Requirement for β-Catenin in Anterior-Posterior Axis Formation in Mice

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Abstract. The anterior-posterior axis of the mouse embryo is defined before formation of the primitive streak, and axis specification and subsequent anterior development involves signaling from both embryonic ectoderm and visceral endoderm. The Wnt signaling pathway is essential for various developmental processes, but a role in anterior-posterior axis formation in the mouse has not been previously established. β-Catenin is a central player in the Wnt pathway and in cadherin-mediated cell adhesion. We generated *β-catenin*–deficient mouse embryos and observed a defect in anterior-posterior axis formation at embryonic day 5.5, as visualized by the absence of *Hex* and *Hesx1* and the mislocation of *cerberus-like* and *Lim1* expression. Subsequently, no mesoderm and head structures are generated. Intercellular adhesion is maintained since plakoglobin substitutes for β-catenin. Our data demonstrate that β-catenin function is essential in anterior-posterior axis formation in the mouse, and experiments with chimeric embryos show that this function is required in the embryonic ectoderm.

Key words: anterior visceral endoderm • Wnt/wingless pathway • cell adhesion • plakoglobin • armadillo

Introduction

The anterior-posterior axis of the mouse embryo becomes explicit morphologically at embryonic day (E)[1] 6.5, when the first mesoderm forms in the primitive streak region at the posterior side of the embryo. However, recent experiments show that anterior-posterior polarity is established at least 1 d earlier (for reviews see Tam and Behringer, 1997; Beddington and Robertson, 1999): first signs of anterior-posterior polarity are detectable before primitive streak formation by the asymmetric expression of *cerberus-like*, *Hex*, and other markers in the prospective anterior portion of the visceral endoderm (Rosenquist and Martin, 1995; Belo et al., 1997; Thomas et al., 1998). Accordingly, genetic evidence in the mouse demonstrates that genes expressed in the visceral endoderm, like *Otx2*, *Lim1*, and *nodal*, are required for anterior development (Shawlot and Behringer, 1995; Varlet et al., 1997; Acampora et al., 1998; Rhinn et al., 1998; Shawlot et al., 1998). In addition, genes expressed in the embryonic ectoderm, such as *cripto*, are also necessary for the establishment of anterior-posterior polarity (Ding et al., 1998). In *Xenopus* and zebrafish, components of the Wnt signaling pathway have been implicated in the induction of embryonic body axis (Harland and Gerhart, 1997; Heasman, 1997; Moon and Kimelman, 1998; Solnica-Krezel, 1999). β-Catenin is a central player of the Wnt signaling pathway (Behrens et al. 1996; Molenaar et al., 1996; Cavallo et al., 1997), and in *Xenopus*, accumulation of β-catenin on the dorso-anterior side of the embryo is the earliest sign of axis formation (Schneider et al., 1996; Larabell et al., 1997). Accordingly, overexpression of β-catenin in *Xenopus* embryos induces formation of an additional embryonic axis (Heasman et al., 1994; Funayama et al., 1995).

Protein stability of β-catenin is controlled through Wnt/wingless signaling. Wnt/wingless activates frizzled receptors, and through dishevelled, induces an increase in cytoplasmic β-catenin by preventing its degradation in proteasomes (for review see Cadigan and Nusse, 1997). The proteins axin and/or conductin, in cooperation with the tumor suppressor gene product adenomatous polyposis coli, are involved in the control of β-catenin degradation, which depends on serine-threonine phosphorylation of β-catenin by GSK3β and subsequent ubiquitination (Rubinfeld et al., 1996; Yost et al., 1996; Aberle et al. 1997; Zeng et al., 1997; Behrens et al., 1998; Ikeda et al., 1998; Jiang and Struhl, 1998). The increased levels of β-catenin allow interaction with transcription factors of the lymphoid enhancer factor (LEF)/T-cell factor (TCF) family and activation of gene expression (Behrens et al., 1996; Huber et al., 1996; Molenaar et al., 1996; He et al., 1998; Tetsu and McCor-

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[1]*Abbreviations used in this paper:* BMP, bone morphogenetic protein; E, embryonic day; ES, embryonic stem; LEF, lymphoid enhancer factor; TCF, T-cell factor.

mick, 1999). In *Xenopus*, target genes of this pathway such as *Siamois*, *Twin*, and *goosecoid* have been identified, which play a role in axis formation (Heasman et al., 1994; Brannon et al., 1997; Fan and Sokol, 1997; Laurent et al., 1997). In addition, inactivating mutations of adenomatous polyposis coli or activating mutations of β-catenin, which result in constitutive nuclear signaling and gene activation, have been identified in tumor progression (Morin et al., 1997; Rubinfeld et al., 1997; He et al., 1998; Tetsu and McCormick, 1999; for review see Polakis, 1999).

β-Catenin contains repeated elements, the armadillo repeats, that are flanked by unique NH_2- and COOH-terminal domains (McCrea et al., 1991; Peifer, 1993; Huelsken et al., 1994a). The central armadillo repeats of β-catenin bind directly to the LEF/TCF transcription factors, and the COOH-terminal domain of β-catenin largely mediates transcriptional activation (Behrens et al., 1996; van de Wetering et al., 1997; Vleminckx et al., 1999). β-Catenin is also located in adherens junctions, where it binds cadherins through the armadillo repeats and establishes a link to the cytoskeleton via NH_2-terminally bound α-catenin (Butz and Kemler, 1994; Hinck et al., 1994; Huelsken et al., 1994b; Aberle et al., 1996; Nieset et al., 1997). Plakoglobin, the closest relative of β-catenin in vertebrates, can also bind cadherins and α-catenin, and in addition, mediates the interaction between desmosomal cadherins and the intermediate filament system (Huelsken et al., 1994b; Troyanovsky et al., 1994; Sacco et al., 1995; Kowalczyk et al., 1997). Ablation of mouse genes that encode components of the cadherin–catenin system results in adhesion defects during embryogenesis: in *E-cadherin*– and *α-catenin*–deficient embryos, the epithelium of the trophectoderm disintegrates at the blastocyst stage (Larue et al., 1994; Riethmacher et al., 1995; Torres et al., 1997). Plakoglobin-deficient embryos exhibit abnormal adhesion of myocardial cells that result in heart rupture at midgestation (Ruiz et al., 1996). At late gestation, adhesion defects are also observed in the skin of plakoglobin-deficient mice (Bierkamp et al., 1996).

We report here the effect of β-catenin–null mutations in mice. In embryos that lack β-catenin, we observe a block in anterior-posterior axis formation at E6.0. At this stage, *cerberus-like* and *Lim1* expressing cells are mislocated in the distal visceral endoderm, and other anterior markers like *Hex* and *Hesx1* are not induced. Subsequently, no mesoderm and head structures are formed, and the mutant embryos retain the differentiation of the early egg cylinder stage but continue to grow. Using chimeric embryos, we show that β-catenin function is required in the embryonic ectoderm. *β-Catenin*–deficient embryos contain well-developed adherens junctions, in which plakoglobin substitutes for β-catenin. In analogy to *Xenopus* and zebrafish, the observed block in axis formation of *β-catenin*–deficient mouse embryos appears to reflect a signaling function of β-catenin.

Materials and Methods

Generation of β-Catenin–deficient Mice

For construction of the targeting vectors, genomic fragments isolated from a λGEM-12 129/Ola library were introduced into the pTV0 vector (Riethmacher et al., 1995). The β-cat^{del} vector contained at the 5′ arm genomic sequence from the end of the first intron to the beginning of the third exon, i.e., encodes only for the first 14 amino acids of β-catenin. The 3′ arm contained exons 5 to 16. Targeting vector β-cat^{lacZ} contained a β-galactosidase cDNA with a nuclear localization signal fused to the ATG translation initiation codon of β-catenin. The 3′ arm contained sequences from the end of the sixth intron to exon 16. In the vector β-cat^{lacZ}(hyg), neomycin was replaced by a hygromycin resistance cassette. Homozygous mutant embryonic stem (ES) cells were generated by the consecutive electroporation of the two targeting vector variants, β-cat^{lacZ}(neo) and β-cat^{lacZ}(hyg). Two independent, heterozygous ES cell clones (derived from E14 ES cell line) for the loci β-cat^{del} and β-cat^{lacZ}(neo) were used to generate chimeric mice by blastocyst injection as described (Riethmacher et al., 1995). Heterozygous mutant animals were bred on a mixed 129×C57Bl6 background, and showed no developmental abnormalities. PCR genotyping was performed using the primers TGG CTT CTT CAG GTA GCA TTT TCA GTT C, CAT TCA TAA AGG ACT TGG GAG GTG T, and GCC TTC TAT CGC CTT CTT GAC G (β-cat^{del}), or CAT GGA CAG GGG TGG CCT GA, TGT TTT TCG AGC TTC AAG GTT CAT, and AGA ATC ACG GTG ACC TGG GTT AAA (β-cat^{lacZ}).

Microscopic Analysis and In Situ Hybridization

β-Galactosidase staining of embryos, immunofluorescence analysis, and EM were performed as described (Hogan et al., 1994; Huelsken et al., 1994b; Riethmacher et al., 1995; Varlet et al., 1997). Embedding in paraffin or plastic was performed according to the manufacturer's protocols (Paraplast, Sherwood Medical; Technovit 7100, Kulzer Heraeus). Sections were stained with hematoxylin/eosin or 0.1% pyroninG (for lacZ-stained embryos). Size and proliferation of embryos between E5.5 and E7.5 were quantified by counting total numbers of embryonic cells and metaphases on consecutive 4,6-diamidino-2-phenylindole–stained sections. In situ hybridizations with digoxygenin-labeled RNA probes (Ding et al., 1998; Liu et al., 1999) were performed according to the manufacturer's protocols (Boehringer Mannheim) followed by PCR genotyping. For immunogold labeling, embryos were embedded in Unicryl (British BioCell Int.), and semithin sections were used for orientation, followed by immunocytochemical labeling of ultrathin sections with antiplakoglobin antibody and 12 nm colloidal gold goat anti–rabbit IgG (Jackson ImmunoResearch Inc.). Sections were contrasted with uranyl acetate and lead citrate.

Generation of Chimeric Embryos

Aggregation chimeras were generated from wild-type embryos and embryos obtained from crossing heterozygous β-cat^{lacZ} and β-cat^{del} mutant mice as described (Hogan et al., 1994). Injection chimeras were produced either by injection of wild-type blastocysts with heterozygous or homozygous β-cat^{lacZ} ES cells or by injection of blastocysts obtained from crossing heterozygous β-cat^{lacZ} and β-cat^{del} mice with wild-type ES cells (Riethmacher et al., 1995). For high or low ES cell contribution, ∼20 or 5 ES cells were injected, respectively. 50 embryos were produced by either method, and three of the most advanced chimeric embryos between E8.0 and E9.5 were analyzed in detail by sectioning. Mutant cells were detected by β-galactosidase staining, followed by PCR genotyping of blue cells for the additional presence of the β-cat^{del} allele.

Results

Phenotype of β-Catenin–deficient Embryos

We generated *β-catenin*–deficient mice by homologous recombination in ES cells using two targeting vectors, β-cat^{del} and β-cat^{lacZ} (Fig. 1, a–c). No truncated β-catenin mRNA was detectable in the targeted ES cells (Fig. 1 d), and Western blotting with an antibody against an epitope of the COOH terminus of β-catenin revealed absence of truncated protein (data not shown). Homozygous β-cat^{del} and β-cat^{lacZ} mutant mouse embryos showed identical phenotypes: a Mendelian ratio of *β-catenin*–deficient embryos was observed up to E7.5 of development, but the ratio was reduced to 9% at E8.5, and no homozygous mutant embryos were found at E9.5 (Fig. 1 e, and data not

shown). Homozygous mutant embryos lacked expression of β-catenin mRNA (as revealed by reverse transcription PCR, data not shown) and protein (Fig. 2, a and b) and were morphologically indistinguishable from wild-type and of identical size at E5.5 of development (Fig. 2, c–f). All embryonic cell layers of the early egg cylinder stage, i.e., embryonic and extraembryonic ectoderm as well as visceral and parietal endoderm were present. At subsequent stages, wild-type and mutant embryos displayed different morphology: at E6.5, wild-type embryos were stretched and had an extended lumen (Fig. 2 g). Mutant embryos were more compact, but nevertheless exhibited well-structured epithelia (Fig. 2 h, see below). At E7.5, wild-type embryos had formed mesoderm at the posterior side and were in the process of gastrulation (Fig. 2 i). Mesoderm formation and gastrulation were not observed in *β-catenin*–deficient embryos (Fig. 2 k), and the early mesoderm and primitive streak markers *Brachyury* and *goosecoid* were not detectable (Fig. 3, a, b, e, and f). The embryonic and extraembryonic ectoderm of the mutant embryo was found to express *Oct4* and *bone morphogenetic protein* (*BMP*) *4*, respectively, i.e., proximal-distal polarity of the embryo was established correctly (Fig. 3, c, d, g, and h). Mutant embryos continued to grow between E5.5 and E7.5: cell numbers increased from 1,500 to 10,000 cells, which corresponds to a 2.5-fold reduction when compared with wild-type embryos (Fig. 3, i and k). After E7.5, apoptosis became prominent in the embryonic ectoderm of β-catenin mutant embryos (as determined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling [TUNEL]-staining) (data not shown).

Defects in Anterior-Posterior Axis Formations in β-Catenin–deficient Embryos

In wild-type embryos at E5.5, the homeobox gene *Hex* is

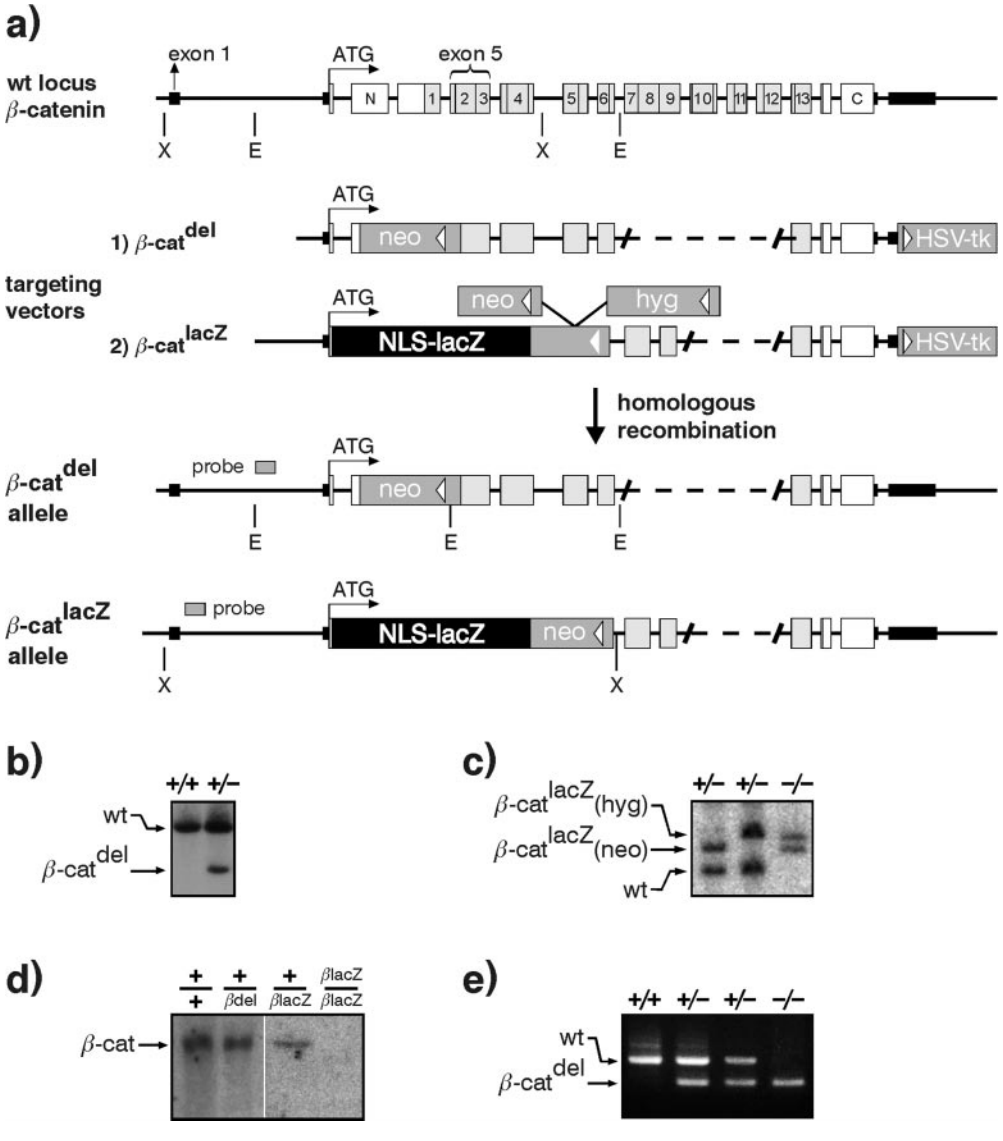

Figure 1. Targeted mutation of the *β-catenin* gene. (a) Scheme of the genomic structure of β-catenin, the targeting vectors, and the targeted alleles. Large boxes indicate coding exons of *β-catenin* (e.g., exon 5), and small black boxes indicate noncoding exons (e.g., exon 1). The encoded β-catenin protein consists of central armadillo repeats (1–13) that are flanked by unique NH_2- and COOH-terminal domains as depicted. In the vector β-cat^{del}, part of exon 3, exon 4, and part of exon 5 were deleted, which encode the NH_2-terminal domain (N) and armadillo repeats 1–3. In the vector β-cat^{lacZ}, a lacZ gene containing a nuclear localization signal was fused in frame to the start codon of the *β-catenin* gene and replaced sequences encoding the NH_2-terminal domain and armadillo repeats 1–4. Two variants of this vector with either a neomycin or a hygromycin resistance cassette were used in consecutive electroporations to generate homozygous mutant ES cells. Mutated alleles are shown below (restriction sites: E, EcoRI; X, XhoI; probes used for Southern analysis are indicated). neo, neomycin resistance cassette; hyg, hygromycin resistance cassette; HSV-tk, herpes simplex virus thymidine kinase. Southern (b and c) and Northern (d) blots of heterozygous and homozygous ES cell lines containing the mutated alleles. Equal amounts of total RNA were used for Northern blotting with full-size β-catenin cDNA as a probe. (e) PCR genotyping of embryos at E6.5 derived from a cross of heterozygous β-cat^{del} mice.

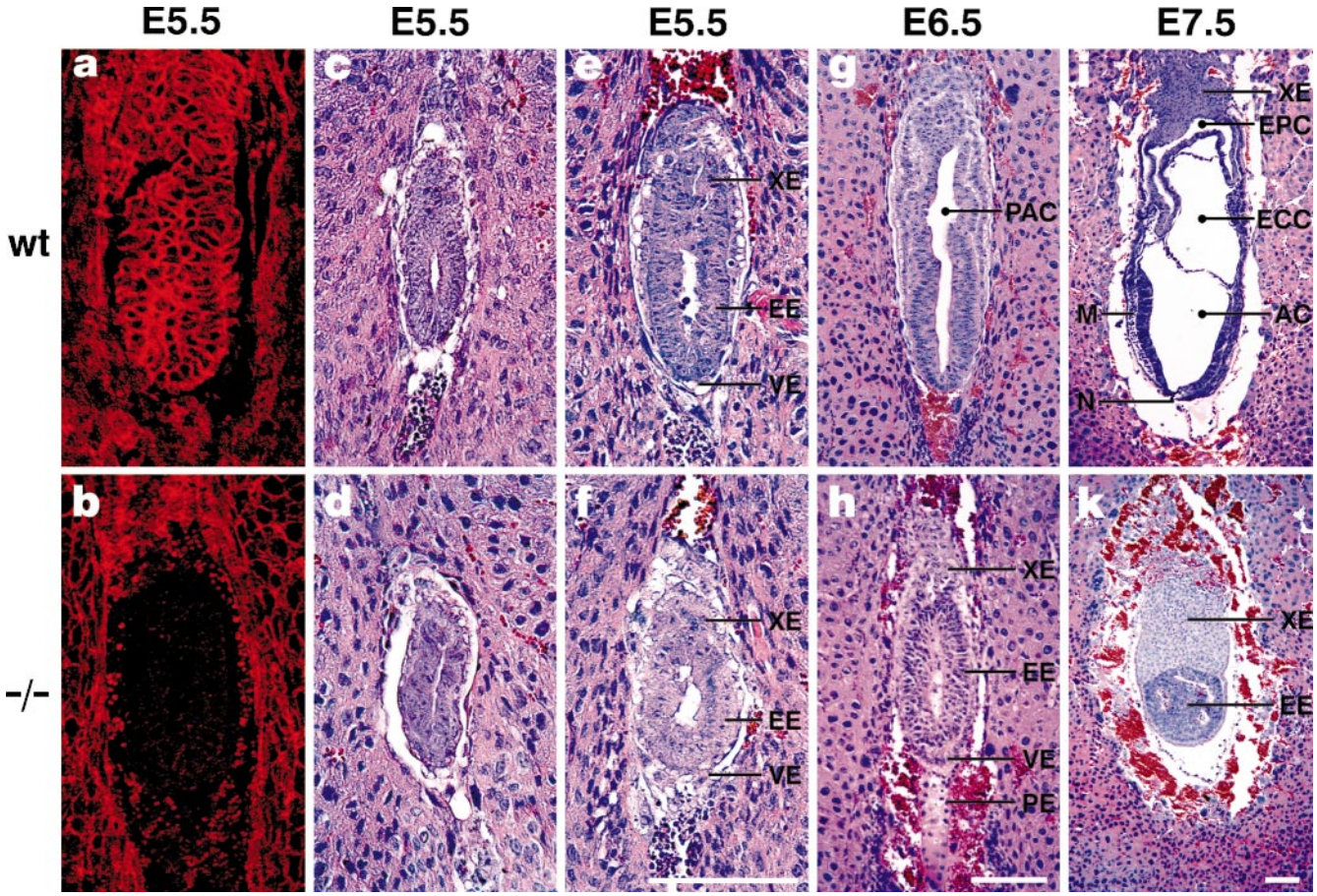

Figure 2. Histological appearance of *β-catenin*–deficient and wild-type embryos. (a and b) β-Catenin expression as determined by immunofluorescence analysis with an antibody against an epitope of the COOH terminus of β-catenin. (c–k) Hematoxylin/eosin-stained sagittal sections of embryos at E5.5 to E7.5. Bars: (a–f) 100 μm; (g and h) 100 μm; and (i and k) 100 μm. AC, amniotic cavity; ECC, exocoelomic cavity; EE, embryonic ectoderm; EPC, ectoplacental cavity; M, mesoderm; N, node; PAC, proamniotic cavity; PE, parietal endoderm; VE, visceral endoderm; and XE, extraembryonic ectoderm.

expressed in the visceral endoderm at the tip of the egg cylinder, and the expression domain moves unilaterally to the prospective anterior side at E6.0 (Thomas et al., 1998). Remarkably, *Hex* is not expressed in *β-catenin*–deficient embryos at E6.0 (Fig. 4, a and b). The secreted factor *cerberus-like* is expressed distally and anteriorly in the visceral endoderm of wild-type embryos at E5.5, and is restricted to the anterior end at E6.0 (Belo et al., 1997). We found that in *β-catenin*–deficient embryos, *cerberus-like* expression is restricted to the distal tip and does not extend anteriorly (Fig. 4, c and d). In situ hybridizations on sections of mutant embryos confirmed symmetrical expression of *cerberus-like* in the distal visceral endoderm (Fig. 4, m and p; compare with the asymmetric distribution in wild-type controls in Fig. 4, l and n). *Lim1* is a marker for anterior-posterior polarity, which is expressed in the wild-type embryo both in the anterior visceral endoderm and in the posterior mesodermal wings at E6.75 (Shawlot and Behringer, 1995; Liu et al., 1999). *Lim1* is located distally in *β-catenin*–deficient embryo (Fig. 4, e and f). Anterior differentiation of mutant embryos could not be observed: *Hesx1* is a marker for anterior differentiation in the wild-type embryo, which is initially expressed in the anterior visceral endoderm and prechordal plate precursor at E7.0, and later in the adjacent ectoderm, which will give rise to the ventral prosencephalon (Hermesz et al., 1996; Dattani et al., 1998). *Hesx1* is not expressed in the *β-catenin*–deficient embryos (Fig. 4, g and h). We also tested the expression of *Otx2*, which becomes restricted to the anterior side of the wild-type embryo at E7.0 (Fig. 4 i) (Acampora et al., 1998). *Otx2* was not expressed in *β-catenin*–deficient embryos at this stage (Fig. 4 k). In addition, the midbrain–hindbrain marker *Engrailed1* was not expressed in the mutant embryos (data not shown).

We also observed a lack of proximal-distal regionalization of the visceral endoderm by ultrastructural analysis: in wild-type embryos, the visceral endoderm of the extraembryonic region is composed of columnar, highly vacuolized cells with dense microvilli, whereas cells in the embryonic region are squamous with low numbers of microvilli (Fig. 5, a and c). This regionalization of the visceral endoderm was not observed in *β-catenin*–deficient embryos; instead, the cells displayed an intermediate morphology with fewer vacuoles and microvilli (Fig. 5, b and d). No morphological differences could be observed in the embryonic or extraembryonic ectoderm between wild-type and *β-catenin*–

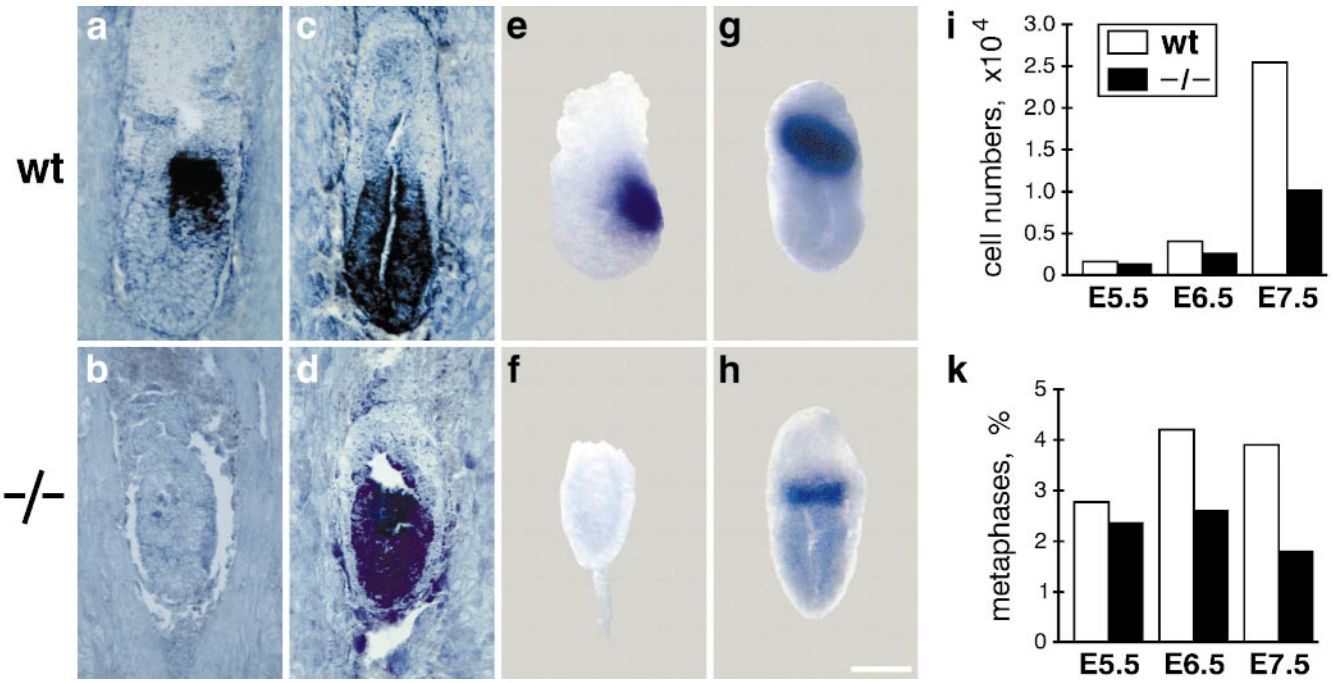

Figure 3. Differentiation and growth in *β-catenin*–deficient embryos. In situ hybridizations on sagittal sections with Brachyury cDNA at E6.5 (a and b), Oct4 cDNA at E6.5 (c and d), goosecoid cDNA at E6.25 (e and f), and BMP4 cDNA at E6.0 (g and h). Bar, 100 μm. (i) The number of cells per embryo and (k) the percentage of cells in metaphase were counted at different stages of embryonic development. The data for three embryos each were averaged.

deficient embryos. These data indicate that a change in the visceral endoderm accompanies defective anterior-posterior axis formation in *β-catenin*–deficient embryos.

Requirement of β-Catenin in the Embryonic Ectoderm and Developmental Potential of β-Catenin–deficient Cells

Homozygous mutant, β-galactosidase–expressing ES cells that lack β-catenin mRNA and protein were generated by the consecutive electroporation of the two targeting vectors β-cat^{lacZ}(neo) and β-cat^{lacZ}(hyg) (Fig. 1, c and d; data not shown). Aggregation chimeras between wild-type and homozygous mutant embryos and injection chimeras with high numbers of homozygous mutant ES cells did not develop beyond the egg cylinder stage, i.e., resembled *β-catenin*–deficient embryos (Fig. 6 b; Fig. 6 a, control; data not shown). Thus, a high contribution of *β-catenin*–deficient cells is not compatible with normal development beyond E6. Moreover, these experiments show that β-catenin function is required in the embryonic ectoderm, since it is known that injected ES cells contribute preferentially to this tissue in chimeric embryos (Beddington and Robertson, 1989; Varlet et al., 1997). However, when low numbers of homozygous mutant ES cells were injected, chimeras continued development beyond the egg cylinder stage, and *β-catenin*–deficient cells contributed to anterior and mesodermal structures (e.g., headfolds and paraxial mesoderm; Fig. 6, c and d). Thus, β-catenin is not required in a cell-autonomous manner for the formation of anterior or mesodermal structures in the early mouse embryo. Reciprocally, chimeric embryos were generated by injecting high numbers of wild-type ES cells into *β-catenin*–deficient blastocysts, i.e., extraembryonic tissues like visceral endoderm are produced from *β-catenin*–deficient cells. In these chimeras, anterior and mesodermal differentiation was observed, as demonstrated by the development of headfolds and heart (Fig. 6, e and f). The extent of rescue in the chimeras correlated with the amount of wild-type cells in the embryonic ectoderm. Taken together, these data indicate that β-catenin function is necessary in the embryonic ectoderm but not in extraembryonic tissues like the visceral endoderm during early postimplantation development of the mouse.

Adherens Junctions Are Maintained in β-Catenin–deficient Embryos

In confocal immunofluorescence analysis, identical staining patterns of E-cadherin and α-catenin were observed in wild-type and mutant embryos at E7.0 (Fig. 7, a and b; data not shown). On an ultrastructural level, intact adherens junctions and desmosomes were detected (Fig. 7, e and g; data not shown). In wild-type embryos, plakoglobin, the closest relative of β-catenin in the armadillo family, is localized preferentially in desmosomes and distributed in a spotty pattern along the membrane (Fig. 7 c; data not shown). In contrast, in homozygous mutant embryos, plakoglobin was detected in increased amounts and was distributed uniformly along the membrane (Fig. 7 d). Immunogold localization confirmed redistribution of plakoglobin to lateral membranes in the mutant embryos (Fig. 7, f and h). Thus, plakoglobin appears to substitute for β-catenin in adherens junctions of mutant embryos. In

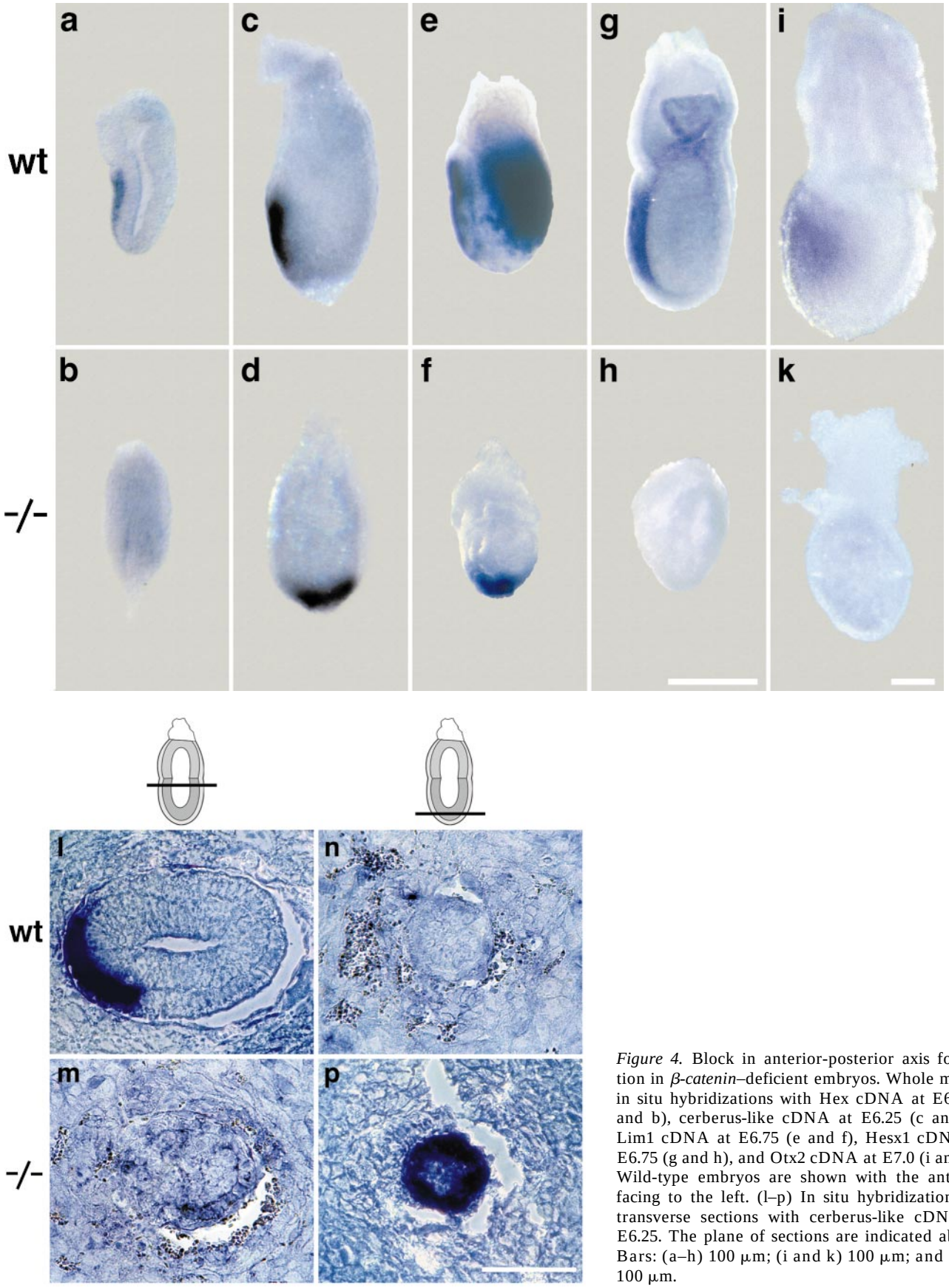

Figure 4. Block in anterior-posterior axis formation in *β-catenin*–deficient embryos. Whole mount in situ hybridizations with Hex cDNA at E6.0 (a and b), cerberus-like cDNA at E6.25 (c and d), Lim1 cDNA at E6.75 (e and f), Hesx1 cDNA at E6.75 (g and h), and Otx2 cDNA at E7.0 (i and k). Wild-type embryos are shown with the anterior facing to the left. (l–p) In situ hybridizations on transverse sections with cerberus-like cDNA at E6.25. The plane of sections are indicated above. Bars: (a–h) 100 μm; (i and k) 100 μm; and (l–p) 100 μm.

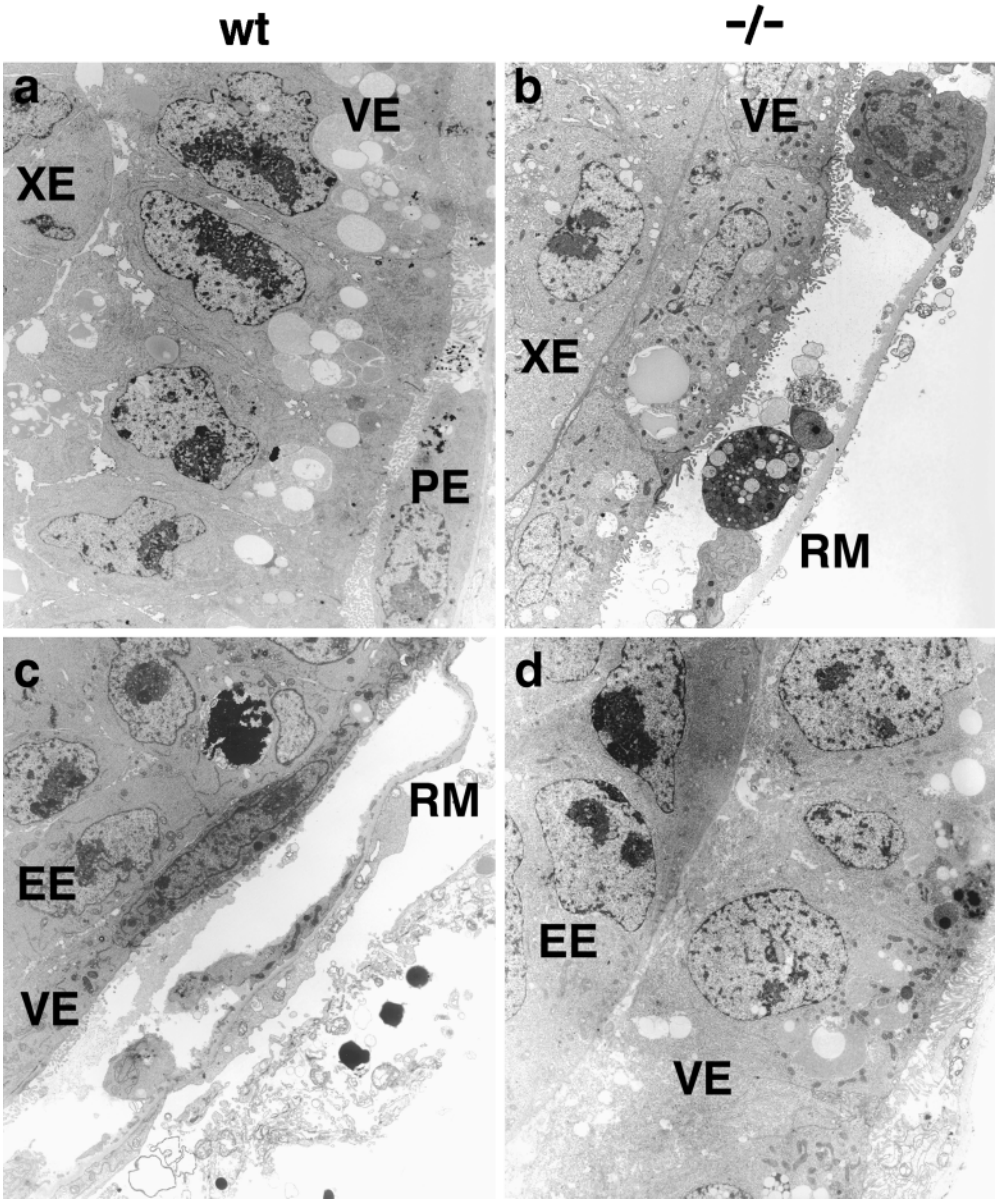

Figure 5. Ultrastructural changes in the visceral endoderm of *β-catenin*–deficient embryos at E6.25. The visceral endoderm in the extraembryonic (a and b) and the embryonic (c and d) region is shown. EE, embryonic ectoderm; PE, parietal endoderm; RM, Reichert's membrane; VE, visceral endoderm; and XE, extraembryonic ectoderm.

vitro data had shown earlier that plakoglobin can mediate the interaction between E-cadherin and α-catenin (Huelsken et al., 1994b).

Discussion

Here we show that anterior-posterior axis does not form appropriately in *β-catenin*–deficient mouse embryos. Axis formation in the mutant embryos is blocked, and the prospective anterior visceral endoderm is mislocated, as judged by the expression of the markers *cerberus-like* and *Lim1* at the distal tip. Subsequently, no mesoderm and head structures form, and markers of posterior, mesodermal differentiation like *Brachyury* and *goosecoid*, as well as markers of anterior differentiation like *Hex*, *Hesx1*, *Otx2*, and *Engrailed1*, are not expressed in the mutant embryos. Intercellular adhesion is maintained in *β-catenin*–deficient embryos, since plakoglobin substitutes for β-catenin. Our data implicate the signaling capacity of β-catenin, and thus the Wnt pathway, in early axis formation of mammalian embryos.

In *Xenopus*, the role of β-catenin and the Wnt pathway in body axis formation and dorso-anterior specification has been well-established (Funayama et al., 1995; Harland and Gerhart, 1997; Heasman, 1997; Moon and Kimelman, 1998). Axis formation precedes gastrulation as indicated by the enrichment of endogenous β-catenin in nuclei on the prospective dorso-anterior side of the blastula (Schneider et al., 1996; Larabell et al., 1997), and a block of β-catenin expression results in failure of axis development (Heasman et al., 1994). β-Catenin is required as a signaling molecule in this process, since a fusion protein consisting of the DNA-binding domain of the transcription factor LEF-1 and the COOH-terminal transcriptional activation domain of β-catenin is sufficient to induce an additional axis in the frog (Vleminckx et al., 1999). Also in

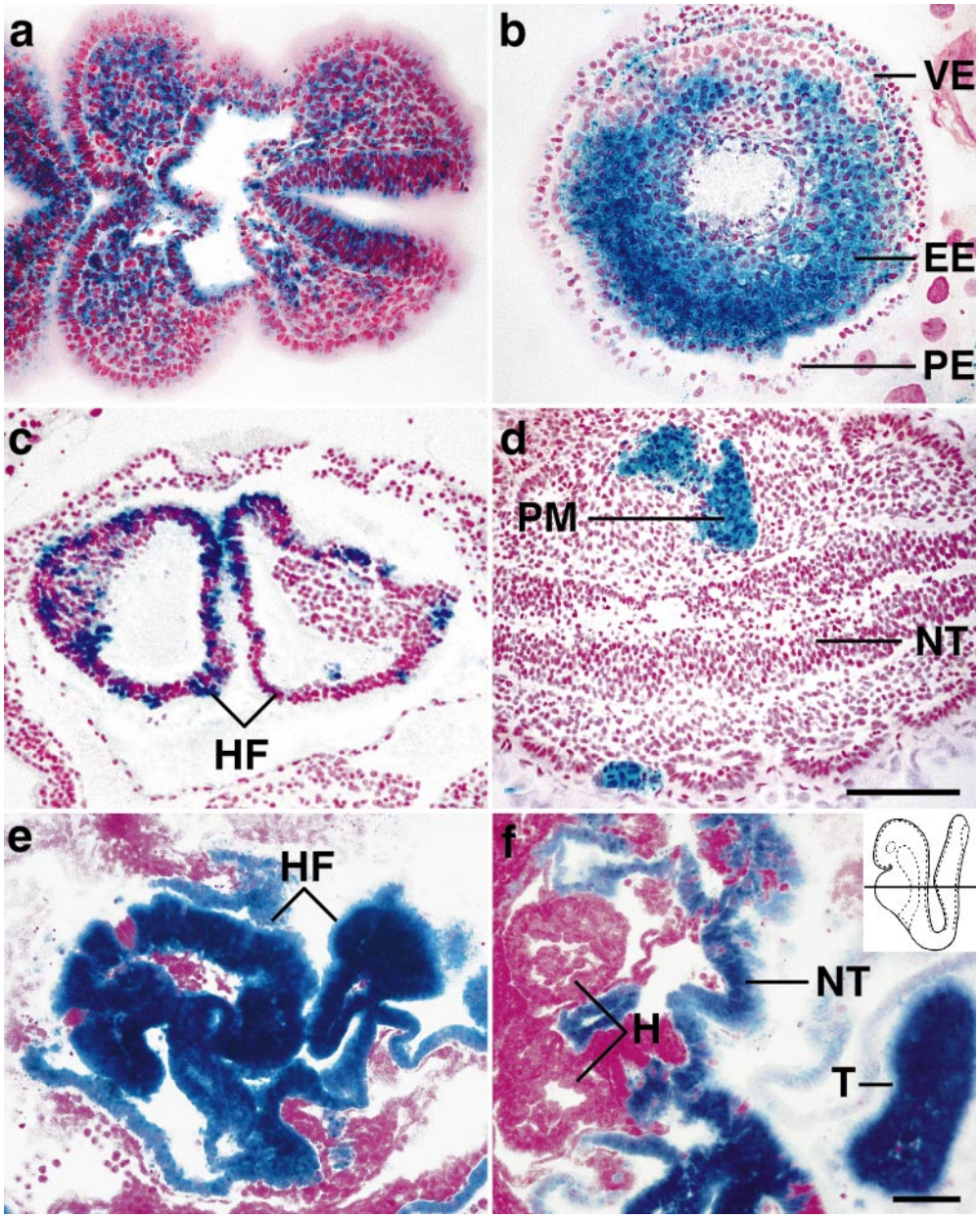

Figure 6. Developmental potential of *β-catenin*–deficient (β-gal stained) cells in chimeric embryos. Aggregation chimeras between (a) wild-type and heterozygous (+/β-cat^{lacZ}) and (b) wild-type and homozygous mutant (β-cat^{del}/β-cat^{lacZ}) embryos, as shown by transverse sections at E8.5. Chimeras obtained from injection of low numbers of homozygous mutant ES cells: (c) transverse section through nonfused headfolds at E8.0 and (d) frontal section through trunk at E9.5. Chimeras generated by injection of wild-type ES cells into homozygous mutant blastocysts (β-cat^{del}/β-cat^{lacZ}): (e) transverse sections through nonfused headfolds and (f) heart at E8.0. Inset indicates level of sectioning. EE, embryonic ectoderm; H, heart; HF, head folds; NT, neural tube; PE, parietal endoderm; PM, paraxial mesoderm; T, tail; and VE, visceral endoderm. Bar, 100 μm.

zebrafish, signaling mediated by β-catenin and the Wnt pathway is essential for embryonic axis formation (Nasevicius et al., 1998; Peleari and Maischein, 1998; Sumoy et al., 1999). Our ablation of the *β-catenin* gene in the mouse produced a defect in anterior-posterior axis formation at E6, i.e., earlier than gastrulation. Moreover, *goosecoid*, a target gene of β-catenin–mediated signaling in *Xenopus* (Laurent et al., 1997; Peleari and Maischein, 1998; Roeser et al., 1999), is not expressed in *β-catenin*–deficient mouse embryos. Therefore, we suggest that in the mouse β-catenin is also required as a signaling molecule for anterior-posterior axis formation. Interestingly, *Engrailed2* has been also characterized as a target gene of β-catenin–mediated signaling in *Xenopus* (McGrew et al., 1999), and we found that the related gene *Engrailed1* is not expressed in β-catenin–deficient embryos. β-Catenin function at the egg cylinder stage may depend on interaction with high mobility group box transcription factors such as LEF-1 or TCF3, which are expressed at this stage (Roose, 1999). A genetic analysis of the mouse TCF3 function has not been reported; other LEF/TCF family members function during later developmental stages (van Genderen et al., 1994; Verbeek et al., 1995; Korinek et al., 1998). Another mutation of the *β-catenin* gene in mice was reported previously (Haegel et al., 1995), but anterior-posterior axis formation was not examined in these mutants.

The phenotype of *β-catenin*–deficient embryos is distinct from other mutant mice that display a defective anterior-posterior axis. Mutations in *Smad2*, *Smad4*, or *ActRIB*, which block signaling of members of the transforming growth factor (TGF) β family, affect embryonic differentiation at the egg cylinder stage before gastrulation (Gu et al., 1998; Sirard et al., 1998; Waldrip et al., 1998; Weinstein et al., 1998). In the absence of *Smad2*, *cer-*

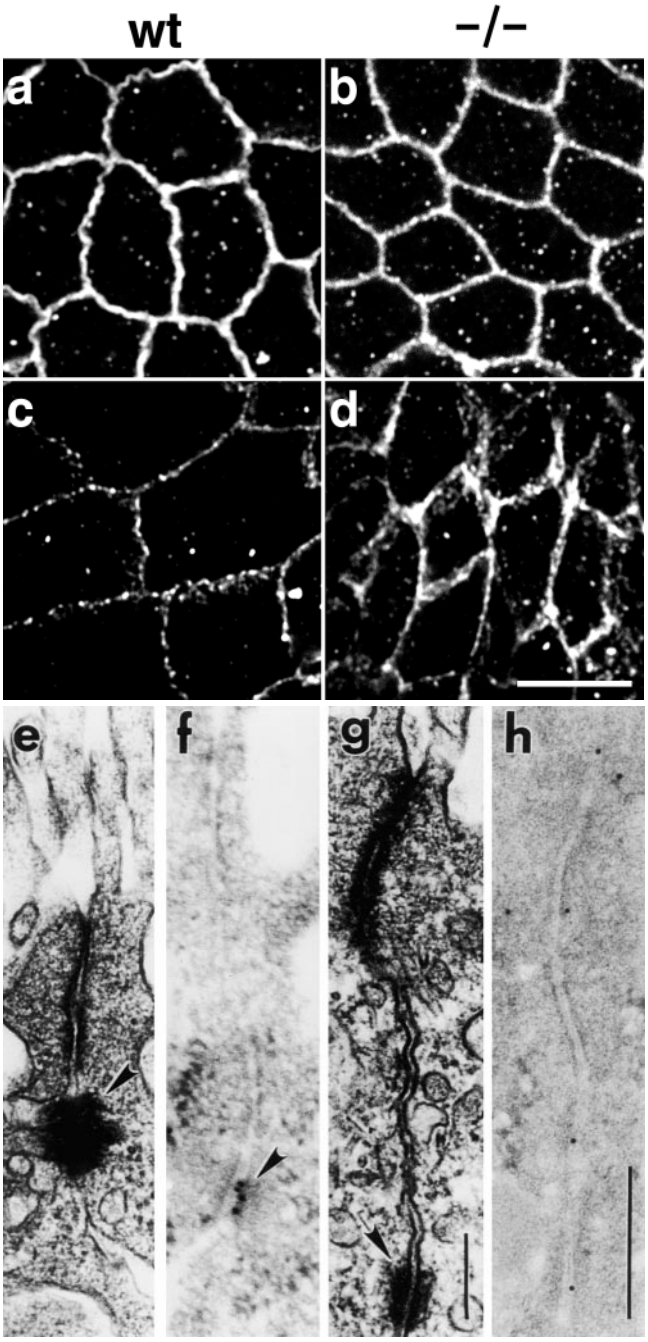

Figure 7. Intact cell adhesion in *β-catenin*–deficient embryos. Confocal immunofluorescence analysis of proteins located in cell adhesion junctions at E7.5: expression of α-catenin (a and b) and plakoglobin (c and d). EM (e and g) and immunogold labeling (f and h) of plakoglobin in wild-type (e and f) and *β-catenin*–deficient embryos (g and h) at E7.5. Desmosomes are marked by arrowheads. Visceral endoderm is shown, but similar results were obtained in the embryonic ectoderm. Bars: (a–d) 10 μm; (e and g) 250 nm; and (f and h) 250 nm.

berus-like is not expressed, and the epiblast exclusively forms extraembryonic mesoderm (Waldrip et al., 1998), indicating that Smad2 is required for the initial generation of anterior-posterior organizing centers. This phenotype is more severe than the β-catenin mutant, and suggests that signaling of members of the TGFβ/BMP family may precede or cooperate with the β-catenin–dependent pathway. In *cripto*$^{-/-}$ embryos, the anterior-posterior axis is initially formed but mislocated, i.e., both *Hex* and *cerberus-like* are expressed distally, whereas *Lim1* is expressed proximally. Anterior neuroectoderm forms at the distal end of the embryo, as assessed by the expression of *Hesx1* (Ding et al., 1998). This is a less severe phenotype than that observed in *β-catenin*–deficient embryos, which also show mislocalization of anterior visceral endoderm markers but completely lack subsequent anterior or posterior differentiation. *Hex* and *Hesx1* are not expressed in *β-catenin*–deficient embryos, suggesting that induction of these genes may depend on β-catenin (Zorn et al., 1999). These data indicate that β-catenin may operate earlier than or cooperate with EGF–cripto, Frl-1, and cryptic (CFC) molecules. Consistent with this, Wnt and β-catenin signaling does not rescue an *oep/cripto* mutant phenotype in zebrafish (Gritsman et al., 1999). Moreover, gene ablation has shown that *Wnt3* is essential for formation or maintenance of the primitive streak (Liu et al., 1999). *Wnt3* is not essential for the formation of the anterior organizing center, since anterior visceral endoderm markers are localized correctly in *Wnt3*-deficient embryos, but no further anterior or posterior differentiation was observed. Hypomorphic mutations of *axin*, which functions as a negative regulator in the Wnt pathway by reducing β-catenin stability (Zeng et al., 1997; Behrens et al., 1998), and overexpression of chicken Wnt8 in the mouse cause duplication of the posterior axis, i.e., a second primitive streak (Zeng et al., 1997; Poepperl et al., 1997). Taken together, these data indicate that signaling by members of the Wnt pathway plays a role for at least two different steps during axis formation in the mouse: (a) for the establishment of initial anterior-posterior polarity (as detected in our *β-catenin* mutants); and (b) for the formation of posterior structures such as the primitive streak (as found in the *Wnt3* mutants) (Liu et al., 1999). *β-Catenin* and *Wnt3* mutants both lack mesoderm and anterior neural ectoderm, probably due to an essential function of β-catenin in Wnt3-dependent signaling. It is not known which *Wnt* genes require β-catenin during the initial formation of polarity in the mouse; several *Wnt* genes are expressed during early stages of mouse embryogenesis (Gavin et al., 1990; McMahon et al., 1992; Bouillet et al., 1996), and might take over important, possibly partially redundant functions. However, β-catenin–mediated signaling is not required for the general specification of all embryonic axes, since markers for the proximal-distal axis like *Oct4* and *BMP4* are unchanged in the mutant embryos.

We have shown that high contribution of *β-catenin*–deficient cells to the epiblast of chimeric embryos leads to developmental arrest. In contrast, absence of β-catenin in extraembryonic tissues like the visceral endoderm allowed anterior and mesodermal differentiation. This indicates that β-catenin function is required in the embryonic ectoderm for axis formation, and that signals from the embryonic ectoderm may contribute to the patterning of the underlying endoderm. To further localize the cells that depend on β-catenin, we carried out two types of experiments. First, we used a mouse reporter strain that carries a lacZ transgene under the control of multiple LEF/TCF

binding sites (Roose, 1999) (a generous gift of Dr. H. Clevers, University of Utrecht, Utrecht, The Netherlands). Whereas β-galactosidase activity was detected at E6.5 (Roose, 1999), we could not locate activity at E5.5 and E6.0. However, it should be noted that this promoter appears not to respond to all β-catenin–mediated signals in vivo or in cell culture. Second, we attempted to identify nuclear β-catenin at the egg cylinder stage by confocal immunofluorescence, but could not detect such a signal. Moreover, experiments with chimeric embryos that have a low contribution of *β-catenin*–deficient cells show that β-catenin is not required cell-autonomously for mesodermal differentiation. This is in accordance with experiments in *Xenopus* that demonstrate that *β-catenin*–deficient marginal zones can be instructed by β-catenin–overexpressing animal caps to form dorsal mesoderm (Wylie et al., 1996).

We showed that cellular adhesion of epithelial cells in the early mouse embryo is not grossly disturbed in the absence of β-catenin. Epithelia in the mutant embryos are well-developed, and the cells are connected by well-defined adherens junctions and desmosomes. Furthermore, *β-catenin*–deficient cells in chimeric embryos contributed to various epithelia such as head and limb bud ectoderm. α-Catenin was found to be located along lateral cell membranes in mutant embryos, although β-catenin is normally required to connect α-catenin to classical cadherins. Instead, the protein level of plakoglobin was found to be enhanced, and plakoglobin was redistributed to adherens junctions in *β-catenin*–deficient embryos. Apparently, plakoglobin can take over the function of β-catenin in cell adhesion of mutant embryos, a function which has been investigated by in vitro experiments (Huelsken et al., 1994b). This prevents the early disintegration of epithelia due to defective adhesion that is observed at the blastocyst stage in mice mutant for the *E-cadherin* or *α-catenin* genes (Larue et al., 1994; Riethmacher et al., 1995; Torres et al., 1997). Cell detachment from the ectodermal cell layer at E7 was reported in the previously generated mutation of the *β-catenin* gene (Haegel et al., 1995). It was not rigorously shown that this earlier mutation corresponds to a null allele. Given the structure of the used targeting vector in Haegel et al. (1995), it is possible that an NH_2-terminally truncated β-catenin protein is produced from the mutant allele described previously, which could bind to cadherins but not to α-catenin. Such a molecule would act in a transdominant manner and disturb adhesion, in contrast to the rescue of adhesion we observe in our null-mutants. It should be noted that no β-catenin mRNA or protein is produced by both our $\beta\text{-cat}^{del}$ or $\beta\text{-cat}^{lacZ}$ alleles, which therefore represent null mutations of the *β-catenin* gene. Thus, our data suggest that plakoglobin can substitute for the adhesive function of β-catenin at the egg cylinder stage. Apparently, plakoglobin cannot compensate for the proposed signaling function of β-catenin in axis formation. This is in accordance with previous findings in *Xenopus*, which indicate that plakoglobin does not significantly participate in Wnt signaling (Kofron et al., 1997; Miller and Moon, 1997; Ben-Ze'ev and Geiger, 1998).

The early postimplantation lethality caused by null mutations in *β-catenin* precludes the analysis of its function in many tissues and events at later developmental stages that depend on Wnt signaling. A function of β-catenin in hair formation and in skin tumors such as pilomatricomas has been identified by the overexpression of an activated form of β-catenin in the skin of mice (Gat et al., 1998). Conditional gene ablation will also allow to study functions of β-catenin at later developmental stage.

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References

Aberle, H., H. Schwartz, H. Hoschuetzky, and R. Kemler. 1996. Single amino acid substitutions in proteins of the armadillo gene family abolish their binding to α-catenin. *J. Biol. Chem.* 271:1520–1526.

Aberle, H., A. Bauer, J. Stappert, A. Kispert, and R. Kemler. 1997. β-Catenin is a target for the ubiquitin-proteasome pathway. *EMBO (Eur. Mol. Biol. Organ.) J.* 16:3797–3804.

Acampora, D., V. Avantaggiato, F. Tuorto, P. Briata, G. Corte, and A. Simeone. 1998. Visceral endoderm-restricted translation of Otx1 mediates recovery of Otx2 requirements for specification of anterior neural plate and normal gastrulation. *Development.* 125:5091–5104.

Beddington, R.S., and E.J. Robertson. 1989. An assessment of the developmental potential of embryonic stem cells in the midgestation mouse embryo. *Development.* 105:733–737.

Beddington, R.S., and E.J. Robertson. 1999. Axis development and early asymmetry in mammals. *Cell.* 96:195–209.

Behrens, J., J.P. von Kries, M. Kuhl, L. Bruhn, D. Wedlich, R. Grosschedl, and W. Birchmeier. 1996. Functional interaction of β-catenin with the transcription factor LEF-1. *Nature.* 382:638–642.

Behrens, J., B.A. Jerchow, M. Wurtele, J. Grimm, C. Asbrand, R. Wirtz, M. Kuhl, D. Wedlich, and W. Birchmeier. 1998. Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK3β. *Science.* 280: 596–599.

Belo, J.A., T. Bouwmeester, L. Leyns, N. Kertesz, M. Gallo, M. Follettie, and E.M. De Robertis. 1997. Cerberus-like is a secreted factor with neutralizing activity expressed in the anterior primitive endoderm of the mouse gastrula. *Mech. Dev.* 68:45–57.

Ben-Ze'ev, A., and B. Geiger. 1998. Differential molecular interactions of β-catenin and plakoglobin in adhesion, signaling and cancer. *Curr. Opin. Cell Biol.* 10:629–639.

Bierkamp, C., K.J. Mclaughlin, H. Schwarz, O. Huber, and R. Kemler. 1996. Embryonic heart and skin defects in mice lacking plakoglobin. *Dev. Biol.* 180:780–785.

Bouillet, P., A.M. Oulad, S.J. Ward, S. Bronner, P. Chambon, and P. Dolle. 1996. A new mouse member of the Wnt gene family, mWnt-8, is expressed during early embryogenesis and is ectopically induced by retinoic acid. *Mech. Dev.* 58:141–152.

Brannon, M., M. Gomperts, L. Sumoy, R.T. Moon, and D. Kimelman. 1997. A β-catenin/XTcf-3 complex binds to the siamois promoter to regulate dorsal axis specification in *Xenopus*. *Genes Dev.* 11:2359–2370.

Butz, S., and R. Kemler. 1994. Distinct cadherin-catenin complexes in Ca(2+)-dependent cell-cell adhesion. *FEBS Lett.* 355:195–200.

Cadigan, K.M., and R. Nusse. 1997. Wnt signaling: a common theme in animal development. *Genes Dev.* 11:3286–3305.

Cavallo, R., D. Rubenstein, and M. Peifer. 1997. Armadillo and dTCF: a marriage made in the nucleus. *Curr. Opin. Genet. Dev.* 7:459–466.

Dattani, M.T., J.P. Martinez-Barbera, P.Q. Thomas, J.M. Brickman, R. Gupta, I.L. Martensson, H. Toresson, M. Fox, J.K. Wales, P.C. Hindmarsh, et al. 1998. Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse. *Nat. Genet.* 19:125–133.

Ding, J., L. Yang, Y.T. Yan, A. Chen, N. Desai, B.A. Wynshaw, and M.M. Shen. 1998. Cripto is required for correct orientation of the anterior-poste-

rior axis in the mouse embryo. *Nature.* 395:702–707.
Fan, M.J., and S.Y. Sokol. 1997. A role for Siamois in Spemann organizer formation. *Development.* 124:2581–2589.
Funayama, N., F. Fagotto, P. McCrea, and B.M. Gumbiner. 1995. Embryonic axis induction by the armadillo repeat domain of β-catenin: evidence for intracellular signaling. *J. Cell Biol.* 128:959–968.
Gat, U., R. DasGupta, L. Degenstein, and E. Fuchs. 1998. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated β-catenin in skin. *Cell.* 95:605–614.
Gavin, B.J., J.A. McMahon, and A.P. McMahon. 1990. Expression of multiple novel Wnt-1/int-1-related genes during fetal and adult mouse development. *Genes Dev.* 4:2319–2332.
Gritsman, K., J.J. Zhang, S. Cheng, E. Heckscher, W.S. Talbot, and A.F. Schier. 1999. The EGF-CFC protein one-eyed pinhead is essential for nodal signaling. *Cell.* 97:121–132.
Gu, Z., M. Nomura, B.B. Simpson, H. Lei, A. Feijen, J. van den Eijnden-van Raaij, P.K. Donahoe, and E. Li. 1998. The type I activin receptor ActRIB is required for egg cylinder organization and gastrulation in the mouse. *Genes Dev.* 12:844–857.
Haegel, H., L. Larue, M. Ohsugi, L. Fedorov, K. Herrenknecht, and R. Kemler. 1995. Lack of β-catenin affects mouse development at gastrulation. *Development.* 121:3529–3537.
Harland, R., and J. Gerhart. 1997. Formation and function of Spemann's organizer. *Annu. Rev. Cell Dev. Biol.* 13:611–667.
He, T.C., A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da-Costa, P.J. Morin, B. Vogelstein, and K.W. Kinzler. 1998. Identification of c-MYC as a target of the APC pathway. *Science.* 281:1509–1512.
Heasman, J. 1997. Patterning the *Xenopus* blastula. *Development.* 124:4179–4191.
Heasman, J., A. Crawford, K. Goldstone, H.P. Garner, B. Gumbiner, P. McCrea, C. Kintner, C.Y. Noro, and C. Wylie. 1994. Overexpression of cadherins and underexpression of β-catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. *Cell.* 79:791–803.
Hermesz, E., S. Mackem, and K.A. Mahon. 1996. Rpx: a novel anterior-restricted homeobox gene progressively activated in the prechordal plate, anterior neural plate and Rathke's pouch of the mouse embryo. *Development.* 122:41–52.
Hinck, L., I.S. Nathke, J. Papkoff, and W.J. Nelson. 1994. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J. Cell Biol.* 125:1327–1340.
Hogan, B., R.S. Beddington, F. Costantini, and E. Lacy. 1994. Manipulating the Mouse Embryo. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 198–201.
Huber, O., R. Korn, J. McLaughlin, M. Ohsugi, B.G. Herrmann, and R. Kemler. 1996. Nuclear localization of β-catenin by interaction with transcription factor LEF-1. *Mech. Dev.* 59:3–10.
Huelsken, J., J. Behrens, and W. Birchmeier. 1994a. Tumor-suppressor gene products in cell contacts: the cadherin-APC-armadillo connection. *Curr. Opin. Cell Biol.* 6:711–716.
Huelsken, J., W. Birchmeier, and J. Behrens. 1994b. E-cadherin and APC compete for the interaction with β-catenin and the cytoskeleton. *J. Cell Biol.* 127: 2061–2069.
Ikeda, S., S. Kishida, H. Yamamoto, H. Murai, S. Koyama, and A. Kikuchi. 1998. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3β and β-catenin and promotes GSK-3β-dependent phosphorylation of β-catenin. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:1371–1384.
Jiang, J., and G. Struhl. 1998. Regulation of the Hedgehog and Wingless signalling pathways by the F-box/WD40-repeat protein Slimb. *Nature.* 391: 493–496.
Kofron, M., A. Spagnuolo, M. Klymkowsky, C. Wylie, and J. Heasman. 1997. The roles of maternal α-catenin and plakoglobin in the early *Xenopus* embryo. *Development.* 124:1553–1560.
Korinek, V., N. Barker, P. Moerer, E. van Donselaar, G. Huls, P.J. Peters, and H. Clevers. 1998. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* 19:379–383.
Kowalczyk, A.P., E.A. Bornslaeger, J.E. Borgwardt, H.L. Palka, A.S. Dhaliwal, C.M. Corcoran, M.F. Denning, and K.J. Green. 1997. The amino-terminal domain of desmoplakin binds to plakoglobin and clusters desmosomal cadherin-plakoglobin complexes. *J. Cell Biol.* 139:773–784.
Larabell, C.A., M. Torres, B.A. Rowning, C. Yost, J.R. Miller, M. Wu, D. Kimelman, and R.T. Moon. 1997. Establishment of the dorso-ventral axis in *Xenopus* embryos is presaged by early asymmetries in β-catenin that are modulated by the Wnt signaling pathway. *J. Cell Biol.* 136:1123–1136.
Larue, L., M. Ohsugi, J. Hirchenhain, and R. Kemler. 1994. E-Cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc. Natl. Acad. Sci. USA.* 91:8263–8267.
Laurent, M.N., I.L. Blitz, C. Hashimoto, U. Rothbacher, and K.W. Cho. 1997. The *Xenopus* homeobox gene twin mediates Wnt induction of goosecoid in establishment of Spemann's organizer. *Development.* 124:4905–4916.
Liu, P.T., M. Wakamiya, M.J. Shea, U. Albrecht, R.R. Behringer, and A. Bradley. 1999. Requirement for Wnt3 in vertebrate axis formation. *Nat. Genet.* 22:361–365.
McCrea, P.D., C.W. Turck, and B. Gumbiner. 1991. A homolog of the armadillo protein in *Drosophila* (plakoglobin) associated with E-cadherin. *Science.* 254:1359–1361.
McGrew, L.L., K. Takemaru, R. Bates, and R.T. Moon. 1999. Direct regulation of the *Xenopus* engrailed-2 promoter by the Wnt signaling pathway, and a molecular screen for Wnt-responsive genes, confirm a role for Wnt signaling during neural patterning in *Xenopus*. *Mech. Dev.* 87:21–32.
McMahon, A.P., B.J. Gavin, B. Parr, A. Bradley, and J.A. McMahon. 1992. The Wnt family of cell signalling molecules in postimplantation development of the mouse. *Ciba Found. Symp.* 165:199–212.
Miller, J.R., and R.T. Moon. 1997. Analysis of the signaling activities of localization mutants of β-catenin during axis specification in *Xenopus*. *J. Cell Biol.* 139:229–243.
Molenaar, M., M. van de Wetering, M. Oosterwegel, M.J. Peterson, S. Godsave, V. Korinek, J. Roose, O. Destree, and H. Clevers. 1996. XTcf-3 transcription factor mediates β-catenin-induced axis formation in *Xenopus* embryos. *Cell.* 86:391–399.
Moon, R.T., and D. Kimelman. 1998. From cortical rotation to organizer gene expression: toward a molecular explanation of axis specification in *Xenopus*. *Bioessays.* 20:536–545.
Morin, P.J., A.B. Sparks, V. Korinek, N. Barker, H. Clevers, B. Vogelstein, and K.W. Kinzler. 1997. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. *Science.* 275:1787–1790.
Nasevicius, A., T. Hyatt, H. Kim, J. Guttman, E. Walsh, S. Sumanas, Y. Wang, and S.C. Ekker. 1998. Evidence for a frizzled-mediated wnt pathway required for zebrafish dorsal mesoderm formation. *Development.* 125:4283–4292.
Nieset, J.E., A.R. Redfield, F. Jin, K.A. Knudsen, K.R. Johnson, and M.J. Wheelock. 1997. Characterization of the interactions of α-catenin with α-actinin and β-catenin/plakoglobin. *J. Cell Sci.* 110:1013–1022.
Peifer, M. 1993. Cancer, catenins, and cuticle pattern: a complex connection. *Science.* 262:1667–1668.
Peleari, F., and H.M. Maischein. 1998. Function of zebrafish β-catenin and TCF-3 in dorsoventral patterning. *Mech. Dev.* 77:63–74.
Poepperl, H., C. Schmidt, V. Wilson, C.R. Hume, J. Dodd, R. Krumlauf, and R.S. Beddington. 1997. Misexpression of Cwnt8C in the mouse induces an ectopic embryonic axis and causes a truncation of the anterior neuroectoderm. *Development.* 124:2997–3005.
Polakis, P. 1999. The oncogenic activation of β-catenin. *Curr. Opin. Genet. Dev.* 9:15–21.
Rhinn, M., A. Dierich, W. Shawlot, R.R. Behringer, M. Le Meur, and S.L. Ang. 1998. Sequential roles for Otx2 in visceral endoderm and neuroectoderm for forebrain and midbrain induction and specification. *Development.* 125: 845–856.
Riethmacher, D., V. Brinkmann, and C. Birchmeier. 1995. A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. *Proc. Natl. Acad. Sci. USA.* 92:855–859.
Roeser, T., S. Stein, and M. Kessel. 1999. Nuclear β-catenin and the development of bilateral symmetry in normal and LiCl-exposed chick embryos. *Development.* 126:2955–2965.
Roose, J. 1999. HMG box transcription factors in development. Thesis. University of Utrecht, Utrecht, The Netherlands. 89–106.
Rosenquist, T.A., and G.R. Martin. 1995. Visceral endoderm-1 (VE-1): an antigen marker that distinguishes anterior from posterior embryonic visceral endoderm in the early post-implantation mouse embryo. *Mech. Dev.* 49:117–121.
Rubinfeld, B., I. Albert, E. Porfiri, C. Fiol, S. Munemitsu, and P. Polakis. 1996. Binding of GSK3β to the APC-β-catenin complex and regulation of complex assembly. *Science.* 272: 1023–1026.
Rubinfeld, B., P. Robbins, M. El-Gamil, I. Albert, E. Porfiri, and P. Polakis. 1997. Stabilization of β-catenin by genetic defects in melanoma cell lines. *Science.* 275:1790–1792.
Ruiz, P., V. Brinkmann, B. Ledermann, M. Behrend, C. Grund, C. Thalhammer, F. Vogel, C. Birchmeier, U. Gunthert, W.W. Franke, and W. Birchmeier. 1996. Targeted mutation of plakoglobin in mice reveals essential functions of desmosomes in the embryonic heart. *J. Cell Biol.* 135:215–225.
Sacco, P.A., T.M. McGranahan, M.J. Wheelock, and K.R. Johnson. 1995. Identification of plakoglobin domains required for association with N-cadherin and α-catenin. *J. Biol. Chem.* 270:20201–20206.
Schneider, S., H. Steinbeisser, R.M. Warga, and P. Hausen. 1996. β-Catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* 57:191–198.
Shawlot, W., and R.R. Behringer. 1995. Requirement for Lim1 in head-organizer function. *Nature.* 374:425–430.
Shawlot, W., J.M. Deng, and R.R. Behringer. 1998. Expression of the mouse cerberus-related gene, Cerr1, suggests a role in anterior neural induction and somitogenesis. *Proc. Natl. Acad. Sci. USA.* 95:6198–6203.
Sirard, C., J.L. de la Pompa, A. Elia, A. Itie, C. Mirtsos, A. Cheung, S. Hahn, A. Wakeham, L. Schwartz, S.E. Kern, et al. 1998. The tumor suppressor gene Smad4/Dpc4 is required for gastrulation and later for anterior development of the mouse embryo. *Genes Dev.* 12:107–119.
Solnica-Krezel, L. 1999. Pattern formation in zebrafish—fruitful liaisons between embryology and genetics. *Curr. Top. Dev. Biol.* 41:1–35.
Sumoy, L., J. Kiefer, and D. Kimelman. 1999. Conservation of intracellular Wnt signaling components in dorsal-ventral axis formation in zebrafish. *Dev. Genes Evol.* 209:48–58.
Tam, P.P., and R.R. Behringer. 1997. Mouse gastrulation: the formation of a mammalian body plan. *Mech. Dev.* 68:3–25.
Tetsu, O., and F. McCormick. 1999. β-Catenin regulates expression of cyclin

D1 in colon carcinoma cells. *Nature.* 398:422–426.
Thomas, P.Q., A. Brown, and R.S. Beddington. 1998. Hex: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. *Development.* 125:85–94.
Torres, M., A. Stoykova, O. Huber, K. Chowdhury, P. Bonaldo, A. Mansouri, S. Butz, R. Kemler, and P. Gruss. 1997. An α-E-catenin gene trap mutation defines its function in preimplantation development. *Proc. Natl. Acad. Sci. USA*. 94:901–906.
Troyanovsky, S.M., R.B. Troyanovsky, L.G. Eshkind, V.A. Krutovskikh, R.E. Leube, and W.W. Franke. 1994. Identification of the plakoglobin-binding domain in desmoglein and its role in plaque assembly and intermediate filament anchorage. *J. Cell Biol.* 127:151–160.
van de Wetering, M., R. Cavallo, D. Dooijes, M. van Beest, J. van Es, J. Loureiro, A. Ypma, D. Hursh, T. Jones, A. Bejsovec, et al. 1997. Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell.* 88:789–799.
van Genderen, C., R.M. Okamura, I. Farinas, R.G. Quo, T.G. Parslow, L. Bruhn, and R. Grosschedl. 1994. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev.* 8:2691–2703.
Varlet, I., J. Collignon, and E.J. Robertson. 1997. Nodal expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. *Development.* 124:1033–1044.
Verbeek, S., D. Izon, F. Hofhuis, M.E. Robanus, H. te Riele, M. van de Wetering, M. Oosterwegel, A. Wilson, H.R. MacDonald, and H. Clevers. 1995. An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature.* 374:70–74.
Vleminckx, K., R. Kemler, and A. Hecht. 1999. The C-terminal transactivation domain of β-catenin is necessary and sufficient for signaling by the LEF-1/β-catenin complex in *Xenopus laevis. Mech. Dev.* 81:65–74.
Waldrip, W.R., E.K. Bikoff, P.A. Hoodless, J.L. Wrana, and E.J. Robertson. 1998. Smad2 signaling in extraembryonic tissues determines anterior-posterior polarity of the early mouse embryo. *Cell.* 92:797–808.
Weinstein, M., X. Yang, C. Li, X. Xu, J. Gotay, and C.X. Deng. 1998. Failure of egg cylinder elongation and mesoderm induction in mouse embryos lacking the tumor suppressor smad2. *Proc. Natl. Acad. Sci. USA*. 95:9378–9383.
Wylie, C., M. Kofron, C. Payne, R. Anderson, M. Hosobuchi, E. Joseph, and J. Heasman. 1996. Maternal β-catenin establishes a 'dorsal signal' in early *Xenopus* embryos. *Development.* 122:2987–2996.
Yost, C., M. Torres, J.R. Miller, E. Huang, D. Kimelman, and R.T. Moon. 1996. The axis-inducing activity, stability, and subcellular distribution of β-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* 10:1443–1454.
Zeng, L., F. Fagotto, T. Zhang, W. Hsu, T.J. Vasicek, W.L. Perry, J.J. Lee, S.M. Tilghman, B.M. Gumbiner, and F. Costantini. 1997. The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell.* 90:181–192.
Zorn, A.M., K. Butler, and J.B. Gurdon. 1999. Anterior endomesoderm specification in *Xenopus* by Wnt/β-catenin and TGF-β signalling pathways. *Dev. Biol.* 209:282–297.