Dosage of *Fgf8* determines whether cell survival is positively or negatively regulated in the developing forebrain

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FGF8 is known to be an important regulator of forebrain development. Here, we investigated the effects of varying the level of *Fgf8* expression in the mouse forebrain. We detected two distinct responses, one that was proportionate with *Fgf8* expression and another that was not. The latter response, which led to effects on cell survival, displayed a paradoxical relationship to *Fgf8* dosage. Either eliminating or increasing *Fgf8* expression increased apoptosis, whereas reducing *Fgf8* expression had the opposite effect. To explain these counterintuitive observations, we suggest that an FGF8-dependent cell-survival pathway is negatively regulated by intracellular inhibitors produced in proportion to FGF8 concentration. Our data provide insight into the function of FGF8 in forebrain development and underscore the value of using multiple alleles and different experimental approaches to unravel the complexities of gene function in vertebrate development.

FGF inhibitor | *Bmp4* | *Foxg1* | sprouty | cell death

It is now well established that the fibroblast growth factor (FGF) family of intercellular signaling molecules plays a central role in vertebrate embryogenesis. At present, 22 different mouse/human genes are classified as FGF family members because the proteins they encode contain a conserved core sequence of ≈120 aa that includes FGF receptor-binding and heparin-binding domains (1). Most FGFs are secreted proteins that bind to high-affinity receptor tyrosine kinases, leading to the activation of multiple signal transduction pathways, including the RAS/MAPK, PLC-γ, PI3 kinase, and STAT1 pathways (2, 3). One response to FGF receptor activation is the production of antagonists of FGF signaling (4–7). Thus, cellular responses to FGF depend on which signaling pathways are activated and the degree to which they are affected by FGF-induced inhibitors.

Fgf8 is an FGF family member that is essential for normal development of the forebrain. At early stages, *Fgf8* is expressed along the apex of the anterior neural ridge (ANR), the rostralmost portion of the neural plate, which contains the progenitors of much of the anterior forebrain (telencephalon; ref. 8). After neural tube closure, *Fgf8* expression is localized in a domain that encompasses the rostral midline of the telencephalon (9, 10). Surgical removal of the ANR or treatment of forebrain explants with inhibitors of FGF signaling causes a loss of expression of molecular markers of the telencephalon, and beads soaked in FGF8 protein can prevent this effect (11, 12). Moreover, experimentally changing the level of *Fgf8* expression at later stages of forebrain development alters telencephalic patterning, suggesting that FGF8 plays a role in specifying positional information in the developing forebrain (13). Analysis of *Fgf8* mutants also demonstrates an important role for FGF8 in forebrain development. Although mouse embryos homozygous for an *Fgf8*-null allele fail to gastrulate and die without forming organs (14, 15), embryos carrying an *Fgf8* hypomorphic allele survive to birth and have telencephalic defects (14, 35). Likewise, zebrafish embryos with reduced *Fgf8* function have an abnormal telencephalon, with striking defects at the midline (16, 17).

In this study, we set out to determine the consequences of inactivating *Fgf8* in the ANR at an early stage of mouse development by using a Cre-based recombination approach. The results showed that FGF8 is required for cell survival in the telencephalon. However, when we compared the effects of genetically eliminating, genetically reducing, or experimentally increasing *Fgf8* expression in the forebrain, we found that cell survival was affected in unexpected ways. It decreased when *Fgf8* expression was either eliminated or increased, and increased when *Fgf8* expression was reduced. We discuss a possible explanation for this dosage sensitivity based on the hypothesis that antagonist(s) of FGF signaling expressed in response to FGF8 specifically inhibit an *Fgf8*-dependent cell-survival pathway.

Materials and Methods

Production, Genotyping, and Analysis of Embryos. Mutant embryos were generated by the crosses illustrated in Fig. 1. For normal controls, we used littermates that either did not inherit $Foxg1^{cre}$ or inherited a wild-type allele of *Fgf8*. Noon of the day on which a vaginal plug was observed was considered E0.5. Embryos were genotyped by PCR using DNA from tail or other tissues. $Fgf8^{neo}$, $Fgf8^{\Delta 2,3}$, and *cre* were detected as described (14, 18). $Foxg1^{lacZ}$ was detected by using primers for *lacZ* sequences: 5′-GTCTCGTTGCTGCATAAACC-3′ and 5′-TCGTCTGCTCATCCATGACC-3′.

Wax embedding, sectioning (7 μm), and whole-mount RNA *in situ* hybridization were performed according to standard protocols by using probes from published sources. Nonradioactive section *in situ* hybridization was performed by using prehybridization steps essentially as described by Neubüser *et al.* (19) and posthybridization steps essentially as described by Storm *et al.* (20). In the whole-mount *in situ* hybridization assays, two normal and two mutant embryos were analyzed by using the *Fgf8*-ex2,3 probe (Fig. 2 *B* and *C*); between three and five embryos of each genotype were analyzed by using each of the other probes (*Foxg1*, *Bmp4*, *Fgf8*-FL, *Spry1*, *Emx2*; Figs. 2 and 3), except that only one $Foxg1^{lacz/+}$ hypomorph was analyzed by using the *Fgf8*-FL probe (Fig. 3*M*). In each experimental group, the results were similar. TUNEL reactions were performed on wax sections by using an *in situ* cell death kit (Roche Applied Science) and detected with nickel-enhanced diaminobenzidine, according to manufacturer's protocols (Vector Laboratories). For each genotype studied, serial sections from between three and five embryos were assayed by TUNEL.

Abbreviations: FGF, fibroblast growth factor; ANR, anterior neural ridge.

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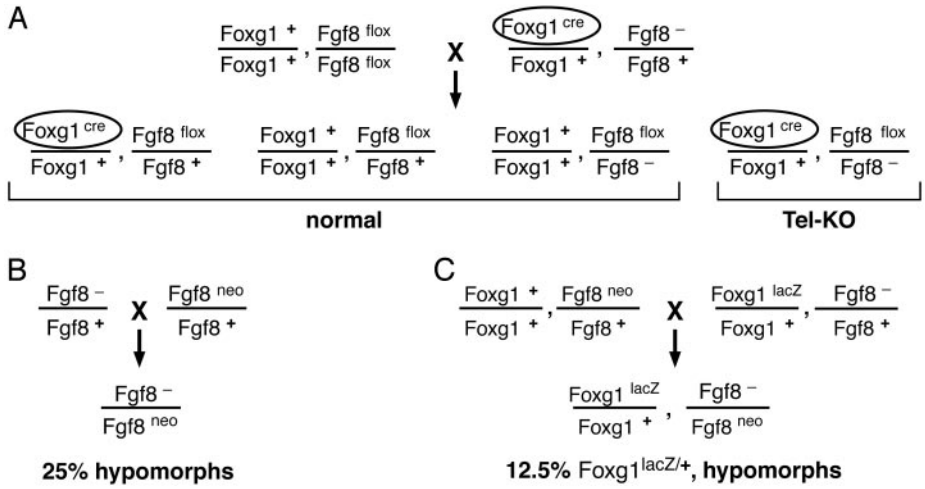

Fig. 1. Crosses used to generate mutant embryos. In these crosses, the *Fgf8*^{flox} allele has wild-type activity, *Fgf8*^{neo} is a hypomorphic allele, and *Fgf8*$^{-}$ is a null allele described as *Fgf8*$^{\Delta 2,3}$ (14). *Foxg1*^{cre} and *Foxg1*^{lacZ} are both null alleles.

Ectopic Gene Expression. Telencephalic explants were prepared as described in the legend to Fig. 2*A* and were either fixed immediately for *in situ* hybridization analysis or electroporated within 1 h of isolation. Expression vectors were generated by cloning an *Fgf8* cDNA (isoform 1 in ref. 9 and isoform B in ref. 21), a *Spry1* cDNA (from 5′HamSpry1/Ires-neo, generously provided by Lesley Jarvis and Mark Krasnow, Stanford University), or a *lacZ* cDNA (from an IRES-*lacZ* plasmid, pDB1, kindly provided by Doris Brown, University of California, San Francisco) into pCAGGS/ES, an expression plasmid containing a chick β-actin promoter and rabbit β-globin poly(A) (22). We also used the *Gfp* expression vector described by Stuhmer *et al.* (22). Plasmid DNA for electroporation was diluted to 1.75–2.5 μg/μl in water before use. Electroporation was performed on explants from CD-1 embryos (Charles River Laboratories) dissected at room temperature in Hibernate E (GIBCO/BRL). They were placed individually, mesenchyme side down, on Whatman nucleopore filters (no. 110414, Fisher), which were then floated on fresh Hibernate E. Electroporation was performed as described with minor modifications (22). One microliter of DNA solution was pipetted onto the explant, and six 5-ms pulses at 30 V were applied. After electroporation, each explant on its filter was floated on fresh Hibernate E until all explants had been processed. They were then transferred to culture medium (75% Optimem I/25% HAM F12, supplemented with 1% FBS/40 mM glucose/Glutamax 1/penicillin-streptomycin; GIBCO/BRL) and incubated at 37°C in 5% CO_2. After 24 h of culture, the explants were photographed, fixed overnight in 4% paraformaldehyde, and either processed in whole mount or serially sectioned.

Results

Fgf8 is expressed from early stages of forebrain development, first in a group of cells at the rostral boundary of the neural plate and then, following neural tube closure, in the rostral midline of the telencephalon (9, 10). To obtain embryos in which *Fgf8* function was eliminated in the telencephalon at early stages (hereafter referred to as Tel-KO mutants; see Fig. 1*A*), we used Cre-mediated recombination to delete essential *Fgf8* sequences (14), thereby circumventing the early lethality of *Fgf8*-null homozygotes (15). The Cre protein was produced by *Foxg1*^{cre}, a null allele generated by inserting a Cre recombinase gene into the *Foxg1* coding sequence, which is expressed in the telencephalon, olfactory placodes, and midbrain/hindbrain region (23). To determine the extent of *Fgf8* inactivation in the forebrain, we assayed telencephalic explants (Fig. 2*A*) by whole-mount *in situ* hybridization by using a probe, *Fgf8*-ex2,3, that detects *Fgf8* sequences deleted by Cre (14). Because *Fgf8* expression normally commences before *Foxg1* (11), we assume that *Fgf8* was transiently expressed in Tel-KO mutants at very early stages of telencephalic development. However, at embryonic day (E) 9.25 (data not shown) and E10.5 (Fig. 2 *B* and *C*), *Fgf8* RNA was not detected in the telencephalon of Tel-KO mutants.

To examine the effect of inactivating *Fgf8* on cell survival in the forebrain, we analyzed serial sections of E10.5 embryos by using a TUNEL assay. As reported (24), a high level of apoptosis was detected in the telencephalic midline of normal embryos (Fig. 2*D* and data not shown). In sections of Tel-KO mutants taken at a similar rostrocaudal level, TUNEL-positive cells were detected in a much wider domain than normal (Fig. 2*E* and data not shown). This effect was not observed in *Foxg1*$^{cre/+}$, *Fgf8*$^{+/-}$ embryos, indicating that the increase in TUNEL-positive cells was not caused by *cre* expression (data not shown). Given the observed increase in apoptosis in Tel-KO embryos, we expected to detect increased apoptosis in embryos with reduced *Fgf8* expression. However, in *Fgf8*$^{neo/-}$ embryos (hereafter referred to simply as "hypomorphs"; see Fig. 1*B*), in which transcripts that code for FGF8 protein are produced at a substantially lower than normal level (14), we observed a decrease rather than an increase in TUNEL-positive cells in the telencephalic midline (Fig. 2*F* and data not shown). Similar results were obtained at E9.5, when the morphology of the rostral telencephalon was similar in normal embryos, Tel-KO mutants, and hypomorphs (data not shown), suggesting that the differences in cell survival were unlikely to be secondary to morphological differences observed at E10.5 (Fig. 2 *D–F*).

To determine how increasing *Fgf8* expression affects apoptosis, we explanted the telencephalon from normal E10.5 embryos and electroporated it with an *Fgf8* expression vector (+*Fgf8*, n = 4) or a *lacZ* expression vector (control, n = 3). After 24 h of culture, the explants were serially sectioned. Assays for *lacZ* and *Fgf8* expression identified regions of ectopic gene expression (Fig. 2*O*). In sections of explants electroporated with *Fgf8*, there were more TUNEL-positive cells than in control explants (Fig. 2*G*), a phenotype qualitatively similar to what was observed in Tel-KO embryos (compare Fig. 2 *G* and *E*). Thus, either eliminating or increasing *Fgf8* expression decreased cell survival, whereas reducing *Fgf8* expression had the opposite effect.

Previous studies have provided evidence for a molecular pathway that regulates cell survival in the forebrain. At neural

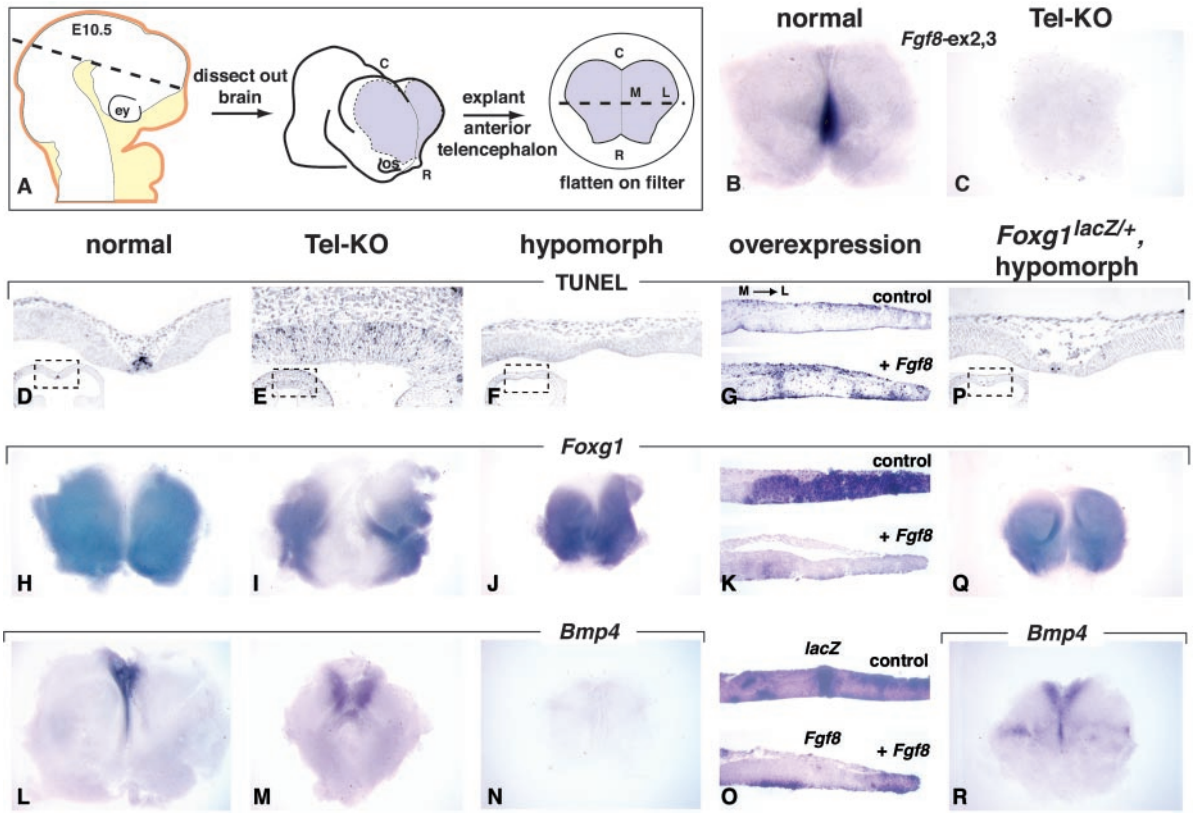

Fig. 2. Response of a *Foxg1*-dependent cell-survival pathway to different levels of *Fgf8* expression. (*A*) Schematic representation of procedure for isolating E10.5 telencephalic explants. (*Left*) Surface ectoderm (orange) and craniofacial mesenchyme (yellow), which were dissected away from the neural tube (white). (*Center* and *Right*) Forebrain region that was subsequently isolated (purple) and then flattened on a Nucleopore filter. The caudal and rostroventral limits of the explant were the approximate telencephalic/diencephalic border and the optic stalks, respectively. (*B* and *C*) RNA *in situ* hybridization of E10.5 explants using a probe, *Fgf8*-ex2,3, that hybridizes to sequences deleted by Cre. (*D–F* and *P*) TUNEL assays for apoptosis in horizontal sections of E10.5 forebrains of the genotypes indicated. (*Lower Left*) Low magnification view of the telencephalon, with a dotted box demarcating the area shown at higher magnification. The plane of section is indicated by the dashed line in *A* (*Left*). (*H–J*, *L–N*, *Q*, and *R*) Analysis by whole-mount RNA *in situ* hybridization of *Foxg1* and *Bmp4* expression in telencephalic explants from E10.5 embryos of the genotypes indicated. (*G*, *K*, and *O*) Explants from E10.5 wild-type embryos were electroporated with *lacZ* (control) or *Fgf8* expression vectors. Near-adjacent sections of control and of +*Fgf8* explants were assayed for TUNEL (*G*), *Foxg1* RNA (*K*), and *lacZ* or *Fgf8* RNA (*O*). The representative plane of the section is illustrated by the dashed line in *A* (*Right*). C, caudal; ey, eye; L, lateral; M, medial; os, optic stalk; R, rostral.

plate stages, FGF8 induces and/or maintains *Foxg1* expression in the ANR (11, 12). *Foxg1* then functions to restrict the expression of *Bmp4* to the midline (25). In turn, BMP4 is thought to induce apoptosis (24). In view of our data showing that FGF8 regulates cell survival in the forebrain, we sought to determine what effect changing the level of *Fgf8* expression had on this pathway, hereafter referred to as the *Foxg1* pathway. Although *Foxg1* expression is detected throughout the ANR at early stages, by E10.5 it is excluded from the midline but is detected at high levels in regions lateral to it (Fig. 2*H*). In Tel-KO mutants, *Foxg1* RNA was excluded from a midline domain that was much wider than normal (Fig. 2*I*). In hypomorphs, however, the opposite effect was observed: *Foxg1* RNA was detected at high levels across the midline (Fig. 2*J*). Increasing *Fgf8* expression by electroporation decreased *Foxg1* expression lateral to the midline, as observed in both serial sections (Fig. 2*K*) and whole-mounts ($n = 6$; data not shown). This phenotype was qualitatively similar to what we observed in Tel-KO mutants (compare Fig. 2 *K* and *I*). We next examined whether changes in *Bmp4* expression correlated with these changes in *Foxg1* expression. At E10.5, *Bmp4* RNA, which is normally expressed in the telencephalic midline (Fig. 2*L*), was detected in a midline domain that appeared wider than normal in Tel-KO mutants (Fig. 2*M*). In contrast, *Bmp4* expression was not detected in hypomorphs (Fig. 2*N*). Analysis of serial sections of forebrains from normal, Tel-KO mutants and hypomorphs confirmed that the *Foxg1* and *Bmp4* expression domains in the midline were complementary and that TUNEL was detected in the *Bmp4* expression domain (data not shown). Thus, the data on *Foxg1* and *Bmp4* expression support the hypothesis that FGF8 regulates cell survival in the telencephalon via the *Foxg1* pathway and show that either eliminating or increasing *Fgf8* expression decreases *Foxg1* pathway activity, whereas reducing *Fgf8* expression increases it.

One potential explanation for the opposite *Foxg1* pathway phenotypes in Tel-KO mutants and hypomorphs is that Tel-KO embryos are heterozygous for a null allele of *Foxg1* (i.e., $Foxg1^{cre}$), whereas hypomorphs are wild type at the *Foxg1* locus. To explore this possibility, we generated hypomorphs heterozygous for $Foxg1^{lacZ}$ (see Fig. 1*C*), a null allele in which *Foxg1* is inactivated by insertion of *lacZ* (26), and assayed *Foxg1* pathway activity. In such embryos, the extent of cell death and the domains of *Bmp4* and *Foxg1* expression (Fig. 2 *P–R*) differed from what was observed in Tel-KO mutants (Fig. 2 *E, I*, and *M*), suggesting that heterozygosity at the *Foxg1* locus is not the reason for the opposite *Foxg1* pathway phenotypes. Interestingly, the phenotype of $Foxg1^{lacZ/+}$ hypomorphs more closely resembled that of normal embryos (Fig. 2 *D*, *H*, and *L*) than of hypomorphs wild type at the *Foxg1* locus (Fig. 2 *F*, *J*, and *N*). This finding that the hypomorphic phenotype is suppressed by lowering *Foxg1* dosage supports the conclusion that increased

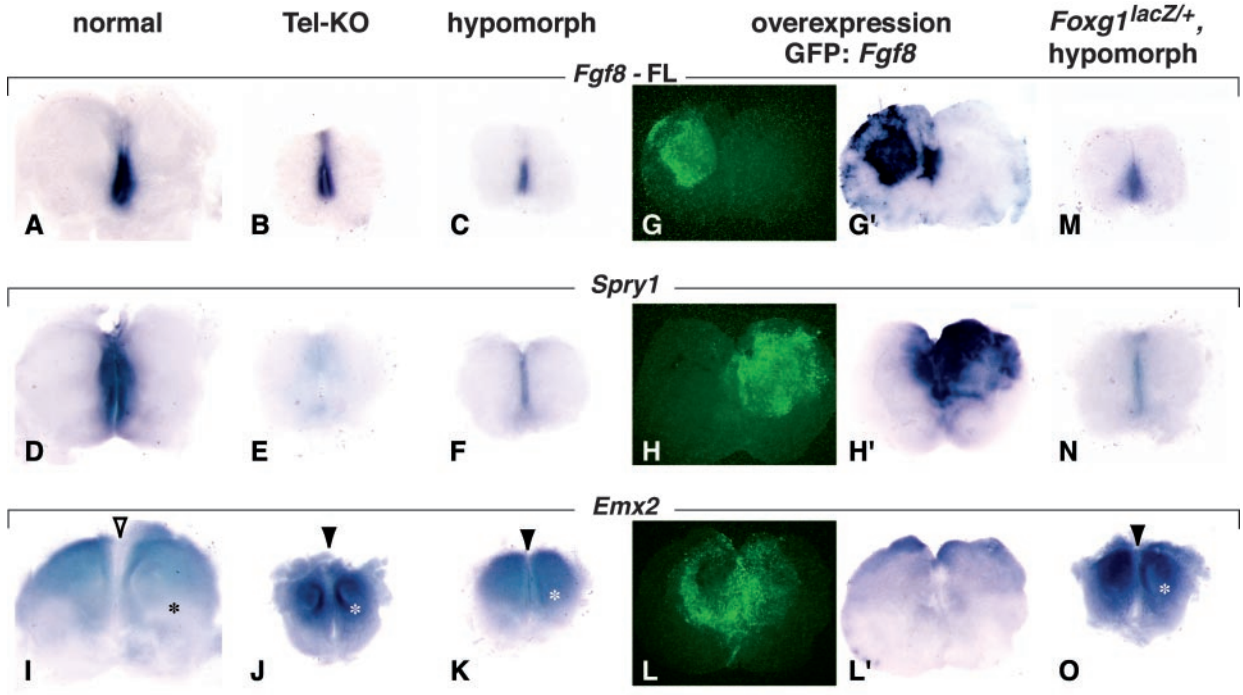

Fig. 3. Response of *Spry1* and *Emx2* to different levels of *Fgf8* expression. Whole-mount RNA *in situ* hybridization assays for *Fgf8, Spry1*, and *Emx2* expression in telencephalic explants from E10.5 embryos of the genotypes indicated. The full-length *Fgf8* probe (*Fgf8*-FL) hybridizes to *Fgf8* sequences not deleted by Cre. The level of *Fgf8* RNA in hypomorphs is lower than in Tel-KO mutants, presumably because the *Fgf8-neo* fusion RNA, which constitutes ≈60% of transcripts produced from the $Fgf8^{neo}$ allele carried by hypomorphs, is unstable (ref. 14; E. Meyers and G.R.M., unpublished results). Black asterisk and open arrowhead (*I*) indicate regions in which *Emx2* expression is low. White asterisks and filled arrowheads (*J*, *K*, and *O*) indicate regions in which *Emx2* expression is increased. To increase *Fgf8* expression, telencephalic explants from E10.5 wild-type embryos were electroporated with a 1:1 mixture of *Fgf8* and *Gfp* (22) expression vectors. GFP fluorescence was monitored to detect regions in which *Fgf8* was ectopically expressed (*G* and *G′*). The effects of ectopic *Fgf8* expression (*H* and *L*) were assayed by using probes for *Spry1* and *Emx2* (*H′* and *L′*). Note that in some regions these effects extend beyond the cells that express *Fgf8*, presumably because FGF8 is secreted. In control experiments (*Gfp* only, data not shown), the expression patterns of *Fgf8*, *Spry1*, and *Emx2* were similar to those observed in normal embryos (*A*, *D*, and *I*).

midline cell survival in hypomorphs is the result of an increase in *Foxg1* pathway activity.

Another potential explanation for the opposite phenotypes is that in Tel-KO mutants, *Fgf8* expression is normal throughout development until Cre-mediated recombination occurs, whereas in hypomorphs, it is lower than normal in all tissues throughout development. To determine whether this early reduction of *Fgf8* function causes the hypomorphic phenotype, we generated $Foxg1^{cre/+}$, hypomorphs by using a cross similar to that illustrated in Fig. 1*C*, except that we used animals carrying a $Foxg1^{cre}$ rather than a $Foxg1^{lacZ}$ allele. Like hypomorphs, these embryos had reduced *Fgf8* expression throughout development. However, because they also carried $Foxg1^{cre}$, they were converted to Tel-KO mutants when the *loxP* sites in their $Fgf8^{neo}$ allele were recombined to yield a null allele (14). The *Foxg1* pathway phenotype of these embryos was qualitatively similar to that of Tel-KO mutants (e.g., increased apoptosis), albeit more severe (not shown). This indicates that early reduction of *Fgf8* expression is not the reason why the midline phenotype of hypomorphs is opposite to that of Tel-KO embryos. Moreover, these data show that the phenotype of hypomorphs is not due to a dominant effect of the *neo* cassette present in the $Fgf8^{neo}$ allele they carry because $Foxg1^{cre/+}$ hypomorphs, which also carry $Fgf8^{neo}$, did not resemble hypomorphs.

Significantly, when we assayed mutant embryos for expression of other genes, we found that they did not respond in the same way to changes in *Fgf8* level as did the *Foxg1* pathway. We first assayed telencephalic explants with a full-length *Fgf8* cDNA probe containing sequences not deleted by Cre. The presence of transcripts in the normal *Fgf8* expression domain (Fig. 3 *A–C*) demonstrated that rostral midline tissue is present in both mutants and that FGF8 activity is not necessary to maintain *Fgf8* transcription, confirming what has been observed in limb buds (18). Expression of *Spry1* (Fig. 3*D*), which encodes a member of the Sprouty family of FGF signaling inhibitors (4, 5, 27, 28) and is positively regulated by FGF8 (5), was not detected in Tel-KO mutants (Fig. 3*E*) and was detected at a lower than normal level in hypomorphs (Fig. 3*F*). As expected, increasing *Fgf8* expression by electroporation (Fig. 3 *G* and *G′*) induced *Spry1* expression ($n = 6$; Fig. 3 *H* and *H′*). *Emx2* expression, which is negatively regulated by FGF8 (10), also displayed a proportionate response to variations in *Fgf8* expression. *Emx2* expression was detected in a larger domain than normal in Tel-KO mutants and hypomorphs (Fig. 3 *I–K*) and in a smaller domain when *Fgf8* was overexpressed ($n = 8$; Fig. 3 *L* and *L′*). Furthermore, unlike the effects on the *Foxg1* pathway, effects on the expression of *Spry1* and *Emx2* were not suppressed when *Foxg1* dosage was lowered (Fig. 3 *M–O*).

These data demonstrate that some responses to FGF8 are proportionate with the level of *Fgf8* expression. Why then does the *Foxg1* pathway respond as it does, with both elimination and increase in *Fgf8* expression causing increased cell survival? One possibility is that the loss-of-function-like phenotypes in the *Foxg1* pathway observed following electroporation of *Fgf8* are caused by the resulting induction of FGF signaling antagonists such as *Spry1*. To explore this possibility, we performed experiments similar to those described above with *Fgf8* and *lacZ* expression vectors (Fig. 2) by using a *Spry1* expression vector (Fig. 4*A*). We found that like ectopic *Fgf8*, ectopic *Spry1* expression caused an increase in the level of apoptosis (Fig. 4*B*, compare with Fig. 2*G*), which was

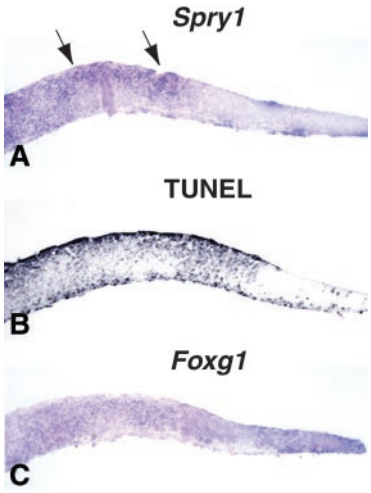

Fig. 4. Effect of ectopic expression of *Spry1* on apoptosis and *Foxg1* expression. In parallel with the experiments shown in Fig. 2 *G*, *K*, and *O*, explants from E10.5 wild-type embryos were electroporated with a *Spry1* expression vector. Near-adjacent sections of each explant were assayed for *Spry1* expression (*A*), TUNEL (*B*), or *Foxg1* expression (*C*). Arrows in *A* indicate regions in which cells expressed the electroporated cDNA. *Spry1* expression resulted in an increase in TUNEL-positive cells (*B*, compare with control explant in Fig. 2*G*) and a decrease in *Foxg1* expression (*C*, compare with control explant in Fig. 2*K*).

correlated with a *Spry1*-induced decrease in *Foxg1* expression (Fig. 4*C*, compare with Fig. 2*K*).

Discussion

In this study, we compared the phenotypes of a series of forebrains with different levels of FGF8 activity. *Fgf8* expression was eliminated in Tel-KO mutants, significantly reduced in hypomorphs that carry both a mild hypomorphic allele and a null allele, and increased in forebrain explants electroporated with an *Fgf8* expression vector. The results of our analysis showed that there are two distinct responses to FGF8. One response, which includes effects on *Spry1* and *Emx2* expression, is proportionate with the level of FGF8 activity (Fig. 5*A Right*). The second response comprises a *Foxg1* pathway that when active leads to cell survival and when inactive leads to apoptosis (Fig. 5*A Left*). The response of this pathway is not proportionate with the level of FGF8 activity. When FGF8 is either absent or high, pathway activity is low and cell survival decreases. In contrast, when FGF8 is low, pathway activity is high and cell survival increases.

We have considered various explanations for why the *Foxg1* pathway responds in this curious fashion and have ruled out heterozygosity at the *Foxg1* locus in Tel-KO mutants and reduced levels of functional *Fgf8* RNA throughout development in hypomorphs. Thus, we favor a model in which the observed *Foxg1* pathway phenotypes result from different levels of FGF8 activity in the telencephalic midline. To explain this dosage sensitivity, we suggest that an FGF8-induced inhibitor(s) produced as part of the proportionate response is induced at a higher threshold of FGF8 than the *Foxg1* pathway. Once induced, the inhibitor(s) specifically blocks the *Foxg1* cell-survival pathway, but not its own expression. According to this model (Fig. 5*B*), when FGF8 is low (as in hypomorphs), little or no inhibitor is produced, resulting in high *Foxg1* expression, low *Bmp4* expression, and high levels of cell survival. When FGF8 is high (following electroporation), inhibitor levels are high enough to block the pathway, resulting in low *Foxg1* expression, high *Bmp4* expression, and decreased cell survival.

We do not know the identity of the proposed *Foxg1* pathway-specific inhibitor(s). However, members of the Sprouty family

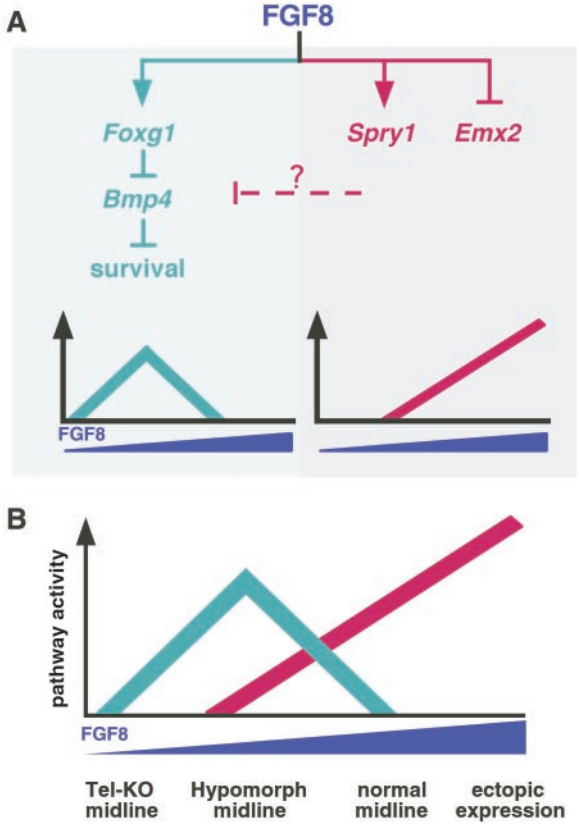

Fig. 5. A model to explain the effects of varying *Fgf8* dosage on cell survival. Our data identify two classes of response to variations in *Fgf8* expression in the developing mouse forebrain, schematically illustrated in *A*. The *x* and *y* axes represent FGF8 concentration and pathway(s) activity, respectively. One response is proportionate with the level of *Fgf8* expression (*Right*), and the other, a *Foxg1* pathway, is not (*Left*). We suggest that genes, expressed as part of the proportionate response, such as *Spry1*, encode inhibitors of the *Foxg1* cell-survival pathway and that their expression is not subject to negative feedback. (*B*) Schematic diagram illustrating the combined responses to variation in FGF8 concentration, in which it is assumed that the proportionate response is activated at a higher level of FGF8 than is the *Foxg1*-dependent response. In the absence of FGF8 (Tel-KO midline), the *Foxg1* pathway is inactive and cell survival is reduced. At very low FGF8 concentrations, there is little or no inhibitor activity, so *Foxg1* pathway activity is not antagonized and cell survival is high. We suggest that the FGF8 concentration is within this range in the midline of hypomorphs. As FGF8 concentration rises, the inhibitor(s) produced as part of the proportionate response becomes active and begins to inhibit the *Foxg1* pathway. Further increases result in more inhibitor activity and decreased cell survival. We suggest that the FGF8 concentration is within this range in the normal midline and in our gain-of-function experiments.

are good candidates because Sprouty genes are expressed in the midline of the telenecephalon (5) (Fig. 3*D* and data not shown), they are induced by FGF signaling (Fig. 3*H′*; refs. 4, 5, and 27), and they function intracellularly as pathway-specific FGF-signaling antagonists (28–31). Furthermore, *sprouty* in *Drosophila* has been implicated as an inhibitor of cell-survival pathways (32). Because our data show that *Spry1* expression in the telencephalon is regulated by FGF8 (Fig. 3 *E*, *F*, and *H′*), SPROUTY1 may play a role in determining the level of cell survival in response to FGF8. In support of this hypothesis, electroporation of a *Spry1* expression vector inhibits *Foxg1* expression and induces cell death in forebrain explants (Fig. 4).

Although FGF8 has been found to be essential for cell survival in other developmental settings, including the first branchial arch (33), limb bud (18, 34), and midbrain/hindbrain boundary region (C. L. Chi, S. Martinez, W. Wurst, and G.R.M., unpub-

lished results), there has been no indication from those studies of the complex relationship between cell survival and *Fgf8* dosage reported here. This might be because a mechanism that is less sensitive to inhibition by intracellular inhibitors is used to regulate cell survival in those regions. Our observations illustrate how variation in gene dosage can lead to very different phenotypes and point to the importance of understanding how pathways downstream of signaling molecules such as FGF8 are integrated during development.

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1. Ornitz, D. M. & Itoh, N. (2001) *Genome Biol.* **2,** http://genomebiology.com/1465-6906/2/REVIEWS3005.
2. Sahni, M., Raz, R., Coffin, J. D., Levy, D. & Basilico, C. (2001) *Development (Cambridge, U.K.)* **128,** 2119–2129.
3. Szebenyi, G. & Fallon, J. F. (1999) *Int. Rev. Cytol.* **185,** 45–106.
4. Hacohen, N., Kramer, S., Sutherland, D., Hiromi, Y. & Krasnow, M. A. (1998) *Cell* **92,** 253–263.
5. Minowada, G., Jarvis, L. A., Chi, C. L., Neubuser, A., Sun, X., Hacohen, N., Krasnow, M. A. & Martin, G. R. (1999) *Development (Cambridge, U.K.)* **126,** 4465–4475.
6. Furthauer, M., Lin, W., Ang, S. L., Thisse, B. & Thisse, C. (2002) *Nat. Cell Biol.* **4,** 170–174.
7. Tsang, M., Friesel, R., Kudoh, T. & Dawid, I. B. (2002) *Nat. Cell Biol.* **4,** 165–169.
8. Cobos, I., Shimamura, K., Rubenstein, J. L., Martinez, S. & Puelles, L. (2001) *Dev. Biol.* **239,** 46–67.
9. Crossley, P. H. & Martin, G. R. (1995) *Development (Cambridge, U.K.)* **121,** 439–451.
10. Crossley, P. H., Martinez, S., Ohkubo, Y. & Rubenstein, J. L. (2001) *Neuroscience* **108,** 183–206.
11. Shimamura, K. & Rubenstein, J. L. (1997) *Development (Cambridge, U.K.)* **124,** 2709–2718.
12. Ye, W., Shimamura, K., Rubenstein, J. L., Hynes, M. A. & Rosenthal, A. (1998) *Cell* **93,** 755–766.
13. Fukuchi-Shimogori, T. & Grove, E. A. (2001) *Science* **294,** 1071–1074.
14. Meyers, E. N., Lewandoski, M. & Martin, G. R. (1998) *Nat. Genet.* **18,** 136–141.
15. Sun, X., Meyers, E. N., Lewandoski, M. & Martin, G. R. (1999) *Genes Dev.* **13,** 1834–1846.
16. Shanmugalingam, S., Houart, C., Picker, A., Reifers, F., Macdonald, R., Barth, A., Griffin, K., Brand, M. & Wilson, S. W. (2000) *Development (Cambridge, U.K.)* **127,** 2549–2561.
17. Shinya, M., Koshida, S., Sawada, A., Kuroiwa, A. & Takeda, H. (2001) *Development (Cambridge, U.K.)* **128,** 4153–4164.
18. Sun, X., Mariani, F. & Martin, G. R. (2002) *Nature* **418,** 501–508.
19. Neubüser, A., Koseki, H. & Balling, R. (1995) *Dev. Biol.* **170,** 701–716.
20. Storm, E. E. & Kingsley, D. M. (1996) *Development (Cambridge, U.K.)* **122,** 3969–3979.
21. MacArthur, C. A., Lawshe, A., Xu, J., Santos-Ocampo, S., Heikinheimo, M., Chellaiah, A. T. & Ornitz, D. M. (1995) *Development (Cambridge, U.K.)* **121,** 3603–3613.
22. Stühmer, T., Anderson, S. A., Ekker, M. & Rubenstein, J. L. (2002) *Development (Cambridge, U.K.)* **129,** 245–252.
23. Hébert, J. M. & McConnell, S. K. (2000) *Dev. Biol.* **222,** 296–306.
24. Furuta, Y., Piston, D. W. & Hogan, B. L. (1997) *Development (Cambridge, U.K.)* **124,** 2203–2212.
25. Dou, C. L., Li, S. & Lai, E. (1999) *Cereb. Cortex* **9,** 543–550.
26. Xuan, S., Baptista, C. A., Balas, G., Tao, W., Soares, V. C. & Lai, E. (1995) *Neuron* **14,** 1141–1152.
27. Furthauer, M., Reifers, F., Brand, M., Thisse, B. & Thisse, C. (2001) *Development (Cambridge, U.K.)* **128,** 2175–2186.
28. Nutt, S. L., Dingwell, K. S., Holt, C. E. & Amaya, E. (2001) *Genes Dev.* **15,** 1152–1166.
29. Casci, T., Vinos, J. & Freeman, M. (1999) *Cell* **96,** 655–665.
30. Gross, I., Bassit, B., Benezra, M. & Licht, J. D. (2001) *J. Biol. Chem.* **276,** 46460–46468.
31. Impagnatiello, M. A., Weitzer, S., Gannon, G., Compagni, A., Cotten, M. & Christofori, G. (2001) *J. Cell Biol.* **152,** 1087–1098.
32. Bergmann, A., Agapite, J., McCall, K. & Steller, H. (1998) *Cell* **95,** 331–341.
33. Trumpp, A., Depew, M. J., Rubenstein, J. L., Bishop, J. M. & Martin, G. R. (1999) *Genes Dev.* **13,** 3136–3148.
34. Moon, A. M. & Capecchi, M. R. (2000) *Nat. Genet.* **26,** 455–459.
35. Garel, S., Huffman, K. J. & Rubenstein, J. L. R. (2003) *Development (Cambridge, U.K.)*, in press.