A disrupted balance between Bmp/Wnt and Fgf signaling underlies the ventralization of the Gli3 mutant telencephalon

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Abstract

Regionalization of the neural plate and the early neural tube is controlled by several signaling centers that direct the generation of molecularly distinct domains. In the developing telencephalon, the anterior neural ridge (ANR) and the roof and floor plate act as such organizing centers via the production of Fgfs, Bmps/Wnts, and Shh, respectively. It remains largely unknown, however, how the combination of these different signals is used to coordinate the generation of different telencephalic territories. In the present study, we report on telencephalic development in Pdn mutant mice, which carry an integration of a retrotransposon in the Gli3 locus. Homozygous mutant animals are characterized by a partial dorsal-to-ventral transformation of the telencephalon and by an increased size of the septum. On a molecular level, these alterations correlate with a reduction and/or loss of Bmp/Wnt expression and a concomitant expansion of Fgf8 transcription. Finally, we provide evidence that the ectopic activation of Fgf signaling in the dorsal telencephalon provides an explanation for the ventralization of the Gli3 mutant telencephalon as application of Fgf8-soaked beads to dorsal telencephalic explants led to the specific induction and repression of ventral marker and dorsal marker genes, respectively. © 2003 Elsevier Inc. All rights reserved.

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Introduction

During neurogenesis, the generation of hundreds of different neuronal cell types has to be temporally and spatially controlled to allow for the formation of a functional nervous system. As a framework for understanding the molecular mechanisms underlying this process, it is thought that the nervous system is organized along two principal axes: an anteroposterior (longitudinal) and a dorsoventral (transverse) axis. Based on this concept of a simple Cartesian coordinate system, neural progenitor cells acquire regional identity and differentiate accordingly. In recent years, enormous advances have been made in deciphering the molecular mechanisms underlying pattern formation along both axes. However, the coordination of both processes remains poorly understood. The developing telencephalon provides an ideal model system for such an analysis as it develops from the rostral end of the neural plate at the anterior neural ridge (ANR) (Couly and LeDouarin, 1985, 1987; Eagleson and Harris, 1990; Eagleson et al., 1995; Rubenstein et al., 1998). The ANR and its related structure in the zebrafish embryo, the anterior neural boundary (ANB), direct development of the forebrain and of adjacent tissues (Shimamura and Rubenstein, 1997; Houart et al., 1998). Gain-of-function experiments implicate Fgf8 in mediating at least some of its effects (Shimamura and Rubenstein, 1997). Moreover, loss of Fgf8 function either in zebrafish (Shanmugalingam et al., 2000) or in mice (Meyers et al., 1998; Ye et al., 1998) leads to a reduction of the forebrain and to a downregulation of some basal telencephalic markers. Suppression of the development of the basal telencephalon is also observed after interference with Fgf signaling in zebrafish (Shinya et al., 2001).

Along the dorsal/ventral axis, the telencephalon is organized into ventral subpallial and dorsal pallial domains which will give rise to the basal ganglia and to the cerebral cortex, respectively, structures which differ in their mor-
phology, axonal connectivity, and neurotransmitter synthesis. An early step in the formation of these distinct territories involves the acquisition of different molecular characteristics. Expression of the *Emx* genes is restricted to dorsal telencephalic progenitors, while ventral progenitors are characterized by the expression of different classes of homeobox genes, namely *Nkx2.1* and members of the *Dlx* gene family (Wilson and Rubenstein, 2000). In addition, the proneural genes *Mash1* and *Ngf2* play important roles in the development of the ventral and dorsal telencephalon, respectively (Casarosa et al., 1999; Fode et al., 2000; Perras et al., 2002; Yun et al., 2002).

Specification of these different dorsal and ventral cell types is under the control of several signaling centers. The expression patterns of several members of the *Bmp* and *Wnt* gene families in the roof plate suggest a role for these factors in the specification of dorsal telencephalic cell types (Furuta et al., 1997; Hebert et al., 2002). Direct evidence for such a role has been obtained by recent knock-out experiments. Mice with either targeted *Wnt3a* or *Left1* alleles, which encodes a transcriptional regulator of the Wnt signaling pathway, show similar defects in the development of the hippocampus (Galceran et al., 2000; Lee et al., 2000). In addition, Bmps and Wnts cooperate in the transcriptional regulation of the dorsal-specific marker *Emx2* (Theil et al., 2002b).

Sonic Hedgehog (*Shh*) was shown to be both necessary and sufficient for the specification of ventral telencephalic cell types. Shh sequentially induces ventral neurons with patterns of gene expression characteristic of the medial and lateral ganglionic eminences (Ericson et al., 1995; Shimamura et al., 1997; Kohtz et al., 1998), while ablation of *Shh* leads to a loss of both structures and to an expanded expression domain of the dorsal-specific marker *Emx1* (Chiang et al., 1996; Ohkubo et al., 2002). *Gli1,-2*, and *-3* encode zinc finger transcription factors, which as mediators of the Shh signaling pathway have been implicated in a number of patterning events during embryogenesis. The best evidence to date for an involvement of *Gli* genes in telencephalic patterning was obtained from the analysis of the *extra-toes* (*Xt*) mouse mutant in which a deletion removes the 3′ end of the *Gli3* gene (Büscher et al., 1998). *Extra-toes* homozygous mutant embryos lack the choroid plexus and the hippocampus, the dorsal most structures of the telencephalon (Johnson, 1967; Franz, 1994; Grove et al., 1998; Theil et al., 1999). These defects correlated with a loss of *Bmp* and *Wnt* expression in the rostrocaudal midline (Theil et al., 1999; Tole et al., 2000) and a concomitant expansion of *Fgf8* expression (Theil et al., 1999; Aoto et al., 2002). In addition, loss of *Emx* gene expression indicated a failure to correctly specify the dorsal telencephalon (Theil et al., 1999; Tole et al., 2000), which subsequently acquires partial ventral characteristics (Tole et al., 2000). The different contributions of the altered *Bmp*/*Wnt* and *Fgf8* expression patterns to this ventral transformation remained to be determined. Unlike this dorsoventral patterning defect in *Xt*/*Xt* embryos, different ventral and dorsal expression domains are established in mutants lacking both *Gli3* and Hedgehog signaling (Aoto et al., 2002; Rallu et al., 2002). These data have been taken to suggest the existence of a yet unknown, Hedgehog-independent pathway which directs patterning of the dorsoventral telencephalon in the absence of *Shh* and *Gli3* (Rallu et al., 2002).

Here, we use a combined genetic and in vitro approach to further study the requirements of *Gli3* for dorsoventral patterning of the telencephalon. We started to analyze telencephalic development in *Pdn* (Polydactyly Nagoya) mutant mice (Schimmang et al., 1994), which carry an integration of a retrotransposon within the *Gli3* locus (Thien and Rüther, 1999). Unlike the *Xt* allele, the *Pdn* allele still produces wildtype *Gli3* transcript though at reduced levels and therefore probably represents a hypomorphic mutation. By intercrossing with *Xt* mutant mice, we were further able to generate an allelic *Gli3* series in which we compared the effect of the different *Gli3* mutations on telencephalic development. We observed a progressive dorsal-to-ventral transformation of the telencephalon which correlated with the extent of ectopic *Fgf8* expression. Furthermore, we provide evidence that ectopic activity of *Fgf8* in a dorsal telencephalic explant culture system induces ventral gene expression and represses the transcription of dorsal marker genes independently of Shh signaling. These results further emphasize the central role of Gli3 in telencephalic development and provide evidence for Fgf8 acting in a Shh-independent pathway in the control of dorsal/ventral patterning of the telencephalon.

**Materials and methods**

**Mice**

*Xt* and *Pdn* heterozygous animals were kept in a mixed C57B16/C3H and C3H/He background, respectively, and were interbred. Embryonic (E) day 0.5 was assumed to start at midday of the day of the vaginal plug discovery. *Xt*/*Xt*, *Xt*/*Pdn*, and *Pdn*/*Pdn* embryos were readily distinguished from heterozygous and wild-type embryos by forebrain and/or limb morphology (Schimmang et al., 1994; Theil et al., 1999). Embryos younger than E12.5 were additionally classified by PCRs on yolk sac DNA (Maynard et al., 2002).

**In situ hybridization and immunohistochemistry**

Whole-mount in situ hybridizations and expression analysis on cryosections were performed as described previously (Theil et al., 2002a) by using the following riboprobes: *Emx1* and *Emx2* (Simeone et al., 1992), *Pax6* (Walther and Gruss, 1991), *Ngf2* (Gradwohl et al., 1996), *Mash1* (Guillemot and Joyner, 1993), *Dlx2* (Bulfone et al., 1993), *Dlx5* (Simeone et al., 1994), *Iis1* (Tsuchida et al., 1994), *Nkx2.1* (Lazzaro et al., 1991), *Wnt2b* (Grove et al., 1994), *Shh* (Aoto et al., 2002), and *Ncad* (Grove et al., 1994). The expression of *Gli3* was confirmed by using DNA extracted from adult forebrain and by immunohistochemistry.
Bryos with marker genes which showed altered expression development, we first performed an in situ hybridization analysis on whole dissected brains of E10.5 to E12.5 embryos with marker genes which showed altered expression domains in Xr/t homozygous embryos. A severe reduction and/or loss in Emx1/2 transcription represented a major defect in the development of the Xr/t/Xr/t forebrain (Theil et al., 1999; Tole et al., 2000). We therefore determined the effects of the Pdn mutation on the expression of these dorsal-specific marker genes. While Emx1 expression was completely abolished in Pdn/Pdn and in Pdn/Xr/t embryos already at E10.5 (Fig. 1A–C), Emx2 transcription appeared to be reduced in both genetic backgrounds with a more severe reduction in Pdn/Xr/t embryos (Fig. 1D–F). In addition, in the most affected Pdn/Xr/t embryos, we were able to detect even a loss of Emx2 expression in the rostral most dorsal telencephalon (data not shown). Thus, the Pdn mutation as well as the Xr/t mutation both lead to a reduction and/or loss of Emx1/2 transcription in the dorsal telencephalon. Interestingly, the effect on Emx2 transcription correlates with the abundance of functional Gli3 protein.

Next, we analyzed whether the Pdn mutation similarly affects the expression of other dorsal telencephalic markers. Pax6 and the proneural gene Ngn2 are expressed throughout the dorsal telencephalic neuroepithelium of control E10.5 to E12.5 mice. Expression of both genes appears unaltered in Pdn homozygous embryos (compare Fig. 1G, H, K, L; and data not shown). In contrast, while Pax6 and Ngn2 expression was unaffected at the earlier stages analyzed (data not shown), the Pdn/Xr/t forebrain showed reduced expression levels for these genes in the rostral most dorsal telencephalon at E12.5 in a domain which appeared to coincide with the region already lacking Emx2 expression 1 day earlier. In addition, expression in the dorsomedial telencephalon appeared patchy (Fig. 1i and M). Interestingly, both alterations were even pronounced in Xr/t/Xr/t embryos (Fig. 1J and N). Therefore, the early defects in Emx expression are followed by later alterations in Pax6 and Ngn2 expression.

The partial and/or complete loss of dorsal characteristic gene expression from the Gli3 mutant telencephalon suggested that these alterations might be accompanied by an ectopic activation of ventral telencephalic marker gene expression as observed in Pax6 and Ngn2 mutant mice (Fode et al., 2000; Stoykova et al., 2000; Toresson et al., 2000; Yun et al., 2001). To address this possibility, the expression patterns of several ventral telencephalic-specific genes were examined in Gli3 mutants. In wild-type E12.5 embryos, the homeobox gene Dlx2 and the proneural gene Mash1 are expressed in the ventral telencephalon (Fig. 2A and E). Although the expression of these genes remained restricted to the basal telencephalon of Pdn homozygous animals (Fig. 2B and F), Pdn/Xr/t and to a larger extent Xr/t/Xr/t forebrains showed an upregulation of both markers in the dorsal telencephalon. Interestingly, this ectopic expression domain predominantly formed along the dorsomedial midline (Fig. 2C, D, G, and H). Unlike these markers, however, expression of the Shh-inducible homeobox gene Nkx2.1 remained confined to the medial ganglionic eminence (MGE) (Fig. 2Q–T).

The above results demonstrate that Gli3 is required to
To determine whether the upregulation of ventral-specific markers in the dorsal Gli3 mutant telencephalon at E12.5 is maintained later in development, we analyzed the expression patterns of different dorsal/ventral marker genes at E14.5. Interestingly, sectioning of control and mutant brains revealed an enlargement of the septal region in Gli3 mutants with a corresponding decrease in the size or even a loss of the medial telencephalic wall which will give rise to the hippocampus (compare Fig. 3A–D). While only subtle effects on Pax6 and Ngn2 expression were observed at E12.5 in Gli3 mutants, expression of both genes is drastically reduced from the ventricular zone (VZ) of the E14.5 dorsal telencephalon. Moreover, the Pdn/Pdn forebrain, which appeared phenotypically normal at E12.5, also showed this loss of Pax6 and Ngn2 expression in E14.5 dorsal progenitor cells (Fig. 3F). In the lateral cortex of all Gli3 mutants, expression of both genes was drastically reduced, but the size of this expression domain varied with the genetic background being smallest in Xr1 homzygous embryos (Fig. 3A–H). In addition, Pax6-positive neurons were
observed in the dorsomedial telencephalon of all Gli3 mutants (Fig. 3B–D). In contrast to this loss of dorsal-specific marker gene expression, the ventral progenitor markers Mash1 and Dlx2 were ectopically expressed in the VZ of the dorsomedial telencephalon (Fig. 3I–P). Interestingly, comparison of adjacent sections indicated that the complementary expression of Mash1 and Ngn2 as observed in control embryos was still maintained in the mutant telencephali. Taken together, this analysis therefore suggests a progressive loss of dorsal characteristics and a concomitantly increase in ventral-specific gene expression in the dorsal telencephalon of Gli3 mutants.

Loss of Bmp/Wnt expression and ectopic Fgf signaling in the dorsal midline of the Gli3 mutant telencephalon

Given this partial dorsal-to-ventral transformation of the Gli3 mutant telencephalon, we were interested in identifying the underlying molecular mechanisms. We and others previously described the loss of Bmp and Wnt expression from the Xr’/Xr’ telencephalon (Grove et al., 1998; Theil et al., 1999, 2002b; Tole et al., 2000), suggesting that the balance between dorsalizing and ventralizing signals is disrupted in the Gli3 mutant telencephalon. We therefore analyzed the expression of members of these gene families as well as that of Shh in the forebrain of the various Gli3 mutants. While between E10.5 and E12.5 Shh expression and that of its target gene Nkx2.1 was unaltered in all mutants (Fig. 2Q–T; and data not shown), we were unable to detect Wnt3a and Bmp4/6 expression in the telencephalon of either E10.5 Pdn homozygous or Pdn/Xr’ embryos (data not shown). In the case of Pdn/Pdn embryos, however, expression of Wnt2b, Wnt3a, and Bmp4 in the cortical hem partially recovered at E12.5 (Fig. 4B and E; and data not shown), although Bmp4 expression appeared rather weak and diffuse (Fig. 4E). Furthermore, a Wnt2b signal was only

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Fig. 2. Induction of ventral-specific marker genes in the dorsal telencephalon of E12.5 Gli3 mutants. Lateral view of (A, E, I, M, Q) wildtype; (B, F, J, N, S) Pdn/Pdn; (C, G, K, O, T) Pdn/Xt J; and (D, H, L, P, R) Xt J /Xt J brains. Mash1 (A, B) and Dlx2 (E, F) expression are confined to the ventral telencephalon of wildtype and Pdn/Pdn forebrains. Ectopic induction of Mash1 (C, D) and Dlx2 (G, H) along the dorsal midline (arrows) of the Pdn/Xt J and Xt J /Xt J telencephalon. Dlx5 (I, J) and Isl1 (M, N) expression are unaltered in the telencephalon of Pdn/Pdn mice, while both genes are ectopically induced in the dorsal forebrain (arrows) of Pdn/Xt J (K, O) and Xt J /Xt J (L, P) embryos. (Q–T) Nkx2.1 expression remains confined to the medial ganglionic eminence in Gli3 mutants. (D, H, L, P) Due to the decreased size of the telencephalic vesicles, the expression of the indicated ventral markers becomes visible around the zona limitans intrathalamica of the Xr’/Xr’ brain.
detected in the caudal most part of the cortical hem (Fig. 4B). In contrast, expression of these marker genes was completely abolished in the *Pdn/Xt* mutant telencephalon. Thus, similar to the *Xt* homozygous forebrain, *Wnt* and *Bmp* expression is severely reduced or even lost in the *Pdn/Pdn* and *Pdn/Xt* mutant brain, respectively.

This loss and/or the reduction of dorsal cell fates specifying signals might provide an explanation for the dorsal-
To this end, we dissected dorsolateral telencephalic mutant telencephalon, we have employed an explant culture system. To gain further evidence that ectopic activation of Fgf signaling might lead to a ventralization of the telencephalon we analyzed Fgf8 as well as Sprouty2 expression. Induction of the latter gene serves as a molecular read out of Fgf signaling (Minowada et al., 1999). In wildtype embryos, Fgf8 and Sprouty2 expression are confined to the commissural plate, the rostral end of the telencephalic midline (Minowada et al., 1999) (Fig. 5A and B). In contrast, expression of both genes is ectopically activated in the dorsomedial midline of the Gli3 mutant brain but to various degrees. While Pdn/Pdn and Pdn/Xt embryos showed a moderate expansion of the Fgf8 and Sprouty2 expression domains (Fig. 5C–F), the complete dorsal midline tissue of the Xr/Xr telencephalon expressed these markers (Fig. 5G and H). Reduction and/or loss of Gli3 function therefore leads to ectopic Fgf8 expression and Fgf signaling.

**Ectopic application of Fgf8 protein to dorsal telencephalic neuroectoderm affects ventral and dorsal telencephalon-specific gene expression**

To gain further evidence that ectopic activation of Fgf signaling might be involved in the ventralization of the Gli3 mutant telencephalon, we have employed an explant culture system. To this end, we dissected dorsolateral telencephalic tissue pieces from E9.5 and E10.5 wildtype embryos according to the criteria proposed by Kohtz (Kohtz et al., 1998). The tissue was maintained for 48 h under in vitro culture conditions in the presence of beads soaked with purified recombinant mouse Fgf8b protein and then analyzed for the expression of several ventral- and dorsal-specific markers. This assay resulted in identical results for both time points. Sprouty2 expression, which served as a positive control for this assay, was specifically induced under these conditions (n = 4/4) (Fig. 6A and E). Furthermore, implantation of Fgf8 beads led to the induction of the ventral markers Mash1 and Dlx2 (n = 15/16; n = 14/16), while BSA-soaked beads had no effect (n = 0/15 for both markers) (Fig. 6B, C, F, and G). Furthermore, Fgf8 specifically inhibited Enxl transcription in dorsal telencephalic neuroectoderm as a zone of downregulation is observed around the Fgf8-soaked bead (n = 9/11 for Enxl) (Fig. 6D and H; and data not shown). Thus, ectopic application of Fgf8 induces subpallial marker gene expression and represses pallial-specific genes in dorsal telencephalic tissue explants.

The above experiments left the possibility that the dorsal telencephalic tissue was exposed to Shh signals before explantation and that Fgf8 was only capable of acting in parallel to Shh signaling to induce ventral-specific marker gene expression in dorsal telencephalic explants. To test for this, we blocked Shh signaling by the addition of cycloamine to the tissue culture medium, a pharmacological inhibitor of Shh signaling (Berman et al., 2002). In a first set of experiments, we determined a cycloamine concentration necessary for the repression of Shh signaling in the explant culture system. Shh readily induces Mash1 expression and

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*Fig. 4. Bmp and Wnt expression are reduced or lost in the dorsal telencephalon of Gli3 mutants. Dorsal view of (A, D) wildtype, (B, E) Pdn/Pdn, and (C, F) Pdn/Xt E12.5 brains processed for in situ hybridization with the indicated probes. (A) Wnt2b is strongly expressed in the dorsomedial telencephalon and in the diencephalic and mesencephalic roof plate (rp) of control embryos. (B) Wnt2b expression in the caudodorsal cortical hem of the Pdn/Pdn telencephalon is specifically reduced and absent in more rostral areas. (C) Wnt2b expression is specifically lost from the dorsomedial telencephalon of Pdn/Xt embryos, while the expression domains in the diencephalic and mesencephalic roof plate remain unaffected. (D) Bmp4 is expressed in the dorsomedial telencephalon and in the dorsal diencephalon (d) of wildtype embryos. (E) Reduced and fuzzy expression of Bmp4 in the dorsomedial telencephalon. (F) Loss of Bmp4 expression in the dorsal telencephalon in Pdn/Xt mutants. Diencephalic expression of Bmp4 is still detected in both mutants (E, F).*
represses *Emx1* transcription in E9.5 dorsal telencephalic explants, while the addition of 10 μM cycloamine abolished these effects (Fig. 6M–P). Next, we applied Fgf8 beads to dorsal telencephalic tissue in the presence of cycloamine. Under these conditions, we observed an induction of *sprouty2* (n = 6/5), *Mash1* (n = 11/13), and *Dlx2* (n = 11/12) expression, while *Emx1* was repressed (n = 9/10) to a similar extent as was found before in the absence of cycloamine (Fig. 6I–L). Taken together, these data indicate that Fgf8 affects dorsal and ventral marker gene expression in this culture assay independently of Shh.

### Discussion

Through a combination of genetic and molecular approaches, we have performed a detailed analysis of regionalization processes in the telencephalon of various *Gli3* mouse mutants. Our observations present indications that a tight balance between Fgf and Bmp/Wnt signaling is required for regionalization of the dorsal and rostral telencephalic vesicles. In addition, our findings provide evidence for a role of Fgfs in the specification of ventral telencephalic cell types independently of Shh signaling. These observations complement findings in zebrafish and chick embryos and point to the importance of a complex, but evolutionary conserved interaction between different signaling centers for the regulation of patterning events in the vertebrate telencephalon.

### Ventralization of the Gli3 mutant dorsal telencephalon

Previous analysis of neurogenesis in the extra-toes mouse mutant indicated that loss of *Gli3* function had profound effects on telencephalic development (Johnson, 1967; Franz, 1994; Theil et al., 1999; Tole et al., 2000). Further analysis of this mutant has been hampered by a rather complex phenotype and by a degeneration of the telencephalon at later developmental stages. To circumvent these limitations, we started with a comparative characterization of the telencephalon of the *Gli3* mouse mutant, *Pdn*. Strikingly, these mutants showed the ectopic expression of ventral telencephalic markers in the dorsal telencephalon as was previously reported for *XrI* homozygous embryos (Tole et al., 2000; Aoto et al., 2002). While *Emx1* and *Emx2* transcription are either completely abolished or drastically reduced throughout the dorsal telencephalon at early stages (E10.5–E11.5), the *Pdn* mutation differently affects the expression of other pallial markers only 1 day later in development. *Pax6* and *Ngn2* expression are reduced along the dorsomedial midline, but are absent from the rostromedial telencephalon, while the ventral progenitor-specific markers *Mash1* and *Dlx2* are ectopically induced in the area devoid of *Pax6* and *Ngn2* transcription, suggesting a ventralization of the *Gli3* mutant telencephalon. This finding is further corroborated by the neuronal expression of *Pax6* in the dorsal *Gli3* mutant telencephalon, which is characteristic of cells forming portions of the claustrum and amygdala (Puelles et al., 1999; Yun et al., 2002). The failure to induce other subpallial marker genes, however, indicates a partial transformation or the activation of a subtype-specific ventral differentiation program in the dorsal telencephalon.

Interestingly, the degree of this ventralization varies between the different *Gli3* alleles with *XrI*/*XrI* mutant brains showing the strongest, and *Pdn/Pdn* mutants the weakest ventralization. Currently, the effect of the *Pdn* mutation on the biochemical properties of the Gli3 protein is unknown. Integration of a retrotransposon leads to the formation of several alternative transcripts, only a fraction of which are predicted to contain the Gli3 zinc finger coding region (Thien and Rüther, 1999). These mutated proteins contain an integration of 56 or 61 aa within the Gli3 N-terminal repressor domain, but it remains to be clarified how this affects the Gli3 transcriptional properties. The gradual effect on telencephalic development in *Pdn/Pdn, Pdn/XrI*, and...
Xt/J embryos, however, indicates that this integration may only have little effects on Gli3 function and that the Pdn mutation may mainly act by reducing the amount of functional Gli3 protein. Thus, the degree of ventralization seems to correlate with the amount of functional Gli3 protein produced by the various alleles.

The ventralization of the Gli3 mutant telencephalon is caused by a disrupted balance between Bmp/Wnt and Fgf signaling.

In the caudal neural tube and in the hindbrain, a tight balance between dorsalizing and ventralizing signals con-
trols the specification and generation of specific cell types along the dorsoventral axis. The ventralization of the Gli3 mutant telencephalon might therefore result from a disruption of this balance. Loss and/or reduction of Gli3 function may lead to the ectopic activation of a Shh-mediated ventralizing pathway. However, we were unable to detect ectopic expression of Shh or of Shh target genes in the dorsal telencephalon of all Gli3 mutants (Theil et al., 1999; Tole et al., 2000; data not shown). Alternatively, Gli3 may be necessary for the establishment or maintenance of the dorsal signaling center. Indeed, Bmp/Wnt expression is completely abolished in the dorsomedial telencephalon at early stages of development in all Gli3 mutants analyzed (E9.5-E10.5) (Grove et al., 1998; Theil et al., 1999; Tole et al., 2000; this study) and only partially recovers in Pdn/Pdn mice at E12.5 (this study). A role for these factors in dorsal patterning of the telencephalon is supported by the fact that development of the dorsalmost structures of the telencephalon, the chorial plexus and the hippocampus, is disrupted in Bmp1ra and Wnt3a mutant mice, respectively (Lee et al., 2000; Hebert et al., 2002). Furthermore, ectopic expression of Bmp4 leads to an activation of the pallial marker Emx2 (Dou et al., 1999; Ohkubo et al., 2002), transcription of which is coordinately regulated by Bmps and Wnts (Theil et al., 2002b).

However, defective Bmp/Wnt expression cannot fully account for the dorsalization defects in the Gli3 mutants. Although all Gli3 mutants lack Bmp/Wnt expression in the dorsomedial telencephalon, the telencephalon appears to be ventralized to various degrees. In this regard, the expansion of Fgf8 expression and ectopic activation of Fgf signaling might provide an additional explanation. The extent and timing of ectopic Fgf8 expression and Fgf signaling correlate with the degree of ventralization in the various Gli3 mutants and with the shape of the ectopic ventral marker gene expression domain. Pdn/Pdn mutant brains are characterized by a weak ectopic activation of this pathway at E12.5 and only develop characteristics of a ventralized brain at E14.5, while Pdn/XtJ and XtJ/XtJ embryos show an increase in both Fgf activation and ventralization of the telencephalon. In addition, we could show in an explant culture assay that Fgf8 induced the ventral markers Mash1 and Dlx2 and locally suppressed Emx1 transcription in telencephalic neuroectoderm consistent with similar findings after inactivation or ectopic activation of Fgf signaling in the vertebrate telencephalon (Crossley et al., 2001; Shinja et al., 2001; Garel et al., 2003; Storm et al., 2003). Furthermore, in all Gli3 mutant embryos, the septum is enlarged while the hippocampal anlage is either reduced or even absent. The opposite effect, a reduction or even loss of septal structures, is observed in Fgf8 mutants (Garel et al., 2003; Storm et al., 2003), indicating that Fgf8 promotes the formation of rostralventral structures at the expense of the dorsomedial telencephalon. In support of this notion, Fgf8 was recently found to anteriorize the cerebral cortex (Fukuchi-Shimogori and Grove, 2001). Taken together, these findings provide evidence for an important role of Fgfs in promoting the ventralization of the Gli3 mutant telencephalon. Ectopic Fgf signaling alone, however, might not be sufficient for this transformation as the effects of ectopic Fgf signaling might only become obvious in the absence of the dorsalizing signals. It therefore seems likely that the enhanced Fgf activity in combination with the defective Bmp/Wnt signaling synergistically acts to cause the ventralization of the Gli3 mutant telencephalon.

Patterning of the telencephalon requires the coordination of different signaling centers

Regionalization of the neural plate and the early neural tube is controlled by several signaling centers that direct the generation of molecularly distinct domains (for review, see Jessel, 2000; Marin and Rubenstein, 2002). It remains largely unknown, however, how the combination of these different signals is used to coordinate the generation of different telencephalic territories. Insights into these questions were obtained from the recent characterisation of Shh\(^{-/-}\);Gli3\(^{-/-}\) compound mutant embryos. Surprisingly, dorsoventral patterning of the telencephalon is established in these embryos, while the specification of ventral and dorsal telencephalic territories is disturbed in Shh and Gli3 single mutants, respectively (Litingtung and Chiang, 2000; Aoto et al., 2002; Rallu et al., 2002). These findings suggested a main role for Gli3 in repressing Shh signaling and led to the idea that a second Shh-independent pathway must be involved in patterning the telencephalon (Rallu et al., 2002). The nature of the signal(s), however, remains unknown. Interestingly, it was recently shown that Shh mutant embryos fail to maintain Fgf8 expression, while an Fgf8 upregulation was observed in Shh\(^{-/-}\);Gli3\(^{-/-}\) compound embryos similar to XtJ/XtJ embryos (Ohkubo et al., 2002; Aoto et al., 2002). In addition, we provide evidence that Fgf8 can induce the expression of ventral telencephalic markers in vitro in the absence of hedgehog signaling. Although we cannot rule out the possibility that an early transient Shh signal during normal development and in our explant assay is required for Fgf8 to function, taken together these findings suggest that Fgf8 may provide a Shh-independent signal for dorsoventral patterning of the telencephalon. Due to its repressive effects on dorsal marker gene expression, however, additional signal(s) besides Fgf8 are required to allow for the specification of the dorsal telencephalic domain. Furthermore, during normal development, Fgf and Shh signals are likely to act in parallel or to even cooperate in the control of the ventral telencephalon. Both factors are required to maintain each other’s expression in the rostral prosencephalon of mouse and zebrafish embryos (Shanmugalingam et al., 2000; Crossley et al., 2001; Shinya et al., 2001; Ohkubo et al., 2002) and are both sufficient for the induction of ventral telencephalic characteristics in vitro and in vivo (Kohtz et al., 1998; Ohkubo et al., 2002; Rallu et al., 2002; this study). In addition, both signals are antag-
optimized by Bmp signaling (Golden et al., 1999; Ohkubo et al., 2002).

In summary, this study emphasizes the central role of GlI3 in controlling the regionalization of the telencephalon. GlI3 regulates the generation and specification of distinct dorsal and ventral telencephalic domains not only by restricting the dorsal extent of Shh signaling but also by setting up the antagonizing Fgf and Bmp/Wnt signaling centers. This dual mechanism ensures the coordinated development of distinct dorsal and ventral telencephalic progenitor domains.

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