

Cadherins regulate aggregation of pancreatic β -cells in vivo

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SUMMARY

It is thought that the cadherin protein family of cell adhesion molecules regulates morphogenetic events in multicellular organisms. In this study we have investigated the importance of β -cell cadherins for cell-cell interactions mediating the organization of endocrine cells into pancreatic islets of Langerhans. To interfere with endogenous cadherin activity in β -cells during pancreatic development, we overexpressed a dominant negative mutant of mouse E-cadherin, lacking nearly all extracellular amino acids, in pancreatic β -cells in transgenic mice. Expression of the truncated E-cadherin receptor displaced both E- and N-cadherin from pancreatic β -cells. As a result, the initial clustering of β -cells, which normally begins at 13.5-14.5 days postcoitum, was perturbed. Consequently, the clus-

tering of endocrine cells into islets, which normally begins at 17.5-18 days postcoitum, was abrogated. Instead, transgenic β -cells were found dispersed in the tissue as individual cells, while α -cells selectively aggregated into islet-like clusters devoid of β -cells. Furthermore, expression of truncated E-cadherin in β -cells resulted in an accumulation of β -catenin in the cytoplasm. Thus, we have for the first time shown in vivo that cadherins regulate adhesive properties of β -cells which are essential for the aggregation of endocrine cells into islets.

Key words: morphogenesis, cell adhesion, E-cadherin, N-cadherin, dominant negative mutant, pancreatic β -cells

INTRODUCTION

The development of any tissue or organ requires that their cellular components are precisely regulated. The cadherin family of cell adhesion molecules (CAMs) are thought to contribute to this process by mediating cell-cell interactions that facilitate many morphogenetic processes (Edelman, 1986; Takeichi, 1991, 1995). The cadherins constitute a superfamily of transmembrane glycoproteins that mediate Ca^{2+} -dependent homophilic interactions between cells. They have a common structure with a Ca^{2+} -binding extracellular domain consisting of five subdomains, a single transmembrane domain, and a highly conserved cytoplasmic domain (Geiger and Ayalon, 1992).

Members of the cadherin family are organized in cell-cell attachment sites called zonulae adherens or adherens junctions, which contain a cytoplasmic 'undercoat' associated with the actin cytoskeleton. The cytoplasmic domain of cadherins interacts with cytoplasmic components, α -, β - and γ -catenin and p120, that mediate the linkage to the actin filaments (Nagafuchi and Takeichi, 1988; Ozawa et al., 1989, 1990; Reynolds et al., 1994; Shibamoto et al., 1995). Binding studies of mutated E-cadherin and catenins have shown that β -catenin or plakoglobin binds directly to the cadherin, and α -catenin binds to β -catenin or plakoglobin (Aberle et al., 1994; Hulsken et al., 1994). In addition to a function in junction formation, the cadherin-catenin complex is thought to transmit signals from neighboring cells through interactions with growth factor receptors (Hoschuetzky et al., 1994; Williams et al., 1994) and proto-oncogenes (Tsukita et al., 1993). Furthermore, β -catenin,

and its *Drosophila* homologue, armadillo, appears to be involved in signal transduction processes regulating developmental patterning during embryogenesis (Gumbiner, 1995; Peifer, 1995).

Members of the cadherin family show distinct spatial and temporal expression patterns during development. The cadherins appear to be involved in cell migration and formation of cell boundaries in vivo, since the expression of cadherin subclasses correlates with the migration and segregation of different cell layers and cell populations in the embryo (Hatta and Takeichi, 1986; Takeichi, 1995). Transfection experiments have shown that cadherin-mediated cell sorting can be attributed to either differences in the cadherin expressed by cells (Nose et al., 1988), or differences in the levels of expression of the same cadherin (Steinberg and Takeichi, 1994). Targetted disruption of the E-cadherin gene demonstrated that E-cadherin is essential for formation of the first epithelium during embryogenesis, the trophectoderm (Larue et al., 1994). The expression of dominant negative cadherin mutants (Kintner, 1992; Fujimori and Takeichi, 1993; Holt et al., 1994; Dufour et al., 1994; Levine et al., 1994) and overexpression of cadherins (Heasman et al., 1994a) in *Xenopus* have shown that cadherins regulate cell adhesion during embryogenesis. In addition, injection of antisense oligonucleotides of cadherin into *Xenopus* embryos caused dissociation of blastomeres (Heasman et al., 1994b). Recently, the function of cadherins in cell adhesion, cell migration, and apoptosis during organogenesis of the intestine was demonstrated by expression of a dominant negative cadherin in intestinal epithelial cells in transgenic mice (Herminston and Gordon, 1995).

The pancreas is a useful model organ for investigating the significance of cadherins for cell sorting and cell aggregation *in vivo*. The organ consists of an exocrine portion, which makes up most of the organ, and an endocrine part, which is organized into microorgans, the islets of Langerhans, which lie interspersed within the exocrine tissue. During pancreatic development much of the cytodifferentiation occurs prior to morphogenesis (Gittes and Rutter, 1992), and, it is thought that the islets are formed through a series of morphogenetic events involving cell-cell sorting, cell migration, and cell reaggregation. Initially, endocrine cells are present in the primitive pancreatic duct epithelium. These cells eventually sort out from the duct epithelium and begin to aggregate into islets of Langerhans with a distinct cell architecture; non- β -cells (α -, D-, and PP-cells) in the periphery and β -cells in the center (Pictet and Rutter, 1972; Slack, 1995). Perturbation of this architecture in certain forms of diabetes suggest that this organization is essential for the function of this microorgan (Unger and Orci, 1981).

From recent studies with cultured rat islet cells, it appears that E-cadherin mediates most of the Ca^{2+} -dependent cell adhesion between all cell-types in the islets of Langerhans (Rouiller et al., 1991). The same investigators suggest that it is Ca^{2+} -independent CAMs that segregate non- β -cells from β -cells in the islets (Rouiller et al., 1991). However, the picture is complicated since additional cadherins, R- and N-cadherin, whose functions are unknown, are expressed in islets (Hutton et al., 1993). In this study we have examined how cadherins regulate morphogenesis of the islets of Langerhans during pancreatic organogenesis. By generating transgenic mice carrying an E-cadherin cDNA lacking most of its extracellular domain, whose expression is driven by the rat insulin promoter 1 (Rip1), we disrupted cadherin activity in β -cells during pancreatic ontogeny. The initial clustering of β -cells, which normally begins at 13.5-14.5 days postcoitum (dpc), was perturbed. This resulted in an inhibition of the aggregation of endocrine cells into islet-like structures, which normally begins at 17.5-18 dpc. Thus, we have for the first time shown *in vivo* that cadherins regulate cell-cell interactions mediating aggregation of endocrine cells into pancreatic islets of Langerhans.

MATERIALS AND METHODS

Generation of transgenic mice

Cloning procedures and evaluation of gene expression were performed using standard techniques (Sambrook et al., 1989). 0.7 kb (Rip1) of the rat insulin promoter has been shown to direct expression in pancreatic β -cells (Hanahan, 1985). Most of the extracellular domain of mouse E-cadherin cDNA was excised as a *EagI-BstEII* fragment (nucleotides 257-2105). The ends were ligated with an oligonucleotide coding for a 10 amino acid epitope from c-myc (Klar et al., 1992), whereafter the mutant E-cadherin cDNA was isolated as a *HindIII-XbaI* fragment from pBluescript. Rip1-DipA (Palmiter et al., 1987) was similarly digested with *HindIII-XbaI*, excising the DipA cDNA leaving Rip1 and the SV40 t intron and late region polyadenylation signal. The mutant E-cadherin cDNA was inserted into the vector.

Transgenic mice were produced using standard techniques (Hogan et al., 1986). The pRip Δ Ecad plasmid was digested with *AatII-SphI* and the insert was purified by non-denaturing PAGE, cesium centrifugation, and further purified by phenol extraction, ethanol precipitation, and filtration. The DNA was adjusted to approximately 1.8

$\mu\text{g/ml}$. The embryos were derived by intercrossing F₁ hybrids between C57Bl/6J and CBA/J. The transgenic mice have been maintained by backcrossing to C57Bl/6J.

Assessment of genetic transmission was by DNA analyses of tail DNA (Hogan et al., 1986). Southern blot analysis was used on the F₂ generations to estimate transgene copy number but thereafter the offsprings were analysed by PCR.

Cell transfections

COS-7 cells were cultured in DMEM (Gibco BRL) supplemented with 10% fetal bovine serum (Gibco BRL). Cells grown on glass coverslips were transiently transfected with pRip Δ Ecad using the Lipofectamine™ reagent (Gibco BRL) according to the manufacturer's instructions.

For immunofluorescence staining of fixed unpermeabilized cells, cells were fixed in HBS- Ca^{2+} (10 mM Hepes, pH 7.4, 150 mM NaCl, 1 mM CaCl_2) supplemented with 4% paraformaldehyde for 20 minutes, washed, and blocked in HBS- Ca^{2+} supplemented with 5% goat serum (Agrisera, Vännäs, Sweden) for 30 minutes, whereafter the first antibody was applied. For visualization of the first antibody, see below.

Immunoreagents

The monoclonal antibodies ECCD-2 (rat anti-mouse E-cadherin; Shi-rayoshi et al., 1986) and MNCD-2 (rat anti-mouse N-cadherin; Matsunami and Takeichi, 1995) were kind gifts from Dr M. Takeichi. Polyclonal antiserum against β -catenin (Peifer et al., 1992) was generously supplied by Drs P. McCreas and B. Gumbiner. The anti- α -catenin monoclonal antibody was generously supplied by Dr A. Nagafuchi (Nagafuchi et al., 1991). Polyclonal antiserum against rat brain NCAM was a kind gift from Dr E. Bock, Copenhagen, Denmark (Rasmussen et al., 1982). Guinea pig anti-insulin and anti-glucagon antisera were purchased from Linco Research, Inc (St. Louis, MO, USA) and used at 1:500 and 1:1000 dilutions, respectively. FITC-coupled goat anti-guinea pig and anti-rabbit secondary antisera were purchased from Molecular Probes (Eugene, OR, USA) and used at 1:500 dilutions. Biotin-coupled anti-rat IgG, anti-mouse IgG, and anti-rabbit IgG secondary antibodies were purchased from Molecular Probes and used at 1:300 and 1:500 dilutions, respectively. FITC- and rhodamine-coupled streptavidin were purchased from Molecular Probes and used at 1:500 dilutions. The Vectastain ABC kit was from Vector Laboratories, Inc. (La Jolla, CA, USA).

Immunoblotting and immunoprecipitation

For immunoprecipitation, pancreata were homogenized in ice cold lysis buffer (10 mM Tris-HCl, pH 7.4, 1% Triton X-100, 0.4% deoxycholate, 66 mM EDTA with protease inhibitor mix: 10 ng/ml of pepstatin, chymostatin, leupeptin and aprotinin, 1 mM PMSF, 1 μM benzaminidine, and 1 $\mu\text{g/ml}$ σ -phenanthroline) by careful sonication. The homogenates were centrifuged at 15 krpm for 20 minutes. Supernatants from samples in lysis buffer were analyzed for protein concentration (based on the Bradford dye-binding procedure, Bio-Rad). Samples were diluted in lysis buffer and incubated with the first antibody at 4°C overnight. The antigen-antibody complexes were precipitated by Pansorbin® cells (Calbiochem, La Jolla, CA, USA) washed three times in lysis buffer, solubilized by boiling in sample buffer (63 mM Tris, pH 6.8, 1% SDS, 10% glycerol, 5% β -mercaptoethanol, and 10 $\mu\text{g/ml}$ of bromophenol blue) for 5 minutes, separated by SDS-PAGE, and electrophoretically transferred onto nitrocellulose filters (Bio-Rad) in 192 mM glycine, 20% methanol, and 25 mM Tris-HCl. Blocking (overnight) and all antibody incubations were in HBST- Ca^{2+} (10 mM Hepes, pH 7.4, 150 mM NaCl, 1 mM CaCl_2 , and 0.1% Tween 20). The primary and secondary antibodies were applied for 3 hours and 60 minutes, respectively. To visualize the antigen-antibody complexes, filters were incubated with a conjugated secondary antibody which was visualized by chemoluminescence using the ECL-detection kit (Amersham).

Immunocytochemistry

Tissues (pancreata) were collected and fixed at room temperature in HBS-Ca²⁺ supplemented with 4% paraformaldehyde for 2 hours. For cryostat protection tissues were incubated overnight at 4°C in HBS supplemented with 30% sucrose. Tissues were frozen on blocks of dry ice and 6 μ m thick sections were cut and mounted on polylysine

(Sigma)-coated glassslides which were used for immunostainings. Sections were washed in HBS-Ca²⁺, blocked in HBS-Ca²⁺ supplemented with 5% goat serum (Agrisera) for 30 minutes at room temperature, first antibody in DMEM (Gibco BRL, United Kingdom) supplemented with 50 mM Hepes, pH 7.4, and 1% goat serum overnight at 4°C. Biotin-coupled anti-rat, -rabbit, -mouse, FITC-coupled anti-guinea pig and anti-rabbit, and FITC- and rhodamine-streptavidin were each added for 60 minutes.

E-cadherin

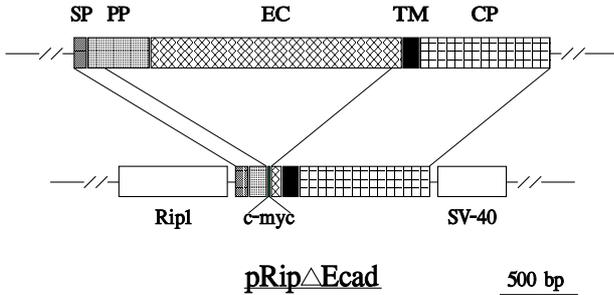


Fig. 1. Fusion gene construct. The rat insulin promoter 1, Rip1 (700 bp), was used to drive the expression of truncated E-cadherin, lacking most of its extracellular amino acids, in frame with one copy of the c-myc epitope (pRip Δ Ecad). For termination sequences the SV40 t intron and late region polyadenylation signal were used. SP, signal peptide; PP, prepeptide; EC, extracellular domain; TM, transmembrane domain; CP, cytoplasmic domain.

RESULTS

Expression of truncated E-cadherin in transgenic mice

As an initial attempt to study the role of cadherins during morphogenesis of the islets of Langerhans, we overexpressed a dominant negative E-cadherin mutant in pancreatic β -cells in transgenic mice (Fig. 1). Since one copy of the human c-myc epitope (Klar et al., 1992) was inserted in the the short extracellular domain of the mutant (Fig. 1), expression of the mutant protein could be detected with the tag-epitope monoclonal antibody MycI-9E10 (Evan et al., 1985). Six transgenic lines, showing similar results, were obtained.

Control experiments showed that the mutant E-cadherin was expressed on the cell surface of transfected COS-7 cells (data not shown), and expression of the truncated E-cadherin

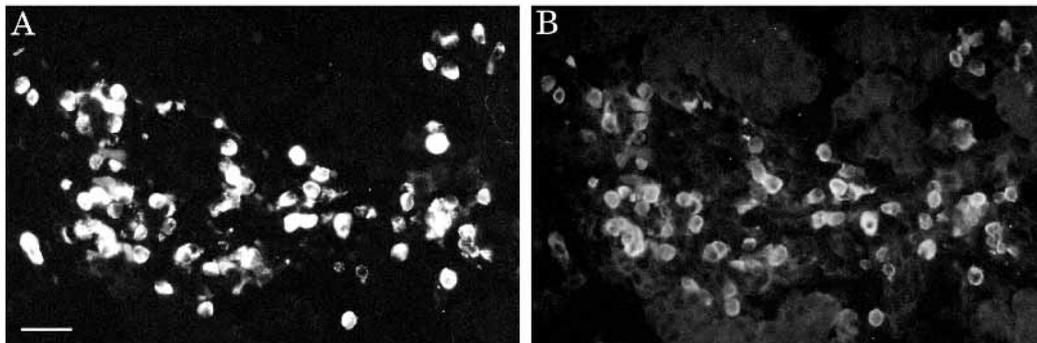


Fig. 2. Expression of truncated E-cadherin in pancreatic β -cells. Double immunofluorescence staining of 17.5 dpc transgenic pancreas with anti-insulin pAb (A) and the tag-epitope mouse mAb 9E10 (B). In transgenic animals the mutant was expressed at high levels in pancreatic β -cells. Bar, 25 μ m.

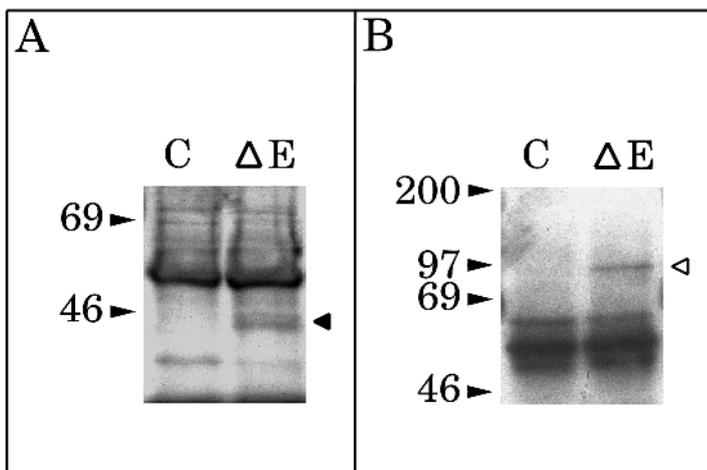


Fig. 3. Interaction between truncated E-cadherin and β -catenin in transgenic β -cells. Similar amounts of extracts from 17.5 dpc pancreata of negative littermates (C) and transgenics (Δ E) were immunoprecipitated with the tag-epitope mAb 9E10, electrophoresed and immunoblotted with the tag-epitope mAb 9E10 (A) anti- β -catenin pAb (B). The filled arrowhead in A indicates the band doublet corresponding to the predicted mutant E-cadherin protein; open arrowhead in B indicates β -catenin.

receptor was first detected immunohistochemically at 13.5 dpc in transgenic mice (data not shown). Furthermore, the truncated receptor was selectively expressed in pancreatic β -cells (Fig. 2A,B). The cytoplasmic staining pattern of the mutant form of E-cadherin most likely represents expression in the ER. Expression of the truncated E-cadherin receptor was also confirmed by immunoprecipitation with the tag-epitope monoclonal antibody (9E10) (Fig. 3A). The band doublet corresponding to the position of the predicted mutant E-cadherin protein was probably due to posttranslational modifications in the cytoplasmic domain similar to endogenous E-cadherin (Behrens et al., 1993).

Truncated E-cadherin inhibits cadherin activity in a dominant negative manner

In order for the truncated E-cadherin mutant to act as a dominant negative cadherin-mutant, interaction with β -catenin is a prerequisite. To examine if the truncated E-cadherin receptor interacts with β -catenin, we immunoprecipitated whole pancreas extracts from negative littermates and transgenics with the tag-epitope antibody (9E10), and analyzed the immunoprecipitates by immunoblotting with anti- β -catenin antibodies (Fig. 3B). The fact that β -catenin coimmunoprecipitated with the mutant E-cadherin indicates that the truncated receptor competes with endogenous cadherins for binding of β -catenin in β -cells. To examine whether expression of the truncated E-cadherin receptor affected E-cadherin function, we investigated whether endogenous E-cadherin was displaced from cell-cell contacts. In fact, no E-cadherin was present in cell-cell contacts between β -cells expressing the mutant E-cadherin (Fig. 4C-F), indicating that expression of truncated E-cadherin inhibits

endogenous E-cadherin activity. Since Kintner (1992) showed that a dominant negative mutant form of N-cadherin competes

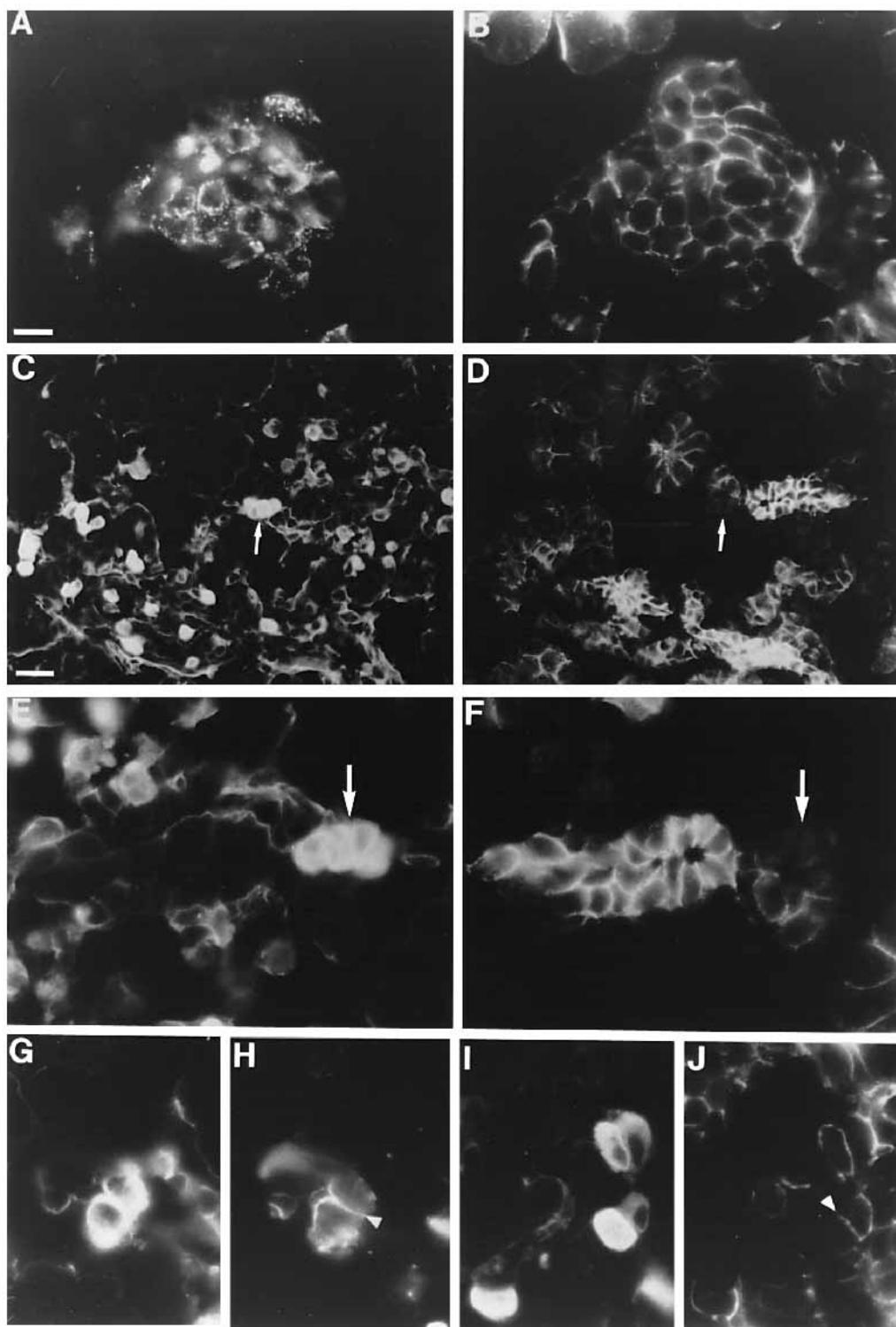


Fig. 4. Displacement of endogenous E-cadherin from cell-cell contacts of β -cells expressing the truncated E-cadherin receptor. Double immunofluorescence stainings of 17.5 dpc pancreata from a negative littermate (A,B) and a transgenic (C-J) with anti-insulin pAb (A), the tag-epitope mAb, 9E10 (C,E,G,I), anti-E-cadherin mAb, ECCD-2 (B,D,F), and anti-NCAM pAb (H,J). E and F are high power and mirror images of C and D. Arrows indicate a group of β -cells expressing truncated E-cadherin and lacking endogenous E-cadherin in cell-cell contacts. Arrowheads indicate expression of NCAM on the cell surface of β -cells expressing the E-cadherin mutant. Bars: A,B,E-I, 10 μ m; C,D, 25 μ m.

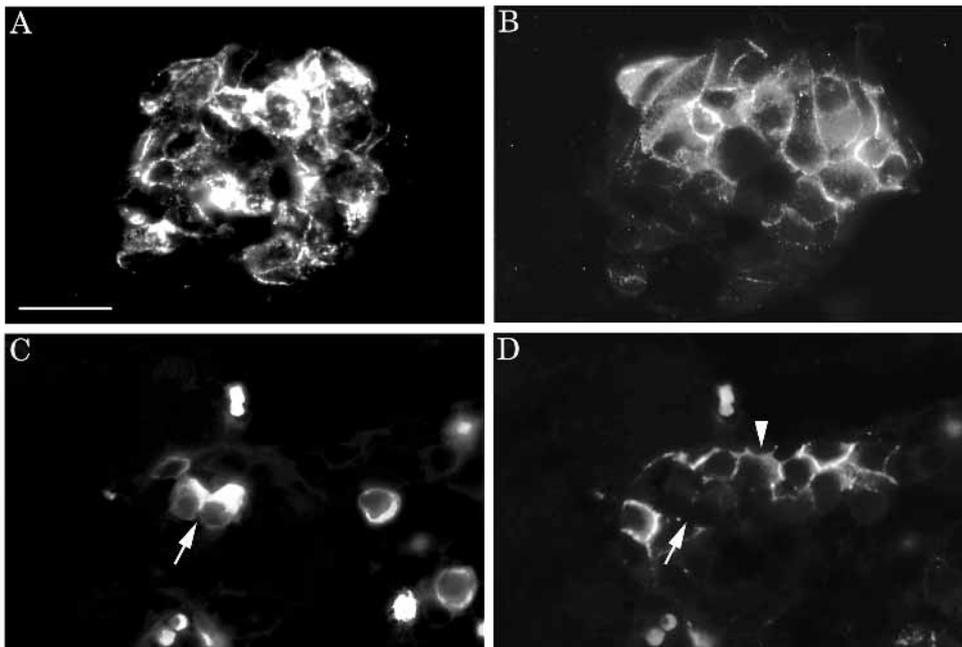


Fig. 5. Displacement of endogenous N-cadherin from cell-cell contacts of β -cells expressing the truncated E-cadherin receptor. Double immunofluorescence stainings of 17.5 dpc pancreata from a negative littermate (A,B) and a transgenic (C,D) with anti-insulin pAb (A), the tag-epitope mAb, 9E10 (C), and anti-N-cadherin mAb (B,D). Arrows indicate two β -cells expressing truncated E-cadherin and lacking endogenous N-cadherin at their cell-cell contact. Arrowhead indicates a group of α -cells with cell-cell contact localization of N-cadherin. Bar, A-D, 20 μ m.

with several cadherins for the binding of cytoplasmic components, it is possible that our effects is also due to an inhibition of cadherins other than E-cadherin. In addition to E-cadherin, pancreatic islets express N- and R-cadherin (Hutton et al., 1993; and our unpublished data). Examination of N-cadherin's expression pattern in transgenic β -cells showed that it was also displaced from cell-cell contacts (Fig. 5C,D). R-cadherin, cannot be a major contributor to cadherin-mediated adhesion of β -cells, since it is normally distributed in the cytoplasm (unpublished data). Expression of NCAM on the cell surface of β -cells expressing truncated E-cadherin showed that the loss of E- and N-cadherin at cell-cell contacts, due to overexpression of the truncated E-cadherin receptor, was not secondary to blockage of membrane trafficking (Fig. 4G-J). Therefore, we believe that truncated E-cadherin acts as a dominant negative mutant and thus inhibits endogenous E- and N-cadherin activities in β -cells.

Expression of truncated E-cadherin perturb initiation of pancreatic β -cell aggregation and islet formation

To examine cadherin function during morphogenesis of islets, we performed a developmental series of comparative analyses of wild-type β -cells and β -cells expressing truncated E-cadherin. At 11.5 dpc, β -cells were found as single cells interspersed in the pancreatic tissue (Fig. 6A,B). However, at 13.5-14.5 dpc, when we first detect significant aggregation of wild-type β -cells (Fig. 6C), expression of the truncated receptor inhibited β -cell aggregation (Fig. 6D). Before 17.5 dpc, endocrine cells are found in interstitial clusters without any well-defined islet cell architecture. However, at 17.5-18 dpc, when negative littermates begin to organize their islets (Fig. 6E) (Herrera et al., 1991), transgenic β -cells were found dispersed in the tissue as individual cells and exceptionally as small loosely clustered aggregates (Fig. 6F). Furthermore, while glucagon-producing cells (α -cells) in negative littermates were found in the periphery of islet-like clusters (Fig.

6E), transgenic α -cells selectively aggregated into islet-like clusters devoid of β -cells (Fig. 6F). Thus, expression of truncated E-cadherin in β -cells perturbed initiation of β -cell aggregation and islet formation.

Expression of truncated E-cadherin affects β -catenin expression in transgenic β -cells

α E- and β -catenin are concentrated at cell-cell contacts in all pancreatic exocrine and endocrine cells (Fig. 7A). However, a significant portion of β -catenin is also freely distributed in the cytoplasm of endocrine cells. To examine whether the expression of the mutant E-cadherin affected the levels of expression and/or intracellular distribution of catenins, we investigated the expression of α E- and β -catenin by immunocytochemistry. Fig. 7B,C shows that expression of the mutant form of E-cadherin resulted in an accumulation of β -catenin in the cytoplasm of β -cells. Expression of α E-catenin was, however, not changed (data not shown).

DISCUSSION

In the pancreas, cytodifferentiation occurs before morphogenesis (Gittes and Rutter, 1992), and, it is thought that endocrine cells, first present in the pancreatic duct epithelium, migrates out of this epithelium and reaggregates into islets with its typical cell architecture; non- β -cells in the periphery and β -cells in the center. Mouse aggregation chimaera experiments have shown that islets are not derived from a few cells that proliferate, but through aggregation of endocrine cells interspersed in the interstitial tissue (Deltour et al., 1991). The purpose of this study was to use the pancreas as a model system for examining the role of cadherins during organogenesis in a multicellular organism. Therefore, we overexpressed (using the rat insulin-promoter 1, Rip1) a mutant form of mouse E-cadherin, in which nearly all its extracellular amino acids had been deleted, in pancreatic β -cells in transgenic mice.

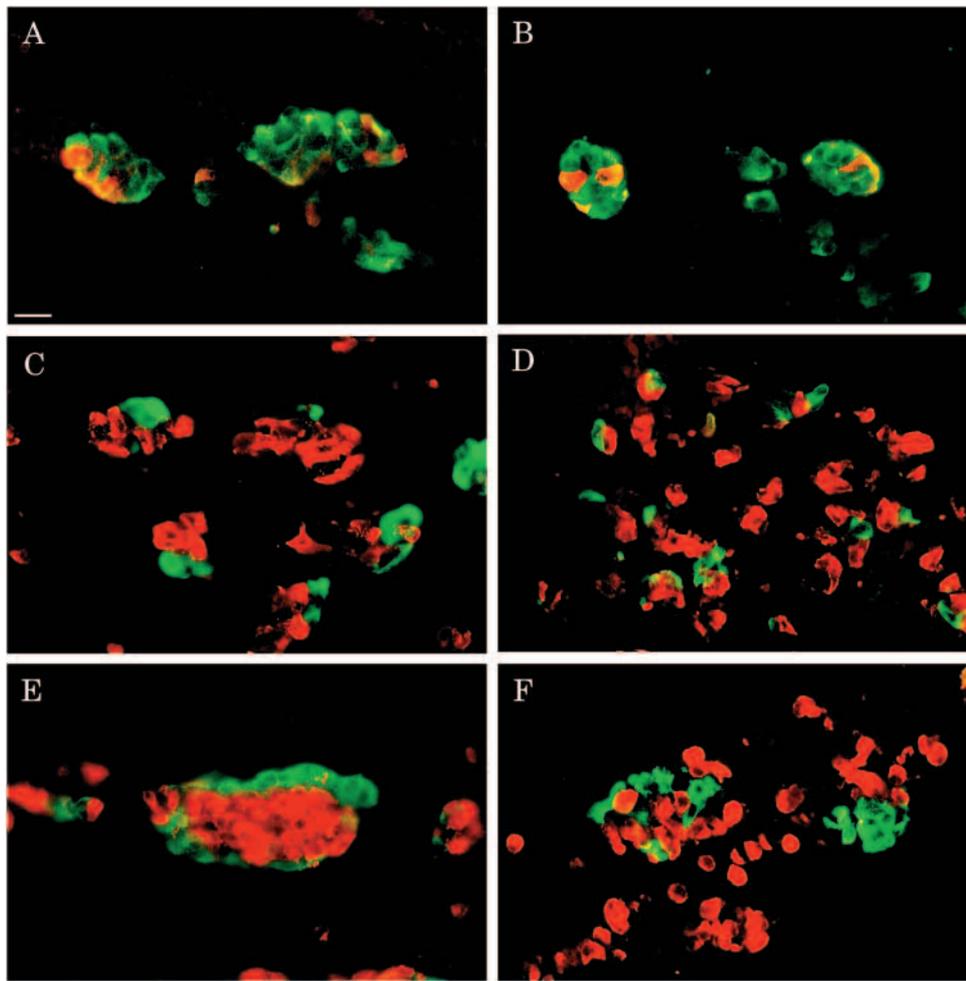


Fig. 6. Cadherins regulate initiation of β -cell aggregation. Double immunofluorescence stainings of 11.5 dpc (A,B), 14.5 dpc (C,D), and 17.5 dpc (E,F) pancreata of negative littermates (A,C,E) and transgenics (B,D,F) with anti-insulin pAb (visualized in red) and anti-glucagon pAb (visualized in green). The initial aggregation of β -cells, which normally begins at 13.5–14.5 dpc, is interfered with in transgenics. Furthermore, the organization of α - and β -cells into islets, which normally begins at 17.5–18 dpc, is perturbed in transgenics. Bar, 20 μ m.

We have for the first time shown *in vivo* that cadherins regulate cell-cell interactions, mediating aggregation of endocrine cells into pancreatic islets of Langerhans. Our evidence for this conclusion is: (1) control experiments showed that truncated E-cadherin protein was only expressed in pancreatic β -cells. The mutant form binds β -catenin and can thus act as a dominant negative mutant for cadherins in pancreatic β -cells. Both E- and N-cadherin were displaced from cell-cell contacts of β -cells expressing high levels of the mutant protein. Furthermore, cell surface expression of NCAM in transgenic β -cells, argues against blockage of membrane trafficking being the explanation for loss of cadherins from cell-cell contacts. Thus, E- and N-cadherin activities were inhibited in β -cells expressing significant levels of truncated E-cadherin. (2) The initial aggregation of pancreatic β -cells, which normally begins at 13.5–14.5 dpc, was abrogated in transgenics. Consequently, the aggregation of endocrine cells into islet-like structures, which normally begins at 17.5–18 dpc, was perturbed in transgenics. While β -cell aggregation was inhibited, non- β -cells (at least α -cells) aggregated into islet-like clusters separate from β -cells. The displacement of E- and N-cadherin from cell-cell contacts could be explained by endocytosis of the cadherins together with other membrane constituents of the adherens junction, as was reported for E-cadherin after inhibition of its activity in MDBK cells (Kartenbeck et al., 1991). Alternatively, if the cadherins are not internalized, their disappearance

from cell junctions could be due to a diffuse redistribution on the cell surface, as was reported in other cases (Navarro et al., 1995).

Our *in vivo* data are in agreement with earlier work *in vitro* on isolated rat islets (Rouiller et al., 1991), namely, that E-cadherin regulates adhesion between all pancreatic endocrine cells. The observation that α -cells aggregated into islet-like clusters devoid of β -cells when β -cell aggregation was perturbed, indicates that cadherin-mediated adhesive properties between all endocrine cells are required for the typical cell organization of islets *in vivo*. The sorting of β -cells from non- β -cells in islets could be explained by differences in either the CAMs expressed or the levels of expression of the same CAM, as has been shown with transfected cells (Nose et al., 1988; Steinberg and Takeichi, 1994). To our knowledge no CAM has been reported to be cell-specifically expressed in the islets. However, Cirulli et al. (1994) recently demonstrated that antibodies against NCAM, a non- Ca^{2+} -dependent CAM which is enriched in non- β -cells in the rat (Rouiller et al., 1990; Møller et al., 1992), inhibited correct histotypical reaggregation of dissociated rat islet cells, suggesting that NCAM is involved in the sorting of non- β -cells from β -cells in the rat. Nevertheless, the fact that no differential expression of NCAM is evident in mouse islet-cells (data not shown), argues against NCAM being involved in the establishment and maintenance of islet architecture in the mouse. Furthermore, NCAM was expressed

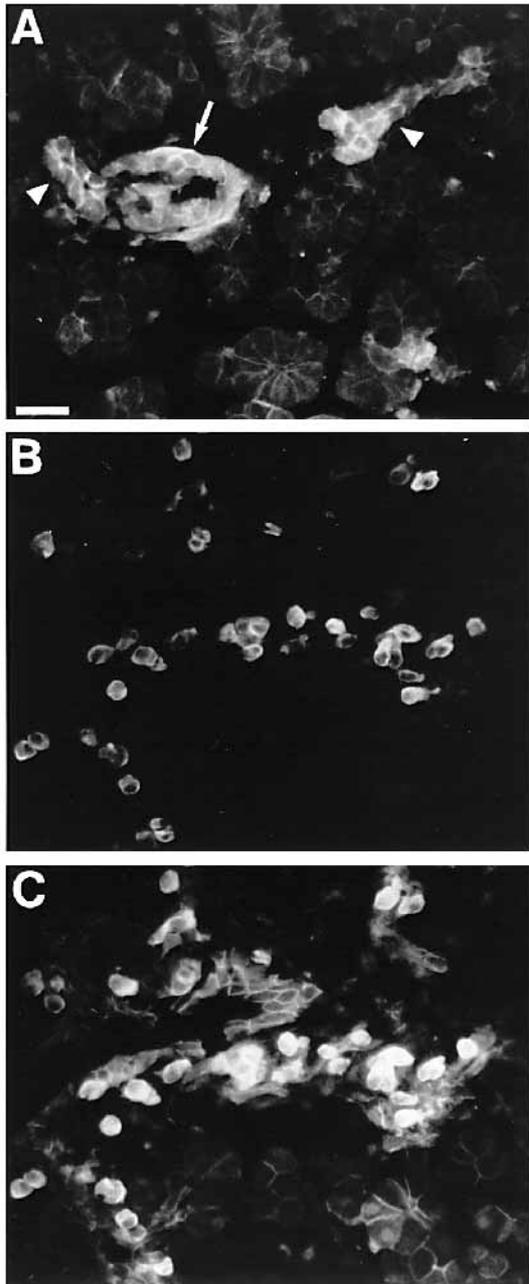


Fig. 7. Cytoplasmic accumulation of β -catenin in transgenic β -cells. (A) Immunofluorescence staining of a 17.5 dpc pancreas from a negative littermate with anti- β -catenin pAb. (B,C) Double immunofluorescence staining of a 17.5 dpc transgenic pancreas with the tag-epitope mAb, 9E10 (B) and anti- β -catenin pAb (C). The arrow indicates a group of β -cells. Arrowheads indicate intralobular ducts. Bar, 25 μ m.

on the cell surface of β -cells lacking E- and N-cadherin, indicating that NCAM does not function as an essential CAM between pancreatic β -cells.

In none of the previous *in vivo* studies of cadherin function by a dominant negative mutant approach were any changes in the expression of catenins reported (Kintner, 1992; Fujimori and Takeichi, 1993; Holt et al., 1994; Dufour et al., 1994; Levine et al., 1994; Herminston and Gordon, 1995). Nonethe-

less, a cell can 'sense' whether the cytoplasmic domain of a cadherin is covered with catenins, because increased synthesis of cadherin stimulates the production of catenins at a post-transcriptional level (Nagafuchi et al., 1991). In our study, expression of the truncated receptor resulted in an accumulation of β -catenin in the cytoplasm of β -cells. Alas, without quantification of the protein, we cannot determine whether β -catenin protein is upregulated or redistributed or both. The colocalization of the truncated E-cadherin receptor and β -catenin in the cytoplasm of β -cells is in agreement with the reported interaction between cadherins and β -catenin already during synthesis and transport of the cadherin (Ozawa et al., 1990; Hinck et al., 1994). Besides functioning in adhesion complex formation, β -catenin interacts with the tumor suppressor gene product, APC (Su et al., 1993), and is involved in signal transduction during developmental patterning in embryos (Gumbiner, 1995; Peifer, 1995). Consequently, we cannot rule out the possibility that the increased levels of β -catenin in β -cells affects cellular processes not related to cell adhesion.

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