Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1

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SUMMARY

Lef1 and other genes of the LEF1/TCF family of transcription factors are nuclear mediators of Wnt signaling. Here we examine the expression pattern and functional importance of Lef1 in the developing forebrain of the mouse. Lef1 is expressed in the developing hippocampus, and Lef1-deficient embryos lack dentate gyrus granule cells but contain glial cells and interneurons in the region of the dentate gyrus. In mouse embryos homozygous for a Lef1-lacZ fusion gene, which encodes a protein that is not only deficient in DNA binding but also interferes with β-catenin-mediated transcriptional activation by other LEF1/TCF proteins, the entire hippocampus including the CA fields is missing. Thus, LEF1 regulates the generation of dentate gyrus granule cells, and together with other LEF1/TCF proteins, the development of the hippocampus.

Key words: LEF1/TCF, Hippocampus, Dentate gyrus, Granule cells, Wnt

INTRODUCTION

Signaling by Wnt/wg proteins regulates multiple cellular and developmental processes that include control of cell proliferation, specification of cell fate and generation of cell polarity (reviewed in Wodarz and Nusse, 1998; Shulman et al., 1998). In addition to short-range effects, Wnt proteins can also influence cells over long distances suggesting that these secreted signaling molecules can function as morphogens in embryonic tissue patterning (Struhl and Basler, 1993; Hoppler and Bienz, 1995). Genetic and biochemical dissection of the Wnt signaling pathway has led to the identification of components and understanding of the mechanism of signal transduction (reviewed in Wodarz and Nusse, 1998). Wnt signaling results in stabilization of β-catenin and in its accumulation in the cytoplasm and nucleus (Hinck et al., 1994; Van Leeuwen et al., 1994). In the nucleus, association of β-catenin with members of the LEF1/TCF family of transcription factors, which alone have no transcription activation potential, mediates a response to Wnt signals and augments expression of target genes (Behrens et al., 1996; Huber et al., 1996; Molenaar et al., 1996; van de Wetering et al., 1997; Hsu et al., 1998). Target genes of LEF1/TCF proteins include the patterning genes Ultrabithorax in Drosophila and siamois and nodal-related gene-3 in Xenopus (Riese et al., 1997; Brannon et al., 1997; McKendry et al., 1997). In addition, the human genes c-myc and cyclin D1, which influence cell proliferation, are regulated by LEF1/TCF proteins in association with β-catenin (He et al., 1998; Tetsu and McCormick, 1999; Shtutman et al., 1999).

The developmental specificities of Wnt signaling can be accounted for, in part, by the cell-type-specific expression of Wnt genes and Lef1/Tcf genes. In the mouse embryo, at least seventeen Wnt genes are expressed in specific but overlapping patterns (Parr et al., 1993; Hollyday et al., 1995; Grove et al., 1998; Wodarz and Nusse, 1998). Likewise, four closely related Wnt-responsive transcription factors, LEF1, TCF1, TCF3 and TCF4, have been identified in the mouse (Travis et al., 1991; van de Wetering et al., 1991; Korinek et al., 1998a). Lef1 and Tcf1, which are expressed in overlapping patterns in the primitive streak and limb buds of the early mouse embryo, regulate paraxial mesoderm formation and limb development in a redundant manner (Oosterwegel et al., 1993; Galceran et al., 1999). Lef1–/– Tcf1–/– mice have a defect in paraxial mesoderm formation that is virtually identical to that observed in Wnt3a-deficient mouse embryos, consistent with the function of LEF1 and TCF1 in Wnt signaling (Galceran et al., 1999). In the developing brain, Lef1 is abundantly expressed in the mesencephalon and in the thalamus (Oosterwegel et al., 1993; van Genderen et al., 1994). Targeted inactivation of Lef1 was noted to impair the generation of the mesencephalic nucleus of the trigeminal nerve (van Genderen et al., 1994). Tcf1 and Tcf4, which are also expressed in the brain, are apparently dispensable for brain development (Verbeek et al., 1995; Korinek et al., 1998b), raising the question as to whether Lef1 acts
Wnt signaling is involved in development of the midbrain and hindbrain and the spinal cord. In particular, Wnt1−/− mice lack derivatives of the isthmic organizer region including the cerebellum (McMahon and Bradley, 1990; Thomas and Capecchi, 1990). Expression of Wnt genes and bone morphogenetic protein (BMP) genes in the dorsal midline (roof plate) of the spinal cord has been implicated in dorsal specification of the spinal cord (Liem et al., 1995, 1997; Ikeya et al., 1997; Chang and Hemmati-Brivanlou, 1998; Lee et al., 1998). Likewise, Wnt and Bmp genes are expressed in the dorsal midline of the brain (reviewed in Tole and Grove, 1999). In regions of the dorsal midline that form the telencephalic choroid plexus, expression of Wnt and Bmp genes tends to be highest in the epithelium known as the fimbria or hem (Grove et al., 1998). In the telencephalon, the fimbria is flanked dorsomedially by the choroid plexus and ventrolaterally by the neuroepithelium, which generates the hippocampal complex. Based on their expression patterns, Wnt and Bmp genes have been hypothesized to regulate hippocampal development (Tole and Grove, 1999). The hippocampal complex consists of the dentate gyrus, the CA fields and the parahippocampal (subicular) cortex (Stanfield and Cowan, 1988). Each of these domains may be derived largely from distinct proliferative zones of the wall of the dorsal telencephalon (medial pallium; Altman and Bayer, 1990a,b). The primordium of the dentate gyrus is postulated to lie adjacent to the Wnt-expressing fimbria, followed by the CA fields and the subicular cortex. Thus, Wnt proteins may form a morphogenetic gradient that could pattern the medial pallium.

With the aim of gaining insight into the functional importance of Wnt signaling for the development of the telencephalon, we analyzed mice carrying two types of mutations in the Lef1 locus. Analysis of the telencephalic phenotype of a Lef1 null mutation indicated that LEF1 regulates the differentiation of a specific cell type in the hippocampus complex, the dentate gyrus granule cells. Mice carrying a mutant allele of Lef1 that also interferes with the function of other Tcf genes, lack the entire hippocampus complex. Thus, the nuclear mediators of Wnt signaling control multiple steps in hippocampal development.

### MATERIALS AND METHODS

#### Generation of an in-frame fusion of Lef1 and lacZ

An in-frame fusion of the murine Lef1 locus and the bacterial lacZ gene was generated by homologous recombination of a targeting construct in murine embryonic stem (ES) cells. For the generation of the targeting construct, a HindIII-XhoI fragment, containing both β-galactosidase-coding (lacZ) sequences and the PGK-neo gene with a polyadenylation signal from the bovine growth hormone gene, was isolated from plasmid pSAβgal (Friedrich and Soriano, 1993). The isolated DNA fragment was inserted into the single HindIII-XhoI site of an 8 kb genomic Salt-XhoI subclone from the murine Lef1 locus containing an HMG domain exon (van Genderen et al., 1994). The resulting gene targeting construct was generated by insertion of an XhoI fragment containing the PGK-tk gene. The gene targeting construct was linearized with Sall and electroporated into D3 ES cells (van Genderen et al., 1994).

Homologous recombinants were identified by DNA blot analysis (van Genderen et al., 1994). The targeted ES clones were injected into C57BL/6 blastocysts and the chimeric animals were screened for germline transmission. Genotyping of mice was performed by PCR amplification as described (Galceran et al., 1999) using the Lef1-specific primers D8: 5′CCGTTCAGTGCGACGCCCTCTCCTCC, and LPP2.2: 5′TGTCCTCTCTTGCCTAGTTC and the lacZ primer LAC1: 5′CGTTCCGATTCTCCGTTGGGAACAAAC. Mice were also genotyped by X-gal staining of tail biopsies from adult animals or from embryonic tissues without previous fixation. X-gal staining was performed in X-gal staining solution (PBS, 2 mM MgCl2, 0.01% (w/v) deoxynucleotide, 0.02% (v/v) Nonident P-40, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 0.5 mg/ml X-gal) at 37°C until signal was developed from control tissues.

#### Transient transfections and reporter gene assays

Neuro2A cells were grown in Eagle’s minimal essential medium with Earle’s balanced salt solution medium containing 5% fetal bovine serum. Transient transfections were performed by the Lipofectamine [Gibco] procedure according to the manufacturer’s protocol. The LEF-CAT reporter gene, containing seven multimerized wild-type LEF1 binding sites linked to the fos-chloramphenicol acetyltransferase (CAT) gene, was transfected alone or in combination with expression plasmids for β-catenin, LEF1 or TCF1 as described (Hsu et al., 1998). The LEF1-BGal expression plasmid was constructed by inserting the Smal fragment from the genomic Lef1-lacZ targeting construct (see above and Fig. 1A) into the Smal site of the expression plasmid for LEF1 to generate an in-frame fusion between LEF1 and β-galactosidase. A RSV-luciferase control plasmid was included in each transfection experiment to control for the efficiency of transfection. The total DNA concentration in each transfection was kept constant and the amounts of expression plasmids encoding LEF1, TCF1 and β-catenin were chosen to yield submaximal transactivation. The accumulation of LEF1-βGal protein was assayed by measuring β-galactosidase activity as described (Hsu et al., 1998). Luciferase activity was measured with the Luciferase Assay System (Promega) according to the manufacturer’s protocol.

#### X-gal staining

For X-gal staining of embryonic brains, embryos were dissected in cold PBS and fixed in freshly prepared 4% formaldehyde in PBS pH 7.4 for 1 hour at 4°C. After extensive washing in cold PBS, embryos were cryoprotected in 30% sucrose in PBS, embedded and oriented in OCT (Miles Scientific) and frozen in a dry ice/ethanol bath. 12-14 μm cryosections were postfixed for 1 minute in freshly made 4% formaldehyde and washed in PBS. β-galactosidase staining was performed in freshly made X-gal staining solution at 37°C overnight or until no more signal was detected. Sections were counterstained with Nuclear Fast Red and mounted in Permount.

For whole-mount X-gal staining of embryonic brains from E9.5 to 12.5 embryos, isolated brains were fixed in freshly prepared 4% formaldehyde in PBS pH 7.4 for 1 hour at 4°C. After extensive washing in PBS, mesenchymal tissue was removed under a dissecting microscope and the brains were stained overnight at 37°C in X-gal staining solution as described above. After staining, tissues were washed in PBS, postfixed in 4% paraformaldehyde overnight, washed and photographed.

#### 5-bromodeoxyuridine incorporation, immunohistochemistry and TUNEL assays

For proliferation assays, pregnant females were injected intraperitoneally with a solution of 5-bromodeoxyuridine (BrdU) in 0.1 M Tris-HCl pH 7.5 at a ratio of 50 μg of BrdU per gram of body weight. After the indicated times, embryos were dissected out in cold...
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modifications: embryos were fixed for one hour prior to labeled riboprobes was done essentially as above with the following (Bulfone et al., 1993). For sections, in situ hybridization using DIG-(Kodak), exposed at 4°C and developed as previously described previously described. Slides were washed, immersed in emulsion at 1:1000 dilution. Rabbit antiserum against Dlx-2 was used at 1:200 Rabbit antibodies against Calretinin and GFAP (Chemicon) were used HCl incubation step and neutralization in borate buffer were omitted. procedure as the BrdU immunohistochemistry, except that the 2 N acids 1 to 243, et al., 1999). In vitro transcribed and DIG-labeled antisense RNA processed for in situ hybridization as previously described (Galceran postfixed overnight in freshly prepared 4% formaldehyde and heads were prepared under a dissecting microscope by removing all in situ hybridization as follows: after extensive washing in cold PBS, in freshly prepared 4% formaldehyde. Heads were processed for in Plus-Peroxidase, and FITC. Oncor). Sections were counterstained treatment following the manufacturers protocol (in situ Apoptag for cryoprotection and embedding, proteinase K treatment was omitted and development of the signal was as described for whole-mount in situ hybridization.

RESULTS

Targeted insertion of a lacZ gene into the Left locus

With the aim of visualizing individual cells that express the Left1 gene and generating a mutation in Left1 that may interfere with the function of co-expressed LEF1/TCF proteins, we inserted a lacZ gene into the Left1 locus by homologous recombination. Previous experiments suggested that two types of alterations in LEF1 protein impair its function and that of co-expressed LEF1/TCF proteins. First, deletion of the β-catenin interaction domain of LEF1 generates a protein that binds DNA but is unresponsive to Wnt signals. At high concentration, this mutant protein interferes with transcriptional activation by β-catenin in transfection assays even in the presence of other intact TCF proteins (Molenaar et al., 1996; van de Wetering et al., 1997; Brannon et al., 1997; Fan et al., 1998; Vleminkx et al., 1999). Second, deletion of sequences that comprise both the DNA-binding domain of LEF1, referred to as the HMG domain, and a nuclear localization sequence (NLS) results in a mutant protein that is predominantly cytoplasmic and has the ability to interact with β-catenin (Behrens et al., 1996; Priev et al., 1998). However, the function of this mutant protein has not been examined.

We generated a targeting construct that allowed for in-frame insertion of the lacZ gene into one of the exons encoding the HMG domain of LEF1 (Fig. 1). This insertion inactivates the DNA-binding domain similar to the previously generated neo allele of the Lef1 locus (van Genderen et al., 1994). In contrast to the Lef1-neo allele, which does not generate any detectable LEF1 polypeptide (van Genderen et al., 1994), expression of the Left1-lacZ allele yields a stable LEF1-βGAL fusion protein that can be detected by X-gal histochemistry and immunoblot analysis (Fig. 2 and data not shown). Moreover, the fusion protein is located predominantly in the cytoplasm (data not shown), consistent with the removal of the nuclear localization signal (Priev et al., 1998). Thus, mice heterozygous for the Left1-lacZ allele should allow for an easy visualization of LEF1-expressing cells. In addition, mice homozygous for the Left1-lacZ mutation may also interfere with the function of co-expressed TCF proteins and may therefore have a more severe phenotype than the Left1-neo allele.

The LEF1-βGAL fusion protein interferes with β-catenin-mediated activation of transcription in transfected cells

To examine the possibility that the mutant LEF1-βGAL fusion protein interferes with β-catenin-mediated transcriptional activation by LEF1/TCF proteins, we generated a cDNA construct encoding the same fusion protein that is encoded by the targeted genomic Left1-lacZ allele (Fig. 1A). As anticipated, transfection of a LEF1 expression plasmid into Neuro 2A cells, together with a β-catenin expression plasmid, activates a fos-CAT reporter gene containing multimerized LEF1 binding sites. Transfection of increasing amounts of a LEF1-βGAL expression plasmid, together with the LEF1 and β-catenin plasmids impairs transcriptional activation in a
dose-dependent manner (Fig. 1B). This inhibition of transcriptional activation by LEF1-βGAL can be overcome by increasing the amount of transfected β-catenin plasmid, demonstrating the specificity of inhibition. A similar effect of the LEF1-βGAL fusion protein on β-catenin-mediated transcription is also observed in the presence of a transfected TCF1 expression plasmid, suggesting that LEF1-βGAL can interfere with the function of multiple members of the LEF1/TCF family of transcription factors.

Lef1 is expressed in the primordium of the hippocampus and in putative migratory granule cell precursors

To determine whether the Lef1-lacZ allele is expressed in the same pattern as the wild-type Lef1 allele, we compared the whole-mount in situ hybridization pattern of Lef1 in wild-type mouse brains at embryonic day 11.5 (E11.5) with the pattern of β-galactosidase activity in equivalent whole mounts from heterozygous Lef1-lacZ (b/+) mice (Fig. 2Aa,b,d,e). The expression patterns of Lef1 RNA and the LEF1-βGAL product are indistinguishable in all brain regions, including the telencephalon (T), mesencephalon (M), diencephalon (D), hypothalamus (Hy) and the dorsomedial telencephalon (medial pallium, MP), which includes the primordium of the hippocampus. Mice homozygous for the Lef1-lacZ allele (b/b) showed a similar pattern of expression, although the level of β-galactosidase activity, as judged by the intensity of X-gal staining, is consistently higher than in heterozygous embryos (Fig. 2Ac,f).

Analysis of the expression patterns during forebrain development using X-gal histochemistry on brain sections from heterozygous Lef1-lacZ mice revealed initial expression in the dorsomedial telencephalon at E10.5 (data not shown). By E11.5, a gradient of expression is detected in the medial pallium (Fig. 2Ba,h). The levels of expression are high immediately adjacent to the fimbria (Fi), which itself is negative for expression and progressively decrease toward the dorsal cerebral cortex. Based on morphological criteria, the region of strongest expression is the ventricular zone of the dentate gyrus anlage (Dvz) (Stanfield and Cowan, 1988; Altman and Bayer, 1990). This interpretation is confirmed by analysis of heterozygous Lef1-lacZ mice at E14.5 and E16.5, which express LEF1-βGAL in the ventricular zone adjacent to the fimbria and in cells that migrate from the Dvz to form a secondary proliferative population (SPP; Fig. 2Be,g,n). This secondary proliferative zone is thought to generate the dentate
gyrus (Altman and Bayer, 1990). Hippocampal expression of LEF1-βGAL protein and Lef1 RNA decreases at the end of gestation and later can be detected only in a few cells of the dentate gyrus (data not shown). LEF1-βGAL is also expressed in several other developing brain regions, including the choroid plexus (CP), the dorsal thalamus (Th), epithalamus (Fig. 2Bl,m), and tectum, all of which have been reported to contain Lef1 RNA (Oosterwegen et al., 1993; van Genderen et al., 1994). In homozygous Lef1-lacZ mice, we note a dorsal expansion of lacZ expression relative to heterozygous Lef1-lacZ mice (Fig. 2Ba,b). This expanded lacZ expression domain could be due to the increase in signal strength, which may allow detection of an otherwise weak signal. Alternatively, the absence of LEF1 may result in an expanded domain of expression.

Comparison of the effects of the Lef1-neo/neo and Lef1-lacZ/lacZ mutations on development of the telencephalon

Based on the cellular expression patterns of the Lef1 locus at various stages of hippocampal development, which include expression in cells that appear to migrate towards the dentate gyrus, we analyzed the hippocampal phenotypes of homozygous Lef1-neo/neo (n/n) and Lef1-lacZ/lacZ (b/b) embryos. Analysis of Nissl-stained sections of Lef1-neo/neo mice at E16.5, E18.5, postnatal day 0 (P0) and P5, shows an absence of a morphologically detectable dentate gyrus (DG), and an abnormal appearance of the CA fields (Fig. 3b,e,h,k, and data not shown). In contrast, comparable sections of E16.5 Lef1-lacZ/lacZ embryos show a complete lack of all hippocampal structures (Fig. 3c,f,i). In addition, the brains of these mutant embryos have cystic defects in external capsule (asterix in Fig. 3c,f) which may be due to vascular abnormalities because Lef1 is expressed in the developing blood vessels (see Fig. 2Bf). Other forebrain regions of the mutant embryos that express Lef1 (e.g. choroid plexus and dorsal thalamus) appear normal. Embryos that are heterozygous for the Lef1-neo allele or the Lef1-lacZ allele show no apparent mutant phenotype (data not shown). Thus, the expression of LEF1-βGAL protein from two mutant alleles results in a mutant phenotype that is more severe than that caused by the Lef1-neo/neo null mutation.

Lef1-neo/neo mutants do not form dentate gyrus granule cells

To confirm the defect in the formation of dentate gyrus in Lef1-neo/neo embryos, we examined the expression of genes that mark distinct hippocampal regions and cell types (Fig. 4A). The SCIP homeobox gene is expressed in the neocortex, subiculum and the CA1 field (Tole et al., 1997). Neuruplinin 2 (NP2), a receptor for semaphorin E and IV (Chen et al., 1997), is thought to be expressed in the entire hippocampus, with a boundary that approximates the transition of the parahippocampal gyrus (e.g., subiculum) with the medial limbic neocortex (e.g., cingulate and retrosplenial cortex). The Prox1 homeobox gene is expressed in the medial pallium, in the secondary proliferative population (SPP) of the dentate gyrus and fimbria, and in immature cells of the dentate gyrus-proper (Oliver et al., 1993 and this study). Finally, Calretinin, a calcium-binding protein, is expressed in immature dentate granule cells and in the Cajal-Retzius cells of the hippocampal outer marginal zone (Super et al., 1998).

In E16.5 Lef1-neo/neo embryos, expression of SCIP and NP2 appear normal in the parahippocampal and CA regions (Fig. 4Ab,d). However, in the region of the dentate gyrus, expression of both Prox1 and Calretinin is reduced to a small area in the marginal zone (see asterisk in Fig. 4Af,h). Expression of Prox1 is maintained in the thalamus and in a thin subpial strip, which might correspond to the migratory stream from the hippocampal and fimbrial ventricular zones. Because Prox1 and Calretinin are molecular markers for the immature granule cells in the dentate gyrus, the loss of these markers in the dentate gyrus of Lef1-neo/neo embryos could suggest that LEF1 may be required for the generation of the dentate gyrus granule cells. However, loss of these markers could also result from mis specification of the progenitors, rather than a block in the differentiation and/or proliferation of granule cells.

In principle, the loss of granule cell markers in the dentate gyrus could be due to defects in the specification, migration, proliferation and/or survival of granule cells. Dentate granule cell precursors are specified in the ventricular zone that is intercalated between the primordia of the fimbria and the CA fields (Altman and Bayer, 1990a). Immature dentate gyrus cells migrate from this ventricular zone and form a secondary proliferative population that follows a migratory stream to the hilus of the dentate gyrus. There, cells continue to divide, even in adults, and undergo further migration to the granule layer of the dentate gyrus (Stanfield and Cowan, 1988; Altman and Bayer, 1990b; Eriksson et al., 1998; Gage et al., 1998). TUNEL assays to detect apoptotic cells did not reveal any significant difference between wild-type and Lef1-neo/neo embryos at E17.5 (Fig. 4Bg,h). To examine the proliferation and migration of cells, mitotically active cells were labeled by injecting pregnant mice with a single pulse of bromodeoxyuridine (BrdU) 1 hour prior to the isolation of embryos at E18.5. Immunohistochemical detection of BrdU-labeled cells in wild-type and Lef1-neo/neo littersmates revealed a 3.4-fold decrease in the number of BrdU-labeled cells in the dentate gyrus of mutant embryos (Fig. 4Ba,b). This decrease in the number of proliferating cells was also confirmed by immunohistochemical detection of proliferating cell nuclear antigen (PCNA; Fig. 4Be,f). However, no significant change in the number of proliferating cells is observed throughout the ventricular zone of the hippocampal and fimbrial regions, and in the area of cells migrating towards the dentate gyrus (Fig. 4Ba,b). Because mitotically active glial precursors follow a similar migration pathway as immature granule cells (Sievers et al., 1992), the decrease in the number of migrating mitotically active granule cells could be underestimated by the presence of dividing glia cells.

To further address the role of LEF1 in the migration of granule cells, we performed a BrdU pulse experiment by injection of pregnant mice with BrdU at E16.5 and analysis at E18.5. A clear reduction in the number of BrdU-labeled cells was observed in the dentate gyrus of homozygous mutant embryos relative to wild-type embryos, but not in the ventricular zone, secondary proliferative zone and migratory stream (Fig. 4Bc,d). Some BrdU-labeled cells are present in the dentate gyrus remnant of mutant embryos, consistent with the presence of some cells in Nissl-stained specimens in this
The BrdU pulse experiment suggests that the second phase of migration may not take place. Although granule cells represent the majority of the neurons in the dentate gyrus, inhibitory interneurons and astroglia are also present in this region (Rickmann et al., 1987; Sievers et al., 1992). **Lef1-neo/neo** embryos have roughly normal numbers of DLX2-expressing interneurons in the region of the dentate gyrus (Fig. 4Ca,b). In mutant embryos, we also detected expression of the glial cell marker GFAP in the region of the ventricular zone and fimbria, and in an apparent stream of cells that co-localizes with the migratory stream of dentate granule cell precursors (Fig. 4Bc-f). In addition, we detected **Mash1** expression in a similar pattern, suggesting that **Mash1** may also mark glial cell precursors. Taken together, these data suggest that dentate granule cells, but not interneurons and astroglia, are specifically lost in the **Lef1-neo/neo** mutants.

**Lef1-lacZ/lacZ** mice lack the entire hippocampal field due to defects in patterning and proliferation

The morphological analysis of **Lef1-lacZ/lacZ** embryos indicated that the entire hippocampus is missing. To determine whether the morphologically abnormal dorsomedial

Fig. 2. Expression patterns of **Lef1** and **Lef1-lacZ** at various stages of brain development. (A) Lateral and dorsal views of the expression patterns of **Lef1** RNA (a,d) and LEF1-βGAL protein (b,c,e,f) in the brain of E11.5 embryos. **Lef1** RNA is detected by whole-mount in situ hybridization of wild-type (wt) embryos and LEF1-βGAL protein is visualized by X-gal staining of **Lef1-lacZ/wt** (b/+ ) embryos and **Lef1-lacZ/lacZ** (b/b) embryos. **Lef1** and LEF1-βGAL expression is detected in the telencephalon (T), diencephalon (D) and mesencephalon (M). Within the telencephalon, the dorsal view shows restricted expression in the medial pallium (MP). Stronger expression of LEF1-βGAL is observed in (b/b) embryos. Other abbreviation: Hy, hypothalamus. (B) LEF1-βGAL expression in rostral and caudal sections of (b/+ ) and (b/b) embryos at E11.5 (a,b,h,i), E14.5 (c,d,j,k) and E16.5 (e,f,g,l,m,n). Rostral sections of E11.5 embryos show LEF1-βGAL expression in the hippocampal primordia dorsal to the fimbria (Fi), which is seen more clearly in the enlarged insets corresponding to the boxed areas (a,b). In homozygous mutant embryos, the area of LEF1-βGAL expression is extended and the level of expression is increased. LV, lateral ventricle; Th, thalamus. (h) The arrow in the E11.5 caudal section indicates hippocampal expression of LEF1-βGAL. The area of the developing hippocampus (H) in E14.5 (b/+ ) embryos (e) is enlarged in g and further magnified in n. Abundant expression can be detected in the ventricular zone of the dentate gyrus (Dvz) and in some positive cells in the secondary proliferative population (SPP). Lower levels of expression are also found in the choroid plexus (CP). The boxed area in f is enlarged in the inset and shows LEF1-βGAL expression in blood vessels. Scale bar: (A) a-f, 0.9 mm; (B) a,b, 0.9 mm; c,d, 0.7mm; e,f, 0.8mm; g, 0.2mm; h, 0.02mm.
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telencephalon also lacks molecular characteristics of the developing hippocampus, we analyzed the expression pattern of SCIP, NP2 and Prox1 at E16.5 (Fig. 5A). Unlike the Lef1-neo/neo mutants, Lef1-lacZ/lacZ embryos lack molecular properties of the subiculum and CA fields (expression of NP2; Fig. 5Ac,d) and the dentate gyrus (expression of Prox1; Fig. 5Ac,d). Note that a small remnant of expression of both genes is present adjacent to the fimbria (asterisks in Fig. 5Ad,f). SCIP expression is extended to the transition of the cortex with the fimbria in the Lef1-lacZ/lacZ mutants, consistent with the loss of the entire hippocampal field (Fig. 5Ab).

The absence of the hippocampal region at E16.5 could result from improper regional specification, cell death and/or decreased proliferation of the primordium. To examine whether cells in the hippocampal primordium are re-specified in Lef1-lacZ/lacZ embryos, we analyzed the expression of several regulatory genes at E11.5 and E12.5 by in situ hybridization (Fig. 5B and data not shown). Bf1 encodes a winged-helix transcription factor that is required for patterning and growth of the telencephalon (Xuan et al., 1995; Dou et al., 1999). In wild-type embryos, Bf1 is expressed in a decreasing gradient through the hippocampal area and, like Lef1, is not expressed in the fimbria (Fig. 5Bc,d; Furuta et al., 1997). Prox1 is expressed both in the hippocampal primordium and the fimbria (Oliver et al., 1993), which also expresses high levels of bone morphogenetic proteins that inhibit Bf1 expression (Furuta et al., 1997). The fimbria also expresses high levels of Wnt2, Wnt3a, Wnt5a and Wnt7b (Fig. 5; Parr et al., 1993; Hollyday et al., 1995; Tole et al., 1997; Grove et al., 1998; Tole and Grove, 1999). The Lef1-lacZ/lacZ embryos show defects in the expression of Lef1, Bf1 and Prox1. Expression of the Lef1-lacZ allele is significantly increased relative to the wild-type allele and is graded with its highest level adjacent to the fimbria (Fig. 5Bb). In the mutant embryos, Bf1 loses its graded expression towards the fimbria, but rather is expressed at a constant level up to a sharp boundary at the fimbria (Fig. 5Bd). Moreover, Prox1 expression is maintained in the fimbria, but is lost in the region of the hippocampal primordium (Fig. 5Bf). In contrast, the expression of genes encoding the signaling molecules Wnt7b (Fig. 5Bh) and Bmp6 and Bmp7 (not shown) did not show any obvious changes. Thus, molecular defects in the hippocampal primordium can be detected prior to the appearance of obvious morphological abnormalities in this region, suggesting that the regional specification of the hippocampus may be abnormal in Lef1-lacZ/lacZ embryos.

Analysis for apoptosis at E11.5, E12.5 and E14.5, stages before the appearance of a morphological defect, did not provide evidence for increased cell death in the medial pallium (Fig. 5Ce,f and data not shown). Analysis of cell proliferation following a single injection of BrdU at E11.5 showed a 30%
Fig. 4. Characterization of the dentate gyrus defect in Lef1-neo/neo embryos. (A) In situ hybridization and immunohistochemistry of markers that distinguish regions of the hippocampus (SCIP, Neuropilin2 (NP2), Prox1 and Calretinin) in coronal sections of brains from E16.5 Lef1 wild-type (wt) embryos (a,c,e,g) and Lef1-neo/neo (n/n) embryos (b,d,f,h). In both wt and (n/n) mutant embryos, SCIP is expressed in the neocortex and CA1 field of the hippocampus but not in CA3 field (arrow in a,b). NP2 expression is absent in mutant embryos in the region of the dentate gyrus (DG; arrowhead in d) whereas normal expression can be detected in the CA fields. Expression of Prox1 is reduced in the area of the dentate gyrus in mutant embryos (asterisk in f), but is normal throughout the hippocampal ventricular zone (Hvz), the area of the migratory stream (MS) and the thalamus (Th). Calretinin expression is also severely impaired in the area where the dentate gyrus is normally located (asterisk in h). (B) Analysis of cell proliferation and apoptosis at E16.5 and E18.5. Wild-type embryos pulsed with 5’-bromodeoxyuridine (BrdU) 1 hour or 48 hours before sacrificing at E18.5. The numbers of BrdU-positive cells in the region of the dentate gyrus (DG; asterisk in b,d) were determined as 134±20 (n=8) for wild-type and 39±16 (n=11) for n/n mutant embryos. In contrast, in the dentate ventricular zone (Dvz), the numbers were 120±22 for wild-type and 171±17 for mutant embryos (see Materials and methods for calculation of the numbers). Antibody against PCNA (proliferating cell nuclear antigen) also identifies fewer proliferating cells in the DG region of mutant embryos (asterisk in f). TUNEL assay to detect apoptotic cells in wt and (n/n) embryos at E17.5 (g,h). Similar numbers of apoptotic cells (small arrows) are detected in multiple sections in the DG region of wt and mutant embryos. (C) In situ hybridization and immunohistochemistry of the cell type markers DLX2, Mash1 and glial filament associated protein (GFAP) to detect interneurons and glia in wild-type embryos (a,c,e) and Lef1-neo/neo embryos (b,d,f). DLX2-expressing interneurons can be detected throughout the hippocampal complex of mutant embryos (asterisk in b). Mash1 is expressed normally in the fimbria (Fi) and in cells of the migratory stream in wt and mutant embryos (arrows in c,d). In wt and mutant embryos, GFAP labels glial cells that are emanating from the transition of the fimbria with the hippocampal complex and populate the migrating stream (e,f). Scale bars: (A) a,b, 1.1 mm; c-h, 0.5 mm; (B) a,b, 0.43 mm; c,d, 0.5 mm; e-h, 0.3 mm; (C) a-f, 0.7 mm.
reduction in the number of mitotically active cells in the region of the hippocampal primordium closer to the fimbria (Fig. 5Ca,d).

**Overlapping expression of Lef1 and Tcf3 in the medial pallium**

The more severe phenotype of the Lef1-lacZ/lacZ mutation relative to the phenotype of the Lef1-neo/neo mutation could be accounted for by an interference of LEF1-βGAL protein with the function of other members of the LEF1/TCF family. Therefore, we examined potential co-expression of Lef1 with Tcf1, Tcf3 and Tcf4 by whole-mount in situ hybridization analysis (Fig. 6). At E10.5, Lef1 is clearly co-expressed with Tcf3 in the medial pallium, and probably also with low levels of Tcf1. At E11.5 and E12.5, expression of Lef1 and Tcf3 can be detected in an overlapping pattern in the medial pallium (Fig. 6c,d,k,l). We also note a reciprocal gradient for Lef1 and Tcf3. In contrast, Tcf1 and Tcf4 are expressed at a low level in the

Fig. 5. Characterization of hippocampal development in Lef1-lacZ/lacZ embryos.

(A) In situ hybridization of SCIP, Neuropilin2 (NP2) and Prox1 in the medial pallium region of wild-type (wt), Lef1-lacZ heterozygous (b/+) and Lef1-lacZ homozygous (b/b) embryos at E16.5. (b) SCIP expression, is maintained in b/b mice, but now extends to the fimbria (indicated by arrow). (d) In the (b/b) mutant, NP2 expression is severely impaired throughout the CA fields and subiculum (Sub), except for a small area designated by an asterisk. (f) Hippocampal expression of Prox1 is absent in the ventricular zone and dentate gyrus (DG) but is detected in a small area next to the fimbria in the DG. Other abbreviations: Ci, cingulate cortex; Hvz, hippocampal ventricular zone; NC, neocortex; Th, thalamus. (B) In situ hybridization of Lef1, Bf1, Prox1 and Wnt7b in (b/+) embryos (a,c,e,g) and (b/b) embryos (b,d,f,h) at E12.5. The boxes highlight the medial pallium and are shown at higher magnification below of each panel. Lef1 expression is detected in the developing hippocampus (H) but is absent in the fimbria (Fi; a,b). The b/b mutant shows Lef1 expression in an area, designated X, that corresponds to the region where the hippocampus normally forms (b). Bf1 is expressed in a gradient within the hippocampus in (b/+) embryos (c) but its expression is uniform in region X of (b/b) mutant embryos (d). In homozygous mutant mice, Prox1 expression is abrogated in region X, but is detected in the fimbria (f). Wnt7b expression is restricted to the mantle zone (MZ) and the fimbria in both the (b/+) and (b/b) embryos (g,h). (C) Proliferation and apoptosis assays in (b/+) embryos (a,c,e) and (b/b) mutant embryos (b,d,f). BrdU was injected at E11.5 and embryos were harvested 1 hour later. BrdU-positive cells are observed in the ventricular zone of the thalamus and hippocampal primordia (Th, thalamus; H, hippocampus) with a 27±4% (11 sections) decrease of mitotically active cells in the mutant (a,b). Higher magnification view of the boxed area is shown in c,d. TUNEL assays at E11.5 show no difference between b/+ control and b/b mutant embryos. Other abbreviation: CP, choroid plexus. Scale bars: (A) a,b, 0.8 mm; c, 0.9 mm; d-f, 0.7 mm; (B) a-h, 0.6 mm; insets, 0.19 mm; (C) a,b, 0.6 mm; c,d, 0.34 mm; e,f, 0.1 mm.
Fig. 6. In situ hybridization of the brain of E10.5, E11.5 and E12.5 wild-type embryos to detect RNA from the transcription factor genes Lefi, Tcf1, Tcf3 and Tcf4. Lateral views (a,e,i,m) and frontal sections (b,d,f,h,j,l,n,p) of the expression pattern of Lefi (a-d), Tcf1 (e-h), Tcf3 (i-l) and Tcf4 (m-p). Lefi is detected in a gradient in the hippocampus of E11.5 embryos (c), Tcf3 is detected in the same region as Lefi, but it can also be detected in the fimbrial region and presents a gradient of expression in the medial pallium that is the inverse of that of Lefi (k). Tcf1 and Tcf4 are expressed at very low levels in the medial pallium (g,h). Large arrows indicate expression in the medial pallium (MP). Labeling is the same as in Fig. 2; R, rhombencephalon. Scale bars: a,b,e,f,i,j,m,n, 0.5 mm; c,g,k,o, 0.3 mm. Insets 0.5 mm; d,h,l,p, 0.9 mm.

medial pallium at E11.5 and E12.5 (Fig. 6g,h,o,p). Thus, in Lefi-neo/neo mutants, TCF3 protein may compensate for the loss of LEF1, whereas the LEF1-βGAL protein may inhibit the function of all TCF proteins expressed in the medial pallium.

DISCUSSION

Our study shows that LEF1, a nuclear mediator of Wnt signaling that is expressed in the primordium of the hippocampus, is required for the generation of granule neurons of the dentate gyrus. In addition, LEF1, together with other members of the LEF1/TCF family, has an earlier role in the specification of the entire hippocampus. Thus, signaling by Wnt proteins, which are expressed in the region of the fimbria, may control multiple steps in the development of the hippocampus.

Role of Wnt and BMP signaling in patterning of the medial pallium

Wnt signaling has been implicated in regulating the function of the isthmic organizer (McMahon and Bradley, 1990; Thomas and Capecchi, 1990), patterning of the dorsal spinal cord and cell proliferation (Ikeya et al., 1997). Bmp genes are often expressed in the same region as Wnt genes and have been implicated in patterning of the spinal cord (Liem et al., 1995, 1997; Lee et al., 1998), cell type specification (Lee et al., 1998) and cell survival (Graham et al., 1994). With regard to the dorsoventral axis of the central nervous system, the medial wall of the dorsal telencephalon (medial pallium) is topographically equivalent to the dorsal parts of the spinal cord. The medial pallium is located in the area in which the primordia of the choroid plexus, fimbria and hippocampal complex (dentate gyrus, CA fields and subicular complex) develop (reviewed in Rubenstein et al., 1999). In the medial pallium, Wnt and Bmp genes are expressed in the fimbria (Grove et al., 1998). Lower levels of Wnt and Bmp gene expression are found in the adjacent neuroepithelium and Bmp genes are additionally expressed in the epithelium of the choroid plexus (Tole et al., 1997; Furuta et al., 1997; Grove et al., 1998).

In homozygous mutant Lefi-lacZ/lacZ embryos, in which Wnt-mediated activation of LEF1 is abrogated and that of TCF3 may be attenuated, we observe three molecular defects in medial patterning of the telencephalon (see scheme in Fig. 7). First, the expression of the Lefi-lacZ locus is increased, both at the RNA and protein level. This result suggests that LEF1 protein normally downregulates expression of its own gene, either directly or indirectly by antagonizing pathways that activate LEF1 gene expression. The increase in Lefi-lacZ expression in homozygous mutant mice is likely to be important for the attenuation of Wnt signaling by other members of the LEF1/TCF family. In the heterozygous mutant mice, no obvious mutant phenotype is observed, suggesting that the Lefi-lacZ gene does not function as a dominant negative allele and that the concentration of the encoded fusion protein may be critical for its interference with Wnt signaling. Homozygous Lefi-lacZ mice also show an expanded domain of lacZ expression, which could account for the dorsal expansion of the hippocampal defects in this mutant background relative to the Lefi-neo/neo background. In addition, Lefi is expressed in a gradient, consistent with an activation of Lefi expression by BMP signals (Figs 5, 7). BMP4 has been shown to induce the expression of Lefi in the mesenchyme of dental organ cultures from E11.5 embryos (Kratochvil et al., 1996). However, a simple correlation of Bmp and Lefi expression is unlikely because the BMP-rich fimbria does not express Lefi.

The second molecular defect in Lefi-lacZ/lacZ embryos is the loss of a gradient of Bf1 expression in the medial pallium. Targeted mutation of the transcription factor gene Bf1 has been
Pallium. For instance, is also expressed in the basal patterns of these genes have been simplified to focus on the medial neocortex that develops in this region is unknown. Fimbria; H (CA), CA fields of the hippocampus; NC, neocortex; choroid plexus; DG, dentate gyrus; EMT, eminentia thalami; Fi, locations for putative primordia of brain regions are indicated: CP, BMP molecules are also expressed in the choroid plexus. The telencephalon and different Bmp and Wnt genes have distinct expression of Prox1 (black stripes) on the lefthand side and embryos at E12.5. Coronal hemisections show expression of Lef1 (gradient of blue) and Prox1 (black stripes) on the lefthand side and expression of Wnt and Bmp genes (red) on the righthand side. The expression patterns of these genes have been simplified to focus on the medial pallium. For instance, Prox1 is also expressed in the basal telencephalon and different Bmp and Wnt genes have distinct expression patterns that include expression in the cerebral cortex. BMP molecules are also expressed in the choroid plexus. The locations for putative primordia of brain regions are indicated: CP, choroid plexus; DG, dentate gyrus; EMT, eminentia thalami; Fi, Fimbria; H (CA), CA fields of the hippocampus; NC, neocortex; NC*, neocortical region of the Lef1-lacZ/lacZ mutant (the type of neocortex that develops in this region is unknown).

Fig. 7. Schematic representation of patterns of gene expression in the medial pallium of wild-type (top) and Lef1-lacZ/lacZ (bottom) embryos at E12.5. Coronal hemisections show expression of Lef1 (gradient of blue) and Prox1 (black stripes) on the lefthand side and expression of Bf1 (gradient of green) and representative expression of Wnt and Bmp genes (red) on the righthand side. The expression patterns of these genes have been simplified to focus on the medial pallium. For instance, Prox1 is also expressed in the basal telencephalon and different Bmp and Wnt genes have distinct expression patterns that include expression in the cerebral cortex. BMP molecules are also expressed in the choroid plexus. The locations for putative primordia of brain regions are indicated: CP, choroid plexus; DG, dentate gyrus; EMT, eminentia thalami; Fi, Fimbria; H (CA), CA fields of the hippocampus; NC, neocortex; NC*, neocortical region of the Lef1-lacZ/lacZ mutant (the type of neocortex that develops in this region is unknown).

reported to result in hypoplasia of the entire telencephalon, apparently due to premature cell differentiation and decreased cell proliferation (Xuan et al., 1995). In addition, the telencephalon of Bf1 mutants is dorsalized, as reflected by a wide-spread ectopic expression of BMP4 (Dou et al., 1999). Therefore, Bf1 protein may downregulate expression of Bmp genes. Furthermore, BMP proteins have also been shown to repress Bf1 expression (Furuta et al., 1997). This reciprocal negative feedback loop may explain why Bf1 is expressed in the medial pallium in a gradient that decreases in the vicinity of the BMP-rich fimbria. This change in Bf1 expression may be directly related to reduced Wnt signaling. Finally, expression of the Prox1 homeobox gene in Lef1-lacZ/lacZ embryos is not detectable in the hippocampal primordium, in which Prox1 is co-expressed with Lef1. The function of Prox1 in the vertebrate central nervous system has not been fully elucidated, although Prox1 may be required for neurogenesis and expression of Prox1 can be induced by Mash1 (Torii et al., 1999). The Drosophila orthologue of Prox1, Prospero, has an essential role in neuronal specification (Broadus and Doe, 1997). Therefore, the absence of Prox1 expression in both the hippocampal primordium of Lef1-lacZ/lacZ embryos and dentate gyrus granule cells of Lef1-neo/neo embryos, raises the possibility that Prox1 is a downstream target of Lef1. Together, these defects reflect an abnormal patterning of the hippocampal domain of the medial pallium, which results in a morphological and molecular loss of the dentate gyrus, CA fields and subiculum complex. In addition to the patterning defects, Lef1-lacZ/lacZ embryos show a proliferative defect in the developing medial pallium, which shows a 30% decrease in the number of dividing cells. Thus, nuclear mediators of Wnt signaling, Lef1/TCF proteins, may participate in the regulation of patterning and outgrowth of the entire hippocampal primordium.

Role of LEF1 in the differentiation of a specific hippocampal cell type: dentate granule cells

Lef1/Tcf genes appear to be required during multiple stages of hippocampal development. In addition to the regulation of early hippocampal development, Lef1 is essential to generate dentate granule cells. Lef1 is expressed in the proliferating precursors of granule cells in the ventricular zone and in the migratory secondary proliferative population. Consistent with this expression pattern, Lef1-neo/neo embryos lack molecular markers of dentate granule cells (Prox1 and Calretinin) in the region of the dentate gyrus. In addition, the dentate gyrus remnant of Lef1-neo/neo embryos contains approximately 2.5-fold reduced number of proliferating cells. BrdU-pulse experiments suggest that granule cells, or their precursors, do not arrive at the dentate gyrus. Therefore, the observed decrease in the number of proliferating cells may reflect the absence of dentate granule cells. The residual proliferating cells are likely glia, which are present in the region of the dentate gyrus remnant and are known to be mitotically active in the SSP and migratory stream (Sievers et al., 1992).

The absence of a specific cell type, the dentate gyrus granule cells, in Lef1-neo/neo mice provides insight into the generation of granule cells, glia and interneurons. We hypothesize that the hippocampal ventricular zone adjacent to the fimbria, which expresses the highest levels of LEF1, contains the granule cell progenitors. In Lef1-neo/neo embryos, these cells are incapable of differentiating into granule cells as evidenced by the loss of Prox1 expression in the dentate gyrus remnant. In contrast, the ventricular zone flanking this region is able to produce pyramidal cells of the CA field. In addition, GFAP-expressing glia cells continue to be produced in the mutant mice, consistent with the model that these cells are derived from the fimbria, which does not express Lef1 (Altman and Bayer, 1990). Finally, the distribution of Dlx2-expressing immature interneurons is not affected by the Lef1-neo/neo mutation, consistent with the model that cortical (including hippocampal) interneurons are derived from the basal ganglia (Anderson et al., 1997, 1999).

Transcriptional control of hippocampal development

Inroads into understanding the genetic hierarchy that controls hippocampal development have been made possible through targeted mutagenesis of several transcription factors in mice. To date, seven mutations have been reported that affect hippocampal patterning and/or proliferation. Mutations of the LIM homeodomain genes Lhx2 (Porter et al., 1997) and Lhx5 (Zhao et al., 1999) eliminate the entire hippocampus. Eno2 mutants lack the dentate gyrus and show hypoplasia of the CA fields (Pellegrini et al., 1996; Yoshida et al., 1997). Targeted inactivation of the transcription factor gene Gli3 results in the absence of the choroid plexus and a dysmorphic, but poorly characterized hippocampus (Frantz, 1994; Grove et al., 1998).
Gli3 has been implicated as a regulator of Wnt signaling and in Gli3-deficient mice, Wnt expression is abrogated in the cortical hem (Grove et al., 1998). Otx1+/−/Otx2+/− mice have a form of holoprosencephaly that includes loss of medial pallial tissues (Matsuo et al., 1995; Acampora et al., 1997). Finally, NeuroD-deficient mice lack the dentate gyrus and cerebellar granular neurons apparently due to increased cell death (Miyata et al., 1999).

The Lef1-neo/neo and Lef1-lacZ/lacZ mutations are distinct from each of these mutations in several aspects. First, Lef1 is expressed relatively late in brain development, compared to Lhx2, Lhx5, Emx2, Otx1 and Otx2, which are all known to be expressed in the neural plate (Simeone et al., 1992; Sheng et al., 1997; Porter et al., 1997). Second, within the telencephalon, Lef1 has the most restricted domain of expression, whereas Lhx2, Emx2, Otx1, Otx2 and Gli3 are more broadly expressed. The expression of Lhx5 resembles that of Lef1 with the exception that Lhx5 is also expressed in the fimbria, which may account for the absence of fimbrial Wnt5a and Bmp7 expression in Lhx5-deficient embryos (Zhao et al., 1999). The loss of these patterning signals may have a primary role in the Lhx5-deficient phenotype. The overexpression of Lef1 with other Lef1/Tcf genes in regions of the lateral neural plate suggest that multiple members of this family of transcription factors may regulate hippocampal development in an, at least partially, redundant manner. Although the analysis of Lef1-lacZ/lacZ mice supports this view, the analysis of hippocampal development in Lef1+/−/Tcf1−/− mutants is hampered by the early lethality around E9.5 (Galceran et al., 1999).

The mechanism by which Lef1-lacZ/lacZ mice exhibit a stronger mutant phenotype than a homozygous null allele is still unclear. Although our transfection data indicate that the LEF1-BGAL protein can interfere with β-catenin-mediated transcriptional activation, an approximately 20-fold molar excess of LEF1-BGAL relative to LEF1 or TCF1 is needed. It is possible that a smaller amount of β-catenin is involved in transcriptional activation in vivo relative to transfection experiments. Such a difference could account for the requirement of a molar excess of LEF1-BGAL in vitro, which has also been observed for the inhibition of β-catenin-mediated transcriptional activation by SOX17β in Xenopus embryos (Zorn et al., 1999). However, we cannot rule out that the LEF1-BGAL protein interferes with β-catenin function by some other mechanism.

The Lef1-neo/neo mutation eliminates a specific hippocampal cell type: dentate granule cells. We are unaware of other reported mutations that affect the generation of a specific hippocampal cell type. The dentate granule cells are among the few neurons that continue to be produced in adult animals (Stanfield and Cowan, 1988; Gage et al., 1998). Moreover, the granule cells of the dentate gyrus have been recently shown to represent a stem-cell-like population that maintains proliferative capacity in human adults (Eriksson et al., 1998). Notably, Tcf4, a member of the LEF1/TCF family that is expressed in colon epithelia, has been shown to be required for the maintenance of the crypt stem cells of the small intestine (Korinek et al., 1998b). Thus Wnt signaling may be involved in the maintenance of cells that have stem cell characteristics in both brain and colon. The renewal and survival of dentate gyrus granule cells have been implicated in the formation of new memories, and dysfunction of these cells may be related to epilepsy. Thus, further studies are warranted to elucidate the mechanism(s) through which Lef1 controls the generation and/or maintenance of dentate gyrus granule cells.

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