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The hem of the embryonic cerebral cortex is defined by the expression of multiple *Wnt* genes and is compromised in *Gli3*-deficient mice

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SUMMARY

In the developing vertebrate CNS, members of the *Wnt* gene family are characteristically expressed at signaling centers that pattern adjacent parts of the neural tube. To identify candidate signaling centers in the telencephalon, we isolated *Wnt* gene fragments from cDNA derived from embryonic mouse telencephalon. In situ hybridization experiments demonstrate that one of the isolated *Wnt* genes, *Wnt7a*, is broadly expressed in the embryonic telencephalon. By contrast, three others, *Wnt3a*, *5a* and a novel mouse *Wnt* gene, *Wnt2b*, are expressed only at the medial edge of the telencephalon, defining the hem of the cerebral cortex.

The *Wnt*-rich cortical hem is a transient, neuron-containing, neuroepithelial structure that forms a boundary between the hippocampus and the telencephalic choroid plexus epithelium (CPe) throughout their embryonic development. Indicating a close developmental relationship between the cortical hem and the CPe, *Wnt* gene expression is upregulated in the cortical hem both before and just as the CPe begins to form, and persists until birth. In addition, although the cortical hem does not show features of differentiated CPe, such as expression of transthyretin mRNA, the CPe and cortical hem are linked by shared expression of members of the *Bmp* and *Msx* gene families.

In the *extra-toes*^{J} (*Xt*^{J}) mouse mutant, telencephalic CPe fails to develop. We show that *Wnt* gene expression is deficient at the cortical hem in *Xt*^{J}/*Xt*^{J} mice, but that the expression of other telencephalic developmental control genes, including *Wnt7a*, is maintained. The *Xt*^{J} mutant carries a deletion in *Gli3*, a vertebrate homolog of the *Drosophila* gene *cubitus interruptus (ci)*, which encodes a transcriptional regulator of the *Drosophila Wnt* gene, *wingless*. Our observations indicate that Gli3 participates in *Wnt* gene regulation in the vertebrate telencephalon, and suggest that the loss of telencephalic choroid plexus in *Xt*^{J} mice is due to defects in the cortical hem that include *Wnt* gene misregulation.

Key words: Choroid plexus, Cortical hem, Extra-toes, *Gli3*, Telencephalon, *Wnt2b*, Mouse

INTRODUCTION

The telencephalon is the largest, most complex part of the mammalian CNS (Nauta and Feirtag, 1986), and must be patterned during development into numerous functional subdivisions. Patterning of the developing telencephalon requires the division of the cerebral cortex into different types of cortex, such as archicortex and neocortex, and the subdivision of these large cortical regions into many functionally specialized areas. Broader divisions include those between cerebral cortex and subcortical nuclei, and between two strikingly different types of tissue that develop in the medial wall of the telencephalon, the medial cerebral cortex and the non-neuronal, secretory epithelium of the choroid plexus (CPe). What are the mechanisms by which these divisions are set up in the developing telencephalon? One approach to this question is to isolate from the embryonic telencephalon members of developmental control gene families implicated in patterning elsewhere in the embryo.

The *Wnt* family of developmental control genes encodes secreted proteins that participate in tissue patterning and morphogenesis (Parr and McMahon, 1994). The extent to which Wnt proteins operate as morphogens themselves, directly acting to pattern adjacent tissues, or serve to establish local signaling centers from which other morphogens act, remains uncertain (Zecca et al., 1996). Nonetheless, in both vertebrate and invertebrate species, *Wnt* gene expression marks sites of morphogenetic signaling, by appearing at boundaries between developmental compartments and at the edges of morphogenetic fields (Lawrence and Struhl, 1996; Parr et al., 1993). In *Drosophila* development, expression of *wingless*, which is the canonical member of the *Wnt* gene family, distinguishes the boundaries between parasegments, the border between dorsal and ventral compartments in the wing imaginal disc, and the dorsal and ventral perimeters of the developing optic lobe (Diaz-Benjumea and Cohen, 1995; Kaphingst and Kunes, 1994). In vertebrate development, *Wnt* gene expression marks the sites of neuroectoderm signaling centers that control

dorsal-ventral patterning in the spinal cord, and rostral-caudal patterning at the junction of the midbrain and hindbrain (Bally-Cuif et al., 1995; Parr et al., 1993).

To gain insight into how the telencephalon is patterned, we searched for members of the *Wnt* gene family whose expression might mark signaling centers within the embryonic telencephalon. A well-established PCR procedure (Gavin et al., 1990) was used to isolate several members of the *Wnt* gene family, including the previously unreported mouse ortholog of the human *WNT13* gene (Katoh et al., 1996), from embryonic day 12.5 (E12.5) mouse telencephalon. E12.5 is early in the growth and development of the telencephalon, when macroscopic patterning is likely to be still underway. In situ hybridization was employed to determine the patterns of expression of the isolated *Wnt* genes within the embryonic telencephalon. Three *Wnt* genes, including the novel mouse *Wnt* gene, were found to be expressed selectively at the medial margin of the telencephalon, defining a zone that we term the *Wnt*-rich 'cortical hem'.

The *Wnt*-rich cortical hem forms a boundary between two major components of the medial telencephalon throughout their embryonic development. The hippocampus, a part of the medial cerebral cortex, develops dorsal to the cortical hem, and the CPe differentiates ventral to the cortical hem. By birth, much of the growth and basic patterning of both the CPe and hippocampus is complete (Sturrock, 1979; Tole et al., 1997), and the cortical hem, as defined by multiple *Wnt* gene expression, disappears. The cortical hem is therefore positioned to provide patterning signals to both the developing hippocampus and the choroid plexus. In the present study, we have focused on the developmental relationship of the cortical hem to the telencephalic CPe.

Previous histological studies indicate that the CPe is generated from a specialized part of the neuroepithelium of the medial telencephalon that is distinguished from the rest of the telencephalic neuroepithelium by becoming progressively effaced (MacKenzie et al., 1991; Maruyama and D'Agostino, 1967; Nicholson-Flynn et al., 1996; Sturrock, 1979; Zaki, 1981). The presumptive CPe, or 'choroid plaque', first appears at the dorsal midline of the telencephalic vesicle as the midline invaginates to form the medial walls of the two telencephalic hemispheres (Sturrock, 1979). At E10.5 in the mouse, the choroid plaque is a small zone of thinning neuroepithelium at the midline, identifiable by the presence of many pyknotic cells (Sturrock, 1979; Zaki, 1981). As the medial walls continue to invaginate, differentiated CPe appears in the position of the choroid plaque. On either side of the plaque, a part of the medial wall of each hemisphere also begins to show evidence of cell death and to thin (Furuta et al., 1997; Sturrock, 1979). CPe continues to ramify ventral to the region of thinning neuroepithelium, until, by birth, the CPe is histologically mature (Sturrock, 1979), and the region of thinning neuroepithelium has disappeared.

In the present study, we show that the region of thinning neuroepithelium in the medial telencephalon is identical with the *Wnt*-rich cortical hem. Although several other genes and gene products, such as members of the *Bmp* and *Msx* gene families, as well as high molecular mass tropomyosins (Furuta et al., 1997; Nicholson-Flynn et al., 1996; MacKenzie et al., 1991, 1992; present study), are expressed in the cortical hem, they are each expressed elsewhere in the medial telencephalic wall as well. To date, only the expression pattern of multiple *Wnt* genes uniquely distinguishes the cortical hem – the part of the neuroepithelium suggested by histological studies to participate in generating the CPe. These observations suggest that Wnt signaling could play a specific role in the initial division of the medial wall neuroepithelium into a part that generates the medial cerebral cortex, and another part that forms the CPe.

Franz (1994) has reported that the *extra-toes* mutant mouse does not develop telencephalic choroid plexus, and that this appears to be due directly to the *extra-toes* mutation rather than indirectly to general forebrain dysmorphology. Intriguingly, the *extra-toes* mutant carries an intragenic deletion of a gene, *Gli3* (Hui and Joyner, 1993; Schimmang et al., 1992), that is homologous to a *Drosophila* gene implicated in the regulation of *wingless*. In *Drosophila*, the zinc-finger transcription factor, *cubitus interruptus* (*ci*), is a transcriptional regulator of *wingless*, mediating response to a hedgehog signal (Von Ohlen et al., 1997). In vertebrates, three *ci* homologs, *Gli*, *Gli2* and *Gli3*, have been identified (Hui et al., 1994; Orenic et al., 1990; Ruppert et al., 1990), one of which, Gli, has been shown to be a target of sonic hedgehog signaling (Lee et al., 1997). In an analysis of the *extra-toes*^{J} mutant mouse, we have tested the hypotheses that Gli3 may participate in *Wnt* gene regulation in the vertebrate telencephalon, and that the defect in telencephalic choroid plexus in *extra-toes* mice is accompanied by *Wnt* gene misregulation in the cortical hem.

MATERIALS AND METHODS

Mice

Outbred CD-1 timed pregnant mice were obtained from the University of Chicago Cancer Research Center Transgenic Facility. Xt^J mutant mice in a C3HeB/FeJ background were obtained as heterozygotes from the Jackson Laboratory (Bar Harbor, ME), and were interbred. Midday of the day of vaginal plug discovery was considered embryonic (E) day 0.5. Homozygous Xt^J embryos and their littermates were recovered for gene expression analysis from Xt^J/+ intercrosses at E10.5, E12.5 or E16.5. Homozygote embryos were readily distinguished from heterozygote and wild-type embryos by their appearance (Hui and Joyner, 1993; Johnson, 1967). We checked our classifications by processing selected litters for whole-mount in situ hybridization demonstrating *Gli3* gene expression, which proved to be undetectable in phenotypically homozygote embryos and present in heterozygote and wild-type animals (data not shown). 20% of the embryos recovered at E12.5, and 16% of those recovered at E16.5, were classified as homozygote (Table 1). That these percentages are slightly lower than 25% may be due to early lethality of the Xt^J mutation; consistent with this interpretation, many embryos (21 in 32 litters) appeared to be in the process of being resorbed at the time of killing (these embryos were not included in the total number recovered).

Isolation of telencephalic *Wnt* gene fragments

cDNA was prepared from E12.5 telencephalon total RNA isolated by guanidinium-acid phenol extraction and employed as substrate for the

Table 1. Classification of Xt^J/Xt^J embryos recovered at E12.5 and E16.5

Age of embryos	Exencephalic Xt^J/Xt^J embryos	Non-exencephalic Xt^J/Xt^J embryos	Total embryos recovered	Percentage Xt^J/Xt^J
E12.5	23	10	161 (23 litters)	20
E16.5	4	5	55 (8 litters)	16

Wnt gene fragment PCR amplification scheme of Gavin et al. (1990). Gel-purified PCR products were subcloned into *Eco*RI-*Xba*I-digested pBluescript II KS(+) plasmid (Stratagene). 45 recombinant clones were sorted by *Hae*III, *Hin*fI and *Rsa*I fingerprinting, and representatives of each group were sequenced. Five distinct mouse *Wnt* genes were identified, expression of one of which (*Wnt4*) was not reproducibly detected with in situ hybridization experiments on E12.5 telencephalon. *Wnt7b*, which is expressed in the early embryonic telencephalon (Parr et al., 1993), was not among the *Wnt* genes recovered, suggesting that our screen of telencephalic *Wnt* genes was not exhaustive.

One of the recovered fragments identified a novel mouse *Wnt* gene, *Wnt2b*. This fragment was employed in high-stringency cDNA library screens (E12.5 mouse λEX*lox* library, Novagen; E11.5 mouse λgt10 library, Clontech) as previously described (Ragsdale et al., 1989). Nucleotide sequencing was done by the dideoxynucleotide method (Sequenase kit, US Biochemical) and with the Applied Biosystems Prism 377 and 377XL DNA sequencers (University of Chicago Cancer Research Center), and was analyzed with GeneWorks software.

Histology

Harvested mouse embryos were immersed in 4% paraformaldehyde in phosphate-buffered saline and processed for two-color wholemount non-radioactive in situ hybridization with a modification of the method of Nieto et al. (1996). Significant changes in the protocol include replacement of proteinase K digestion with detergent treatment (Rosen and Beddington, 1993) and use of the chromagens nitroblue tetrazolium (Boehringer; 350 μg/ml) and tetranitroblue tetrazolium (Sigma; 350 μg/ml), which were selected from a range of chromagens tested for sensitivity and color separation (T. A. Sanders and C. W. Ragsdale, unpublished data). Some embryo brains were cryoprotected after fixation, sectioned into 40 μm coronal sections using a sledge microtome (Leica), and processed for in situ hybridization using a method described previously (Tole et al., 1997; Tole and Patterson, 1995).

Riboprobes incorporating digoxygenin- or fluorescein-labeled nucleotides were synthesized from linearized plasmids with T7 or SP6 polymerase (Boehringer). Probes for *Wnt3a*, *Wnt5a* and *Wnt7a* were derived from the subcloned PCR fragments. *Wnt2b* gene expression was demonstrated with cDNA clone pRK (1 kb insert in pEX*lox* vector; linearization by *Eco*RI digestion, antisense riboprobe transcription with SP6 polymerase). *Class III β-tubulin* gene expression was detected with cDNA clone p82-2, in which a 321 bp insert derived from a 3′ untranslated region of the mouse β_6-*tubulin* gene (Burgoyne et al., 1988) was subcloned in the pBluescript II SK(+) vector (*Bam*HI digestion, T7 transcription). Other probes employed were derived from a 1 kb mouse *Gli2* cDNA, a 0.8 kb mouse *Gli3* cDNA (Hui et al., 1994), a 0.6 kb rat *transthyretin* cDNA (Duan et al., 1989), a 2.2 kb mouse *neurogenin2* genomic fragment (Sommer et al., 1996), a 0.7 kb mouse *Msx1* cDNA, a 0.85 kb mouse *Msx2* cDNA (MacKenzie et al., 1991, 1992), a 1.2 kb mouse *Bmp2* cDNA, a 2 kb mouse *Bmp6* cDNA, a 0.8 kb mouse *Bmp7* cDNA (Furuta et al., 1997), a mouse *Bmp4* cDNA (IMAGE Consortium, GenBank number AA473799), and a 0.8 kb mouse *Fgf8* cDNA (Crossley and Martin, 1995).

Dividing cells in mouse embryos were labeled with 5-bromo-2′-deoxyuridine (BrdU) (100 mg/kg) delivered intraperitoneally to pregnant mice 2 hours before killing. Nucleotide incorporation in tissue sections was detected with antibody M0744 (DAKO) followed by diaminobenzidine peroxidase immunohistochemistry.

RESULTS

Multiple *Wnt* genes define a boundary zone between the developing hippocampus and the choroid plexus

To identify members of the *Wnt* gene family that are specifically expressed in the developing telencephalon, a PCR procedure (Gavin et al., 1990) was employed to isolate *Wnt* gene fragments from cDNA derived from the cerebral hemispheres of E12.5 CD-1 mice. Sequencing the PCR products showed that cDNA fragments from five different *Wnt* genes had been isolated: *Wnt3a*, *4*, *5a*, *7a* and a previously unreported mouse *Wnt* gene. cDNA library screens employing the novel *Wnt* gene fragment yielded overlapping cDNA clones providing 711 base pairs of coding sequence (GenBank database accession number AF038384). Sequence comparisons establish that this novel mouse *Wnt* gene is the ortholog of the recently described human *WNT13* gene (Katoh et al., 1996), with 98% identity over the 236 amino acid C-terminal fragment for which we have sequence data. Like human *WNT13*, this mouse *Wnt* gene is closely related to the *Wnt2* subfamily, with amino acid identity scores of 69% to mouse *Wnt2* (McMahon and McMahon, 1989) and 79% to Xenopus *Wnt2b* (Landesman and Sokol, 1997). Following the *Wnt* gene nomenclature suggestions of Cadigan and Nusse (1997) we identify this new mouse *Wnt* gene as mouse *Wnt2b*.

In situ hybridization experiments showed that four of the isolated *Wnt* genes, *Wnt2b*, *3a*, *5a* and *7a*, are strongly expressed in E12.5 telencephalon. Moreover, three *Wnt* genes, *Wnt2b*, *3a* and *5a*, show a striking, similar pattern of expression in the telencephalon. The expression of *Wnt2b*, representative of the three, is shown in Fig. 1. *Wnt2b* is strongly expressed in a band of tissue along the medial telencephalic wall, adjacent to the lateral ventricle (Fig. 1A). Views of the medial face of the telencephalic hemisphere (Fig. 1B) or coronal sections through the telencephalon (Fig. 1D-F) show the close association of the *Wnt2b* expressing zone with the newly forming CPe of the lateral ventricle (Fig. 1B,D). The band of *Wnt2b* expression is dorsal to the forming CPe at rostral levels (Fig. 1B,D), and curves ventrally at caudal levels to surround the caudal end of the CPe (Fig. 1B,E,F). Expression of *Wnt3a* surrounding the developing telencephalic CP has been reported previously (Roelink and Nusse, 1991); we found that both *Wnt3a* and *5a* show the same characteristic curved band of expression along the medial face of the telencephalon as *Wnt2b* (Fig. 3A,H). Multiple *Wnt* genes are therefore expressed at the continuous, curving line of attachment between the differentiating CPe and adjacent telencephalic neuroepithelium.

The same zone of tissue that is delineated by the expression of *Wnt2b*, *3a* and *5a* is also distinguished by the absence of detectable expression of a fourth isolated *Wnt* gene, *Wnt7a*. At E12.5, *Wnt7a* is strongly expressed in the lateral and dorsal cerebral cortex (Fig. 1C), but not at the medial margin of the cerebral hemisphere (Fig. 1G). Expression of *Wnt7a* thus appears complementary to that of *Wnt2b*, *3a* and *5a*.

The *Wnt*-rich boundary tissue is neuron-containing neuroepithelium and forms the cortical hem

What type of tissue makes up the *Wnt*-rich boundary zone between the differentiating CPe and adjacent neuroepithelium? Is it CPe at an early stage of differentiation, cortical neuroepithelium or a third type of tissue? Differentiating CPe forms a simple columnar epithelium, then matures into cuboidal epithelium (Sturrock, 1979). Cortical neuroepithelium is a pseudostratified epithelium in which the nuclei of dividing cells translocate within the ventricular zone

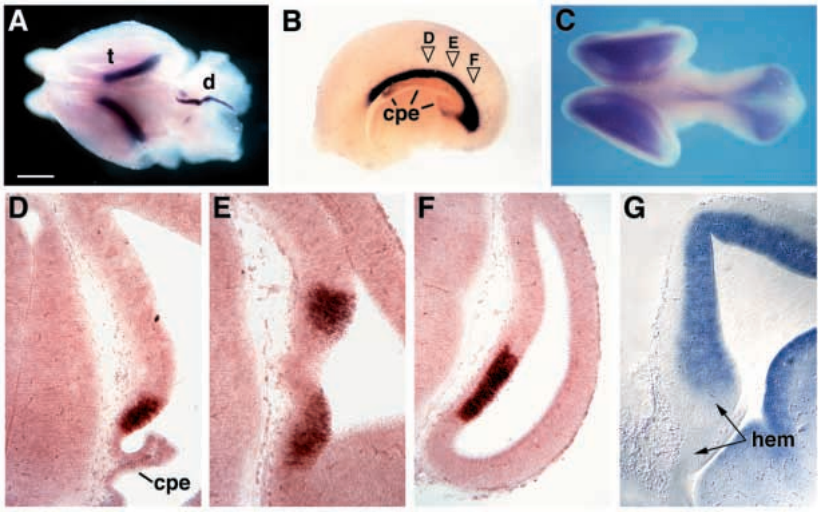

Fig. 1. The cortical hem is marked by the complementary expression of *Wnt2b* and *7a*. (A) Dorsal view of a CD-1 mouse forebrain at E12.5. Rostral is to the left. *Wnt2b* is strongly expressed along the caudal two-thirds of the medial wall of the telencephalon (t), next to the lateral ventricle. In the diencephalon (d), *Wnt2b* expression marks the dorsal midline, and two patches on either side of the midline. (B,D-F) A medial view of a telencephalic hemisphere at E12.5 (B, rostral is to the left) and coronal sections through a similar hemisphere (D-F, midline is to the left). *Wnt2b* expression defines the hem of the embryonic cerebral cortex (B), and abuts the developing choroid plexus epithelium (cpe) dorsally (B,D,E), caudally (B,F), and caudoventrally (B,E). (C,G) Dorsal view of a mouse forebrain at E12.5 (C, rostral to the left). Coronal section through a telencephalic hemisphere at E12.5 (G, midline to the left). *Wnt7a* is expressed in most of the embryonic cerebral cortex (C), but not in the cortical hem (hem, arrows in G). Bar in A, 550 μm (A); 420 μm (B); 700 μm (C); 130 μm (D); 110 μm (E); 170 μm (F); 130 μm (G).

(VZ) between the ventricular and pial surfaces of the cortex (Bayer and Altman, 1991). CPe cells are characterized as soon as they begin to differentiate by the strong expression of transthyretin (TTR), a transport protein for thyroxine and retinols (Thomas and Dziadek, 1993). Differentiating cortical neurons can be identified by the expression of a neuronal marker, *class III β-tubulin* mRNA (Lee et al., 1990; Tole et al., 1997). These neurons migrate away from the VZ towards the pial surface to form the preplate and cortical plate (Bayer and Altman, 1991).

At E12.5, cells expressing *Wnt2b*, *3a* and *5a* at the boundary between the cortex and CPe do not form a simple columnar or cuboidal epithelium, nor do they express *TTR* (Fig. 2A,C). Like adjacent cortical neuroepithelium, the *Wnt*-rich tissue is organized as a pseudostratified epithelium, in which dividing cells labeled with BrdU form a broad VZ (Fig. 2G). At E12.5, moreover, the *Wnt*-rich tissue contains a preplate-like layer of neurons that express *class III β-tubulin* (Fig. 2F). Finally, expression of the putative neuronal determination factor, *neurogenin2* (*Ngn2*)/*MATH4A* (Gradwohl et al., 1996; Sommer et al., 1996) distinguishes the VZ of the entire cerebral cortical neuroepithelium, including the *Wnt*-rich tissue, but avoids the CPe (Fig. 2B,D). The *Wnt*-rich tissue is therefore embryonic cortical neuroepithelium, rather than CPe, and thus represents the hem of the developing cerebral cortex.

Between the *Wnt*-rich cortical hem and the CPe, a junctional epithelium, a few cells wide, can be identified. Junctional epithelium does not express, or expresses weakly, *TTR*, *Wnt2b*, *3a*, *5a* and *Ngn2* (Fig. 2B,C, and data not shown), and does not contain a VZ or preplate (data not shown). Thus, at E12.5, four tissues can be identified in the medial wall of the telencephalon

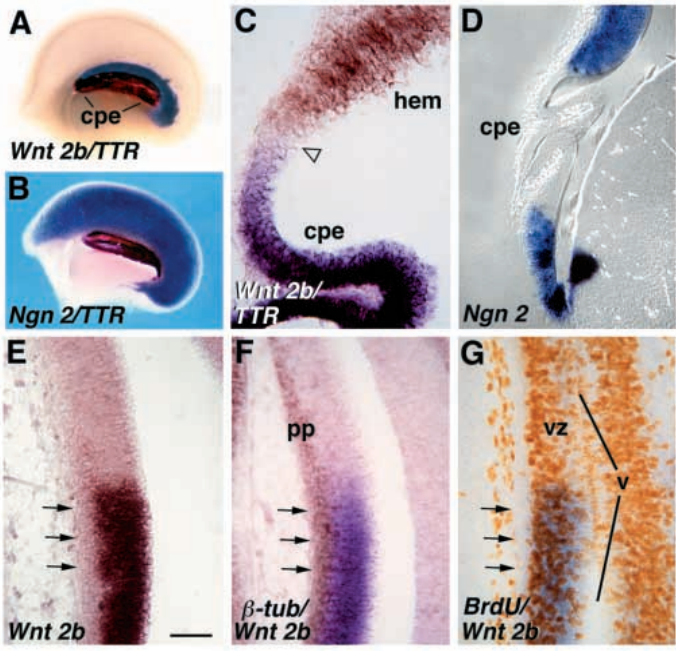

Fig. 2. The cortical hem shows molecular and morphological features of cortical neuroepithelium. Medial views of telencephalic hemispheres at E12.5 (A,B) and coronal sections through E12.5 hemispheres (C-G). Hemispheres oriented with rostral to the left. (A-D) Choroid plexus epithelium (cpe) strongly expresses *TTR* (brown in A, purple in B and C). The cortical hem (hem), marked by *Wnt2b* expression (blue in A, brown in C), abuts the cpe (A,C), but does not contain *TTR*-expressing cells (C). The junctional epithelium between the cpe and hem (open arrowhead in C) expresses neither *TTR* nor *Wnt2b*. Expression of *Ngn2* (blue in B and D) marks cerebral cortex neuroepithelium, including the hem but avoiding the cpe. The junctional epithelium does not express *Ngn2* (thin line of unstained cells just dorsal to the cpe in B). (E-G) Sections through the caudal hem and adjacent embryonic cerebral cortex at a level similar to that shown in Fig. 1F. Medial to the left. *Wnt2b* (brown in E, purple in F, blue in G) is expressed at the ventricular side of the hem, but not in cells close to the pial surface (E, arrows indicate the layer of *Wnt2b*-negative cells in E-G). The latter cells express the neuronal marker, *class III β-tubulin* (brown in F), and represent a continuation of the developing preplate (pp) of the embryonic cerebral cortex into the hem (F). Dividing precursor cells, labeled with BrdU (brown in G) injected into the mother 2 hours before killing, are organized in a broad ventricular zone (vz) adjacent to the ventricle (v), in both the *Wnt2b*-positive hem and adjacent embryonic cortex (G). Bar in E, 510 μm (A,B); 40 μm (C); 170 μm (D); 90 μm (E-G).

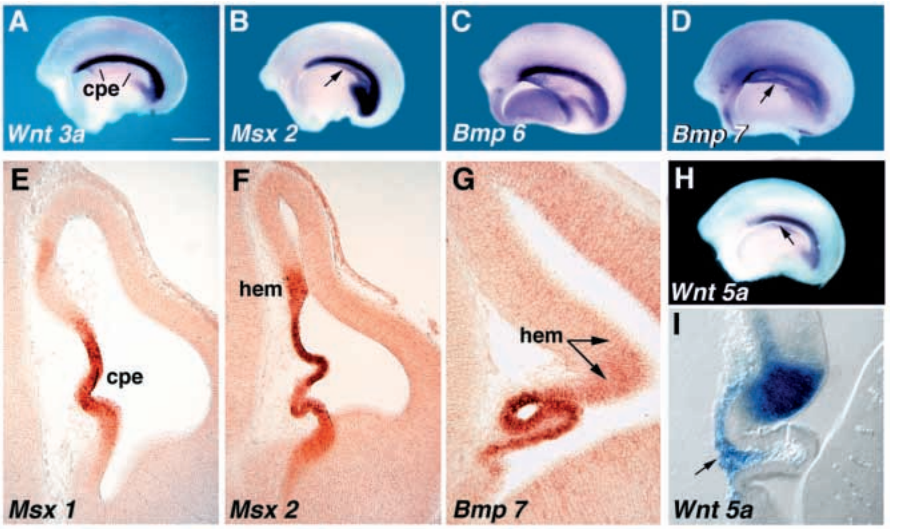

Fig. 3. The cortical hem and choroid plexus epithelium share strong expression of *Bmp* and *Msx* genes, but not of *Wnt* genes. Medial views of telencephalic hemispheres at E12.5 (A-D,H, rostral to the left), and coronal sections through E12.5 hemispheres (E-G,I, medial to the left). (A-C,H) Expression of *Wnt3a*, *Msx2*, *Bmp6* and *Wnt5a* marks the same curved band of tissue in the medial telencephalon, the cortical hem. (B,F) *Msx2* is additionally expressed in the choroid plexus epithelium (cpe, arrow in B). (D,E,G) *Bmp7* and *Msx1* are expressed strongly in the cpe (arrow in D), and in the ventral part of the cortical hem. (H,I) *Wnt5a* is additionally expressed in the mesenchymal cells (arrows) that are invading the medial wall of the telencephalon to form the stromal layer of the choroid plexus. Bar in A, 550 μm (A-D,H); 140 μm (E,F); 70 μm (G,I).

with respect to the morphological and molecular features indicated in Fig. 2. Moving from dorsal to ventral, these are: embryonic cerebral cortex (*Ngn2*+, *Wnt2b/3a/5a*–, *TTR*–, neuron-containing neuroepithelium), the cortical hem (*Ngn2*+, *Wnt2b/3a/5a*+, *TTR*–, neuron-containing neuroepithelium), junctional epithelium (*Ngn2*–, *Wnt2b/3a/5a*–, *TTR*– epithelium) and CPe (*Ngn2*–, *Wnt2b/3a/5a*–, *TTR*+, columnar epithelium).

The *Wnt*-rich cortical hem shows subtle differences with neighboring embryonic cortex. The cortical hem becomes progressively thinner than adjacent neuroepithelium (Fig. 5A), as described previously for the neuroepithelium that gives rise to CPe (Sturrock, 1979). Suggesting that fewer neurons are generated in the cortical hem at E12.5 compared with adjacent cortex, or that neurons are being removed by cell death, *class III β-tubulin* expression is weaker in the cortical hem than in adjacent cortex (data not shown), and cells immunoreactive for MAP2, another neuronal marker, could not be detected in the same region in the rat at a comparable embryonic age (Nicholson-Flynn et al., 1996). Perhaps the most dramatic difference between the cortical hem and adjacent cortex, however, is that the former shares with the developing CPe the expression of several members of the *Msx* and *Bmp* gene families.

The cortical hem expresses genes implicated in epithelial/ mesenchymal inductive interactions

Early in choroid plexus morphogenesis, head mesenchyme cells invade the developing CPe to form the second, mesenchymal or stromal layer of the choroid plexus (CPm) (Birge, 1961; Sturrock, 1979). The invasion by mesenchyme appears to be one of the motive forces that pushes the CPe out

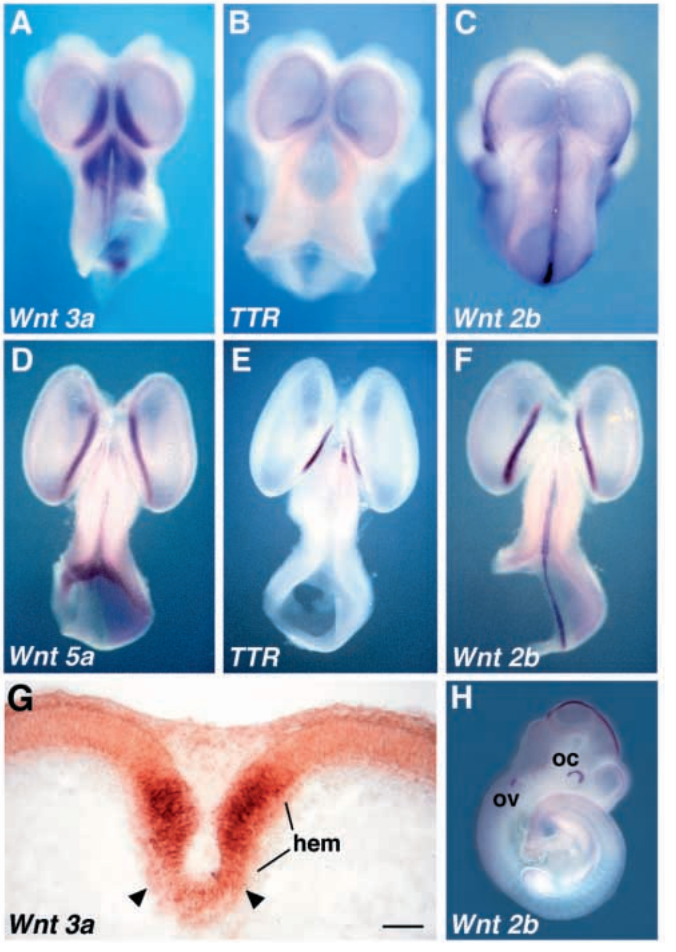

Fig. 4. *Wnt* gene expression is upregulated in the cortical hem both before, and just as, choroid plexus begins to form. Dorsal views of forebrain at E10.5 (A-C) and E11.5 (D-F). Coronal section through midline of telencephalon at E10.5 (G). E10.5 whole embryo (H). (A-H) In the medial wall of the telencephalon at E10.5, *Wnt3a* is already expressed strongly in the cortical hem (A,G), but no *TTR*-expressing choroid plexus epithelium is detectable (B), and no *Wnt2b* is expressed (C,H). *Wnt2b* is already expressed at the dorsal midline of the diencephalon and midbrain (C,H). The choroid plaque is evident at the midline of the telencephalon (between arrowheads in G), and expresses *Wnt3a* only weakly. A day later, at E11.5, *TTR* expression marks differentiating telencephalic choroid plexus epithelium (E), and *Wnt5a* and *2b* expression has been upregulated in the hem (D,F). (H) Sites of strong*Wnt2b* expression at E10.5 include the optic cup (oc), and the otic vesicle (ov). Bar in G, 380 μm (A-C); 540 μm (D-F); 70 μm (G); 900 μm (H).

into the ventricles (Birge, 1961, 1962). Further, inductive interactions between neuroepithelium and invading head mesenchyme appear to be required for at least some aspects of CPe differentiation (Birge, 1961, 1962; Cavallaro et al., 1993). We accordingly sought evidence that the *Wnt*-rich cortical hem is involved in such interactions. Members of the *Msx* and *Bmp* gene families are characteristically expressed at sites of epithelial/mesenchymal interactions elsewhere in the embryo, including the developing kidney and tooth (MacKenzie et al., 1991, 1992; Thesleff et al., 1995). Moreover, expression of both gene families has been previously reported in the dorsal and medial telencephalon between E9.5 and E13.5 (Furuta et al., 1997; MacKenzie et al., 1991). We therefore examined the expression of *Msx1* and *2*, and *Bmp4*, *6* and *7* with respect to the *Wnt*-rich cortical hem.

At E12.5, *Msx*2 and *Bmp6* show the same characteristic curved band of strong expression along the medial face of the telencephalon as *Wnt2b*, *3a* and *5a* (Figs 1B, 3A-C,H), neatly distinguishing the cortical hem from adjacent embryonic cortex. *Msx1* and *Bmp4* and *7* are also expressed in the cortical hem, but strong expression is restricted to the ventral part of the hem (Fig. 3D,E,G, and data not shown). At E12.5, *Msx 1* and *2*, and *Bmp 4*, *6* and *7* are also expressed in the CPe itself (Fig. 3B-G, and data not shown), the junctional epithelium (Fig. 3E-G, and data not shown), and the head mesenchyme that invades the CPe to form the CPm (MacKenzie et al., 1991, 1992; Furuta et al., 1997; data not shown). By contrast, among the *Wnt* genes examined, only *Wnt5a* was detected in the CP. *Wnt5a* expression appears in the CPm, but not the CPe (Fig. 3H,I). Thus, whereas the expression of multiple *Wnt* genes uniquely distinguishes the cortical hem, the more extensive expression of *Bmp* and *Msx* genes in the cortical hem and CPe suggests a close relationship between the two tissues, and implicates the cortical hem in the inductive interactions that shape development of the choroid plexus.

The cortical hem is detectable by *Wnt* gene expression before telencephalic CPe appears, and persists throughout CPe morphogenesis

The temporal pattern of *Wnt* gene expression in the medial telencephalon supports the involvement of *Wnt* genes in the formation of two different tissue districts in the medial wall. At E10.5, the dorsal midline of the telencephalon has just begun to invaginate, creating the medial walls of the two telencephalic hemispheres (Fig. 4G). No differentiated CPe can be detected by TTR expression in the medial wall at E10.5 (Fig. 4B), but *Wnt3a* is already strongly expressed in a medial band marking the cortical hem (Fig. 4A,G). The choroid plaque at the telencephalic midline (between arrowheads in Fig. 4G) expresses *Wnt3a*, but weakly compared with the cortical hem. By E11.5, telencephalic CPe has begun to express *TTR* (Fig. 4E), and the cortical hem is now delineated by the expression of the three *Wnt* genes, *Wnt2b*, *3a* and *5a* (Fig. 4D,F). A previous study reported the expression of *Wnt3a* along the dorsal midline of the telencephalon as early as E9.5 (Parr et al., 1993). In the present study, however, we relate the expression of *Wnt3a* and two other *Wnt* genes to the onset of differentiation of the CPe. We find that both before, and just as the CPe begins to differentiate in the telencephalon, *Wnt* gene expression is upregulated in the immediately adjacent cortical hem.

Comparisons of gene expression patterns at E10.5 and E12.5 (Figs 3 and 4) refine the subdivisions that can be identified in the medial wall of the developing telencephalon. First, differentiating CPe is identifiable by its strong expression of *TTR*, and is thereby distinguished from the choroid plaque and its probable continuation, the junctional epithelium. The latter two divisions are likely to contain precursor cells that directly generate the CPe (Maruyama and D'Agostino, 1967; Sturrock, 1979; Zaki, 1981). Second, strong expression of *Wnt* genes uniquely distinguishes the cortical hem from adjacent cortical neuroepithelium, CPe, junctional epithelium and the choroid plaque. Third, there may be subdivisions within the cortical hem itself. Although *Bmp6* and *Msx2* are expressed throughout the cortical hem, *Bmp4*, *7* and *Msx1* are strongly expressed only in the ventral part that adjoins the CPe, suggesting a difference between ventral and dorsal parts of the cortical hem.

As choroid plexus morphogenesis continues, the cortical hem, as defined by the expression of multiple Wnt genes, maintains its position relative to the developing CPe, but shrinks. Thus, by E15.5-16.5, overlapping expression of *Wnt2b*, *3a* and *5a* marks a few cells along the lateral margin of the hippocampal fimbria-fornix (Fig. 5B,D), which remains the dorsal point of attachment of the CPe to the neuroepithelium. At birth, when CPe is histologically mature (Sturrock, 1979), intense expression of multiple *Wnt* genes next to the CPe has disappeared (data not shown). Likewise, the territory of expression of *Msx2* and *Bmp6*, two other markers of the cortical hem, shrinks as CPe matures (data not shown).

The expression in the medial telencephalon of *Wnt3a* and several *Bmp* and *Msx* genes has previously been described as marking prospective archicortex, or hippocampus, as well as choroid plexus (Furuta et al., 1997; Roelink and Nusse, 1991; Yoshida et al., 1997). However, by the age at which a hippocampal anlage is identifiable by morphology (about E14.5) the *Wnt*-rich cortical hem is clearly separate from regions of the neuroepithelium thought to generate hippocampal neurons (Fig. 5A) (Altman and Bayer, 1990).

As an exception to the circumscribed expression of multiple *Wnt* genes in the cortical hem, *Wnt5a* is newly expressed outside the cortical hem as the medial telencephalon matures (Fig. 5C,D). At E13.5, *Wnt5a* is expressed in the cortical plate of the entire medial cerebral cortex, which includes the hippocampus and adjacent limbic cortical areas (Fig. 5C). Subsequently, *Wnt5a* expression retreats back along the medial telencephalic wall, and by E16.5 is largely confined to the hippocampal dentate gyrus (Fig. 5D). Expression of *Wnt5a* in these neuronal cell layers implies that *Wnt5a* is expressed in postmitotic neurons. Telencephalic *Wnt5a* expression is, therefore, broader than that of *Wnt2b* and *3a*, marking not only the cortical hem, but also the CPm, and developing neurons of the medial cortex. Similar to *Wnt3*, which is implicated in several stages of cerebellar development (Salinas et al., 1994), *Wnt5a* may play a variety of roles in the development and differentiation of the medial telencephalon.

Expression of *Wnt2b* at other sites in the embryonic nervous system

Choroid plexus also develops in the hindbrain, where CPe differentiates by E10.5 (Thomas and Dziadek, 1993). *Wnt1* and *Wnt3a* are expressed next to the hindbrain site of choroid plexus generation from E9.5 onwards (Parr et al., 1993), and

Wnt2b is more weakly expressed in the same region by E11.5 (data not shown). Expression of *Wnt2b* elsewhere in part resembles that of *Wnt3a* (Roelink and Nusse, 1991). At E10.5, *Wnt2b*, like *Wnt3a*, is expressed at the dorsal midline of the neural tube and in the otic vesicle (Fig. 4H). However, at E10.5, *Wnt2b* expression at the dorsal midline of the neural tube does not continue caudal to the isthmus (Fig. 4H), whereas *Wnt3a* expression extends into the spinal cord (Roelink and Nusse, 1991). Further, *Wnt2b*, unlike *Wnt3a*, appears to function in the developing eye. By E10.5 *Wnt2b* is expressed in the pigmented epithelium of the retina (Fig. 4H). Other sites of *Wnt2b* expression include the nasal epithelium, and a part of the diencephalon (Fig. 1A, and data not shown.)

Wnt2b, 3a and *5a* expression is deficient in the telencephalon of the *extra-toes^J* mutant, and telencephalic CPe fails to form

Homozygous Xt^J embryos and their littermates were recovered from Xt^J/+ intercrosses at E10.5, E12.5 or E16.5, ages that span the period of normal telencephalic choroid plexus development. Consistent with a previous report (Hui and Joyner, 1993), no *Gli3* expression was detectable by in situ hybridization in Xt^J/Xt^J embryos. By contrast, in wild-type CD-1 or C3H mice between E9.5 and E12.5, *Gli3* is readily detected throughout telencephalic neuroepithelium (Fig. 6A,B and data not shown), including the cortical hem, but not the choroid plexus epithelium (Fig. 6B and data not shown). Thus, *Gli3* is expressed appropriately to affect the development of the cortical hem.

Consistent with a previous description of the Harwell strain of *extra-toes* mice, many Xt^J/Xt^J embryos (28/43 Xt^J/Xt^J embryos recovered) showed an exencephaly, probably due to delayed closure of the anterior neural tube (Franz, 1994; Johnson, 1967). In exencephalic embryos, a massive overgrowth of the midbrain partially enveloped the forebrain, and the morphology of the telencephalon was severely disrupted. Also consistent with previous descriptions (Franz, 1994), however, about one third of Xt^J/Xt^J embryos (15/43 recovered) showed no exencephaly, and no marked overgrowth of the midbrain.

At E12.5, the telencephalon appeared smaller than normal in non-exencephalic Xt^J/Xt^J mice (Fig. 7A-D), but showed several normal features of morphology and gene expression. For example, the embryonic cerebral cortex of non-exencephalic Xt^J/Xt^J mice was defined, as in wild-type animals, by the strong expression of *Ngn2* (data not shown) and *class III β-tubulin* (Fig. 7E), and the formation of a neuronal preplate (Fig. 7E). Further, the dorsal midline had begun to invaginate to form the medial walls of the telencephalic hemispheres (Fig. 7E). Invagination at E12.5 appeared less complete than in wild-type mice, so that in most Xt^J/Xt^J embryos, the two medial walls of the telencephalon did not appose one another at the dorsal midline (compare Figs 1A,C and 7B,D). Instead, the original roof of the telencephalon formed a broad 'bridge' region between the two hemispheres (marked 'b' in Fig. 7B; see also Fig. 7D). A somewhat similar morphology has been described in mice deficient in *Emx2* expression (Yoshida et al., 1997). To control for the effects of grossly abnormal brain morphology on telencephalic development, we compared exencephalic and non-exencephalic Xt^J/Xt^J mice, and present a detailed analysis of non-exencephalic Xt^J/Xt^J mice only. Brains from 15 non-exencephalic Xt^J/Xt^J embryos, 28 exencephalic embryos, and 35 littermate controls were assayed with in situ hybridization for expression of *Wnt1, 2b, 3a, 5a, 7a, Fgf8* and *TTR*.

At E12.5, the medial telencephalic wall in non-exencephalic Xt^J/Xt^J mice was composed of a curved, cortical structure (Fig. 7E), ending in a small wedge of tissue (Fig. 7E-G) that resembled the junctional epithelium at the base of the CPe in wild-type mice. However, no *TTR*-expressing, cuboidal epithelium extruded from this wedge in any Xt^J/Xt^J embryo examined (Fig. 7F). Nor could expression of *Wnt2b, 3a* or *5a* be detected in adjacent tissue that might correspond to the cortical hem (Fig. 7G). Expression of *Wnt2b, 3a* or *5a* elsewhere in the telencephalon was either undetectable (Fig. 7A), or weak and diffuse (Fig. 7B). Nonetheless, *Wnt2b, 3a* and *5a* were strongly expressed at other appropriate embryonic sites, such as the otic vesicle (Fig. 7H), or nasal epithelium (data not shown). *Wnt1, 2b* and *3a* were additionally expressed next to the fourth ventricle where *TTR*-expressing choroid plexus did form in Xt^J/Xt^J mice (Fig. 7A, and data not shown). *TTR, Wnt2b* and *3a* were also expressed in some mutant mice at the dorsal midline of the diencephalon (Fig. 7A, and data not shown). Thus, although *Wnt* gene expression was downregulated in the medial telencephalon of Xt^J/Xt^J mice, it was strikingly maintained at other sites, such as the hindbrain, at which choroid plexus was successfully generated.

The deficiency of *Wnt* gene expression and the absence of CPe in the medial telencephalon of Xt^J/Xt^J mice at E12.5 did not appear to represent a simple developmental delay. Five non-exencephalic Xt^J/Xt^J embryos examined at E16.5 still showed neither CPe, assayed by *TTR* expression, nor detectable expression of *Wnt2b, 3a* and *5a* in the medial telencephalon (data not shown).

Observations of Xt^J/Xt^J mice at E12.5 and E16.5 indicate that *Wnt* gene expression is deficient in the medial telencephalon of the mutant mice during the normal period of CPe formation. In wild-type mice, *Wnt3a* is expressed earlier, before the onset of CPe differentiation. In Xt^J/Xt^J mice at E10.5, *Wnt3a* expression in the medial telencephalon was weak or undetectable (Fig. 7K), indicating that *Wnt* gene expression is compromised by the time the choroid plaque is forming and the medial telencephalon begins to invaginate.

Finally, observations of exencephalic Xt^J/Xt^J embryos were consistent with those of non-exencephalic mutants. In 27 exencephalic brains, no telencephalic choroid plexus formed at either E12.5 or E16.5, as assessed by morphology or *TTR* expression, and no telencephalic expression of *Wnt2b, 3a* or *5a* was detected (data not shown). Exencephalic Xt^J homozygotes can show severe malformations of the brainstem as well as forebrain, yet choroid plexus develops in the fourth ventricle and in the diencephalon, and *Wnt2b* and *3a* are expressed at these sites (data not shown).

Telencephalic expression of Fgf8 and Wnt7a persists in the *extra-toes^J* mutant

The deficiency of Wnt gene expression in the medial telencephalon does not reflect a general failure of developmental control gene expression in the telencephalon of Xt^J/Xt^J mice. For example, expression of *Fgf8*, which may be involved in directing regionalization in the forebrain (Shimamura and Rubenstein, 1997), was at least partially maintained in Xt^J/Xt^J embryos (Fig. 7D). In wild-type mice at E12.5, *Fgf8* is expressed in the medial wall of the

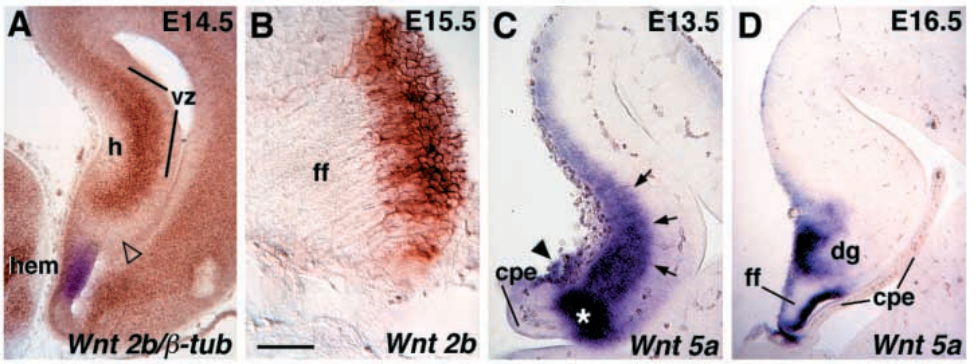

Fig. 5. Late embryonic expression of *Wnt2b* and *5a* in the medial wall of the telencephalon. Coronal sections through the medial telencephalon from E13.5 – E16.5. Medial is to the left. (A) At E14.5, *Wnt2b* expression (purple) marks the shrinking cortical hem. The curving line of the developing hippocampal pyramidal cell layer (h) is marked by expression of *class III β-tubulin* (brown). Pyramidal neurons are generated in the underlying ventricular zone (vz), and an arrowhead points to the source of the first dentate granule neurons (Altman and Bayer, 1990). The *Wnt*-rich hem therefore does not overlap with known sources of hippocampal neurons at E14.5. (B) High magnification of developing fimbria fornix (ff) at E15.5. Ventricle is to the left. *Wnt2b* expression marks a thin layer of cells along the ventricular side of the ff. (C,D) Between E13.5 and E16.5, *Wnt5a* expression marks the shrinking hem (asterisk in C, and see label along the ff in D), but also labels head mesenchymal cells (arrowhead in C), postmitotic hippocampal neurons in the developing pyramidal cell layer (arrows, C), and the dentate gyrus (dg in D). Bar in B, 220 μm (A); 55 μm (B); 110 μm (C); 140 μm (D).

telencephalon just rostral to the site of multiple *Wnt* gene expression (data not shown). In Xt^J/Xt^J embryos, *Fgf8* was strongly expressed in a comparable position along the dorsal midline of the telencephalon in the 'bridge' region between the two hemispheres (Fig. 7D).

Perhaps most striking was that expression of *Wnt7a*, which normally appears in the lateral and dorsal telencephalon (Fig. 1C), persisted in the telencephalon of E12.5 Xt^J/Xt^J embryos (Fig. 7C). Thus, of the several *Wnt* genes examined, all and only those normally expressed in the cortical hem are deficient in Xt^J/Xt^J embryos. Further, expression of these *Wnt* genes is markedly deficient in the telencephalon, but not at several other normal sites of expression, such as the hindbrain. Finally, correlating with these observations, choroid plexus is missing in the telencephalon, but not in the hindbrain.

Cells accumulate at the medial margin of the telencephalon in the *extra-toes*^{J} mutant, but do not develop a CPe identity

In several non-exencephalic Xt^J/Xt^J mice, an amorphous tissue extruded from the medial edge of the telencephalon (Fig. 7J), indicating that cells continue to accumulate at this site, presumably by cell proliferation, but that the cells do not develop as CPe. By contrast with developing wild-type CPe (Fig. 7I), this extruding tissue did not show a simple columnar or cuboidal morphology, nor was it observed to be invaded by mesenchymal cells (Fig. 7J). No *TTR* expression was observed at this site, and neither *Ngn2* nor *class III β-tubulin* were consistently expressed within the extruding tissue.

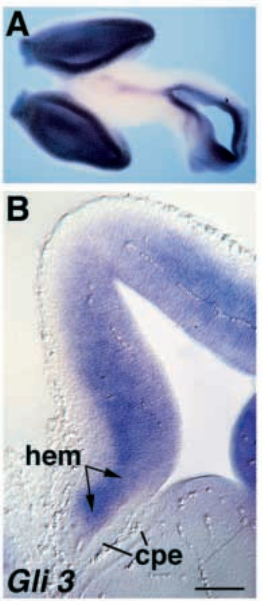

Fig. 6. Telencephalic expression of *Gli3* at E12.5. (A) Dorsal view of a CD-1 mouse brain at E12.5. *Gli3* is strongly expressed throughout the embryonic cerebral cortex. (B) A coronal section through the same brain. *Gli3* is expressed throughout the neuroepithelium of the medial telencephalic wall, including the cortical hem (hem), but not in the choroid plexus epithelium (cpe). Bar in B, 850 μm (A); 130 μm (B).

DISCUSSION

The *Wnt*-rich cortical hem and its relationship to the CPe

In vertebrate development, *Wnt* gene expression marks signaling centers that regulate patterning in the spinal cord and brainstem (Parr et al., 1993; Bally-Cuif et al., 1995; McMahon and Bradley, 1990). In the present study, we have drawn on this observation to identify a potential source of patterning signals within the embryonic telencephalon. We find that the expression of multiple *Wnt* genes, including a previously unreported mouse *Wnt* gene, *Wnt2b*, marks out a longitudinal strip of neuroepithelium in the medial telencephalon, which we term the cortical hem. The *Wnt*-rich cortical hem forms the boundary between the developing hippocampus, the most medial part of the cerebral cortex, and the telencephalic choroid plexus. We show that, in the Xt^J mouse mutant, a defect in the cortical hem that includes downregulation of *Wnt* gene expression is associated with the loss of at least one of these adjacent structures, the choroid plexus. Determining if the hippocampus is also missing or mispatterned in the Xt^J mouse mutant remains for a future study that will employ molecular markers of the hippocampal subfields (Tole et al., 1997).

The *Wnt*-rich cortical hem is a transient structure, in that it appears to shrink as development proceeds, and cannot be identified by *Wnt* gene expression in the postnatal animal. The shrinkage of the cortical hem, as defined by gene expression, is likely to be due at least in part to progressive cell loss. Apoptotic cell death is increased, and cell proliferation is decreased in the region of the *Wnt*-rich cortical hem compared with adjacent cortical neuroepithelium (Furuta et al., 1997; Maruyama and D'Agostino, 1967; Sturrock, 1979; Zaki, 1981). Additionally,

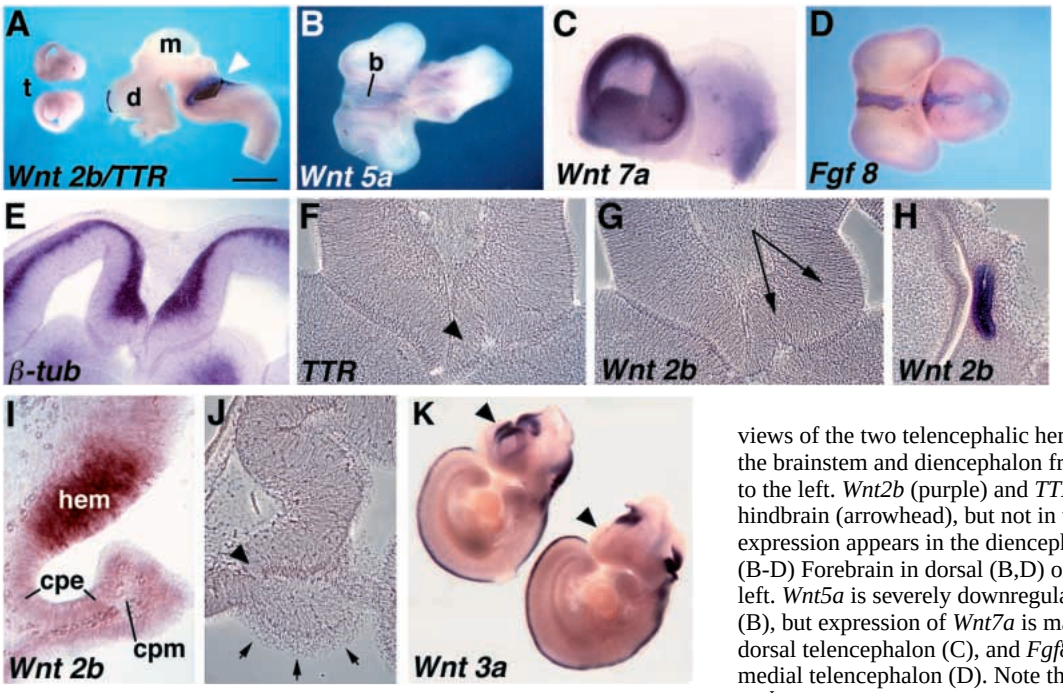

Fig. 7. In the medial telencephalon of Xt^J/Xt^J mice, *Wnt* gene expression is deficient, and *TTR*-expressing choroid plexus epithelium does not form. (A-H,J) Brains of non-exencephalic Xt^J/Xt^J mice at E12.5. (I) Section through the cortical hem and choroid plexus epithelium (cpe) of a wild-type CD-1 mouse brain at E12.5. (K) A wild-type mouse embryo at E10.5 (left), and an Xt^J/Xt^J littermate (right). (A) Medial views of the two telencephalic hemispheres (t), and lateral view of the brainstem and diencephalon from the same mouse. Rostral is to the left. *Wnt2b* (purple) and *TTR* (brown) are expressed in the hindbrain (arrowhead), but not in the telencephalon. *TTR* expression appears in the diencephalon (d) as well. (B-D) Forebrain in dorsal (B,D) or lateral (C) views, rostral to the left. *Wnt5a* is severely downregulated in the medial telencephalon (B), but expression of *Wnt7a* is maintained in the lateral and dorsal telencephalon (C), and *Fgf8* expression is maintained in the medial telencephalon (D). Note that in some non-exencephalic Xt^J/Xt^J brains (B,D), the partially invaginated roof of the telencephalon forms a broad 'bridge' (b) between the two hemispheres. (E-H) Coronal sections from a single Xt^J/Xt^J mouse; dorsal is up. A curving cortical structure has developed in the medial wall of the telencephalon (E), ending in a wedge-shape (arrowhead, F) similar to the base of the cpe in wild-type mice. Differentiating neurons have formed a preplate and express *class III β-tubulin* (purple in E). Neither *TTR* (F) nor *Wnt2b* (G) are expressed at the margin of the medial telencephalic wall. Arrows in G indicate the site that may correspond to the cortical hem in wild-type mice. By contrast, *Wnt2b* is strongly expressed in the inner ear (H). (I,J) Sections through the hem/cpe transition in a wild-type mouse (I), and the comparable region in an Xt^J/Xt^J mutant mouse (J). In the wild-type mouse, two layers of the choroid plexus are developing: the cpe, and the mesenchymal layer (cpm). By contrast, in the Xt^J/Xt^J mouse, an amorphous tissue (arrows) extrudes from the wedge (arrowhead) at the base of the medial telencephalic wall. (K) At E10.5, an Xt^J/Xt^J embryo (right) shows relatively normal *Wnt3a* expression in the spinal cord, hindbrain and diencephalon, compared with a littermate control embryo (left). The control embryo shows strong expression of *Wnt3a* in the cortical hem (arrowhead), but in the comparable region in the Xt^J/Xt^J embryo, *Wnt3a* expression is barely detectable (arrowhead). Bar in A, 1.4 mm (A); 900 μm (B,D); 700 μm (C); 220 μm (E); 110 μm (F-H); 55 μm (I); 150 μm (J); 1.2 mm (K).

however, the Wnt-rich cortical hem may shrink by progressively contributing cells to adjacent structures.

Modern techniques of fate mapping will be required to determine whether the *Wnt*-rich cortical hem contributes cells to the CPe, the hippocampus, or both. However, classical morphological studies suggest that at least some cells from the cortical hem are recruited into the developing CPe (Maruyama and D'Agostino, 1967; Sturrock, 1979; Zaki, 1981). The cortical hem appears to be the thinning neuroepithelium. described in these studies as giving rise to the CPe, and gene expression patterns support a close developmental relationship between the CPe and the cortical hem. The entire stretch of the medial wall that includes the CPe, junctional epithelium and the cortical hem expresses *Bmp* and *Msx* genes (Furuta et al., 1997; MacKenzie et al., 1991; present study), as well as high molecular mass tropomyosins, which may regulate the cell shape changes and cell movements of choroid plexus morphogenesis (Nicholson-Flynn et al., 1996).

Wnt gene downregulation and loss of telencephalic CPe in the Xt^J mouse mutant

In non-exencephalic Xt^J/Xt^J mice, we observed some thinning of the neuroepithelium in the medial telencephalon, and an accumulation of cells, perhaps by proliferation, at the medial edge of the telencephalon. Wnt signaling therefore may not be required for these processes. However, cells with a specific CPe identity fail to develop. Could the absence of Wnt signaling at the cortical hem underlie this failure? Several observations from the present study implicate *Wnt* gene function in CPe development: the expression of multiple *Wnt* genes in the cortical hem surrounding the developing CPe; the cumulative expression of *Wnt* genes in the cortical hem before and just as the CPe begins to appear; and the tight correlation in the Xt^J mutant between *Wnt* gene expression and CPe generation at different sites. However, the Xt^J mutant is not equivalent to a mouse line generated in a gene targeting experiment in which *Wnt2b*, *3a* and *5a* expression is selectively depleted in the medial telencephalon. That is, the loss of CPe in the telencephalon of Xt^J/Xt^J mice could be due to the *Gli3* deficiency directly, or to a consequence of the *Gli3* deficiency other than the loss of Wnt signaling in the cortical hem.

Gli3 has not been detected in the embryonic CPe itself (Hui et al., 1994; present study), therefore the development of telencephalic CPe appears unlikely to depend on *Gli3* expression within that tissue. However, *Gli3* is expressed in embryonic head mesenchyme (Hui et al., 1994), as is *Wnt5a* (present study). Therefore, a *Gli3* deficiency could disrupt inductive interactions between the developing CPe and head mesenchyme. Suggesting that this is not the primary cause of the loss of telencephalic CPe in the Xt^J/Xt^J mouse, the CPe can

develop into a *TTR*-expressing, cuboidal epithelium, although not a convoluted plexus, in the absence of mesenchymal interactions (Birge, 1962; Thomas and Dziadek, 1993). In the Xt^J/Xt^J mouse, CPe development appears to have stalled at an early stage, before cuboidal, TTR-expressing epithelium is detected, and therefore perhaps before signals from the mesenchyme become important.

Due to the low yield of non-exencephalic Xt^J/Xt^J mice (15 out of 225 embryos recovered), we have not explored other possible gene expression defects at the cortical hem that might follow from the *Gli3* deficiency. For example, the expression of *Bmp* and *Msx* genes remains to be examined in Xt^J/Xt^J mice. Given the links between Wnt and Bmp signaling in other systems, the two gene families appear likely to interact in the development of the medial telencephalon too. In *Drosophila*, *wingless* is implicated in patterning the optic lobe, operating at least in part through regulation of the expression of the *Drosophila Bmp* family member, *dpp* (Kaphingst and Kunes, 1994). In the vertebrate neural tube, signaling from the ectoderm overlying the dorsal spinal cord, probably mediated by Bmp proteins, induces *Wnt1* expression, as well as other markers of dorsal cell identity (Dickinson et al., 1995; Liem et al., 1995; Marcelle et al., 1997). If expression of *Bmp* family members is disrupted in Xt^J/Xt^J mutants too, it will be important to test whether Wnt proteins are required to regulate *Bmp* expression in the cortical hem, or vice versa, and where Gli3 might operate in this pathway.

Gli3 regulation of cortical hem *Wnt* gene expression

A parsimonious explanation for the deficiency of *Wnt* gene expression in the Xt^J/Xt^J mouse is that it is due to a direct action of the Xt^J mutation. In *Drosophila* parasegment development, ci activates *wingless* expression in response to a hedgehog signal (Alexandre et al., 1996; Dominguez et al., 1996; Von Ohlen et al., 1997). Could Gli3 similarly respond to a hedgehog signal to regulate *Wnt* gene expression in the cortical hem? Of the three identified vertebrate *hedgehog* genes, only *sonic hedgehog* has been reported to be expressed near the cortical hem, but its expression in the choroid plexus appears after the *Wnt*-rich cortical hem has been established (Bitgood and McMahon, 1995). The present study therefore raises the possibility that a *Gli* family member is required for *Wnt* gene expression in the absence of hedgehog signaling. Consistent with this possibility, several *wingless* expression boundaries in *Drosophila* appear not to be established in response to hedgehog signaling (Kaphingst and Kunes, 1994; Lawrence and Struhl, 1996). Further, a genetic analysis of wingless autoregulation during segment polarity determination suggests a hedgehog-independent requirement for *ci* function in *wingless* expression (Hooper, 1994).

Why is the expression of *Wnt2b*, *3a* and *5a* downregulated in the telencephalon of Xt^J/Xt^J mice, but not at other sites? Hui and colleagues (1994) have shown that some regions that express high levels of *Gli3*, such as the spinal cord, appear not to be morphologically affected in Xt^J mutants, and suggest that *Gli2*, which shares an almost identical expression pattern with *Gli3*, might functionally substitute for *Gli3* in these unaffected regions. The expression of *Gli2* appears slightly weaker in the cortical hem than in immediately adjacent cortical neuroepithelium (data not shown). In the Xt^J mutant, therefore, *Gli2* expression levels might be insufficient to maintain the expression of *Wnt* genes at the cortical hem.

Conclusion

Because the medial walls of the telencephalic hemispheres are formed by the invagination of the telencephalic vesicle, the cortical hem arises from the dorsal midline of the telencephalic vesicle. The dorsalmost cells of the telencephalon, the CPe and the hippocampus, are generated on either side of the cortical hem. In the developing spinal cord and brainstem, the roofplate, which also lies at the dorsal midline of the neural tube, directs development of adjacent dorsal cell groups via secreted peptides encoded by members of the *Wnt* and *Bmp* gene families (Ikeya et al., 1997; Liem et al., 1995). Findings from the present study suggest that the cortical hem should be investigated as a potential, analogous source of midline cues that direct development of the dorsal telencephalon.

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