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

Jörg Huesken, Regina Vogel, Volker Brinkmann, Bettina Erdmann, Carmen Birchmeier, and Walter Birchmeier

Max-Delbrueck-Center for Molecular Medicine, 13125 Berlin, Germany

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 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) 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 of an additional embryonic axis (Heasman et al., 1995; Molenaar et al., 1996; He et al., 1998; Shawlot et al., 1998). Accordingly, genetic evidence in the mouse demonstrates that genes expressed in the visceral endoderm (Rosenquist and Martin, 1995; Belo et al., 1997; Thomas et al., 1998) are essential for various developmental processes, but a role in anterior-posterior axis formation has not been previously established. 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 X enopus, 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 X enopus 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; Aker et al., 1997; Zeng et al., 1997; Behrens et al., 1998; Iida 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 McCormick, 1997).
mick, 1999). In X enopus, 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; Fan and Sokol, 1997; Laurent et al., 1997). Ablation of mouse genes that encode components of the cadherin–catenin system results in adhesion defects during embryogenesis: in 99% of the cadherin–catenin system results in adhesion defects during embryogenesis: in anterior-posterior axis formation at E6.0. At this stage, phenotypes: a Mendelian ratio of 20 or 5 ES cells were analyzed in detail by sectioning. Mutant cells were detected by PCR genotyping of blue cells 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 digoxigenin-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 immuneocytochemical labeling of ultrathin sections with antiplakoglobin antibody and 32 nm colloidal gold goat anti–rabbit IgG (Jackson Immunoresearch Inc.). Sections were contrasted with uranyl acetate and lead citrate.

### Materials and Methods

#### Generation of β-Catenin–deficient Mice

For construction of the targeting vectors, genomic fragments isolated from a XGEM-12 129/Ola library were introduced into the pTV0 vector (Riedelkamp 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. Ablation of mouse genes that encode components of the cadherin–catenin system results in adhesion defects during embryogenesis: in anterior-posterior axis formation at E6.0. At this stage, β-catenin–deficient embryos exhibit abnormal adhesion of myocardial cells that result in heart rupture at midgestation (Rulik et al., 1996). A t late gestation, adhesion defects are also observed in the skin of plakoglobin-deficient mice (Bierkamp et al., 1996).

We show that the 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 X enopus and zebrafish, the observed block in axis formation of β-catenin-deficient mouse embryos appears to reflect a signaling function of β-catenin.

### Results

#### Microscopic Analysis and In Situ Hybridization

β-Galactosidase staining of embryos, immunofluorescence analysis, and EM were performed as described (Hogan et al., 1994; Huesken et al., 1994b; Riedtmacher et al., 1995; Varlet et al., 1997). Embossing in paraffin or plastic was performed according to the manufacturer’s protocols (Paraplast, Sherwood Medical; Technovit 7100, Kulzer H eraeus). 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 digoxigenin-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 32 nm colloidal gold goat anti–rabbit IgG (Jackson Immunoresearch Inc.). Sections were contrasted with uranyl acetate and lead citrate.

#### Generation of Chimeric Embryos

A generation of wild-type embryos and embryos obtained from crossing heterozygous β-catenin+/- and β-catenin+/- mutant mice as described (Hogan et al., 1994). Injection chimeras were produced either by injection of wild-type blastocysts with heterozygous or homozygous β-catenin+/- E5 cells or by injection of blastocysts obtained from crossing heterozygous β-catenin+/- and β-catenin+/- mice with wild-type E5 cells (Riedtmacher et al., 1995). For high or low E5 cell contribution, ~20 or 5 E5 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-catenin allele.

#### Materials and Methods

For construction of the targeting vectors, genomic fragments isolated from a XGEM-12 129/Ola library were introduced into the pTV0 vector (Riedelkamp et al., 1996). The β-catenin+/- 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-catenin+/- contained a β-galactosidase cDNA with a nuclear localization signal fused to the A5G 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-catenin+/- (hyp), neomycin was replaced by a hygromycin resistance cassette. Homozygous mutant animals were bred on a mixed 129/Sv/C57Bl6 background, and showed no developmental abnormalities. PCR genotyping was performed using primers TGG GTT CTT CAG GTA GCA TTT TCA GTT C, CAT TCA TAA AAG ACT TGG GAG GTG T, and GCC TTC TAT CGC CCT TTT GAC C (β-catenin), or CAT GGA CAG GGG TGG CCA GTG, TGT TTT TCG AGC TTT TCC AAG GTT CAT, and AGA ATC ACG GTT ACC TGG GTT AAA (β-catenin+/-).
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
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 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). A 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-
deficient embryos. These data indicate that a change in the visceral endoderm accompanies defective anterior-posterior axis formation in \( \beta \)-catenin-deficient embryos.

**Requirement of \( \beta \)-Catenin in the Embryonic Ectoderm and Developmental Potential of \( \beta \)-Catenin–deficient Cells**

Homozygous mutant, \( \beta \)-galactosidase–expressing ES cells that lack \( \beta \)-catenin mRNA and protein were generated by the consecutive electroporation of the two targeting vectors \( \beta \)-catlacZ(neo) and \( \beta \)-catlacZ(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 \( \beta \)-catenin–deficient embryos (Fig. 6 b; Fig. 6 a, control; data not shown). Thus, a high contribution of \( \beta \)-catenin-deficient cells is not compatible with normal development beyond E6. Moreover, these experiments show that \( \beta \)-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 \( \beta \)-catenin-deficient cells contributed to anterior and mesodermal structures (e.g., headfolds and paraxial mesoderm; Fig. 6, c and d). Thus, \( \beta \)-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 \( \beta \)-catenin-deficient blastocysts, i.e., extraembryonic tissues like visceral endoderm are produced from \( \beta \)-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 \( \beta \)-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 \( \beta \)-Catenin–deficient Embryos**

In confocal immunofluorescence analysis, identical staining patterns of E-cadherin and \( \alpha \)-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 \( \beta \)-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 \( \beta \)-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.
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. A xis 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 Hox, 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; H blosson, 1997; Moon and Kimelman, 1998). A xis 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 (H blosson 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 (V leminckx et al., 1999). Also in
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 X. laevis (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 X. laevis (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; Körinek 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) 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 6. Developmental potential of β-catenin-deficient (β-gal stained) cells in chimeric embryos. A gestation chimeras between (a) wild-type and heterozygous (+/β-catlacZ) and (b) wild-type and homozygous mutant (β-catdel/β-catlacZ) 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-catdel/β-catlacZ): (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.
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 crypto–/– embryos, the anterior-posterior axis is initially formed but misplaced, i.e., both Hex and cerberus-like are expressed distally, whereas Lim1 is expressed proximally. A posterior neuroectoderm forms at the distal end of the embryo, as assessed by the expression of Hex1 (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 Hex1 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 (CF) molecules. Consistent with this, Wnt and β-catenin signaling does not rescue an oep/cripto mutant phenotype in zebrafish (Grinstein 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

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. D esmosomes 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.
binding sites (Roos, 1999) (a generous gift of Dr. H. Cleevers, University of Utrecht, Utrecht, The Netherlands). Whereas β-galactosidase activity was detected at E6.5 (Roos, 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 X. laevis 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. A presumably, 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; Riemmacher 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 (Haegele 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 Haegele et al. (1995), it is possible that an NH₂-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 β-cateΔdel or β-cateΔIVZ 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 X. laevis, which indicate that plakoglobin does not significantly participate in Wnt signaling (Kofron et al., 1997; Miller and Mool, 1997; Ben-Ze’ev and Giger, 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 pilomatrixomas 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.

We thank Dieter Riemmacher (Max-Delbrueck-Center, Berlin, Germany and Centre for Molecular Neurobiology, Hamburg, Germany) for advice in generating mutant mice; Martin Blum (Institute of Genetics, Forschungszentrum, Karlsruhe, Germany) for the introduction to early mouse embryogenesis and in situ hybridization probes; Kurt Herrenknecht (EISAI Company, London) for providing the antisense against plakoglobin; and Rosa Beddington (Medical Research Council’s National Institute for Medical Research, Mill Hill, London), Tewes Bouwmeester (European Molecular Biology Laboratory, Heidelberg, Germany), and Maki Wakamiya and Richard Behringer (M. A. Anderson Cancer Center, University of Texas at Houston, Houston, TX) for providing further probes for in situ hybridization.

This work was supported in part by a grant of the Volkswagen-Stiftung to J. Huelsken and W. Birchmeier. Submitted: 9 November 1999
Revised: 23 December 1999
Accepted: 23 December 1999

References

Acampora, D., V. Avantaggiato, F. Tuorto, P. Briata, G. Corte, and A. Simioni. 1997. Vis- ceral 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. Roberts. 1989. A new assay of developmental potential of embryonic stem cells in the midgestation mouse embryo. Development. 105:733–737.

Beddington, R. S., and E. J. Roberts. 1999. A xis development and early asymmetry in mammals. Cell. 96:195–209.

Behrens, J., P. von Kries, M. Kuhl, L. Bruhn, D. Wedlich, R. Grosschedi, 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. Wurtz, J. Grimm, C. A. Brand, R. Wirtz, M. Kuhl, D. Wedlich, and W. Birchmeier. 1998. Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK-3β. Science. 280:596–599.

Belo, J. A., T. Bouwmeester, L. Leys, N. Kertesz, M. Gallo, M. Folletti, 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. Mch. Dev. 68:45–57.

Ben-Ze’ev, A., and B. Giger. 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. 180:780–795.

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, Wnt8, is expressed during early embryogenesis and is ectopically induced by retinoic acid. Mch. Dev. 38:141–152.

Branconn, M., M. Gomperts, L. Sumoy, R. T. Mool, and D. K. Gimbleman. 1997. A β-catenin/TCF-3 complex binds to the samei promoter to regulate dorsal axis specification in X. laevis. Genes Dev. 11:2359–2370.

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

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

Cavallaro, R., D. Rubenstein, and M. Pfeifer. 1997. A radiallo and dfCf: a marriage made in the nucleus. Curr. Opin. Genet. Dev. 7:459–466.

Dattani, M. T., J. P. Martinez-Barbera, P. O. Thomas, J. M. Brickman, R. Gupta, I. L. Martinson, H. Toresson, M. Fox, J. K. Wales, P. C. Hindmarsh, et al. 1998. Mutations in the homeobox gene Hes5 in the UK enl associated with septo-optic dysplasia in human and mouse. Nat. Genet. 19:125–133.

Ding, J., L. Yang, T. Y. Yan, A. Chen, N. Desai, B. A. Wynshaw, and M. M. Shen. 1998. Crito is required for correct orientation of the anterior-poste-
rior axis in the mouse embryo. Nature. 395:702–707.
Fan, M.J., and S. Sokol. 1997. A role for Siamois in Spemann organizer formation. Development. 124:2581–2589.
Funayama, N., F. Fagotto, P.M. Ceresa, and B.M. Gumbiner. 1995. Embryonic axis induction by the armadillo repeat domain of β-catenin: evidence for interaction with a Tcf/RBP-Jk-dependent pathway. J. Cell Biol. 128:959–968.
Gat, U., R. Daigiupta, 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. McA. ahon. 1990. Expression of multiple novel Wnt-Lnt-1-related genes during fetal and adult mouse development. Genes Dev. 4:2319–2332.
Gritsmans, K., J.J. Zhang, S. Cheng, E. Hecksher, W.S. Talbot, and A.F. Schier. 1999. The EGF-CFC protein one-eyed pinhead is essential for budgastrulation. O. Cell Biol. 18:391–399.
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 A cTRIB is required for egg cylinder formation 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. 121:3926–3937.
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 A PC pathway. Science. 281:1509–1512.
Heasman, J. 1997. Patternning the Xenopus blastula. Development. 142:4179–4191.
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. 79:793–803.
Hendry, S.M., J. Haessig, 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.
Hindk, L.I., S. Nathke, J. Papkoff, and W.J. Nelson. 1994. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of apicobasal cell polarity. Development. 121:3926–3937.
Huelsen, 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.
Huelsen, J., W. Birchmeier, and J. Behrens. 1994b. E-cadherin and A PC compete for the interaction with β-catenin and the cytoskeleton. J. Cell Biol. 127:1327–1340.
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, D., 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.
Korinek, V., N. Barker, P. Moerer, E. van Donselaar, G. Huls, P.J. Peters, and K.W. Kinzler. 1996. Nuclear localization of β-catenin in Anterior-Posterior Axis Formation and function of Spemann’s organizer. Thesis. University of Utrecht, Utrecht, The Netherlands. 89–106.
Koorn, M.A., A. Spagnuolo, M. Kljmowski, 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, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
Korinek, V., N. Barker, M. Ohsugi, 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.
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 N-cadherin 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. 88:2691–2703.

van Genderen, C., R.M. Okamura, I. Farinas, R.G. Quo, T.G. Parslow, L. Bruhn, and R. G rosschedl. 1994. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Proc. Natl. Acad. Sci. USA. 91:9378–9383.

Vlieghe, A. L., 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. Proc. Natl. Acad. Sci. USA. 94:1921–1925.

Waldrip, W.R., E.K. Bikoff, P.A. Hoodless, J.L. Wran a, 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.

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 Xe-nopus 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 Xe-nopus embryos by glycogen synthase kinase 3. Genes Dev. 10:1443–1454.

Zeng, L., F. Fatogho, T. Zhang, W. Hsu, 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. Proc. Natl. Acad. Sci. USA. 94:1921–1925.

Zorn, A.M., K. Butler, and J.B. Gurdon. 1999. A posterior endomesoderm specification in Xe-nopus by Wnt/β-catenin and TGF-β signalling pathways. Dev. Biol. 209:282–297.