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Wnt7b Regulates Placental Development in Mice

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Secreted Wnt proteins regulate many developmental processes in multicellular organisms. We have generated a targeted mutation in the mouse *Wnt*7b gene. Homozygous *Wnt*7b mutant mice die at midgestation stages as a result of placental abnormalities. *Wnt*7b expression in the chorion is required for fusion of the chorion and allantois during placental development. The α4 integrin protein, required for chorioallantoic fusion, is not expressed by cells in the mutant chorion. Wnt7b also is required for normal organization of cells in the chorionic plate. Thus, Wnt7b signaling is central to the early stages of placental development in mammals.

Key Words: **Wnt7b; placenta; chorion; α4 integrin.**

INTRODUCTION

The mammalian placenta is a complex structure requiring the coordinated growth and differentiation of maternal and fetal tissues. The amnion, yolk sac, chorion, and allantois are the extraembryonic tissues most closely associated with the embryo during early stages of mouse development. Amniotic folds appear at 7 days post coitum (d.p.c.), and a sheet of amnion overlies the embryo by 7.5 d.p.c. The yolk sac provides a second supportive layer that encircles the amnion and the embryo. The mesodermally derived allantois projects from the posterior end of the embryo at 7.5 d.p.c. and contacts the chorion around 8.25 d.p.c. to initiate placenta formation. The allantois and chorion subsequently fuse to each other. At early stages, the chorion includes multiple cell populations such as the diploid trophoblasts and mesoderm cells of the chorionic plate (which contacts the allantois), the spongiotrophoblasts, and the trophoblast giant cells (furthest from the allantois).

It is not surprising that extraembryonic tissues produce many secreted factors that regulate interactions required for placental development. When normal cell signaling is disrupted, a variety of placental defects are observed. For example, mutations in the genes for scatter factor, the EGF receptor, or the LIF receptor produce trophoblast cell abnormalities in the placenta (Threadgill *et al.*, 1995; Uehara *et al.*, 1995; Ware *et al.*, 1995).

A number of mouse mutants with defects in the chorionic component of the placenta have been identified. For instance, in early stages of chorion development, *Mash*-2 mutants lack diploid spongiotrophoblasts, and the tetraploid giant cell populations are expanded (Guillemot *et al.*, 1994). The production of diploid trophoblast cells is impaired in mutants for the *estrogen-receptor-related receptor* β, or in embryos that carry compound mutations for the *retinoid X receptors* α and β (Luo *et al.*, 1997; Wendling *et al.*, 1999). At a slightly later developmental stage, normal growth and morphogenesis of the labyrinthine layer of the chorion requires the retinoid X receptors, secreted Wnt2, and the transcription factors Dlx-3 and Esx1 (Li and Behringer, 1998; Monkley *et al.*, 1996; Morasso *et al.*, 1999; Wendling *et al.*, 1999).

A key step in the formation of the chorioallantoic placenta is the binding and fusion of the allantois to the chorion. Integrin $\alpha 4\beta 1$, which is expressed on the chorion, binds VCAM-1 on the allantois. This interaction is required for normal development, as mutations in the *VCAM*-1 or *α4 integrin* genes cause similar placental abnormalities characterized by a failure of the allantois and chorion to properly bind and/or fuse to each other (Gurtner *et al.*, 1995; Kwee *et al.*, 1995; Yang *et al.*, 1995).

This paper reports the generation of mouse embryos with a targeted mutation in the *Wnt*7b gene. *Wnt* genes encode

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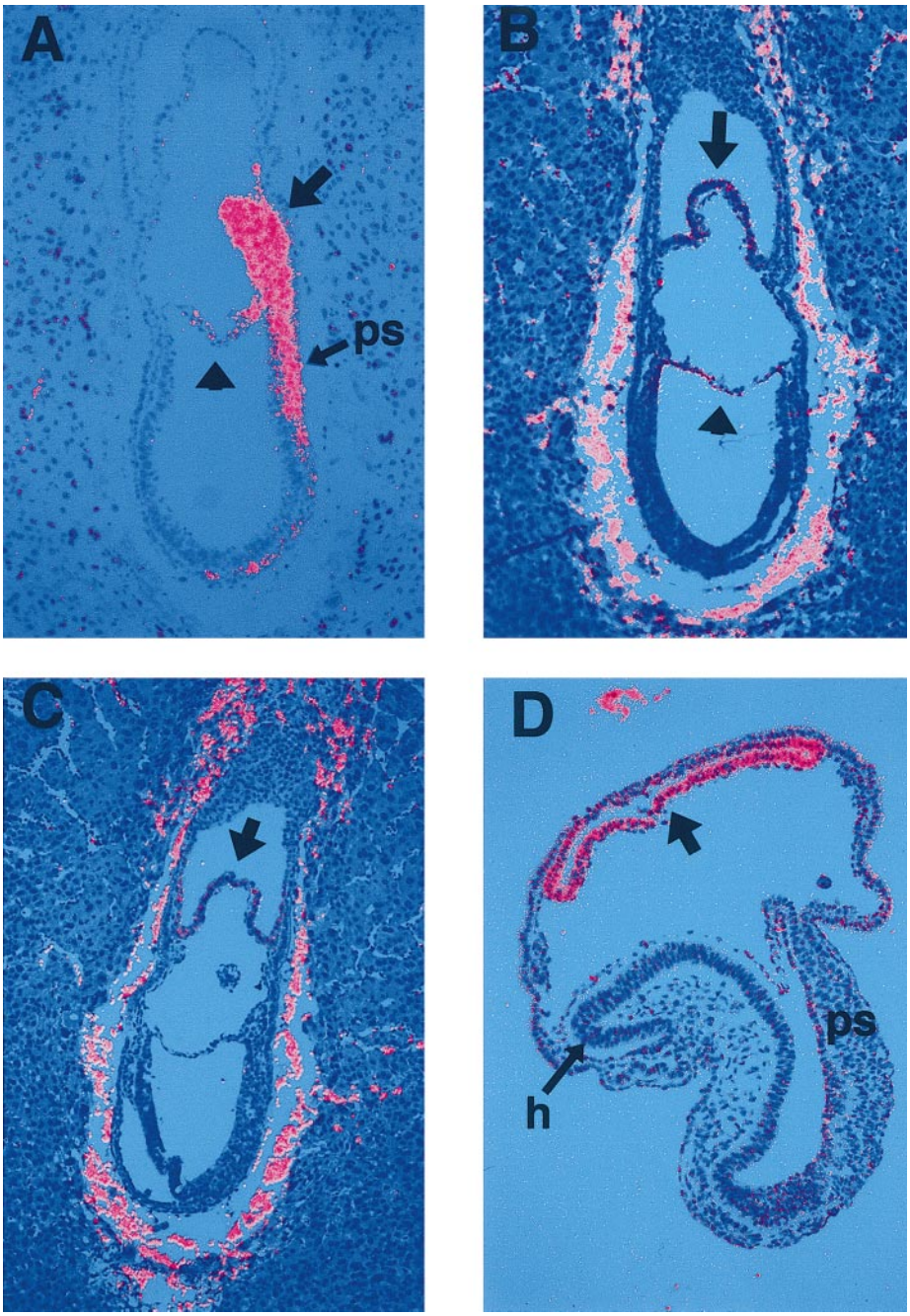

FIG. 1. (A–D) *Wnt* RNA expression in the extraembryonic membranes of 7.5 d.p.c. and 8.5 d.p.c. mouse embryos. *Wnt*5a is expressed in the allantois (arrow), amnion (arrowhead), and primitive streak at 7.5 d.p.c. (A), *Wnt*6 is expressed in the amnion (arrowhead) and chorion (arrow) at 7.5 d.p.c. (B), and Wnt7b is expressed in the chorion (arrow) at 7.5 d.p.c. (C) and 8.5 d.p.c. (D). In (B) and (C), the bright staining surrounding the embryo is due to background signal produced by blood cells. h, head; ps, primitive streak.

secreted signaling molecules that play important roles in intercellular communication during many developmental processes (for review, see Wodarz and Nusse, 1998). Previous papers demonstrated that *Wnt*7b is expressed in the central nervous system, kidneys, and lungs during embryogenesis (Kispert *et al.*, 1996; Parr *et al.*, 1993; Pepicelli *et al.*, 1998). Here, we show that *Wnt*7b is expressed in the chorion during early stages of placental development. A

mutation in the *Wnt*7b gene leads to a failure of normal chorionic development and the absence of fusion between the allantois and chorion. As a consequence, embryos die at midgestation stages.

MATERIALS AND METHODS

Wnt7b Gene Targeting

*Wnt*7b genomic clones were isolated from a 129/Sv library (Stratagene) by using standard methods (Sambrook *et al.*, 1989). We designed a gene targeting construct that inserted a neomycin resistance gene into the 3rd exon of the *Wnt*7b gene and deleted part of the 3rd exon and 3rd intron. The linearized construct was electroporated into CJ7 embryonic stem (ES) cells (Swiatek and Gridley, 1993). A total of 120 G418/FIAU resistant colonies were picked and screened by PCR. The five positive colonies were confirmed by Southern blots using probes at both 5′ and 3′ ends of the targeting construct. The targeted clones were injected into C57Bl6/J blastocysts to generate chimeric mice. The targeted allele was transmitted through the germline in four of the clones, and all four independent targeting events produced identical phenotypes in homozygous mutant embryos.

In Situ Hybridization

The ^{35}S section *in situ* hybridization protocol has been published (Parr *et al.*, 1993); the digoxigenin section protocol is derived from a previously published whole-mount protocol (Parr *et al.*, 1993). Prior to hybridization with digoxigenin probes, embryos were embedded in paraffin and sectioned at 10 μm. The sections were dewaxed in toluene, rehydrated through an ethanol series, fixed in 4% paraformaldehyde, treated with proteinase K, and refixed in paraformaldehyde. After treatment with 0.6% H_2O_2 and washing in PBS, the sections were hybridized overnight at 65°C with the digoxigenin-labeled RNA probe.

On the second day, the sections were washed at 65°C in 0.2× SSC. After treatment with RNase A (10 μg/ml) and washing in PBS, the slides were blocked with 10% sheep serum/2% Boehringer blocking reagent for 1 h. After a 1-h incubation with an alkaline phosphatase-conjugated anti-digoxigenin antibody, the slides were stained with BM purple solution overnight.

Histology and Antibody Staining

For histological analysis, embryos were fixed in Bouin's fixative, embedded in paraffin wax, sectioned at 10 μm, and stained with haematoxylin and eosin.

Staining of sections with antibodies to VCAM-1, α4 integrin, and β1 integrin followed published procedures (Gurtner *et al.*, 1995). To visualize β-catenin protein, chorions were fixed in Bouin's fixative, embedded in paraffin wax, sectioned at 10 μm, and stained with a mouse monoclonal antibody (Transduction Laboratories) at a 1:100 dilution and an Alexa 488-conjugated goat anti-mouse secondary antibody (Molecular Probes) at a 1:200 dilution.

RESULTS

Wnt Gene Expression in the Extraembryonic Membranes

The early stages of placental formation in the mouse occur between 7.0 and 8.5 d.p.c. Multiple *Wnt* genes, including *Wnt*5a, *Wnt*6, and *Wnt*7b, are expressed in overlapping patterns in the extraembryonic membranes by 7.5 d.p.c. (Fig. 1 and unpublished results; also see Takada *et al.*, 1994). *Wnt*6 is expressed in the chorion and amnion. *Wnt*6 expression in these extraembryonic membranes is immediately adjacent to a region of *Wnt*6 expression in the embryonic ectoderm. *Wnt*5a expression in the amnion and allantois is contiguous with a domain of *Wnt*5a expression in the primitive streak. *Wnt*6 expression appears confined primarily to the ectodermal cells of the amnion and chorion, while *Wnt*5a is expressed in both mesodermal and ectodermal cells in the extraembryonic membranes. *Wnt*7b expression in the extraembryonic membranes at 7.5 and 8.5 d.p.c. is restricted to cells that form the chorionic plate. Thus, it is found in the region of the chorion that is contacted by the allantois, but is not detected in cell populations such as spongiotrophoblasts or giant cells. Notably, *Wnt*7b is expressed in the chorion well before interactions occur between the chorion and allantois.

The Chorion and Allantois Do Not Fuse in Wnt7b Mutant Embryos

To investigate the function of the Wnt7b gene product, we used gene targeting in ES cells to generate a likely null allele of the *Wnt*7b gene (Fig. 2). When *Wnt*7b heterozygous mice were intercrossed, no *Wnt*7b homozygous embryos survived to term. The analysis of midgestation litters from heterozygote intercrosses revealed Mendelian ratios (+/+: 7; +/−: 19; −/−: 11) at 10.5 d.p.c., but *Wnt*7b−/− embryos did not survive beyond 11.5 d.p.c.

Examination of Wnt7b mutant embryos revealed extensive necrosis throughout much of the embryo by 10.5 d.p.c. (Fig. 3A). From 8.5 to 9.5 d.p.c., the first external indication of developmental abnormalities is the appearance at the posterior end of the embryo of a ball of allantoic cells that has not fused with the chorion (Fig. 3B). This observation suggests that *Wnt*7b mutants have deficiencies in formation of the chorio-allantoic placenta. It is worth noting that mice lacking both Tcf-1 and Lef-1, transcription factors that transduce Wnt signals, are reported to have placental abnormalities and die around 10.5 d.p.c. (Galceran *et al.*, 1999). It would be interesting to see a more detailed analysis of the *Tcf-1/Lef-1* mutant mice and how their placental defects compare with the *Wnt*7b phenotype.

Although some defects observed in Wnt7b mutant embryos could result from a requirement for localized Wnt7b function in the embryo proper, it is more likely that these defects are secondary to an inadequate nutrient and/or oxygen supply due to the placental abnormalities. For this

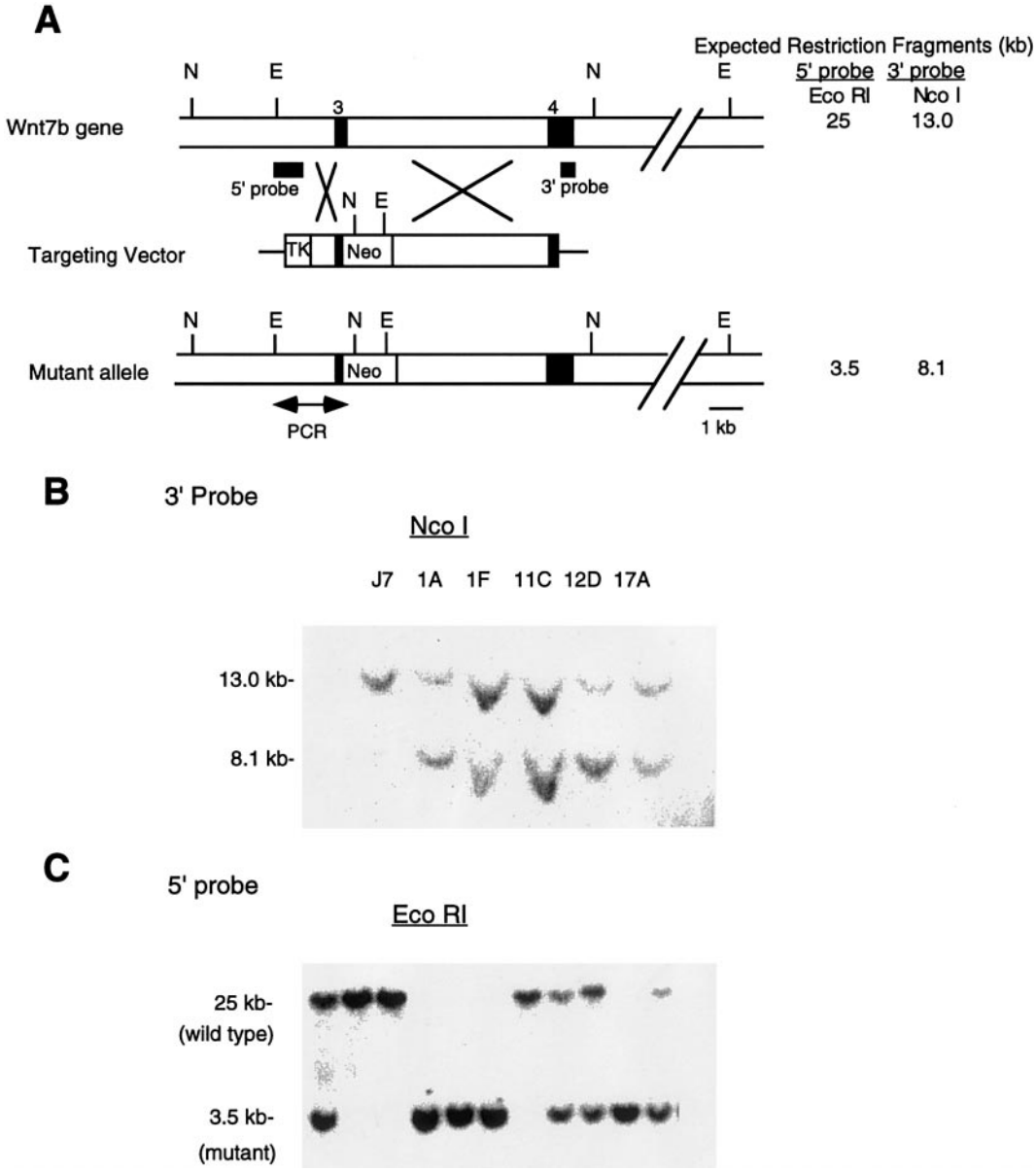

FIG. 2. (A) Strategy for targeting the 3rd exon of the *Wnt*7b gene. The location of the PCR reaction and the restriction enzymes *Nco*I (N) and *Eco*RI (E) used in the analysis of targeted clones are shown. (B) Southern blot using the 3′ probe and DNA from the parental ES cells (J7) and the five positive clones. (C) Southern blot of a representative litter of embryos derived from a heterzygote intercross using the 5′ probe.

reason, our analysis of the *Wnt*7b mutants focuses on the placental abnormalities.

We observed several defects in the development of the chorion in *Wnt*7b mutant embryos. As noted above, the chorion fails to bind and fuse with the allantois. Normally, binding of the allantois is observed around the 6-somite (8.25–8.5 d.p.c.) stage (Downs and Gardner, 1995). Although the allantois grows across the exocoelom toward the chorion in *Wnt*7b mutants, it never connects with the chorion. By 8.5 d.p.c., the tip of the allantois begins to round up and dying cells are observed within the allantois (not shown). Allantoic cells may die in the absence of chorionic signals subsequent to fusion, and/or as a consequence of the general lack of nutrient supply from the mother.

A very similar phenotype is observed in mutants lacking VCAM-1 or α4 integrin activities, which appear to regulate chorioallantoic binding and/or fusion (Gurtner *et al.,* 1995; Kwee *et al.,* 1995; Yang *et al.,* 1995). Therefore, we analyzed

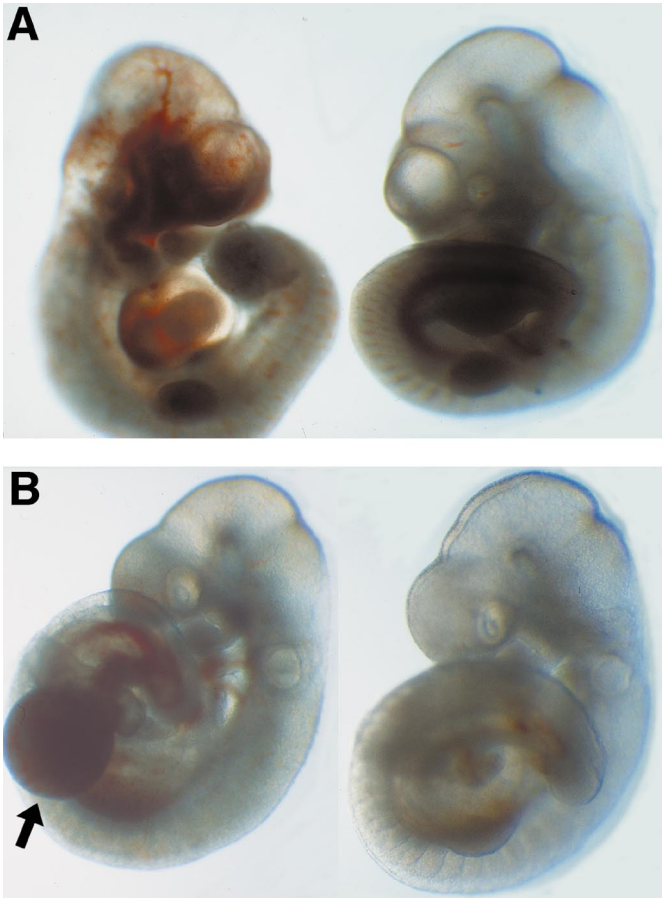

FIG. 3. The *Wnt*7b mutant phenotype demonstrated by *Wnt*7b null (left) and wild-type littermates at 10.5 d.p.c. (A) and 9.5 d.p.c. (B). Note the ball of allantoic cells (arrow) at the posterior end of the 9.5-d.p.c. embryo.

the expression of the $\alpha 4\beta 1$ integrin and VCAM-1 proteins in the *Wnt*7b mutant embryos (Fig. 4). VCAM-1 protein was present on the surface of the allantois in *Wnt*7b mutants, and its distribution did not differ from wild-type embryos during early stages of placental development. In wild-type embryos, the $\alpha 4\beta 1$ integrin heterodimer is present on the surface of the chorionic plate (chorionic mesoderm) and binds VCAM-1. Interestingly, $\alpha 4$ integrin was absent from the chorionic plate of *Wnt*7b mutants, but $\beta 1$ integrin was still detected, albeit at a somewhat lower level than in wild-type embryos. This result suggests that Wnt7b may have a specific effect on the regulation of $\alpha 4$ integrin expression in the chorion. The absence of $\alpha 4$ integrin expression may explain the failure of chorioallantoic fusion.

Wnt7b Mutants Exhibit Multiple Defects in Chorion Development

In addition to a failure of chorioallantoic fusion, *Wnt*7b mutants exhibit a second defect in chorion development. Upon dissection of wild-type embryos at 8.0–8.25 d.p.c., the chorionic plate appears as a disc-shaped structure. An obvious disc-shaped chorion is not evident in *Wnt*7b mutant embryos. This difference could be explained by a decrease in the size of the mutant chorion, or by alterations in the organization of the chorion. To help distinguish between these possibilities, we examined the expression of several genes that mark different cell populations in the chorionic region. *Placental lactogen*-1 is expressed by the giant cells, *4311* is expressed by the spongiotrophoblasts, *Esx*1 is expressed by diploid trophoblasts, and *Mash*-2 is expressed in several cell populations including the spongiotrophoblasts and ectoplacental cone (Colosi *et al.*, 1988; Guillemot *et al.*, 1994; Lescisin *et al.*, 1988; Li *et al.*, 1997). Expression of all four markers appeared normal in *Wnt*7b mutants from 8.5 d.p.c. (Fig. 5) through 10.5 d.p.c. (not shown), indicating that no significant changes in cell number or cell fate had occurred in these populations.

We stained sectioned embryos with an antibody against the β-catenin protein to further characterize the organization of the chorion in *Wnt*7b mutants (Fig. 6). β-Catenin functions in the canonical Wnt signaling pathway (McCrea *et al.*, 1993; Peifer *et al.*, 1991) and also regulates cell adhesion at adherens junctions. The general pattern of β-catenin staining did not change in *Wnt*7b mutants, but it illustrated an interesting change in the organization of the mutant chorion. In wild-type embryos, a layer of elongated diploid trophoblast cells forms a regular, brick-like structure in the chorion immediately below the surface of the chorionic plate (Figs. 6A and 6C). In the *Wnt*7b mutants, this layer of elongated cells is replaced by a layer of round cells that are less regularly organized than the cells in the wild-type chorion (Figs. 6B and 6D). Although these round cells in the mutant chorion somewhat resemble the adjacent spongiotrophoblasts in appearance, the analysis of marker gene expression indicated normal zones of spongiotrophoblast and diploid trophoblast cells, arguing against a cell fate transformation. In particular, *Esx*1 expression, which marks the diploid trophoblast cell population, is not changed in the *Wnt*7b mutants. Thus, it is likely that the affected diploid trophoblast cell population is present but morphologically abnormal in the absence of Wnt7b signaling.

DISCUSSION

Our results suggest that Wnt7b has multiple functions in placental development. First, it is required for the fusion of the allantois and chorion. This function may be mediated by Wnt7b regulation of $\alpha 4$ integrin, as $\alpha 4$ integrin is essential for chorio-allantoic fusion and is absent from the chorionic plate of *Wnt*7b mutants. The regulation of $\alpha 4$ integrin appears quite specific. $\beta 1$ integrin, the dimerization partner of $\alpha 4$ integrin, is still present in the *Wnt*7b mutant chorion. Detectable $\beta 1$ integrin indicates that the absence of $\alpha 4$ integrin is not due to the loss of $\alpha 4$-expressing

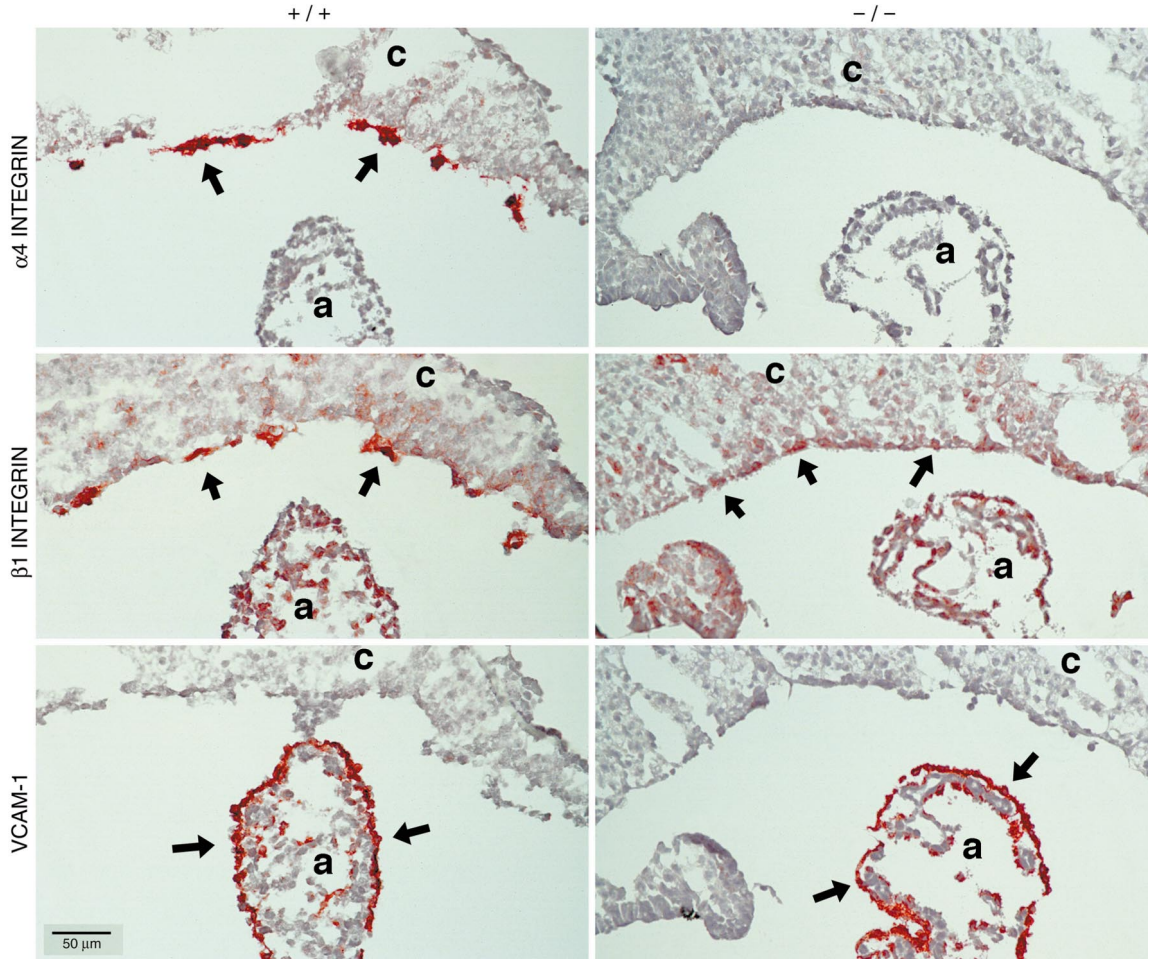

FIG. 4. Antibody staining of proteins required for chorioallantoic fusion. α4 integrin protein is not detected on the surface of the chorion in Wnt7b−/− embryos, while its dimerization partner β1 integrin still can be detected in the mutants. VCAM-1 expression in the allantois is unchanged in the mutants. Arrows illustrate regions of antibody staining (red dots). a, allantois; c, chorion.

cells in the *Wnt*7b mutants. Rather, it suggests that regulation of α4 integrin may be a fairly direct target of Wnt7b signaling in the chorion.

The absence of α4 integrin in the *Wnt*7b mutant chorion likely explains the failure of chorioallantoic fusion and subsequent lethality for the most part. However, it should be noted that some α4 integrin-deficient embryos achieve chorioallantoic fusion (Yang *et al.,* 1995). Moreover, a few α4 integrin mutants and a few *VCAM*-1 mutants survive to the end of gestation (Gurtner *et al.,* 1995; Kwee *et al.,* 1995; Yang *et al.,* 1995). In contrast, we have never observed chorioallantoic fusion in Wnt7b mutant embryos, even when the mutant allele was examined on a number of different genetic backgrounds (data not shown). Thus, the *Wnt*7b phenotype is more severe than the *α4* phenotype, suggesting that Wnt7b plays additional roles in placental development.

Indeed, Wnt7b is required for normal morphogenesis of the chorionic plate. The morphology and laminar organization of cells in the chorionic plate, especially the diploid trophoblast layer, is altered in the *Wnt*7b mutant chorion. Expression of molecular markers suggests that no cell fate transformations have occurred. Rather, these alterations might reflect additional changes in the cell adhesion properties of mutant cells. These differences in the chorion are apparent by the time that the chorion normally fuses to the allantois, so they are unlikely to be secondary consequences of the lack of fusion. Examination of other mouse mutants suggests that the processes of chorion morphogenesis and chorioallantoic fusion are to some extent separable. For example, *Mash*-2 or *retinoid X* α/β mutants possess defective labyrinthine zones in the chorion, but still undergo chorioallantoic fusion (Guillemot *et al.,* 1994; Wendling *et al.,* 1999). It remains possible that both aspects of the *Wnt*7b placental phenotype (absence of α4 integrin expression and chorionic disorganization) have a single underlying

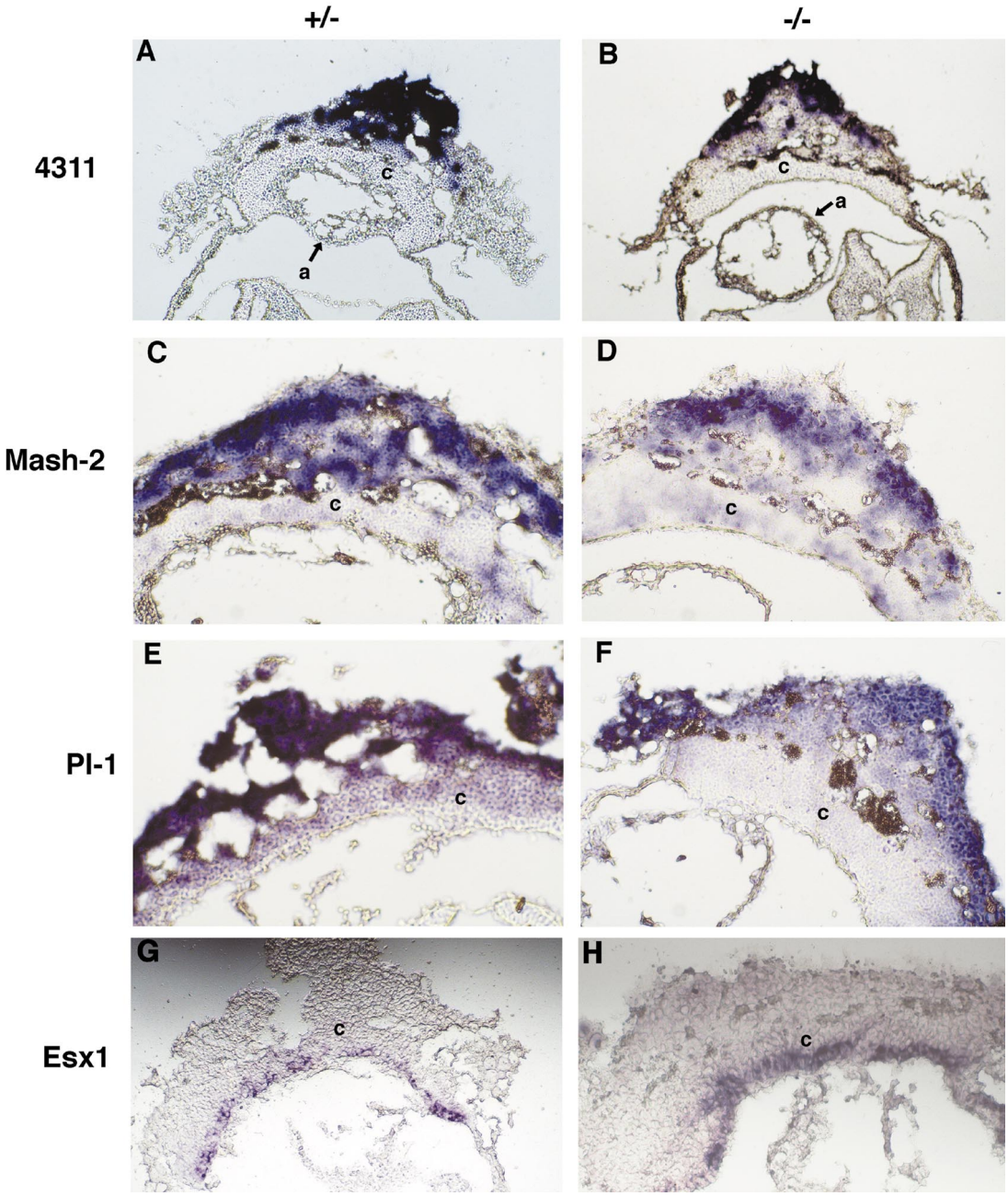

FIG. 5. *In situ* hybridization analysis of cell type-specific markers in the 8.5-d.p.c. chorion. The left panels show sections from heterozygous (wild-type) embryos, while the right panels show sections from *Wnt*7b mutant embryos. All four markers exhibit equivalent expression in wild-type and mutant embryos. The sections are oriented so that the chorionic component of the placenta is toward the top of the figures and the allantois is towards the bottom. a, allantois; c, chorionic plate.

cause. This possibility needs to be investigated in future studies.

In sum, Wnt7b function appears to integrate the development of the chorionic plate with its ability to bind the allantois. It is interesting that both aspects of Wnt7b function in placental development may involve alterations in cell adhesion properties. Further work is required to identify the specific targets of Wnt7b signaling in the placenta, but the current analysis suggests that α4 integrin is an attractive possibility.

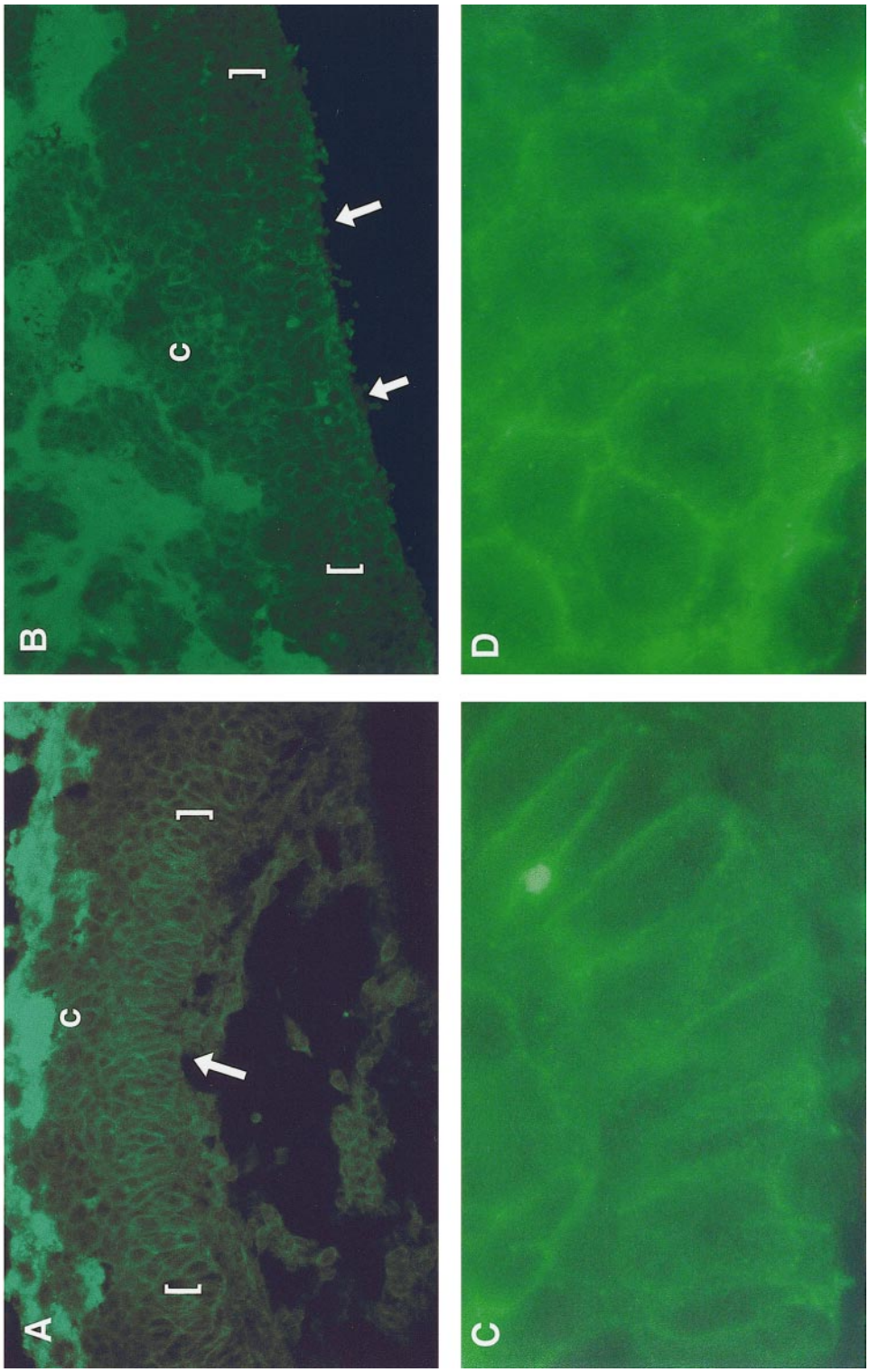

FIG. 6. Organization of cells in the chorionic plate is altered in *Wnt7b* mutants at 8.5 d.p.c. Wild-type (A) and Wnt7b mutant (B) chorions were sectioned and stained with an antibody against β-catenin. Diploid trophoblast cells in the chorion of wild-type embryos appear much more elongated than the cells in the mutant chorion. The brackets indicate the approximate location of the affected cells. The sections are oriented similarly to Figs. 4 and 5, so that allantois and embryo would be located at the bottom of the figures. Arrows indicate the surface of the chorionic plate. (C) and (D) show the affected area of the chorion at higher magnification. c, chorion.

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REFERENCES

Colosi, P., Swiergiel, J. J., Wilder, E. L., Oviedo, A., and Linzer, D. I. (1988). Characterization of proliferin-related protein. *Mol. Endocrinol.* **2,** 579–586.

Downs, K. M., and Gardner, R. L. (1995). An investigation into early placental ontogeny: Allantoic attachment to the chorion is selective and developmentally regulated. *Development* **121,** 407–416.

Galceran, J., Farinas, I., Depew, M. J., Clevers, H., and Grosschedl, R. (1999). Wnt3a−/−-like phenotype and limb deficiency in Lef1(−/−)Tcf1(−/−) mice. *Genes Dev.* **13,** 709–717.

Guillemot, F., Nagy, A., Auerbach, A., Rossant, J., and Joyner, A. L. (1994). Essential role of Mash-2 in extraembryonic development. *Nature* **371,** 333–336.

Gurtner, G. C., Davis, V., Li, H., McCoy, M. J., Sharpe, A., and Cybulsky, M. I. (1995). Targeted disruption of the murine VCAM1 gene: Essential role of VCAM-1 in chorioallantoic fusion and placentation. *Genes Dev.* **9,** 1–14.

Kispert, A., Vainio, S., Shen, L., Rowitch, D. H., and McMahon, A. P. (1996). Proteoglycans are required for maintenance of Wnt-11 expression in the ureter tips. *Development* **122,** 3627–3637.

Kwee, L., Baldwin, H. S., Shen, H. M., Stewart, C. L., Buck, C., Buck, C. A., and Labow, M. A. (1995). Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (VCAM-1) deficient mice. *Development* **121,** 489–503.

Lescisin, K. R., Varmuza, S., and Rossant, J. (1988). Isolation and characterization of a novel trophoblast-specific cDNA in the mouse. *Genes Dev.* **2,** 1639–1646.

Li, Y., and Behringer, R. R. (1998). Esx1 is an X-chromosome-imprinted regulator of placental development and fetal growth. *Nat. Genet.* **20,** 309–311.

Li, Y., Lemaire, P., and Behringer, R. R. (1997). Esx1, a novel X chromosome-linked homeobox gene expressed in mouse extraembryonic tissues and male germ cells. *Dev. Biol.* **188,** 85–95.

Luo, J., Sladek, R., Bader, J. A., Matthyssen, A., Rossant, J., and Giguere, V. (1997). Placental abnormalities in mouse embryos lacking the orphan nuclear receptor ERR-beta. *Nature* **388,** 778–782.

McCrea, P. D., Brieher, W. M., and Gumbiner, B. M. (1993). Induction of a secondary body axis in Xenopus by antibodies to beta-catenin. *J. Cell Biol.* **123,** 477–484.

Monkley, S. J., Delaney, S. J., Pennisi, D. J., Christiansen, J. H., and Wainwright, B. J. (1996). Targeted disruption of the Wnt2 gene results in placentation defects. *Development* **122,** 3343–3353.

Morasso, M. I., Grinberg, A., Robinson, G., Sargent, T. D., and Mahon, K. A. (1999). Placental failure in mice lacking the homeobox gene Dlx3. *Proc. Natl. Acad. Sci. USA* **96,** 162–167.

Parr, B. A., Shea, M. J., Vassileva, G., and McMahon, A. P. (1993). Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. *Development* **119,** 247–261.

Peifer, M., Rauskolb, C., Williams, M., Riggleman, B., and Wieschaus, E. (1991). The segment polarity gene armadillo interacts with the wingless signaling pathway in both embryonic and adult pattern formation. *Development* **111,** 1029–1043.

Pepicelli, C. V., Lewis, P. M., and McMahon, A. P. (1998). Sonic hedgehog regulates branching morphogenesis in the mammalian lung. *Curr. Biol.* **8,** 1083–1086.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Swiatek, P. J., and Gridley, T. (1993). Perinatal lethality and defects in hindbrain development in mice homozygous for a targeted mutation of the zinc finger gene Krox20. *Genes Dev.* **7,** 2071–2084.

Takada, S., Stark, K. L., Shea, M. J., Vassileva, G., McMahon, J. A., and McMahon, A. P. (1994). Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* **8,** 174–189.

Threadgill, D. W., Dlugosz, A. A., Hansen, L. A., Tennenbaum, T., Lichti, U., Yee, D., LaMantia, C., Mourton, T., Herrup, K., Harris, R. C., and et al. (1995). Targeted disruption of mouse EGF receptor: Effect of genetic background on mutant phenotype. *Science* **269,** 230–234.

Uehara, Y., Minowa, O., Mori, C., Shiota, K., Kuno, J., Noda, T., and Kitamura, N. (1995). Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* **373,** 702–705.

Ware, C. B., Horowitz, M. C., Renshaw, B. R., Hunt, J. S., Liggitt, D., Koblar, S. A., Gliniak, B. C., McKenna, H. J., Papayannopoulou, T., Thoma, B., and et al. (1995). Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development* **121,** 1283–1299.

Wendling, O., Chambon, P., and Mark, M. (1999). Retinoid X receptors are essential for early mouse development and placentogenesis. *Proc. Natl. Acad. Sci. USA* **96,** 547–551.

Wodarz, A., and Nusse, R. (1998). Mechanisms of Wnt signaling in development. *Annu. Rev. Cell. Dev. Biol.* **14,** 59–88.

Yang, J. T., Rayburn, H., and Hynes, R. O. (1995). Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. *Development* **121,** 549–560.

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