* marks borders between frontal/motor and somatosensory areas, and somatosensory with visual areas (Bulchand et al., 2003) (Fig. 3*A*,*B*,*C*). We analyzed *Lmo4* expression by *in situ* hybridization in *Spry1-2*$^{-/-}$ at E18.5 on sagittal and coronal sections; *Lmo4* expression shifted caudally (Fig. 3*B*′) and ventrally (Fig. 3*D*′). This result was further confirmed by studying expression of *Id2*, a member of the inhibitor of DNA binding (ID) family, which is strongly expressed in layers 2/3 of rostrodorsal neocortex (Rubenstein et al., 1999) (Fig. 3*E*, arrowhead), in a domain that corresponds to the motor area. As with *Lmo4*, the *Id2*$^{+}$ domain shifted ventrally in the *Spry1-2* mutant rostral cortex (Fig. 3*E*′). Thus, *Spry1-2* mutants show rostroventral expansion of molecular features of the frontal cortex; these findings are consistent with the molecular patterning changes and increased proliferation-neurogenesis in the rostral cortical progenitor domains at E12.5 (Figs. 1, 2).

Spry1-2 are expressed in different telencephalic domains at different times

By E15.5 *Spry1-2* expression showed important changes. *Spry1* expression, analyzed by using *Spry1*^{lacz} transgenic mice (Thum et al., 2008), showed strong expression in the septum (Fig. 4*A*, arrowheads), the cortical hem-medial pallium (Fig. 4*A*, arrows), and in diencencephalic and subcortical structures. Whereas *Spry2* expression was now low in the remnant of the RPC (the septum), and its expression was robust in the cortical ventricular/subventricular zone (Fig. 4*B*, arrow), with rostrocaudal and ventrodorsal gradients. In addition, *Spry2* RNA was present in the deep layer of the cortical plate (arrowhead).

We compared *Spry1-2* expression at this stage to the pattern of Erk phosphorylation (a readout of Fgf signaling, see next paragraph). Strikingly, *Spry2* and Erk phosphorylation (pErk) followed the same gradient in the ventricular/subventricular zone (VZ/SVZ) (Fig. 4, compare *B* and *C*), consistent with hypothesis that *Spry2* and pErk expression both reflect positive responses to Fgf signaling. *Spry2* in turn would attenuate Fgf signaling and thereby may shape the gradient of MAPK activation. Finally, we compared *Spry2* and pErk expression patterns to *Coup-TFI*, a gene repressed by Fgf signaling. Intriguingly, the pattern of *Coup-TFI* expression was similar to pErk in the ventrodorsal axis, whereas it was opposite in the rostrocaudal axis.

In sum, comparing the E12.5 and E15.5 expression data suggests that *Spry1-2* may have different regional functions at different times. In the next section we studied the *Spry2*$^{-/-}$ cortical phenotype at E15.5, when *Spry2* has its ventrodorsal expression gradient in the pallial progenitors.

Spry2 negatively regulates Erk phosphorylation and proliferation and regulates dorsoventral molecular patterning of pallial progenitors

Activation of the Erk1/2 MAPK is a general response that can be mediated by all FgfRs. Sprouty family members act intracellularly to negatively regulate Fgf signaling primarily via repressive effects on the MAPK pathway (Kim and Bar-Sagi, 2004). Thus, we examined the levels of activated MAPK (phosphorylated p42-p44, pErk) in *Spry2*$^{-/-}$ using immunofluorescence. While at E12.5 we did not detect a change in Erk phosphorylation in *Spry2*$^{-/-}$ or *Spry1-2*$^{-/-}$ (data not shown), by E15.5 we did detect increased

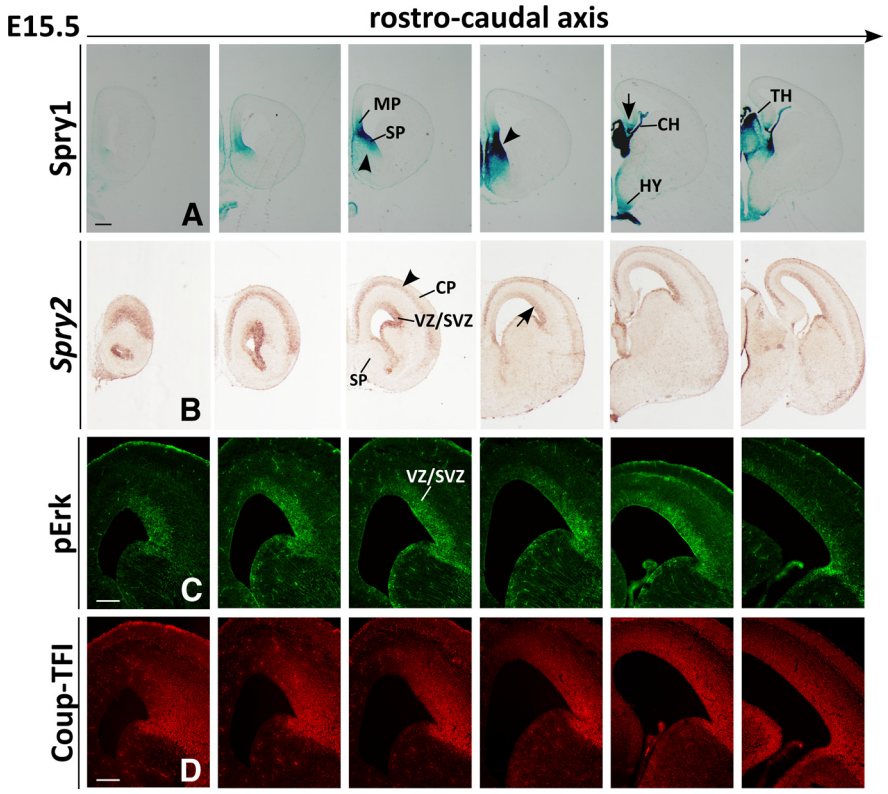

Figure 4. *Spry1*, *Spry2*, pErk, and Coup-TFI expression patterns in the telencephalon at E15.5. ***A***, Assay for β-Gal activity on coronal sections of Spry1 $^{LacZ/+}$ transgenic mice, showing Spry1 expression in the septum, medial pallium, choroid plexus, hypothalamus, and thalamus. ***B***, RNA *in situ* hybridization for *Spry2*. *Spry2* expression is prominent in the cortical ventricular zone (arrow) with a high-ventral low-dorsal gradient. Its RNA is also present in the deep layer of the cortical plate (arrowhead). ***C***, Immunofluorescence analysis of p42/p44 phosphorylation (pErk) pattern in the pallium. The immunostaining shows a high-ventral low-dorsal gradient in the VZ/SVZ similar to *Spry2* expression. ***D***, Coup-TFI immunofluorescence analysis showing the same ventrodorsal gradient as pErk and *Spry2* in the VZ/SVZ. CP, Cortical plate; VZ/SVZ, ventricular zone/subventricular zone; SP, septum; MP, medial pallium; CH, choroid plexus; TH, thalamus. Scale bar, 500 μm.

pErk levels. At E15.5 pErk $^{+}$ cortical progenitors (VZ and SVZ) were present in a ventrodorsal gradient (Figs. 4*C* and 5*A*), a pattern that resembles *Spry2* and *Coup-TFI* expression (Fig. 4). In *Spry2* $^{-/-}$, pallial VZ/SVZ pErk staining was greatly expanded (Fig. 5*A*,*A*′, arrowheads). We measured the dorsal spread of pErk expression in the dorsal pallium; there was a 77 ± 37% SD ($p < 0.03$; $n = 3$) increase in the *Spry2* $^{-/-}$ brains.

Next, we assessed whether alterations in *Spry2* dosage altered Fgf-regulated gene expression in the cortex at E15.5. We examined expression of the Fgf-responsive genes: *Blbp*, *Etv1*, and *Mest*; all three genes were expressed in the pallial VZ with a ventrodorsal gradient in a pattern similar to *Spry2* and pErk (Figs. 4*B*,*C*, 5*B*,*C*,*D*). In the *Spry2* $^{-/-}$ mutants this pallial expression increased for all three genes (Fig. 5*B*′–*D*′, arrowheads). We also tested whether Spry2 controls expression of *Coup-TFI*, a gene repressed by Fgf signaling. As at E12.5 (Fig. 1*D*,*D*′), Coup-TFI was repressed in the absence of Spry2 in the VZ and in the IZ (Fig. 5*E*,*E*′). Quantification of VZ/SVZ fluorescence intensity showed a significant decrease in Coup-TFI expression, which was most robust in the ventral pallium (Fig. 5*F*).

We then assessed whether alterations in *Spry2* dosage and Erk activation had an effect on proliferation. First, we used the M phase marker phospho-histone-3 (PH3) to quantify the mitotic index. PH3 staining labels apical VZ and basal SVZ progenitors. We quantified the number of PH3 $^{+}$ cells in the VZ (Fig. 5*G*,*G*′, arrows) and SVZ (Fig. 5*G*,*G*′, boxes). Removal of *Spry2* increased the mitotic index of SVZ progenitors [Fig. 5*G*,*G*′, boxes; quantification below (see Fig. 8*C*)] ($p < 0.05$, $n = 4$). The VZ appeared to have increased PH3 $^{+}$ cells, although this was not statistically significant [Fig. 5*G*,*G*′, arrows (see Fig. 8*B*)]. Next, to study S phase of the cell cycle, we administered BrdU 1 h before harvesting the embryos (E15.5), and counted the number of BrdU $^{+}$ cells. *Spry2* $^{-/-}$ had increased numbers of S-phase cells [Fig. 5*H*,*H*′ and quantification below (see Fig. 8*E*)] ($p < 0.005$, $n = 3$).

Quantification of VZ length in WT and *Spry1-2* $^{-/-}$ pallium showed a statistically significant increase of VZ extension in *Spry1-2* $^{-/-}$ mutants (~14 ± 6% SD, $p < 0.02$, $n = 3$) (Fig. S5, available at www.jneurosci.org as supplemental material), showing that *Spry2* loss of function caused morphological abnormalities, probably related to increased proliferation in VZ and SVZ. Moreover, in ~30% of the E15.5 brains examined we detected a thinner cortical plate in *Spry2* $^{-/-}$ cortices compared to WT.

In summary, these data show a role for Spry2 as a regulator of transcription and proliferation in the ventrodorsal pallium at the stage of mid-neurogenesis (E15.5).

Coup-TFI overexpression induces *Spry1-2* in dorsomedial cortex

We have previously shown that when *Coup-TFI* is overexpressed in dorsomedial cortex using the D6 enhancer (D6/Coup-TFI), there was a reduction in pErk (Faedo et al., 2008). Given the effects on pErk in *Spry2* $^{-/-}$, we investigated whether *Coup-TFI* overexpression was associated with changes in *Spry1-2* RNA expression at E12.5 and E15.5.

Indeed, *Coup-TFI* overexpression at E12.5 resulted in increased *Spry1-2* expression in the dorsomedial pallium (Fig. 6*A*–*B*′, arrowheads), overlapping with the area of Coup-TFI overexpression (Fig. 6*C*,*C*′). This same region showed reduced pErk immunofluorescence (on adjacent sections) (Fig. 6*D*,*D*′, arrowheads). Next, we performed the same assays at E15.5 when *Spry2* and *Coup-TFI* are expressed in the pallium with similar ventral-dorsal gradients (high-ventral low-dorsal) (Fig. 4). Whereas *Spry1* showed only small changes (Fig. 6*E*,*E*′, arrowhead), *Spry2* expression was increased in the ventral and dorsal pallium (Fig. 6*F*,*F*′, arrowhead), in the region of high Coup-TFI overexpression. pErk immunofluorescence showed a profound reduction in the region in which Coup-TFI protein was overexpressed (Fig. 6*G*–*H*′). We next examined *Coup-TFI* $^{-/-}$-null mice at E12.5 and E15.5. We did not detect changes in *Spry* expression (data not shown), suggesting that other molecules, in addition to Coup-TFI, regulate their expression. On the other hand, we found that expression of *Etv5* and *Etv1* (Fgf-activated genes) were upregulated in the *Coup-TFI* $^{-/-}$ ventral pallium (*Etv5*) and dorsal LGE (*Etv1*) at E12.5 and E15.5, respectively (Fig. S6*A*,*A*′,*C*,*C*′, available at www.jneurosci.org as supplemental material). Moreover, *FgfR3* (Fgf-repressed gene), was downregulated in *Coup-TFI* $^{-/-}$ mutants (Fig. S6*B*,*B*′, available at www.jneurosci.org as supplemental material). These data support the hypothesis that

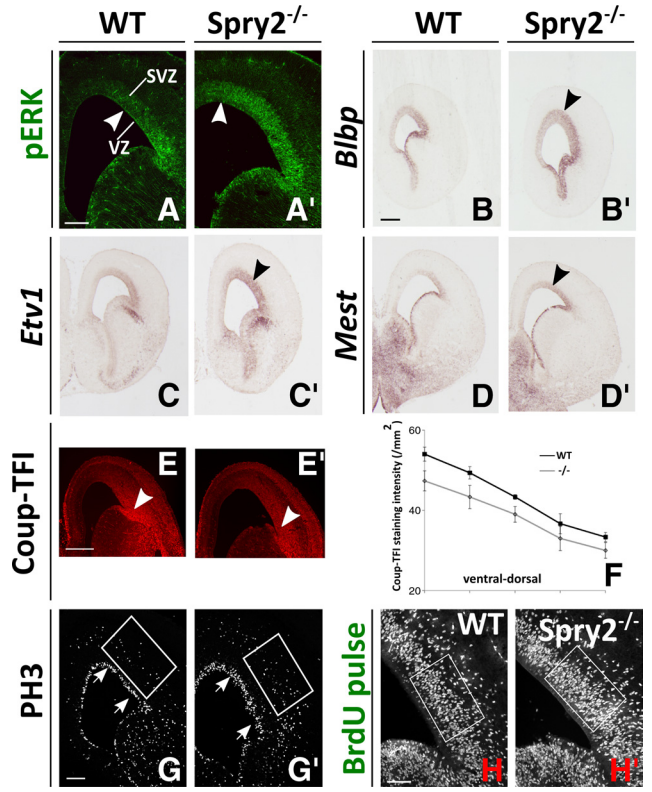

Figure 5. *Spry2* regulates Erk phosphorylation, proliferation, and progenitor identity in pallial proliferative regions at E15.5. ***A***, ***A′***, pErk immunofluorescence in WT (***A***) and *Spry2*$^{-/-}$ (***A′***) brains, showing increased Erk activation in the VZ/SVZ. ***B–D′***, RNA *in situ* hybridization for *Blbp* (***B***, ***B′***), *Etv1* (***C***, ***C′***), and *Mest* (***D***, ***D′***) in WT (***B–D***) and *Spr2*$^{-/-}$ (***B′–D′***) brains, showing the upregulation in the VZ/SVZ region for all three genes (arrowheads). ***E***, ***E′***, Coup-TFI immunofluorescence in WT (***E***) and *Spry2*$^{-/-}$ (***E′***) brains showing downregulation of Coup-TFI expression in the dorsal/ventral pallium. ***F***, Quantification of Coup-TFI staining intensity in the ventral-dorsal pallium in WT and *Spry2*$^{-/-}$ brains in the ventral-dorsal axis. ***G***, ***G′***, PH3 immunofluorescence in WT (***G***) and *Spry2*$^{-/-}$ (***G′***); PH3^{+} cell quantification for VZ (between arrows in ***G***, ***G′***) and SVZ (in the boxes in ***G***, ***G′***) is shown in Figure 8 *B*, *C*. ***H***, ***H′***, 60 min pulse BrdU labeling of WT (***H***) and *Spry1-2*$^{-/-}$ (***H′***) at E15.5. Coronal sections were immunostained with anti-BrdU antibody. More BrdU^{+} cells are present in the dorsal/ventral pallium in *Spry1-2*$^{-/-}$ cortex (***H′***) than in WT (***H***). BrdU^{+} cells quantification is shown in Figure 8 *E*. Scale bars: 500 μm.

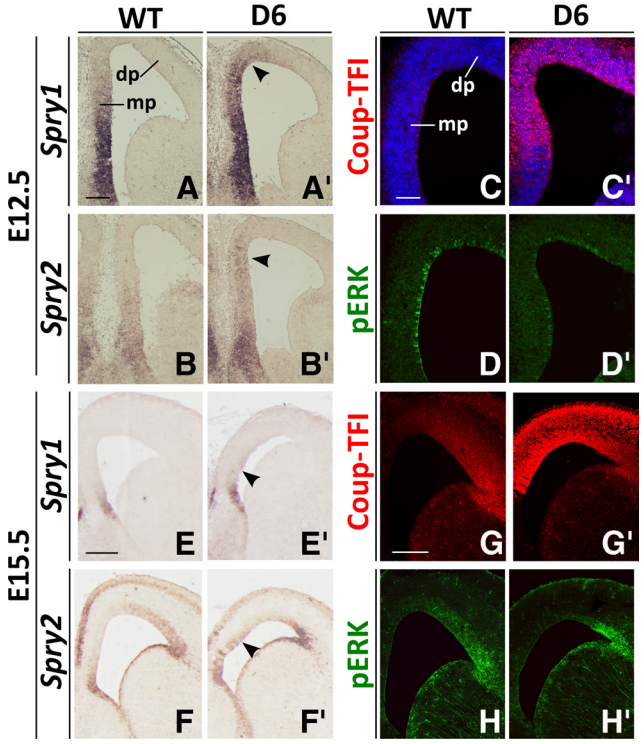

Figure 6. Coup-TFI overexpression induces Spry1-2. ***A–B′***, E12.5 RNA *in situ* hybridization for *Spry1* (***A***, ***A′***) and *Spry2* (***B***, ***B′***) in WT (***A***, ***B***) and D6/Coup-TFI (***A′***, ***B′***) telencephalons showing *Spry1-2* ectopic expression in dorsal pallium (arrowheads in ***A′*** and ***B′***). ***C–D′***, E12.5 immunofluorescence analysis of Coup-TFI (***C***, ***C′***) and pErk (***D***, ***D′***) expression in WT (***C***, ***D***) and D6/Coup-TFI (***C′***, ***D′***) telencephalons. ***C*** and ***D′*** show adjacent sections of ***A*** and ***B′***. ***E–F′***, E15.5 RNA *in situ* hybridization for *Spry1* (***E***, ***E′***) and *Spry2* (***F***, ***F′***) in WT (***E***, ***F***) and D6/Coup-TFI (***E′***, ***F′***) telencephalons showing *Spry2* ectopic expression in dorsal pallium (arrowheads in ***E′*** and ***F′***). ***G–H′***, E15.5 immunofluorescence analysis of Coup-TFI (***G***, ***G′***) and pErk (***H***, ***H′***) expression in WT (***G***, ***H***) and D6/Coup-TFI (***G′***, ***H′***) telencephalons. ***G*** and ***H′*** show adjacent sections of ***A*** and ***B′***. dp, Dorsal pallium; mp, medial pallium. Scale bars: 200 μm (***A–D′***); 500 μm (***E–H′***).

Coup-TFI negatively regulates Fgf signaling in the ventral pallium and subpallium. We suggest that increased levels of Coup-TFI in the D6-COUP-TFI mouse revealed functions of this nuclear receptor that are subtle or masked in the *Coup-TFI*$^{-/-}$ mouse, perhaps because of compensation by another gene.

Spry2 is required for Coup-TFI repression of Fgf-regulated genes

Given our evidence that Coup-TFI promotes *Spry2* expression, we assessed whether removing *Spry2* function altered the effect of overexpressing Coup-TFI in the dorsal pallium. First, we examined Erk phosphorylation; as previously shown (Faedo et al., 2008), Coup-TFI overexpression led to a striking downregulation of pErk (Fig. 7, compare *A* and *A″*). However, eliminating *Spry2* function in the D6/Coup-TFI mutants significantly rescued pErk levels (Fig. 7, compare arrows in *A″* and *A‴*). We measured the length of pErk^{+} domain in the dorsal pallium, and found a 92 ± 39% SD ($p < 0.005$, $n = 3$) increase in the *Spry2*$^{-/-}$;D6/Coup-TFI brains compared to D6/Coup-TFI.

Next, we investigated the expression of the Fgf- and Spry-responsive genes *Etv1*, *Blbp*, and *Mest*. When Coup-TFI was overexpressed, we observed greatly reduced dorsal expression of *Etv1* and *Mest* (Fig. 7*B″*,*D″*, arrowheads) and a reduction of VZ/SVZ thickness (*Blbp*) (Fig. 7*C″*). The introduction of the *Spry2*-null allele into the D6/Coup-TFI line rescued *Etv1*, *Mest* and *Blbp* expression levels in the dorsal pallium (Fig. 7, compare arrowheads in *B″* and *B‴*; *C″* and *C‴*; and *D″* and *D‴*).

Thus, these experiments show that increased levels of Coup-TFI require *Spry2* to reduce cortical Fgf signaling in the dorsal pallium.

Loss of Spry2 rescues proliferation defects in D6/CoupTFI at E15.5

We have previously shown that Coup-TFI promotes cell cycle withdrawal and neuronal differentiation (Faedo et al., 2008). Taking into account the decrease of pErk and the upregulation of *Spry2* (Fig. 6) in D6/Coup-TFI, we investigated whether *Spry2* played a role in regulating proliferation of cortical progenitors when *Coup-TFI* is overexpressed. We used the M phase marker PH3 to quantify the mitotic index. Quantification of PH3^{+} cells showed that removal of *Spry2* in the D6/Coup-TFI cortex largely rescued the VZ and SVZ proliferation defects due to *Coup-TFI* overexpression (Fig. 8*A″*,*A‴*; quantification in *B* and *C*) ($p < 0.05$, $n = 4$).

Next, to study S phase of the cell cycle, we administered BrdU 1 h before harvesting the embryos (E15.5), and counted the number of BrdU^{+} cells in the combined VZ and SVZ. Eliminating

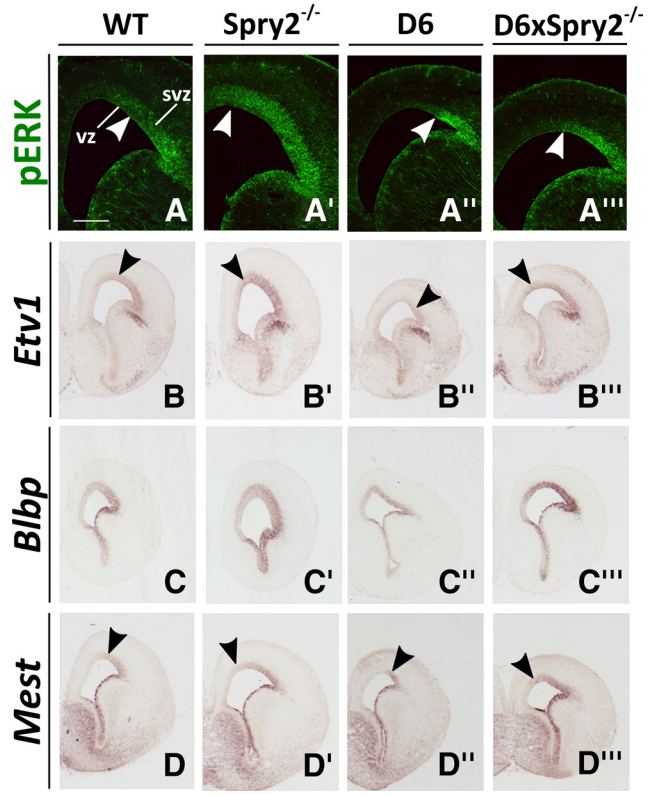

Figure 7. *Spry2*$^{-/-}$ rescues gene expression defects in E15.5 Coup-TFI overexpressing mice. ***A–A‴***, Immunofluorescence at E15.5 showing phosphorylated Erk in WT (***A***), *Spry2*$^{-/-}$ (***A′***), D6/Coup-TFI (***A″***), and D6/Coup-TFI;*Spry2*$^{-/-}$ (***A‴***) (***A*** and ***A′*** are the same panels as in Fig. 5; all the genotypes are littermates). pErk phenotype in *Spry2*$^{-/-}$ is described in Figure 5. Coup-TFI overexpression downregulates Erk activation (***A″***); introduction of the *Spry2*-null allele into the D6/Coup-TFI genotype significantly rescued Erk phosphorylation (***A‴***). Arrows in ***A–A‴*** point to the dorsal extent of high pErk staining. We set the WT length to 100%, and we measured pErk levels in the different genotypes. On average, *Spry2*$^{-/-}$ showed a 177 ± 30% SD increase, D6/Coup-TFI showed a reduction of 50 ± 11% SD, whereas the D6/Coup-TFI; *Spry2*$^{-/-}$ showed levels similar to WT (93 ± 20% SD). ***A–D‴***, RNA *in situ* hybridization at E15.5 for *Etv1* (***B–B‴***), *Blbp* (***D–D‴***), and *Mest* (***C–C‴***) in WT (***B–D***), *Spry2*$^{-/-}$ (***B′–D′***), D6/Coup-TFI (***B″–D″***), and D6/Coup-TFI;*Spry2*$^{-/-}$ (***B‴–D‴***). Fgf positively regulated genes were upregulated in *Spry2*$^{-/-}$ telencephalons (as described in Fig. 5; arrowheads in ***B′–D′*** mark the increase in expression level), and downregulated in D6/Coup-TFI (arrowheads in ***B″–D″*** mark the decrease in expression level). The gene expression defects in D6/Coup-TFI were rescued by the *Spry2*$^{-/-}$ allele (arrowheads in ***B‴–D‴*** point to the increase in expression level). VZ, Ventricular zone; SVZ, subventricular zone. Scale bar, 500 μm.

Spry2 function in the D6/Coup-TFI;Spry2$^{-/-}$ compound mutant partially rescued the number of cells in S phase (Fig. 8*D″*,*D‴*; quantification in *E*) ($p < 0.005$, $n = 3$).

Thus, our data show that *Spry2* negatively regulates cortical progenitor proliferation, and that Coup-TFI's repression of proliferation is largely mediated by Spry2 (Fig. S7, available at www.jneurosci.org as supplemental material).

Discussion

Here we show that Spry1 and Spry2 have distinct early and late functions in cortical development. At early stages (E12.5), Spry1 and Spry2 function together as negative regulators of Fgf signaling from the RPC to control patterning, proliferation, and differentiation of the rostral cortex. By E15.5, Spry2 has a function in regulating patterning and proliferation of the ventrolateral pallium. Finally, we present evidence that Coup-TFI promotes *Spry* expression, which mediates some of its Fgf-repressive properties (inhibition of Erk phosphorylation, proliferation, and Etv gene transcription).

Spry1-2 regulate molecular patterning of rostral cortical progenitors

Fgf signaling emanating from ligands produced by the RPC is perhaps the most important mechanism for specifying rostral identity of the cortex (Fukuchi-Shimogori and Grove, 2003; Garel et al., 2003; Storm et al., 2006; Cholfin and Rubenstein, 2007, 2008).

We found that *Spry1-2* expression in the RPC (through E12.5) negatively regulated several aspects of Fgf signaling in cortical progenitors. *Spry1-2*$^{-/-}$ mutants showed increased rostral molecular properties, based on increased *Etv4*, *Etv5* and *Sp8* expression, and reduced expression of *Coup-TFI* and *FgfR3* (Fig. 1). In addition, the *Spry1-2*$^{-/-}$ mutants showed reduced *Axin2* expression (Fig. 1) indicative of Fgf's antagonistic effect on Wnt signaling in the forebrain (Fukuchi-Shimogori and Grove, 2003; Shimogori et al., 2004; Storm et al., 2006). Furthermore, reduced Wnt signaling is associated with increased cortical neurogenesis (Machon et al., 2007), as found at early stages in *Spry1-2*$^{-/-}$ mutants (Fig. 2). Thus, as at the midbrain-hindbrain patterning center (Basson et al., 2008), *Spry1-2* have key functions in early forebrain patterning. Ongoing studies are aimed at elucidating *Spry1* and *Spry2*'s individual and combined functions in the RPC on development of the septum and adjacent rostral telencephalic structures, which also show molecular phenotypes (e.g., increased Blbp and Mest expression in the septum and rostral LGE) (Fig. 2).

Spry1-2 inhibit neurogenesis and progenitor cell maturation

A recent paper demonstrated that *Fgf10* expression in cortical progenitors contributes to the maturation of neuroepithelial cells into radial glia progenitors (Sahara and O'Leary, 2009). Our results may explain why this study reported that *Fgf10*$^{-/-}$ mutants have a delay in radial glia cells formation that is biased for the rostral cortex (Sahara and O'Leary, 2009). *Spry1-2*, whose early expression is concentrated in rostral regions at early time points (Fig. S1, available at www.jneurosci.org as supplemental material), are excellent candidates for being negative feedback regulators of Fgf10-induced radial glia maturation. Thus, perhaps reducing *Spry* dosage in *Fgf10*$^{-/-}$ mutants would rescue their phenotype.

Consistent with this finding, we found that *Spry1-2* inhibit radial glia maturation, based on increased Blbp expression (Fig. 2). Onset of Blbp is strictly correlated with the start of neurogenesis and radial glia directed neuronal migration, and almost all neurons in the mouse brain derive from Blbp^{+} radial glia (Anthony et al., 2004). In *Spry1-2*$^{-/-}$ mutants the region of Blbp upregulation has increased neuronal differentiation based on increased numbers of basal progenitors (Tbr2^{+}), and increased numbers of preplate neurons (βIII-tubulin^{+}, *Tbr1*$^{+}$ and *Etv1*$^{+}$) (Fig. 2). These findings show that Spry function is required to inhibit progenitor maturation in the dorsal/medial pallium.

Finally, loss of *Spry1-2* function caused an expansion of cortical areas with rostral identity, as shown by the expression of *Lmo4* and *Id2* (Fig. 3). Thus, Spry1-2 negatively regulate Fgf-driven frontal (motor) area specification by modulating progenitor cell identity and differentiation.

Later *Spry* functions in cortical development

While at early developmental stages (through E12.5) *Spry1* and *Spry2* are coexpressed in the RPC, these genes show distinct telencephalic expression patterns by E15.5: *Spry1* is expressed in the septum and cortical hem and *Spry2* in the cortical VZ and cortical plate (Fig. 4). Moreover, at E15.5 *Spry2* has an expression pattern similar to pErk, characterized by a ventrodorsal gradient. At ear-

lier stages (E12.5) pErk is observed throughout the telencephalic VZ; at this stage we could not detect changes in Erk activation in *Spry1-2*$^{-/-}$ brains, suggesting that the *Spry* genes have a stronger role in Erk activation at later developmental stages. Indeed, at E15.5 *Spry2*$^{-/-}$ mutants showed marked upregulation of pErk (Fig. 5), supporting a model that Spry genes have different effects on Erk activation in different spatial and temporal developmental contests.

Thus, here we demonstrated that at E15.5 *Spry2* regulates proliferation, Fgf signaling and molecular patterning in the pallial progenitor zone. *Spry2*$^{-/-}$ mutants showed increased proliferation (particularly in the SVZ), pErk levels and expression of *Blbp*, *Mest* and *Etv1* (Figs. 6, 7). This demonstrates a role for Spry2-mediated inhibition of Fgf signaling within the cortical progenitors.

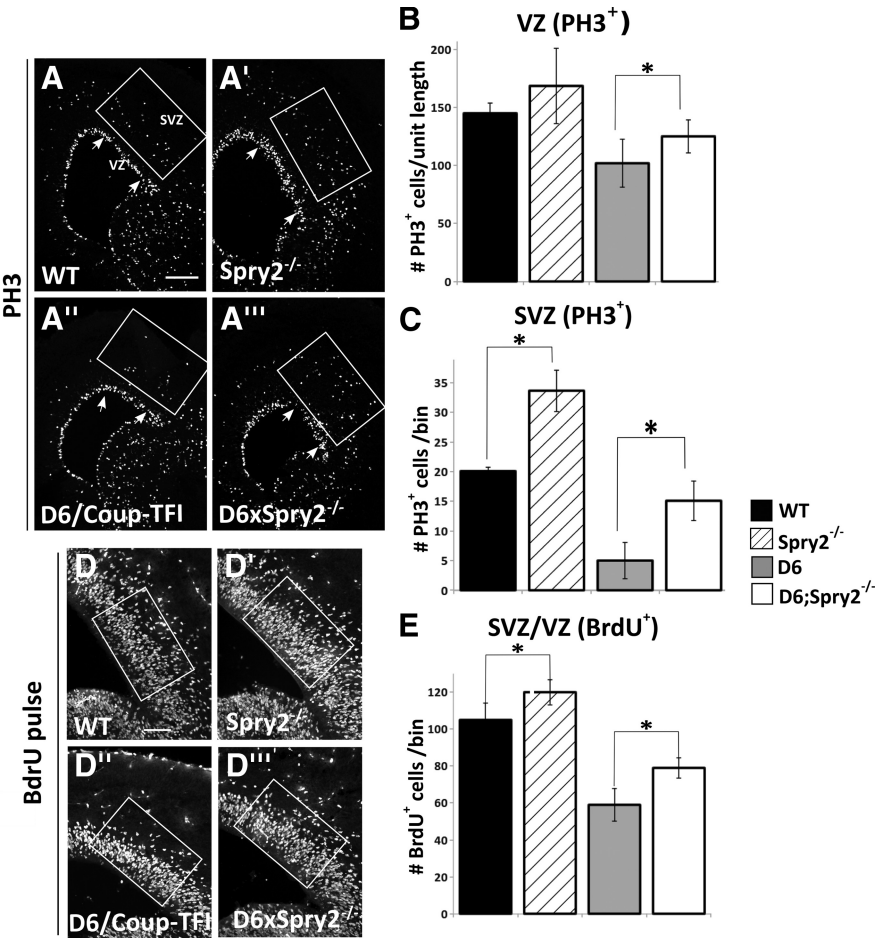

Figure 8. *Spry2* rescues the Coup-TFI-induced proliferation defects at E15.5. ***A–A'''***, PH3 immunofluorescence in WT (***A***), *Spry2*$^{-/-}$ (***A'***), D6/Coup-TFI (***A''***), and in the compound mutant D6;*Spry2*$^{-/-}$ (***A'''***) (***A***, ***A'*** and ***D***, ***D'*** are the same in Fig. 5*G*,*G'*,*H*,*H'*; all the genotypes are littermates). ***B***, Quantification of PH3^{+} cells in the VZ (cells counted in the region between the arrows in ***A–A'''*** and normalized per unit length, 1 mm). No statistically significant difference between WT and *Spry2*$^{-/-}$ were found, although there was a tendency toward an increase ($p = 0.2$, unpaired Student's test, $n = 4$). A statistically significant difference was found between D6/Coup-TFI and the compound mutant D6/Coup-TFI; *Spry2*$^{-/-}$ (unpaired Student's test, $p < 0.005$, $n = 4$). D6/Coup-TFI showed a reduction in PH3^{+} cells as previously reported (unpaired Student's test, $p = 0.01$, $n = 4$). ***C***, Quantification of PH3^{+} cells in the SVZ (cells counted in the boxed region in ***A–A'''***, and normalized for bin, 1 mm^2). A statistically significant difference was found between all the genotypes (WT-*Spry2*$^{-/-}$ $p < 0.05$, WT-D6/Coup-TFI $p < 0.05$, D6/Coup-TFI-D6/Coup-TFI;*Spry2*$^{-/-}$ $p < 0.02$, unpaired Student's test, $n = 4$). ***D–D'''***, BrdU immunofluorescence after 1 h BrdU pulse at E15.5 in WT (***D***), *Spry2*$^{-/-}$ (***D'***), D6/Coup-TFI (***D''***), and in the compound mutant D6;*Spry2*$^{-/-}$ (D'''). ***E***, Quantification of BrdU^{+} cells (cells counted in the boxed region in ***D–D'''***, and normalized for bin, 1 mm^2). A statistically significant difference was found between all the genotypes (WT-*Spry2*$^{-/-}$ $p < 0.005$, WT-D6/Coup-TFI $p < 0.005$, D6/Coup-TFI-D6/Coup-TFI;*Spry2*$^{-/-}$ $p < 0.005$, unpaired Student's test, $n = 3$). VZ, Ventricular zone; SVZ, subventricular zone. Scale bar, 500 μm.

Coup-TFI negatively regulates Fgf signaling through Sprouty

The Coup-TFI orphan nuclear receptor has antagonistic functions to Fgf8 and Fgf17. Coup-TFI represses rostrodorsal cortical identity (Armentano et al., 2007; Faedo et al., 2008), and reduces pErk and pAkt levels while promoting cell cycle arrest and neurogenesis (Faedo et al., 2008). We presented several lines of evidence that Coup-TFI performs some of these functions (reducing pErk levels and promoting cell cycle arrest) at least in part through promoting *Spry* expression when Coup-TFI is overexpressed.

By using Coup-TFI gain-of-function experiments in the dorsal pallium (using the D6/Coup-TFI allele), we found that Coup-TFI can induce expression of *Spry2* and to a lesser extent *Spry1* (Fig. 6). Furthermore, removing *Spry2* expression in the D6/Coup-TFI mutants partially rescued several of D6/Coup-TFI's prominent phenotypes, including decreased proliferation, and reduced expression of pErk, *Blbp*, *Mest*, and *Evt1* (Figs. 7, 8). Thus, *Spry2* function contributes to many of the phenotypes caused by *Coup-TFI* overexpression. An important question is whether the overexpression data are relevant for the physiological *Coup-TFI* expression in the ventral pallium. We analyzed *Coup-TFI*$^{-/-}$-null mice, and while we did not detect changes in *Spry* expression, we found that Coup-TFI alters the expression of three Fgf-regulated genes, *Etv5*, *FgfR3*, and *Etv1* in the ventral pallium and subpallium (Fig. S6, available at www.jneurosci.org as supplemental material). In particular, it is interesting to note that *Etv5* is upregulated and *FgfR3* downregulated at E12.5, in the ventral pallium of both *Spry1-2*$^{-/-}$ (Fig. 1) and *Coup-TFI*$^{-/-}$ mutants (Fig. S6, available at www.jneurosci.org as supplemental material).

The apparently unchanged *Spry2* expression in the *Coup-TFI*$^{-/-}$ mice may reflect the contribution of other transcription factors that can drive *Spry2* expression. Thus, we propose that COUP-TFI overexpression has revealed Coup-TFI functions that are masked in the *Coup-TFI*$^{-/-}$ mutant by compensation.

Together, these data support the hypothesis that Coup-TFI negatively modulates Fgf signaling in the telencephalon, at least in part through promoting *Spry* expression.

Model of Sprouty regulation of cortical patterning

We propose models of Spry regulation of early and late cortical patterning (Fig. S7, available at www.jneurosci.org as supplemental material). At early stages (through E12.5) *Spry1-2* expression in the RPC cooperates to repress Fgf signaling in the rostral cortex, thus controlling *Etv* genes and *Coup-TFI/FgfR3*. Moreover, *Spry1-2* inhibit progenitor maturation: in their absence, there is an increase in radial glial marker expression (Blbp), pro-

liferation, and early differentiation. Later in development (by E15.5), *Spry2* is expressed in cortical progenitors of the ventrolateral pallium, in which it represses Erk activation and proliferation and regulates expression of Fgf-responsive genes. Thus, Spry regulation of Fgf signaling from the RPC and the ventral pallium has potent and temporally distinct roles in modulating cortical development.

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