Liver-Intestine Cadherin: Molecular Cloning and Characterization of a Novel Ca^{2+}-dependent Cell Adhesion Molecule Expressed in Liver and Intestine

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Abstract. A novel member of the cadherin family of cell adhesion molecules has been characterized by cloning from rat liver, sequencing of the corresponding cDNA, and functional analysis after heterologous expression in nonadhesive S2 cells. cDNA clones were isolated using a polyclonal antibody inhibiting Ca^{2+}-dependent intercellular adhesion of hepatoma cells. As inferred from the deduced amino acid sequence, the novel molecule has homologies with E-, P-, and N-cadherins, but differs from these classical cadherins in four characteristics. Its extracellular domain is composed of five homologous repeated domains instead of four characteristic for the classical cadherins. Four of the five domains are characterized by the sequence motifs DXNDN and DXD or modifications thereof representing putative Ca^{2+}-binding sites of classical cadherins. In its NH$_2$-terminal region, this cadherin lacks both the precursor segment and the endogenous protease cleavage site RXKR found in classical cadherins. In the extracellular EC1 domain, the novel cadherin contains an AAL sequence in place of the HAV sequence motif representing the common cell adhesion recognition sequence of E-, P-, and N-cadherin. In contrast to the conserved cytoplasmic domain of classical cadherins with a length of 150–160 amino acid residues, that of the novel cadherin has only 18 amino acids. Examination of transfected S2 cells showed that despite these structural differences, this cadherin mediates intercellular adhesion in a Ca^{2+}-dependent manner. The novel cadherin is solely expressed in liver and intestine and was, hence, assigned the name LI-cadherin. In these tissues, LI-cadherin is localized to the basolateral domain of hepatocytes and enterocytes. These results suggest that LI-cadherin represents a new cadherin subtype and may have a role in the morphological organization of liver and intestine.

C ELL–cell interactions are of fundamental importance for the development and the maintenance of tissues and organs in multicellular organisms. The basic morphogenetic processes involved in organogenesis, including cellular aggregation, segregation, and migration, are mediated and controlled by an increasingly large and complex number of cell adhesion molecules that exhibit a well-regulated spatiotemporal pattern of expression during development and regeneration (for review see Simons and Fuller, 1985; Ekblom et al., 1986; Edelman, 1988; Schachner, 1989; Takeichi, 1991; Hynes and Lander, 1992). Intercellular adhesion molecules identified so far have been classfied into at least four major molecular families, the im-

munoglobulin superfamily, the integrin superfamily, the cadherin family, and the selectin family (Williams and Barclay, 1988; Kemler et al., 1989; Osborn, 1990; Takeichi, 1990; Geiger and Ayalon, 1992; Hynes, 1992). Selectins that are mainly present on blood cells and endothelial cells bind to specific carbohydrate epitopes via their lectin domains (Osborn, 1990), by contrast to the other three major classes of cell adhesion molecules functioning by means of protein–protein interactions. Several of the immunoglobulin superfamily cell adhesion molecules mediate cell–cell adhesion by means of heterophilic interactions, whereas others such as the neural cell adhesion molecule (N-CAM)[1] may

1. *Abbreviations used in this paper:* CAM, cell adhesion molecule; LI-cadherin, liver-intestine cadherin; PB, sodium phosphate; PB/BSA, globulin-free BSA in 60 mM sodium phosphate; PBST, 0.05% (vol/vol) Tween-20 in PBS; PNGase F, peptide-N^4-(N-acetyl-β-glucosaminyl)-asparagine amidase F.

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bind both in a homophilic and heterophilic mode (Cole et al., 1986; Cunningham et al., 1987). Although the majority of the known members of the integrin superfamily constituting a third major family of cell adhesion molecules bind to various molecules of the extracellular matrix and mediate cell matrix binding, a few integrins may act as cell–cell adhesion molecules in a heterophilic fashion (Hynes, 1992). Cadherins are calcium-dependent cell adhesion molecules, and they mediate intercellular adhesion by homophilic binding (Kemler et al., 1989; Takeichi, 1990; Geiger and Ayalon, 1992). Cell adhesion molecules of the cadherin family are particularly relevant for morphogenetic processes (Edelman, 1988; Takeichi, 1991) and have been shown to be essential, e.g., for compaction of preimplantation mouse embryos (Kemler et al., 1977; Hyafil et al., 1980, 1981), the formation of intermediate and tight junctions between epithelial cells (Boller et al., 1985; Gumbiner and Simons, 1986, 1987), neurogenesis (Napolitano et al., 1991), and neurite outgrowth (Bixby and Zhang, 1990; Doherty et al., 1992). Several subclasses of cadherin molecules have been described (for review see Kemler et al., 1989; Takeichi, 1990; Geiger and Ayalon, 1992; Pouliot, 1992). Uvomorulin/E-cadherin primarily expressed in epithelial tissues, N-cadherin abundantly present in the nervous system, skeletal muscle, and cardiac muscle, and P-cadherin originally identified in placenta belong to the group of classical cadherins. These are characterized by a similar domain structure of their extracellular part, a HAV motif in the EC1 domain, and by a highly conserved cytoplasmic domain. M-cadherin expressed in skeletal muscle (Donalies et al., 1991) does not harbor the HAV motif, but is otherwise very similar to classical cadherins. Desmosomal cadherins expressed in epithelia (Koch et al., 1992), as well as protocadherins characterized in the central nervous system (Sano et al., 1993), exhibit a completely different cytoplasmic domain, and the GPI-anchored T-cadherin found in neural and non-neural tissues even lacks both the transmembrane and the cytoplasmic domains (Ranscht and Dours-Zimmermann, 1991).

In developing and regenerating liver, particularly members of the cadherin family and the immunoglobulin superfamily, have been shown to be major mediators of intercellular adhesion. Cell-CAM 105/gp110 belonging to the immunoglobulin superfamily and more specifically to the carcinoembryonic antigen family can mediate adhesion between hepatocytes in a homophilic calcium-independent manner (Ocklind and Öbrink, 1982; Tingström and Öbrink, 1989; Öbrink, 1991; Becker et al., 1993). Cell-CAM 105/gp110 is mainly localized in the bile canalicular domain of the rat hepatocyte where there is no intercellular adhesion, prompting the assumption that cell-CAM 105/gp110 might rather have a role in bile canalicular formation than in hepatocyte–hepatocyte binding (Hixson and McEntire, 1989). Antibodies directed against uvomorulin/E-cadherin have been shown to interfere with cell–cell contact of 14-d-old embryonic hepatocytes (Vestweber and Kemler, 1984). Likewise, inhibition of the function of liver cell adhesion molecule thought to be the avian analogue of uvomorulin/E-cadherin inhibited the aggregation of embryonic chick liver cells (Bertolotti et al., 1980; Gallin et al., 1983). Moreover, the formation of structurally differentiated liver after birth has been shown to coincide with an increased expression of

uvomorulin/E-cadherin and of the desmosomal cadherin desmoglein (Stamatoglou et al., 1992). However, antibodies directed against E-cadherin did not completely inhibit calcium-dependent cell aggregation of hepatocytes prepared from adult mouse liver indicating the presence of additional cadherins in hepatic tissue (Ogou et al., 1983).

To identify novel cell adhesion molecules involved in intercellular adhesion of hepatocytes, a panel of plasma membrane glycoproteins purified from rat liver and hepatoma (Tauber et al., 1983, 1986, 1989) was screened for both adhesive function and localization to the basolateral cell surface of hepatocytes, where adhesive interactions predominate. In the present report, cloning, sequencing, and functional characterization of a novel member of the cadherin multigene family is described. This novel cadherin has an extracellular domain consisting of five homologous repeated domains instead of four characteristic for the classical cadherins. Moreover, by contrast to the classical cadherins having a highly conserved cytoplasmic tail of ∼150 amino acid residues (for review see Takeichi, 1990), this new type of cadherin has a very short cytoplasmic tail of only 18 amino acids. As shown by expression in *Drosophila* S2 cells, the novel cadherin mediates Ca^{2+}-dependent intercellular adhesion, despite the structural differences that make this molecule unique among the known members of the cadherin family. The novel cadherin is solely expressed in hepatocytes of liver, as well as in intestinal enterocytes and goblet cells; this is reflected in its name, LI-cadherin, indicating a possible morphoregulatory role for these two tissues.

Materials and Methods

Immunoscreening and DNA Screening of cDNA Library

For immunoscreening, a preamplified rat liver cDNA expression library in λ-ZAPII (Stratagene, Heidelberg, FRG) was plated on XL1-Blue bacteria with ∼250,000 plaques per 150-mm plate and incubated for 3.5 h at 42°C. Nitrocellulose membranes were soaked in 10 mM isopropyl-β-D-thiogalactopyranoside, dried, and placed on the bacterial lawn. Incubation was continued for another 3.5 h at 37°C. Membranes were lifted, rinsed in PBST (0.05% [vol/vol] Tween-20 in PBS), blocked for 3 h in blocking buffer I (3% [wt/vol] bovine serum albumin in PBST), blocked for another 3 h in blocking buffer II (10% [vol/vol] fetal calf serum in blocking buffer I), and incubated for 16 h with rabbit anti-hgp125 IgG (Tauber et al., 1989) in blocking buffer II. After three washing cycles in PBST, membranes were incubated for 30 min with swine anti-rabbit IgG (Dakopatts, Hamburg, FRG) 1:40 in blocking buffer II, washed three times in PBST, incubated for 30 min with a complex of horseradish peroxidase and rabbit anti-horseradish peroxidase (Dakopatts) 1:200 in blocking buffer II, and developed using 4-chloro-naphthol as substrate. Positive clones TB1 and TB2 were plaque purified by several rounds of rescreening, and the corresponding pBluescript clones (pTB1 and pTB2) were in vivo-excised using the R408-helper phage (Stratagene). Thereafter, the library was additionally screened on the DNA level using the insert of pBluescript clone pTB1 as a probe. The isolated insert was digoxigenin labeled with random primer by the method of Feinberg and Vogelstein (1983) using digoxigenin-11-dUTP and DNA-polymerase I (Klenow fragment) from Boehringer Mannheim (Mannheim, FRG). Hybridization was performed at 42°C followed by washing at high stringency conditions. The digoxigenin-labeled probe bound to the membrane was detected with antidigoxigenin Fab fragments conjugated to alkaline phosphatase and AMPPD® (Boehringer Mannheim) as chemiluminescent substrate using the procedure described by the manufacturer.

DNA Sequencing

Inserts of the in vivo-excised pBluescript clones pTB1, pTB2, pTB3, pTB4, and pTB5 were digested with appropriate restriction enzymes to generate

overlapping fragments. DNA sequencing was carried out by the dideoxy-chain termination method (Sanger et al., 1977) using T7 polymerase (Pharmacia, Freiburg, FRG). In addition to the vector-specific sequencing primers (SK, KS, T7, and T3; Stratagene), synthetic primers were used that bind to internal regions of the cloned inserts. All sequence data were obtained for both strands.

Protein and DNA Sequence Analysis

Nucleotide and deduced amino acid sequences were analyzed using the Mac Molly software package™ (SoftGene, Berlin, FRG), which includes the EMBL and the Swiss-Prot sequence databases.

Isolation of RNA and Northern Blot Hybridization

Total RNA was isolated from rat tissues using the guanidinium isothiocyanate method according to Sambrook et al. (1989). Approximately 10 μg of RNA from each tissue were separated on a 1.2% agarose gel containing formaldehyde, transferred to nylon membranes (Hybond-N; Amersham Buchler, Braunschweig, FRG) and UV cross-linked. Hybridization with ^{32}P-labeled cDNA probes was performed in the presence of 10% (wt/vol) dextran sulfate, 1 M NaCl, 1% (wt/vol) SDS, 100 μg/ml salmon testes DNA at 60°C for 20 h. After hybridization, membranes were washed at a final stringency of 0.1 × SSC/1% (wt/vol) SDS at room temperature. Standardization of RNA levels was achieved by comparing the intensity of the ribosomal RNA bands. Relative integrated optical density levels were calculated from grey levels measured with an Elscript 400 System (Hirschmann, Unterhaching, FRG).

cDNA Probe Purification and Labeling

The EcoRI fragment from the insert of plasmid pTB1 was separated by agarose gel electrophoresis and extracted from the gel by electroelution. The purified fragment was random primer labeled with [α-^{32}P]dCTP using the Random Primer Labeling Kit (Gibco BRL, Eggenstein, FRG). Unincorporated label was removed using NAP-columns (Pharmacia). The purified probe had a specific activity of 1.0 × 10^8 dpm·μg^{-1}.

Protein Expression in Escherichia coli

E. coli strain XL1-Blue (Stratagene) carrying the plasmid pTB2 was grown at 37°C in Luria broth medium containing ampicillin (50 μg/ml) to a density corresponding to an absorption at 600 nm of 0.5. Expression of the β-galactosidase LI-cadherin fusion protein was induced by the addition of iso-propyl-β-D-thiogalactopyranoside to a final concentration of 10 mM and incubation for 2 h at 37°C. Cells were lysed in 5% (wt/vol) SDS, 15% (vol/vol) glycerol, 1 M β-mercaptoethanol, 100 mM Tris-HCl, pH 6.8, and cell debris was removed by centrifugation. Protein concentration of cell extracts was determined with the bicinchoninic acid protein assay reagent (Pierce Europe B.V., Oud-Beijerland, The Netherlands) using bovine serum albumin as a standard.

One- and Two-dimensional SDS-PAGE and Immunoblotting

SDS-PAGE was performed according to Laemmli (1970). For two-dimensional SDS-PAGE, samples were separated in the first dimension by isoelectrofocusing on immobilized pH gradients using the method of Sinha et al. (1990). Proteins were transferred from SDS polyacrylamide gels electrophoretically at constant 110 V for 2 h onto nitrocellulose membranes (BA85; Schleicher & Schuell, Dassel, FRG), according to Towbin et al. (1979). Nitrocellulose membranes were immunostained with rabbit anti-hgp125 IgG as described for immunoscreening of the cDNA library. Tissue samples for SDS-PAGE were prepared by homogenization in SDS sample buffer and boiling for 3 min. Samples of small intestine were in part obtained by freeze clamping in situ in liquid nitrogen followed by homogenization and boiling in SDS sample buffer. Plasma membranes from Morris hepatoma 7777 grown in Buffalo rats and from livers of Buffalo rats were isolated as described (Tauber et al., 1986, 1989). Membrane purity was checked by electron microscopy and by the assay of marker enzymes as described (Tauber and Reutter, 1978).

Enzymatic Deglycosylation

Plasma membrane glycoproteins were N-deglycosylated by incubation with peptide-N^4-(N-acetyl-β-glucosaminyl) -asparagine amidase F (PNGase F) from Flavobacterium meningosepticum (Boehringer Mannheim). Isolated plasma membranes (1 mg/ml) were suspended in 0.4% (wt/vol) SDS, 1.0% (vol/vol) β-mercaptoethanol, 40 mM EDTA, and 500 mM sodium phosphate, pH 8.0, boiled for 3 min at 95°C, and centrifuged for 3 min at 16,000 g. The following were added to 100-μl aliquots of the supernatant: leupeptin, chymostatin, antipain, and pepstatin, each to a final concentration of 65 μg/ml, octylglucopyranoside to a final concentration of 1.9 mM, and 10 U of PNGase F. Samples were incubated for 18 h at 37°C.

## Immunolabeling of Semithin Sections

Small intestine was dissected into 2-mm cubes and fixed for 1 h in 3% (wt/vol) paraformaldehyde/0.25% (vol/vol) glutaraldehyde in 60 mM sodium phosphate, pH 7.3, followed by progressive lowering temperature dehydration from 0°C to −30°C and embedding in LR White® (The London Resin Co. Ltd., Basingstoke, United Kingdom). Semithin (0.5-μm) sections were preincubated for 30 min with 1% (wt/vol) globulin-free bovine serum albumin in 60 mM sodium phosphate, pH 7.3 (PB/BSA), and incubated with anti-hgp125 IgG (12 μg/ml in PB/BSA) at 4°C for 16 h. After washing three times with PB/BSA, sections were incubated for 1 h with goat anti-rabbit IgG coupled to 10 nm colloidal gold (EM-grade; Amersham Buchler) 1:50 in 50 mM Tris/HCl, pH 8.4, 1% (wt/vol) BSA. After washing with 60 mM sodium phosphate, pH 7.3, sections were fixed with 2.5% (vol/vol) buffered glutaraldehyde for 10 min, washed with aqua dest, and the size of gold particles was increased by silver enhancement (Danscher, 1981).

Liver tissue was fixed by perfusion via the portal vein with 3% (wt/vol) paraformaldehyde/0.25% (vol/vol) glutaraldehyde in 60 mM sodium phosphate, pH 7.3. Sections (200-μm) of the fixed liver tissue were incubated first in 0.5% (wt/vol) sodium borohydride/0.1% (wt/vol) glycine in 60 mM sodium phosphate, pH 7.3, for 10 min, then in 0.5% NaN$_3$/0.1% (wt/vol) gelatine in 60 mM sodium phosphate, pH 7.3 (BSA/gelatine), for 30 min, and finally with anti-hgp125 IgG (12 μg/ml in BSA/gelatine) at 4°C for 16 h. After washing in BSA/gelatine for 2 h, sections were incubated with goat anti-rabbit IgG coupled to 1 nm colloidal gold (1:50 in BSA/gelatine) for 5 h at 37°C, washed extensively in 60 mM sodium phosphate, pH 7.3, fixed for 10 min in 2.5% (wt/vol) buffered glutaraldehyde, washed with aqua dest, and silver enhanced. Semithin sections (0.5-μm) were prepared from the sections (200-μm) after embedding in araldite. Sections from liver and intestine were finally stained with toluidine blue. Immunofluorescence microscopy of frozen sections was performed as described (Tauber et al., 1986).

Immunoelectron Microscopy

Small blocks of tissue were fixed in 3% (wt/vol) paraformaldehyde/0.25% (vol/vol) glutaraldehyde in 60 mM sodium phosphate, pH 7.3, for 1 h at 4°C and washed three times in 60 mM sodium phosphate (PB), pH 7.3, followed by progressive lowering temperature dehydration and embedding in LR White®. Ultrathin sections (60–90 nm) were preincubated with 1% (wt/vol) PB/BSA for 30 min, and incubated with anti-hgp125 IgG (12 μg/ml in PB/BSA) at 4°C for 16 h. Washing with PB/BSA was followed by incubation with 10 nm gold-conjugated goat anti-rabbit IgG (1:50 in Tris/HCl, pH 8.4, 1% [wt/vol] BSA) for 1 h. After washing with PB, sections were fixed with 2.5% (vol/vol) buffered glutaraldehyde for 10 min, washed with aqua dest, and the size of gold particles was increased in some sections by silver enhancement. Finally, the sections were stained with 1% (wt/vol) phosphotungstic acid, 2% (wt/vol) osmium tetroxide, and 5% (wt/vol) uranyle acetate for 15 min each.

MH7777 cells grown on Thermanox™ coverslips (Miles Laboratories, Naperville, IL) were fixed and incubated with anti-hgp125 IgG as described above for immunolabeling of semithin sections. After three washing cycles with PB/BSA cells were incubated with goat anti-rabbit IgG coupled to 10 nm colloidal gold (1:50 in 50 mM Tris/HCl, pH 8.4, 1% [wt/vol] BSA) for 1 h at room temperature. Cells were washed three times in PB/BSA, fixed in 2.5% (vol/vol) glutaraldehyde in PB for 10 min, washed and incubated with 2% (wt/vol) osmium tetroxide. After dehydration, the cells were embedded in araldite. Ultrathin sections (60–90 nm) were stained with 5% (wt/vol) uranyl acetate for 15 min and with lead citrate according to the method of Reynolds (1963).
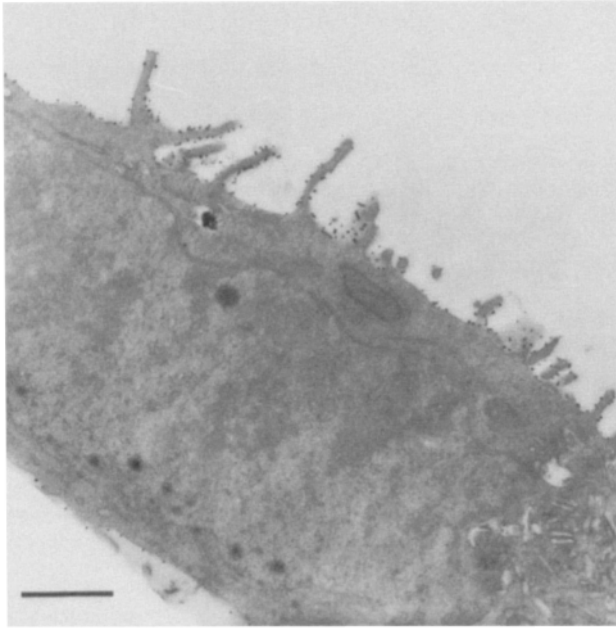
Figure 1. Immunoelectron microscopic localization of hgp125 on MH 7777 cells. Cells grown on Thermanox™ coverslips were fixed and labeled with anti-hgp125 IgG and 10 nm gold-conjugated secondary antibody before embedding and processing for electron-microscopy. Bar, 1 μm.

Cell Adhesion Assay

Cell adhesion of hepatocarcinoma MH 7777 cells (Vedel et al., 1983; Loch et al., 1992) was measured using an assay based on that described by Balsamo et al. (1991) and Walther et al. (1973). MH 7777 cells were cultured as described (Loch et al., 1992). For preparation of labeled probe cells, MH 7777 cells were grown to ~70% confluence. After a 30-min incubation in 5 ml of MEM with Earle's salts without L-methionine/L-cysteine, cells were labeled for 90 min by addition of 5 μl (2.2 MBq) of TRAN ³⁵S-LABEL (37.96 TBq/mmol L-methionine; ICN, Meckenheim, FRG). Cells were washed once with DME containing 10% heat-inactivated fetal bovine serum (Gibco BRL), insulin (3 ng/ml), 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) (complete DME) supplemented with 1 mM unlabeled L-methionine, and chased for 30 min. After removal of medium and washing three times with Dulbecco's PBS, single cells were prepared by brief incubation with 5 ml of 0.25% (wt/vol) trypsin (Biochrom, Berlin, FRG) in Dulbecco's PBS supplemented with 5.5 mM D-glucose. Cells detached from the plate were suspended in ice-cold DME without bovine serum, washed twice by centrifugation (20 g, 5 min), and finally suspended to a cell density of 7 × 10⁵/ml at 4°C. For preparation of target monolayer cultures, cells were seeded into six-well tissue culture plates (7 × 10⁵ cells/well) in complete DME, and cultured for ~24 h until cells formed a confluent monolayer.

To measure cell–cell adhesion, target cells were cooled to 4°C on ice, incubated with 3% (wt/vol) bovine serum albumin in Dulbecco's PBS for 20 min and washed twice with Dulbecco's PBS containing 2 mM CaCl₂. Cells designed for testing the effect of EDTA were washed with Dulbecco's PBS without Ca²⁺. Either one of the following was added to the target cells (2 ml/well): (a) Dulbecco's PBS containing 2 mM CaCl₂; (b) Dulbecco's PBS without Ca²⁺ containing 3 mM EDTA; and (c) Dulbecco's PBS containing 2 mM CaCl₂ and the appropriate concentration of antibody. Target cells were then incubated at 4°C for 15 min, labeled probe cells (4.6 × 10⁵/ml per well) were added, and the incubation was continued for 60 min on a gyratory shaker at 4°C. Unbound cells were removed by aspiration and the wells washed three times with Dulbecco's PBS, 1% (wt/vol) bovine serum albumin, containing either 2 mM CaCl₂ (a and c) or 3 mM EDTA (b). Cells were then lysed in 1 ml of lysis buffer (20 mM Tris/HCl, pH 8.0, 150 mM NaCl, 1% [vol/vol] NP-40), and radioactivity was determined by liquid scintillation counting. Assays with anti-uvomorulin/E-cadherin IgG (anti-gp84) (Ozawa and Kemler, 1990), final concentration 0.1 mg/ml, were performed in 24-well tissue culture plates following the same protocol.
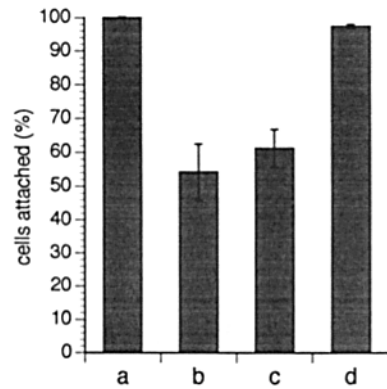
Figure 2. Inhibition of cell–cell adhesion of MH 7777 cells by anti-hgp125 IgG. Adhesion of MH 7777 probe cells metabolically labeled with TRAN³⁵S-LABEL to MH 7777 target cells was assayed as described in Materials and Methods in the presence of either (a) 2 mM CaCl₂, (b) 3 mM ETA; (c) 0.1 mg/ml anti-hgp125 IgG and 2 mM CaCl₂; (d) anti–human transferrin receptor IgG (OKT9; 0.1 mg/ml) and 2 mM CaCl₂. Adhesion was calculated as percent of control values measured in the presence of 2 mM CaCl₂ without antibodies. Data represent means of three experiments.

Antibodies

Antibodies raised in rabbits against plasma membrane glycoproteins gp80, gp140, gp160, hgp85, hgp105, hgp115, hgp125, and hgp175 were those described earlier (Tauber et al., 1986, 1989). For isolation of IgG, rabbit antisera were diluted 1:5 with PBS and loaded onto a protein A-Sepharose column (Pharmacia). Unspecifically bound proteins were removed by extensive washing with PBS. IgG was eluted with 100 mM sodium citrate, pH 6.0, dialyzed against PBS, and stored at −20°C. Anti–human transferrin receptor antibody OKT9 (Sutherland et al., 1981) was prepared as described (Orberger et al., 1992). Anti-gp84 IgG was a gift of Dr. R. Kemler (Max-Planck-Institut für Immunbiologie, Freiburg i. Br., FRG).

Culture, Transfection, and Cloning of Drosophila S2 Cells

Drosophila S2 cells (Schneider, 1972) were grown in complete Schneider's medium containing 5 mM CaCl₂ (Gibco BRL) supplemented with 12.5% heat-inactivated fetal calf serum (Sigma, Deisenhofen, FRG), penicillin (100 U/ml), and streptomycin (100 μg/ml) (Biochrom). The cells were maintained at 25°C with air as the gasphase. For the expression of LI-cadherin in S2 cells, the insert of pTB2 (see Fig. 7) was subcloned into the SmaI site of the Drosophila expression vector pRmHa-3 (Bunch et al., 1988) under the control of the metallothionein promoter. The resultant plasmid pRmHa-LI was cotransfected into S2 cells with the plasmid pPC4 (Jokerst et al., 1989), which confers α-amanitin resistance to the cells. Cell transfection was performed using the Lipofectin™ reagent (Gibco BRL) as described by Nose et al. (1992). After selection, the α-amanitin-resistant cells were cloned in 0.3% agar in the presence of a lethally irradiated feeder layer of pPC4-transfected S2 cells.

Immunofluorescence of Drosophila S2 Cells

After cloning, transfected S2 cells were suspended in culture medium to a density of 1 × 10⁶ cells/ml. Expression of LI-cadherin was induced by addition of CuSO₄ to 0.7 mM for 18–24 h at 25°C. Immunofluorescence microscopy was performed using the protocol of Fehon et al. (1990). Briefly, cells were collected by centrifugation and fixed in 0.6-ml Eppendorf tubes with 0.5 ml of freshly made 2% (wt/vol) paraformaldehyde in PBS for 10 min at room temperature. Cells were then collected by centrifugation, rinsed twice in PBS, and stained for 1 h in anti-hgp125 IgG (12 μg/ml in 0.1% [wt/vol] saponin in PBS and 1% [vol/vol] fetal calf serum in PBS). After being washed twice in PBS cells were stained for 1 h in affinity-purified, FITC-conjugated goat anti-rabbit IgG (Sigma) (1:40 in saponin-FCS-PBS). Cells were rinsed twice in PBS and mounted on slides in 885 mM Tris/HCl, pH 8.0, 0.5% (wt/vol) n-propyl gallate, 10% (vol/vol)
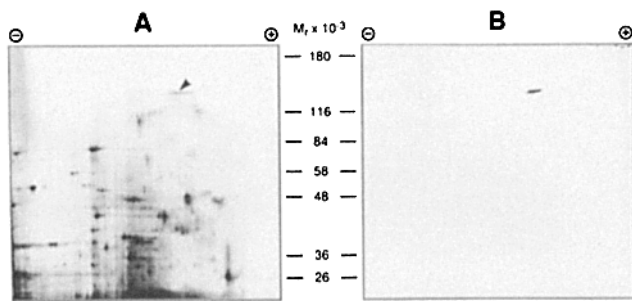
Figure 3. Characterization of hgp125 by two-dimensional SDS-PAGE and immunoblotting. Plasma membranes isolated from rat liver were resolved by two-dimensional SDS-PAGE and were either silver-stained (*A*) or transferred to nitrocellulose membranes (*B*). Membranes were immunostained with rabbit anti-hgp125 IgG as described in Materials and Methods. The antibody recognizes a 120-kD polypeptide with a pI of 4.5 (*A*, *arrowhead*).

glycerol. Cells were examined under epifluorescence on a microscope (Axioplan; Zeiss, Oberkochen, FRG).

Aggregation Assays

S2 cells were collected by centrifugation and resuspended in culture medium to a density of 1 × 10⁶ cells/ml. Expression of LI-cadherin in the
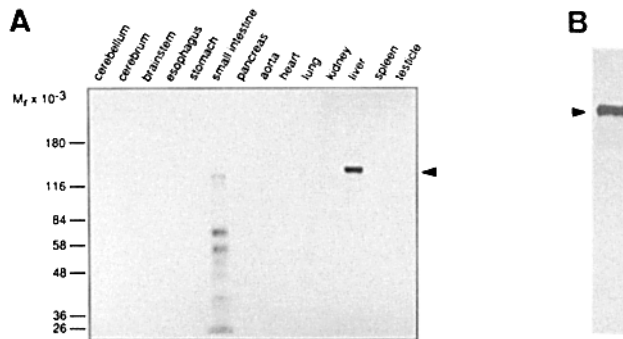

Figure 4. Western blot analysis. (*A*) Protein extracts of tissues solubilized in SDS-sample buffer were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunostained with anti-hgp125 IgG as described in Materials and Methods. (*B*) Samples of small intestine were prepared by freeze clamping in liquid nitrogen and solubilization in SDS-sample buffer, and they were analyzed as in *A*.

transfected cells was induced by addition of CuSO₄ to 0.7 mM for 18–24 h at 25°C. For aggregation assays, cell aggregates formed during the induction period were gently dissociated by slow pipetting through a glass pipette. To allow aggregation, 3 ml of cell suspension were placed into 50-ml conical tubes and agitated at 25°C on a rotary shaker (150 rpm) for the times indi-
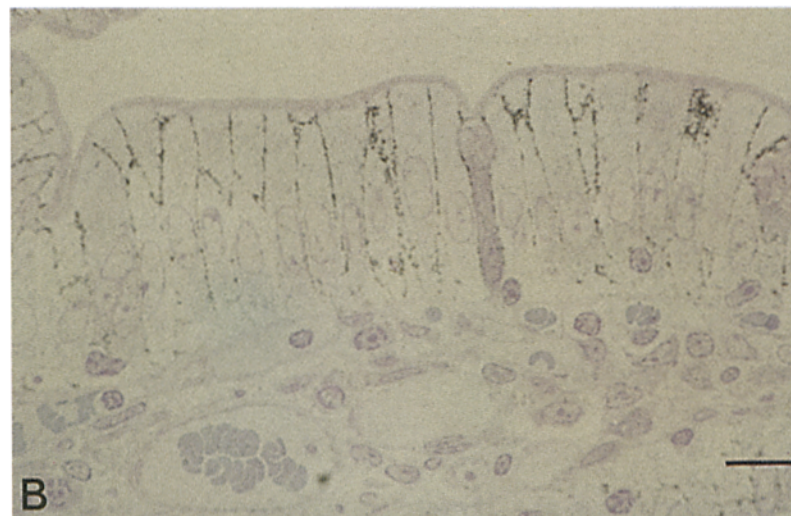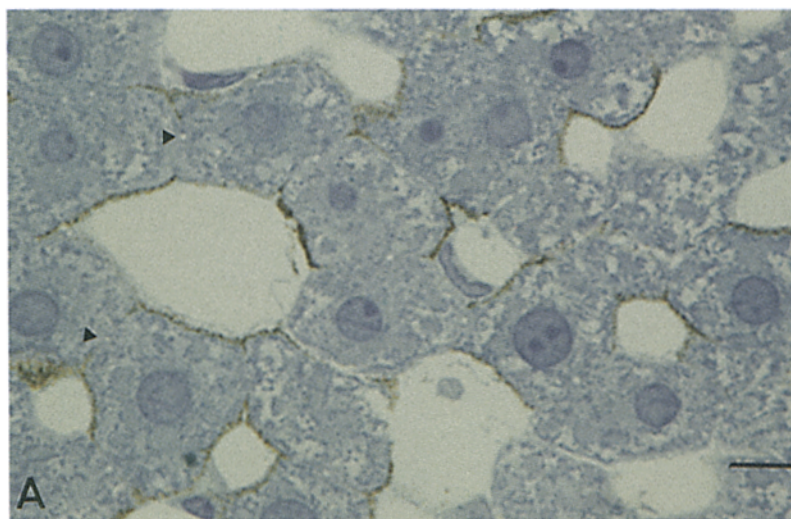
Figure 5. Localization of hgp125 in rat liver and small intestine. Semithin (0.5-µm) sections of rat liver (*A*) and small intestine (*B*) were stained with anti-hgp125 IgG and gold-conjugated secondary antibody followed by silver enhancement as described in Materials and Methods. In *A*, bile canaliculi are indicated by arrowheads. Bars, 12.8 µm in *A* and 20 µm in *B*.
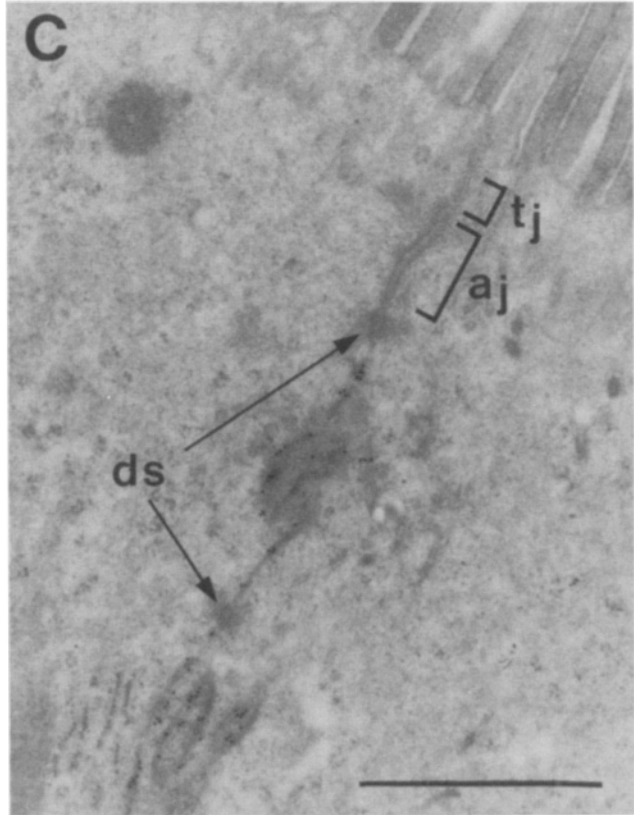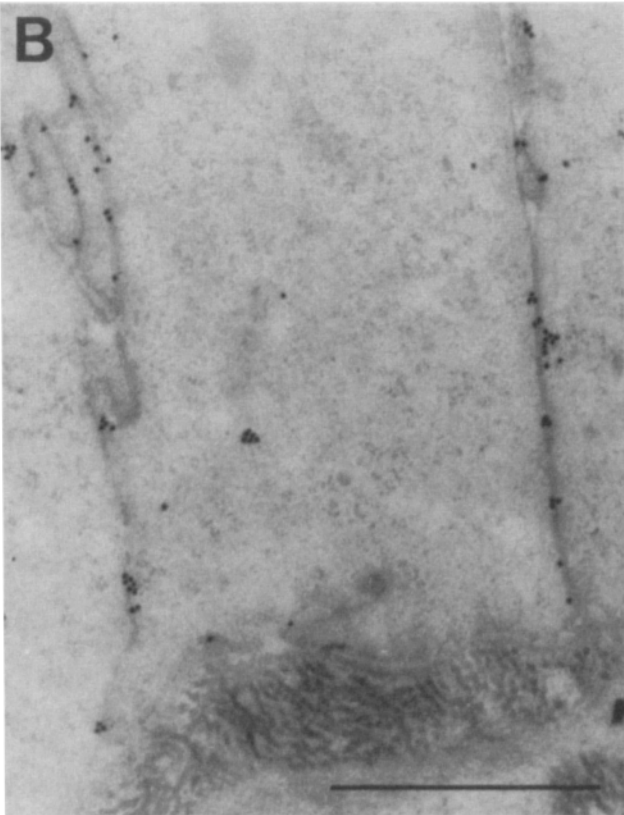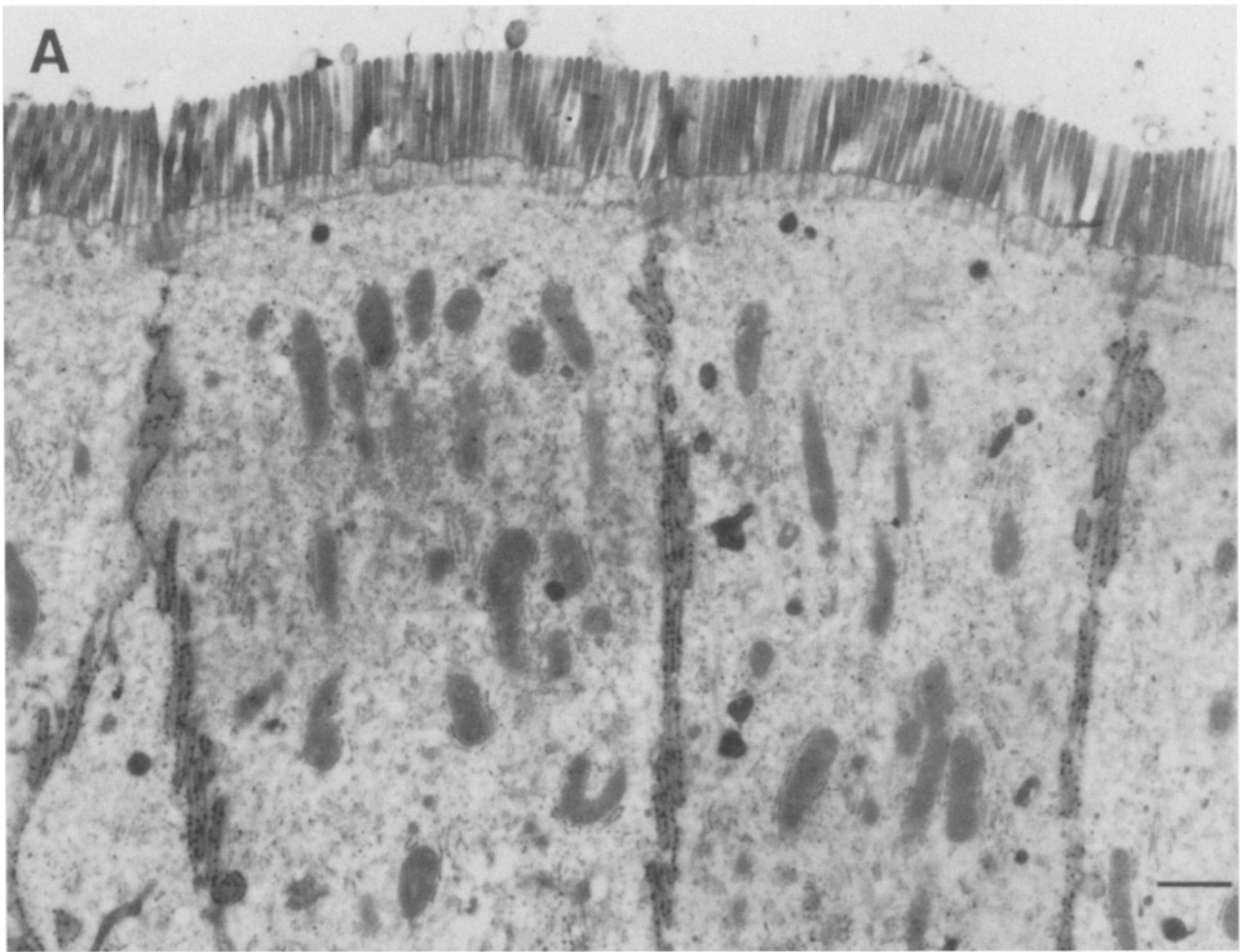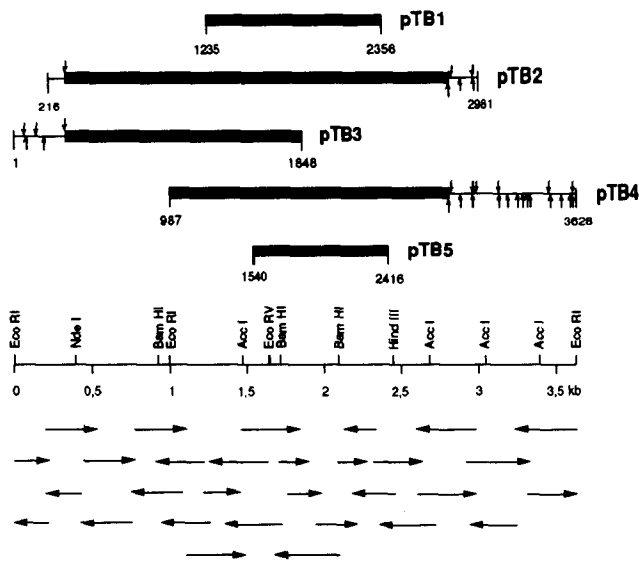
Figure 7. Restriction map and sequencing strategy for cDNA clones of hgp125. The entire inserts of pBluescript clones pTB1, pTB2, pTB3, pTB4, pTB5, and various fragments prepared by appropriate restriction enzyme digestion were sequenced as described in Materials and Methods. (*Black boxes*) Open reading frame of the clones; (*arrows from the top*) start codons and (*arrows from the bottom*) stop codons; (*horizontal arrows*) the extent and the direction of each sequence determination.

cated. In the experiments examining the influence of Ca^{2+} ions or of LI-cadherin–specific antiserum on cell aggregation, aggregation was performed in complete Schneider's medium containing 5 mM $CaCl_2$, or in the same medium supplemented either with 10 mM EGTA or with anti-hgp125 antiserum (1:100), respectively. To measure the extent of cell aggregation, 30-μl aliquots were removed at different times and the number of single cells and aggregates (a cluster of four or more cells) present in the aliquots was counted in a hemocytometer. Small aggregates of less than four cells were regarded as single cells. Aggregation was scored by the index N_t/N_0, where N_t and N_0 are the total numbers of particles at the incubation time t and the incubation time 0, respectively.

Results

Identification of a Plasma Membrane Glycoprotein Involved in Cell–Cell Adhesion

To identify surface proteins involved in cell–cell adhesion of hepatic cells, a panel of antisera directed against eight different plasma membrane glycoproteins—gp80, gp140, gp160, hgp85, hgp105, hgp115, hgp125, and hgp175—purified from rat liver and Morris hepatoma 7777 (Tauber et al., 1983, 1986, 1989) were tested for their inhibitory capacity on cell–cell adhesion of hepatocarcinoma MH 7777 cells. The effect of antibodies on cell–cell adhesion was measured with an assay based on that described by Balsamo et al. (1991) and Walther et al. (1973). MH 7777 cells were used because this cell line has been shown to retain the expression of various hepatocytic surface proteins (Vedel et al., 1983; Loch et al.,

1992). Among the eight antisera that antiserum raised against the plasma membrane glycoprotein hgp125 (Tauber et al., 1989), which was shown to be uniformly distributed on the surface of MH 7777 cells (Fig. 1), inhibited intercellular adhesion of MH 7777 cells by 38% (Fig. 2). In the presence of 3 mM EDTA adhesion was inhibited to a similar extent of 46%. Anti-human transferrin receptor IgG used as a control exhibited no inhibitory effect. MH 7777 cells also expressed uvomorulin/E-cadherin as demonstrated by immunoblotting using polyclonal anti-gp84 IgG raised against the extracellular part of uvomorulin/E-cadherin (Ozawa and Kemler, 1990) (not shown). Antiuvomorulin/E-cadherin inhibited intercellular adhesion of MH 7777 cells by ~28%, indicating that uvomorulin/E-cadherin contributes to the aggregation of MH 7777 cells.

Tissue Distribution and Cellular Localization

Anti-hgp125 IgG immunoreacted specifically with a 120-kD polypeptide with a pI of 4.5 after two-dimensional electrophoresis of rat liver plasma membranes (Fig. 3). Immunoblotting of various tissues with anti-hgp125 IgG showed that apart from the liver, hgp125 is only present in intestine (Fig. 4 A). In homogenates of small intestine, hgp125 was partly cleaved into smaller fragments reacting with the antibody. No fragmentation was observed in samples of small intestine obtained by freeze clamping in situ (Fig. 4 B). In accordance to the results obtained by immunoblotting, hgp125 could be detected by immunomicroscopy of various tissues embedded in LR White® or analyzed as frozen sections solely in liver and intestine. In the liver, hgp125 is localized on the basolateral surface of hepatocytes and could not be detected on the apical, i.e., bile canalicular surface as shown by immunostaining of semithin sections (Fig. 5 A). Likewise, hgp125 was present on the basolateral, but not on the apical surface of enterocytes of small intestine (Fig. 5 B). The same localization was observed in large intestine, whereas hgp125 was not present in the stomach and in the esophagus, as could be shown by both immunoblotting (Fig. 4 A) and immunomicroscopy (not shown).

The surface localization of hgp125 observed at the light microscopic level was confirmed at the ultrastructural level. As shown by immunoelectron microscopy of intestinal epithelial cells, hgp125 is localized at those sites of the lateral surface, where cells are in close contact to each other (Fig. 6 A). On the other hand, hgp125 was almost not detectable on the basal surface, where the cells are in contact with the basal membrane (Fig. 6 B). In addition, hgp125 was absent from the apical surface. No labeling was observed in the junctional complex and in desmosomes (Fig. 6 C).

Molecular Cloning

Anti-hgp125 IgG was used to screen a preamplified rat liver cDNA expression library in λ-ZAPII (2.0 × 10⁶ primary recombinants). In 1.5 × 10⁶ recombinants screened, two

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Figure 6. Immunoelectron microscopic localization of hgp125 in rat intestinal epithelial cells. Sections through LR White®-embedded rat small intestine were labeled with anti-hgp125 IgG and 10 nm gold-conjugated secondary antibody. Section through (*A*) apical and lateral region, (*B*) lateral and basal region, and (*C*) junctional complex of intestinal epithelial cells. *tj*, tight junctions; *aj*, adherens junctions; *ds*, desmosomes. Bars, 1 μm.

GAATTCCATTGGGCATCGTGGGCAAAACTTCCGAACGTCCTCTGCAGAGTCTGGTGTGGAATGACACTGGGCGTGTAGCACCCTGAACTGGCCATCTTGTTTCAGGAAGTGGAAGTCACA 120

GTCTCCCTCCACCGCATGCTCGCCTGTTGCCTCACAGAACAATTTGCTAAGGGGGTTGAAGGGAGAGGTTAGAGGAGAGAGCAGGTTGAGGAAGTGTCTCTTGGGGTTCTTCAGCCACAG 240

CAAGTATCTCCACGGCTGAATGGACGACAACGACTTTTAAGCAGTATTTTCTTACTTACAGAAAGGAGTCCAAGTGAAGAGAGCAACATGGTCTCTGCCCAGCTTCACTTCCTGTGTCTT 360
                                                                                               M V S A Q L H F L C L 11

CTCACGCTTTATTTGACTGGCGCATATGGCCAAGAAGGGAAGTTCAGCGGCCCCCTGAAACCCATGACATTCTCTATTTTTGAAGGCCAAGAACCGAGTCAAATCATATCCAGTTTAAG 480
L T L Y L T G A Y G Q E G K F S G P L K P M T F S I F E G Q E P S Q I I F Q F K 51

GCCAACCCTCCAGCTGTGACTTTTGAGCTAACAGGAGAGACGGATGGTATATTTAAGATAGAAAGGATGGACTTCTTTATCACACAAGAGTCCTGGACAGAGAAACAAGGGCGGTTCAT 600
A N P P A V T F E L T G E T D G I F K I E K D G L L Y H T R V L D R E T R A V H 91
  ⌐→ EC1

CATCTGCAGCTTGCAGCCTTGGATTCTCAGGGAGCTATAGTGGATGGCCCAGTCCCCATCATCATAGAAGTCAAGGACATCAATGACAACCGACCTACATTTCTCCAGACAAAATATGAA 720
H L Q L A A L D S Q G A I V D G P V P I I I E V K D I N D N R P T F L Q T K Y E 131

GGCTCAGTGAGGCAGAACTCTCGCCCAGGAAAGCCTTTCATGTATGTCAATGCTACAGACCTGGATGACCCGGCTACTCCCAATGGCCAGCTTTTTTATCAAATTGTCATCCAGCTTCCC 840
G S V R Q N S R P G K P F M Y V N A T D L D D P A T P N G Q L F I Q I V I Q L P 171
                                        └→ EC1a

AAAATCAACAATGTTATGTACTTCCAGATCGACAACAAAACAGGGGCAATCTCGCTTACCCCAGAAGGTTCTCAGGTATTGGATCCAATAAAGAATCCGTACTACAACCTGGTGGTCTCT 960
K I N N V M Y F Q I D N K T G A I S L T P E G S Q V L D P I K N P Y Y N L V V S 211

GTGAAGGACATGGGGGGCCAGAATGAGAATTCCTTCAGCGATACCACATCTGTGGATATTACTGTCAGAGAGAACATCTGGAAAGCACCAGAGCCTGTGGAGATTCGAGAAAACTTAACT 1080
V K D M G G Q N E N S F S D T T S V D I T V R E N I W K A P E P V E I R E N L T 251

GATCCTCACCCCATCAAAATCACTCAGGTGCAGTGGAATGACCCAGGGGCCCACTATTCCTTAATCAACAAGGAGAAGCTGCCACAGTTTCCATTCTCGATCGACCAAGAAGGAAATATT 1200
D P H P I K I T Q V Q W N D P G A H Y S L I N K E K L P Q F P P F S I D Q E G N I 291
            └→ EC2

TACGTGACTCAGCCTTTGGACCGTGAGGAAAAGGACTCTCATGTTTTCTTTGCCACTGCCAAGGATGAGAATGGAAAACCCCTTGCATATCCACTGGAAATTCGTGTGAAAGTTATTGAC 1320
Y V T Q P L D R E E K D S H V F F A T A K D E N G K P L A Y P L E I R V K V I D 331

ATTAACGACAACCCACCGACATGTCTCTCTCAAGTGACTGTATTTGAAGTCCAGGAGAATGAAGTCTTGGGTAGTAGCATTGGGATCTTTGCTGCCCACGATATGGATGAAGCTAACAAT 1440
I N D N P P T C L S Q V T V F E V Q E N E V L G S S I G I F A A H D M D E A N N 371

ATCAACAGTATTTTGAAGTACAGACTTGTAGACCAAACACCCAAAGTTCCCTCAGATGAACTCTTTCTCATTGACGAATATGGAGGAAAGGTCCAGTTGGGTAAACGGTCCTTGAAGAAG 1560
I N S I L K Y R L V D Q T P K V P S D E L F L I D E Y G G K V Q L G K R S L K K 411
          └→ EC2a

CAAGACAGTCCTCAGTACAACTTAACAGTAGAGGTGTCTGACATAGATTTCAAGACTCTCTGTTCTCTTCAAGTCAACGTTATTGATATCAACGATCAGATTCCCATCTTTGAAAGATCA 1680
Q D S P Q Y N L T V E V S D I D F K T L C S L Q V N V I D I N D Q I P I F E R S 451

GACTATGGAAGCAAGACTCTTTCTGAAGACACAGCCATCGGATCCACCATCTTAATCATCCAAGCTACTGATGATGATGAGCCCTTTACCGGGAGCTCTAAAATTCTGTACAAGATTGTA 1800
D Y G S K T L S E D T A I G S T I L I I Q A T D D D E P F T G S S K I L Y K I V 491
                                                                                   └→ EC3

CAGGGAGACACTGAGGGAAGACTGGAAGTTGTCACAGATCCCATGACCAATACAGGATACGTCAAGATCAGAAAGCCTCTTGATTTTGAAACCGAACCAGTTACTAGCATTGTGTTCAAA 1920
Q G D T E G R L E V V T D P M T N T G Y V K I R K P L D F E T E P V T S I V F K 531

GCAGAAAACCCTGAGCCGCTGGTGAATGGCATAGAGTACAATGCTAGTTCTTTTGCCTCCTTCGAGCTGACCGTGACGGATGTGAATGAAGTGCCTGTATTTCCCAGCAAATATTCCAA 2040
A E N P E P L V N G I E Y N A S S F A S P E L T V T D V N E V P V F P Q Q I F Q 571

GCCAACGTTAGCGAGGATACTGCTATAGGCACTAAAGTGGGCACTGTGACTGCCAGGGATCCGGAAGGCCTGACTGTGAGTTATTCACTGAAAGACAATAAGAGAGGTTGGCTTAAAATC 2160
A N V S E D T A I G T K V G T V T A R D P E G L T V S Y S L K D N K R G W L K I 611
                                               └→ EC4

GACTCCGTCACCGGCGACATATTTAGCACTGCTCCGCTGGACAGGGAAACAGAAAGTGTGTATCGAGTACAAGTGGTGGCCACTGAAGTAGGTGGGTCCTCTCTGAGTTCTACAGCTAT 2280
D S V T G D I F S T A P L D R E T E S V Y R V Q V V A T E V G G S S L S S T A Y 651

TTCCACCTGGTCCTCATGGATGTGAATGACAACCCCCCTCGCCTAGCTAAGGACTACACAGGCTTGTTCTTCTGCCATCCCCTAAGTGCCCCTGGGAGCCTCATCTTTGAAGCCACTGAT 2400
F H L V L M D V N D N P P R L A K D Y T G L F F C H P L S A P G S L I F E A T D 691

GATGACCAGCAGTCAGTTCGGAGGCCCAAGTTTACGTTTGCCCTTGGCAGAGAAAGCTTACAAAGTGACTGGGAAGTGTCCAAAATCAATGGCACACATGCTAGACTCTCCACCAAGCAC 2520
D D Q Q S V R R P K F T F A L G R E S L Q S D W E V S K I N G T H A R L S T K H 731

ACACGCTTTGAGGAACAAGTTTATGACATCCCAATCCTCATCAATGATGGGGGGCCAGCCACCCATGGAAGGCATTGTCTCCTTATCAGTTACTTTCTGCCAGTGTGTGGACGGAAGTTGT 2640
T R F E E Q V Y D I P I L I N D G G Q P P M E G I V S L S V T F C Q C V D G S C 771

TTCCGGCCAGCAGGCAACCAGGTTGGGATACCCACCGTGGGCATGGCAGTTGGTATACTTCTGACCACATTTCTGGTCATTGGTATAATTTTAGCGGTTGTATTCATCCGTATGAGGAAA 2760
F R P A G N Q V G I P T V G H A V G I L L T T F L V I G I I L A V V F I R M R K 811

GATAAAGTTGAAGATCCACAGTCCCCTGAGAATAAGCCTCTGAGGAGCTGAATTTGAACAGAAATGTTCAAGGTTGTACACCAAGCACTGTATCAATGCCTCAGCTGTTTAATTTCATCC 2880
D K V E D P Q S P E N K P L R S *** 827

AAAAATGTGAACTATAATTGGTAGAGTTTATGCATCATGTGCTATATCTGGTCCCCAGCTGTTTAATCTCATAATTTCATCCAAAATGTGAACTATAAATTCTTTGATGGCATGCCTTCTT 3000

GTCCTGCAACACTTAACGCTACTTAGATATTTTATCTCTGTAGACATTAGAGTTACTCTCCATTCCCCCATAACATTTTGCTCCTTTACAAAAGCCTACATAACATGAACTTTCCCTTCT 3120

GGAAAGAAGATGAACTAGGTATAAACCCTGTGTGCTGTTGTTTCAGAGACAAGTCTGTTTTCTCCCAACTGACTTTTCTAGGCTCCACTTATCCCTTTCTGTTTCCACCTGCGCCCCCTG 3240

ATCTCAGCATTACATTAAATTTAAAATGTGTCACACAAAAGAACAAAGTGAAGTCCTTGGGGGGGGTGTTGCTAAGTGGACAGCCTTATTCTTTAGCACAAACAACAGCTTCTCTGTGTT 3360

GTCATCTTTAATAGAGGTCCTCCAGCTTGGCTATGGTGTAGACCCTGGGGAGGTATCAAATACAACTGCCGTGTTTCAAGAAAGACCTACTTCATGAGGCACAGGAACTGACGAGCTGTC 3480

TGGGTTTACTCACTACTCCGTGCTTACATACATGCTGTACATGTTTTATTTGTATATTGAAGTTTTGTTATATATTTATCATGTGGAGGAAAGACGAAAGCATGTACGTGGTGAAGAAAT 3600

CCAAAATAAATGTTGGTTTCAGGAATTC 3628

Figure 8. cDNA and deduced amino acid sequence of rat LI-cadherin. The amino acid sequences determined by direct sequencing of cyanogen bromide fragments are labeled with open boxes. Potential N-glycosylation sites are indicated by circles. The transmembrane domain is underlined with a bold line. The NH2-terminal region is subdivided into six domains termed EC1, EC1a, EC2, EC2a, EC3, and EC4. Cadherin-specific cysteine residues are marked by closed boxes. The putative polyadenylation signal is underlined with a thin line. These sequence data are available from EMBL/GenBank/DDBJ under accession number X78997.
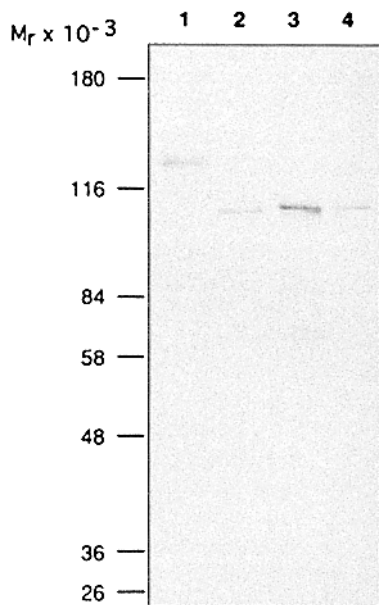
Figure 9. Comparison of fusion protein of clone TB2 expressed in *E. coli* with the glycosylated and N-deglycosylated form of hgp125 from hepatoma plasma membrane. Plasma membranes were isolated from Morris hepatoma 7777 and were either mock digested (lane *1*) or were digested with PNGase F (lane *2*). Lysates of *E. coli* transformants expressing the fusion protein of pTB2 were prepared after induction with isopropyl-β-D-thiogalactopyranoside (lane *3*) and without induction (lane *4*). Samples were separated by SDS-PAGE, transferred to nitrocellulose and were immunostained with anti-hgp125 IgG as detailed in Materials and Methods.

positive clones termed TB1 and TB2 were detected and isolated. Using the insert of clone TB1 as a probe, 1.2 × 10⁶ recombinants of the library were rescreened on the DNA level yielding three additional clones, TB3, TB4, and TB5. Out of λ-phages, the corresponding pBluescript clones (pTB1, pTB2, pTB3, pTB4, and pTB5) were in vivo–excised, and the relationship among the inserts of these plasmids was analyzed by restriction endonuclease mapping (Fig. 7). The five clones were colinear and contained overlapping cDNA inserts, which together spanned a stretch of ∼3.6 kb of cDNA.

Nucleotide and Deduced Amino Acid Sequence Analysis

The inserts of clones pTB1 and pTB2, and those regions of clones pTB3 and pTB4 not contained in clone pTB2 were sequenced on both DNA strands using vector-specific sequencing primers and synthetic primers binding to internal regions of the cloned cDNA. The combined nucleotide sequence of clones pTB1 to pTB5 with a length of 3,628 bp contains an open reading frame of 2,484 bp starting with a methionine codon at nucleotide 328 and terminating with a stop codon at position 2,809. Start codons upstream and downstream the open reading frame are immediately terminated by stop codons. The complete cDNA sequence contains one potential poly(A) acceptor site within its 3'-untranslated region (Fig. 8). The predicted polypeptide encoded by the open reading frame has a calculated molecular weight of 91,857. In line with this prediction, pTB2 containing the full
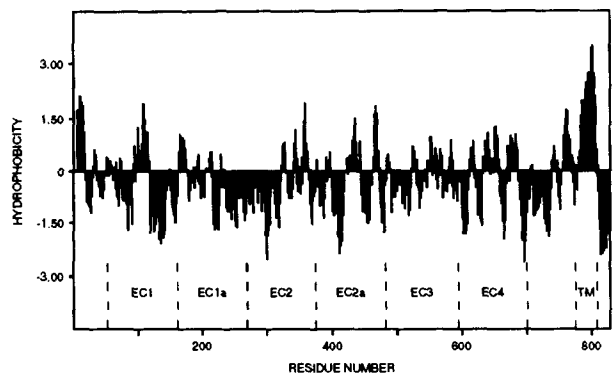
Figure 10. Hydrophobicity plot of the deduced amino acid sequence of LI-cadherin calculated according to Kyte and Doolittle (1982). A window size of 11 residues was used. The relative positions of the extracellular domains EC1, EC1a, EC2, EC2a, EC3, and EC4 and of the transmembrane region TM are indicated.

open reading frame expressed a fusion protein of ∼100-kD in *E. coli* that was recognized by anti-hgp125 IgG (Fig. 9, lanes *3* and *4*). When separated by SDS-PAGE, the fusion protein had a similar molecular mass as compared to the *N*-deglycosylated form of hgp125 from Morris hepatoma 7777 generated by digestion with PNGase F (Fig. 9, lane *2*). The higher molecular mass of the fusion protein as compared to the calculated molecular weight of the predicted protein most likely reflects the remaining 37 amino acid residues of the β-galactosidase part of the fusion protein and the 43 amino acid residues of the noncoding 5'-end of the insert of pTB2.

Hydrophobic analysis (Kyte and Doolittle, 1982) indicates a hydrophobic amino terminal signal sequence of 28 amino acids (Fig. 10) that is followed by a long stretch of a mostly hydrophilic region of 748 amino acid residues, a hydrophobic putative transmembrane sequence of 33 amino acid residues and a short hydrophilic COOH-terminal domain of 18 amino acids. The amino acid sequence deduced from the cDNA completely contains the amino acid sequences of six
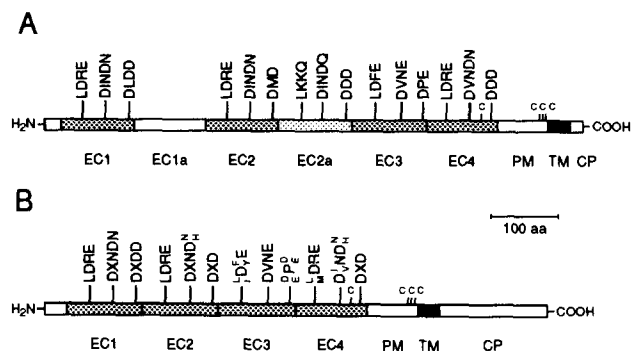
Figure 11. Schematic drawing of the primary structure of LI-cadherin (*A*) and classical E-, P-, and N-cadherins (*B*). Boxes indicate homologous domains. Corresponding extracellular (*EC*), premembrane (*PM*), transmembrane (*TM*), and cytoplasmic (*CP*) domains are shaded in the same pattern. The positions of the four conserved cysteine residues (*C*) are indicated. Conserved sequence motifs are labeled according to the one-letter amino acid code.

Table I. Protein Sequence Comparison of Extracellular Domains of LI-Cadherin

Domains	Percent of amino acid sequence identity				
	EC1a	EC2	EC2a	EC3	EC4
EC1	10	24	17	18	18
EC1a		11	19	12	13
EC2			19	18	23
EC2a				13	17
EC3					17

peptides (Fig. 8) obtained by NH₂-terminal sequencing of CNBr fragments of immunoaffinity-purified hgp125 (Gessner et al., manuscript in preparation) evidencing that the obtained cDNA clones correspond to hgp125. The deduced protein structure contains seven potential N-glycosylation sites. The reduction in the molecular mass of $\sim$20 kD by N-deglycosylation with PNGase F (Fig. 9, lanes *1* and *2*) indicates that all potential N-glycosylation sites are glycosylated assuming a molecular mass of 3 kD per N-linked oligosaccharide (Bartles et al., 1985).

Sequence Homology with Cadherins

Analysis of the deduced protein sequence reveals that the part of the protein NH₂-terminal to the putative transmembrane domain is composed of five domains of internal homology termed EC1, EC2, EC2a, EC3, and EC4, each with a length of 106–111 amino acid residues (Figs. 8 and 11). In line with the definition of a repeated cadherin domain proposed by Ringwald et al. (1987) for uvomorulin/E-cadherin, each of the five domains contains the LDRE motif and the putative calcium-binding sites DXNDN and DXD, or modifications thereof, characteristic for the extracellular repeated domains of cadherin cell adhesion molecules (Takeichi, 1991; Geiger and Ayalon, 1992). The five domains have amino acid sequence identities in between 13 and 24% (Table I). Similar degrees of identity have been reported for the three NH₂-terminal domains of uvomorulin/E-cadherin and P-cadherin (Ringwald et al., 1987; Nose et al., 1987). A domain localized between EC1 and EC2, termed EC1a, had a

similar length of 109 amino acid residues, but neither showed significant homology with the other extracellular domains nor contained one of the three conserved sequence motifs. In addition, four cysteine residues are distributed in a pattern conserved among cadherins. Three cysteine residues are localized in the premembrane domain adjacent to the transmembrane domain, and one in the EC4 domain between the two putative calcium-binding motifs DVNDN and DDD. These structural characteristics indicate that the protein of this study belongs to the cadherin family of cell adhesion molecules. Reflecting its selective expression in liver and intestine, it was termed LI-cadherin. The assumption that the LI-cadherin belongs to the cadherin family is supported by alignment (Fig. 12) and amino acid sequence comparison (Table II) with E-, P-, and N-cadherin from different species. Since the corresponding domains of these cadherins have a different degree of homology, homology was analyzed comparing the four extracellular domains EC1, EC2, EC3, and EC4, as well as the premembrane, the transmembrane, and the cytoplasmic domains (Table II). Among E-, P-, N-, and LI-cadherin, the highest degree of homology is found for the EC1 domain. Averaging the homology of the repeated domains except EC2a, LI-cadherin has the highest degree of overall homology with N-cadherin (Table II).

Despite these similarities, LI-cadherin differs from the classical E-, P-, and N-cadherins in several aspects. First, with respect to the definition of a repeated cadherin domain (Ringwald et al., 1987), the extracellular portion of LI-cadherin is organized into five repeated domains, whereas the classical cadherins have only four homologous repeats. Second, different from E-, P-, and N-cadherin, LI-cadherin has a very short COOH-terminal intracellular domain of 18 amino acid residues (Figs. 11 and 12). Third, the HAV sequence motif in the extracellular EC1 domain involved in the cell-binding function of E-, P-, and N-cadherin (Blaschuk et al., 1990), is replaced by an AAL sequence in LI-cadherin (Fig. 12), similar to a FAL sequence in M-cadherin (Donalies et al., 1991). Fourth, as inferred from the alignment with E-, P-, and N-cadherins, the amino acid sequence of LI-cadherin lacks the prosequence that is present in all other known cadherin precursors (Ringwald et al., 1987; Nose et al., 1987; Hatta et al., 1988; Ranscht and Dours-

Table II. Protein Sequence Comparison of Domains of Individual Members of the Cadherin Family

Comparison*	Percent of amino acid sequence identity							
	EC1	EC2	EC3	EC4	PM‡	TM§	CP‖	av¶
LI:N(c)	42	28	37	34	34	33	39	35.2
LI:N(m)	43	28	39	32	30	33	39	34.9
LI:E(m)	41	25	33	32	16	27	28	28.9
LI:P(m)	39	24	31	32	22	15	33	28.0
N(m):N(c)	95	99	84	87	74	100	99	91.1
N(m):E(m)	58	52	40	46	25	45	64	47.1
N(m):P(m)	53	49	42	47	26	23	57	42.4
N(c):E(m)	57	53	42	45	26	47	63	47.6
N(c):P(m)	52	49	40	45	26	22	57	41.6
E(m):P(m)	68	56	48	53	36	41	80	54.6

* *LI*, LI-cadherin (rat); *N(c)*, N-cadherin (chicken); *N(m)*, N-cadherin (mouse); *E(m)*, E-cadherin (mouse); *P(m)*, P-cadherin (mouse).
‡ Premembrane domain.
§ Transmembrane domain. ‖ Cytoplasmic domain. ¶ Average amino acid sequence identity of EC1, EC2, EC3, EC4, PM, TM, and CP.

```
         Signal peptide
LI-R  MVSAQLHFLCLLTLYLTGAYGQEGKFSG
N-C   MCRIAGTPPRILPPLALMLLAALQQAPI
N-M   MCRIAGGRGTLLPLLAALLQASVEASGE
E-M   MGARCRSFSALLLLLQVSSWLCQELEP.
P-M   MELLSGPHAFLLLLLQVCWLRSVVSEP.
            +        +
```

```
         Precursor segment
LI-R  ..............................................................................................
N-C   KATCEDMLCKMGFPEDV.HSAVVSRSVEG.GQPLLNVRPQSCDENRKIYPGSSEPEDFRVGEDGVVYAERSFQLSAEPTEFVVSARDKETQEKWQMKVKLT.PEPAFTGASEKDQKKIEDIIFPWQQYKDSSHLRQKR
N-M   .....IALCKTGFPEDV.YSAVLPKDVHE.GQPLLNVKFSNCNRKRKVQYESSEPADFKVDEDGTVYAVRSFPLTAEQAKFLIYAQDKETQEKWQVAVNL.SREPTLTREPMKEPHEIEEIVFPRQLAKHSGALQRQKR
E-M   ......ESCSPGFSSEV.YTFFVPEHRLERGHVLGRVRFEGCT.GRPRTAFFSEDSRFKVATDGTITVKRHLKLHKLETSFLVRARDSSHRE.LSTKVTLKSMGHEHHRHEHRDPASESNPELLNFPSVIPG.LRRQKR
P-M   ........YRAGFIGEAGVTLEVEGTDLEPSQVLGKVALAGQG.................................................MHHADNGDIIMLTRGTVQGGKDAMHSPPTRILRRRKR
```

```
         N-terminal domain
LI-R  ....HLKPMTFSIFEGQEFSQ....IIFQFKA...
N-C   DWVIFPINLPENSR.GPFPQELVR.IRSDRDKSLS
N-M   DWVIFPINLPENSR.GPFPQELVR.IRSDRDKNLS
E-M   DWVIFPISCPENEK.GEFPKNLVQ.IKSNRDKETK
P-M   ENVMFPIFVPENGK.GPFPQRLNQ.LKSNKDRGTK
         +        + +    +
```

```
         Extracellular domain 1 (EC1)
LI-R  NPPAVTFELTGET.DGIFKIEKD......GLLYHTRV...LDRE.....TRAVEHLQLAALGHQQAIVDG.........FVPIIEVKIDNDNRPTFLQTKYEGSVRQNSRFGKPFN...YVNATDLGD.FATPNGQ
N-C   LRISVTGPGADQPPTGIFIINPIS.....SQLSVTKP...LDRE.....QIASFHLRAEAVDVNGNQYEN.........FIDIVINYIDNDNRRPEFLEQVWNGTVPEGSKFQTYVN...TFTAIDADD.FNAQNGM
N-M   LRISVTGPGADQPPTGIFIINPIS.....SQLSVTKP...LDRE.....LIARFHLRAEAVDINGNQYEN.........FIDIVINYIDNDNRPEFLEQVWNGSFVPEGSKFQTYVN...TFTAIDADD.FNALNGM
E-M   VFYSITGQGADKPPVGVFIIERET.....GWLKVTQP...LDRE.....AIAKYILYSHAVSSNGEAVED.........PMEIVIFVTDQNDNRPEFTQEVFEGSVAEGAVFGTSVK...NVSATDADDVNTYNAA
P-M   IFYSITGPGADSPPEGVFTIEKES.....GWLLLHMP...LDRE.....KIVKYELYGHAVSENGASVEE.........PMNIEIIVTDQNDNKFKFTQDITRGHVLEGVMFGTSVK...QVTATDEDDAVNTYNGV
         +      +  +  +       +           ++++          +  +     + +        + +   +  +++ +          ++   +       +  +  +  +
```

```
         Extracellular domain 1a (EC1a)
LI-R  LFYQIVIQLPKINNVNYFQIDNKT.....GAISLTPEGSQVLDP....IKNPTYNLVVSVKDMGGQNENSFSD......TTSVDITVRENIWKAPEPVEIRENLTDPHPIKI.TQV.............QWNDPGAH
```

```
         Extracellular domain 2 (EC2)
LI-R  YSLINHEKLFQFFF....SIDQE......GNI.YVTQP..LDRHEKDSH.VFP....ATAKDENGKPLAYPLEI.........RVKVIDINDNRPTCLSQVTVPHVQNNEVLGSSIG...IPAAHSMDE.ANNINSI
N-C   LRYRILSQAPSSPSPNMFTINNET.....GDIITVAAG..LDRE.....KVQQYTLIIQATDMEGNEFTY.GLSN.....TATAVITFTDVNDNPPEFTAMTFYGEVPEH.RVDVIVANL.TVT..DKDQ.FHTPAWN
N-M   LRYRILSQAPSTPSPNMFTINNET.....GDIITVAAG..LDRE.....KVQQYTLIIQATDMEGNEFTY.GLSN.....TATAVITFTDVNDNPPEFTAMTFYGEVPEH.RVDVIVANL.TVT..DKDQ.FHTPAWN
E-M   IAYTIVSQDPELPEKNMFTVNRDT.....GVISVLGSG..LDRE.....SYPTVTLVQAADLQGE....GLST.....TAKAVITFTDVNDNPPEFTNPSTYQGQVPEN.EVNARIATL.KVT..DDDA.PHTPAWK
P-M   VAYSIHSQEFKEFHDLMFTIHKST.....GTISVISSG..LDRE.....KVPEYRLTVQATDMDQE....GSTT.....TAKAVVQILDANDNAPEFEPQKYEAWVPEN.EVGHEVQRL.TVT..DLQV.PHWPAWR
         + +            + +          ++++         + +         + +         + +++ +          + +++ +          + ++       + +
```

```
         Extracellular domain 2a (EC2a)
LI-R  LKYRLVDQTPKVPSDELFLIDEYG.....GKVQLGKRS..LKKQ.....DSPQYNLTVTVEVSDIDFK...........TLCSLQVNVIDINDQIPIFERSDYGSKTLSEDTAIGSTILIIQAT..DDDE.PFTGSSK
```

```
         Extracellular domain 3 (EC3)
LI-R  ILSKIVQGD...TEGKL.EVVFVDVHNT.G.YVKIRKF..LDFE.....TEPVTSIVF.KAE.MFHFLVNHIEIYNASS.FASFELAVTDVNN.VRVFPQQIFQANYSEDTAIGTKVG....TVTAEDPEGL......T
N-C   ARYQHTGGD...PTGQ.FTILEINPNGD.G.LVTVVKF..IDFE.....TNRMFVLTV.AAE.NQVFLAKGIQHFPQE.TATVSIKYIINNK.SFIFVPNPKLVRQEEGLLAGSMLT..TFTAEDPDRIMQQT..S
N-M   AAYRASGQD...PTGR.FAILEINPNGD.G.LVTVVKF..IDFE.....TNRMFVLTV.AAE.NQVFLAKGIQHFPQE.TATVSVTIIINNK.NETFAPNPKIIRQEEGLEAGSMLT..TLTAQDEDRIMQQN...
E-M   AVFTVV.ND...PDQQ.FVVVIDFTNND.G.ILKTA.KG..LDFE.....AKQQYILEV.RVE.NEAPPA.GSLVPS..TATVTVDVVDVNE.APIFMPAERRVEVFEDFGVGQEIT..SYIRAEEDTFNDQ...K
P-M   ATYHIVGGD...DODE.FTITTERKING.QVLTTK.KG..LDFE.....AQDQHTLYV.EVT.NEAPFA.VKLPTA..TATVVHVKDVNE.APFFVPPSKVIEAQEGISIGELVC...IYTAQDKDKE.DQ...K
         +  +          + + +  +   +   + +   +++       +           + +         + +         + ++++ +          +     +     +       ++ +
```

```
         Extracellular domain 4 (EC4)
LI-R  VNYGLK.D...NKRGWKKI...DSVT...GDIFSTAP...LDRETE.SV....YRVQVVAIE.VGGSSLES........TAYFHLVLMDVNDNPPRLAKDYTGLFFCHPLHAPGSL....IFEATDDDQQSVRRFKF
N-C   LRYSKLSD....PANWLKI...DFYN...SQITTTAV...LDRESI.YVQNNMYNATFLASD.NGIPPMSG........TGTLQIYLLDINDNAPQVNPKEATT..QETLQ.FNAI...NITHVDPHIDFNAGFFA
N-M   IRYTKLSD....PANWLKI...DFYN...SQITTIAV...LDRESP.YVQNNIYNATFLASD.NGIPPMSG........TGTLQIYLLDINDNAPQVLPQEAET..QETPE.FNSI...NIAALDYRIDFNAGFFA
E-M   ITYRIWRD....TANWLKI...NPET...GAIFTRAE...MDREDAEHVKNGTFVALIIATD.DQSPIATG........TGTLLLVVLDVNDNAPIPEPRNMQF..CQR.N.FQPH...IITILDPDLPFNTSFFT
P-M   IGTTISRD....PANWKAV...DPDS...GQI.TAAGI...LDRNDEQFVKNNVYEVMVLATD.SENFPTTG........TGTLLLTLTDINDNGFIPEPRQIII..CNQ.S.FVPQ...VLNITHKDLSFNSSFFQ
         + +         ++       +  +         +++      + +         +         + + ++         + + ++ +          +        + +      + +       + +
```

```
         Premembrane domain (PM)
LI-R  TFAL..GRESL.QSDW..EVSKIK..GTHARLET.KHTRFEKQV........YDIPILIND.GGQFFMEGIVSLSVTFCQQV...DGSCFKFAG
N-C   FELPD.SPFSI.KRMW..TIVRIS..EDEAQLEL..RIKFLEAGI........YDVFIVITD.SGNFHASSTSVLKVKVCQQD.ING.DQTDVDR
N-M   FDLPL.SPVTI.KRMW..TINRLM..GDFAQLNL..KIKFLEAGI........YEVFIIITD.SGNFFKSNTSILRVKVCQQD.SNG.DQTDVDR
E-M   AELTH.GAS....VNW..TIEYNDAAQESLILQP..RKDLEIGH........IKIBHLKLAD.NQNKD.QVT.TLDVNVCDCHGTVNN.CMK...
P-M   AQLTH.DS.DI....YW..MAEVSE.KQDTVALSL...KKFLKQDT........YDLHLSLGD.HGNRE.QLT.MIRATVCDCHGQVFNDCFRFWK
         +                   +         +                   +            +  +       + +     +
```

```
         Transmembrane domain (TM)
LI-R  NQ.VGIPTVGMAV.GIILTTF.LVIGIILAVVFIRM
N-C   ..IVMAGLGTGAIIAILLCIIILLILV.LMFVV.WM
N-M   ...VGAGLGTGAIIAILLCIIILLILV.LMFVV.WM
E-M   AGIVAAGLQVPAILGILGGILALLLL.LLLLL.FL
P-M   ....G.GFILP.ILGAV...LALLTLL.LALLL.LV
            +       +
```

```
         Cytoplasmic domain (CP)
LI-R  .RKDK.......................................................................................
N-C   KSRDKERQAKQLLIDPEDDVRDNILKYDEEGGGEEDQDYDLSQLQ.QPDTVEFDAIKFVGIRRLDERPIHAE.PQYFVRSAAP
N-M   KSRDKERQAKQLLIDPEDDVRDNILKYDEEGGGEEDQDYDLSQLQ.QPDTVEFDAIKFVGIRRLDERPIHAE.PQYFVRSAAP
E-M   RERTVVKEP..LLP.PEDDTRDNVYYYDEEGGGEEDQDFDLSQLHRGLDAR.PEVT......RNDVAPTLMSVFQYRPRPA..
P-M   RKKRKVKEP..LLL.PEDDTRDNVFYYGEEGGGEEDQDITQLHRGLEAR.PEVVL.....RNDVVPTFIPTFMYRPRPA..
```

```
         Cytoplasmic domain (CP)
LI-R  .................VEDFQSFENKPLRS*
N-C   HPGDIGDFINEGLKAADNDFTAFFYDSLLVFDYEGSGSTAGSLSSLNSSSSSGGEQDYDYLNDWGPRFKKLADMYGGG.DD*
N-M   HPGDIGDFINEGLKAADNDFTAFFYDSLLVFDYEGSGSTAGSLSSLNSSSSGGDQDYDYLNDWGPRFKKLADMYGGG.DD*
E-M   NPDEIGNFIDENLKAADSDFTAFFYDSLLVFDYEGSGSEAASLSSLNGSESDQDQDYDYLNENGNRFKKLADMYGGGEDD*
P-M   NPDEIGNFIIENLKPANTDFTAFFYDSLMVFDYEGSGSDAASLSSLTTSASDQDQDYNYLNEWGSRFKKLADMYGGGEDD*
         ++    +      +
```

Figure 12. Alignment of the LI-cadherin protein sequence with cadherin cell adhesion molecules. Amino acid residues found in LI-cadherin and in at least one of the other cadherins are shaded. Residues maintained in all of the cadherins are marked with a cross. The HAV sequence motif in the EC1 domain is underlined. *LI-R*, LI-cadherin from rat; *N-C* and *N-M*, N-cadherin from chicken and mouse; *E-M*, E-cadherin from mouse; *P-M*, P-cadherin from mouse.

Zimmermann, 1991; Koch et al., 1991, 1992), with the exception of the recently described protocadherins (Sano et al., 1993).

Northern Blot Analysis

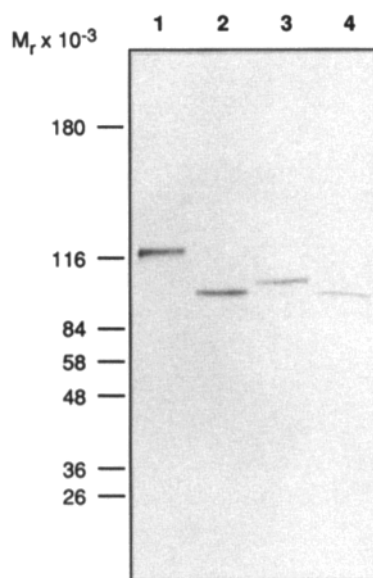The expression of LI-cadherin mRNA in different tissues was examined by Northern blot analysis with the 1.1-kb insert of pTB1 as a probe. LI-cadherin mRNA was detected in the liver and strongly in the intestine, in accordance with the results obtained by immunoblotting and immunomicroscopy. In both tissues, the detected transcript has a size of ~3.9 kb (Fig. 13). Probing of small amounts of RNA showed that the over-exposed band from intestine consists of one single species (not shown).
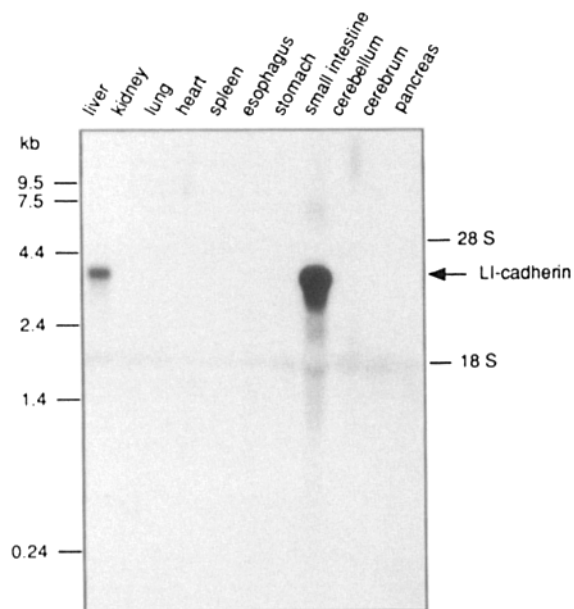
Figure 13. Northern blot analysis. Total RNA (10 μg/lane) isolated from different tissues was size-fractionated on a 1.2% agarose gel containing formaldehyde, blotted onto nylon membranes and hybridized with the random primed insert of clone pTB1. The positions of 18S and 28S ribosomal RNA are also indicated as the fragments of a RNA molecular weight ladder.

Figure 14. Comparison of LI-cadherin expressed in transfected S2 cells with LI-cadherin from hepatoma plasma membranes. Plasma membranes from Morris hepatoma 7777 (lanes *1* and *2*) and membranes from S2/pRmHa-LI cells (lanes *3* and *4*) were either mock-digested (lanes *1* and *3*) or were digested with PNGase F (lanes *2* and *4*). Samples were separated by SDS-PAGE, transferred to nitrocellulose, and stained with LI-cadherin–specific anti-hgp125 IgG as detailed in Materials and Methods.

Heterologous Expression of LI-Cadherin in Drosophila S2 Cells

To characterize its adhesive function, LI-cadherin was expressed in *Drosophila* S2 cells (Schneider, 1972). S2 cells have low endogenous adhesiveness, grow as single, unattached cells, and do not aggregate, even when cell surface glycoproteins known not to function in cell adhesion are overexpressed (Snow et al., 1989). Therefore, S2 cells have been used to study the function of a variety of *Drosophila* cell adhesion molecules and have also been suggested to be used as a tool for the functional characterization of putative vertebrate cell adhesion molecules (Hortsch and Bieber, 1991). The LI-cadherin cDNA insert of pTB2 was subcloned into the pRmHa-3 vector (Bunch et al., 1988) downstream of the metallothionein promoter. This construct (pRmHa-LI) was introduced into S2 cells by cotransfection with the pPC4 plasmid, which contains a *Drosophila* α-amanitin-resistant RNA polymerase II gene (Jokerst et al., 1989). After selection with α-amanitin, resistant cell lines were cloned in soft agar. LI-cadherin expression levels of the clonal cell lines were tested after induction with Cu²⁺-ions by immunoblotting of isolated cell membranes using the LI-cadherin-specific anti-hgp125 IgG. Whereas LI-cadherin was not detectable in untransfected S2 cells (not shown), one clonal line (S2/pRmHa-LI) was isolated expressing LI-cadherin at high levels. LI-cadherin expressed in S2/pRmHa-LI cells had a lower molecular mass of 109 kD (Fig. 14, lane *3*), as compared to LI-cadherin from Morris hepatoma 7777 plasma membranes having a molecular mass of 120 kD (Fig. 14, lane *1*). After removal of N-linked oligosaccharides by digestion with PNGase F, the N-deglycosylated form of LI-cadherin expressed in S2/pRmHa-LI cells comigrated

with the N-deglycosylated form of LI-cadherin from Morris hepatoma 7777 both having a molecular mass of ~100 kD (Fig. 14, lanes *2* and *4*). LI-cadherin expressed in S2 cells is, hence, N-glycosylated in accordance with previous reports that *Drosophila* cells can synthesize and transfer N-linked oligosaccharides onto vertebrate heterologous expressed glycoproteins (Domingo and Trowbridge, 1988; Gibson et al., 1993). The lower molecular mass of 109 kD of LI-cadherin expressed in S2 cells most likely reflects that insect cells are unable to elongate trimmed oligosaccharides to complex side chains (Butters et al., 1981; Hsieh and Robbins, 1984; Kuroda et al., 1990).

LI-cadherin Induces Calcium-dependent Cell Adhesion in S2 Cells

To study whether LI-cadherin mediates cell adhesion, S2/pRmHa-LI cells expressing LI-cadherin were examined for their ability to aggregate. After induction with Cu²⁺ ions, S2/pRmHA-LI cells aggregated in large clusters, whereas uninduced S2/pRmHa-LI cells did not form aggregates even after incubation for 6 h (Fig. 15 *A–C*). To demonstrate that the adhesion of the transfected S2 cells is mediated by LI-cadherin present on the cell surface (Fig. 16), the ability of LI-cadherin–specific antibodies to inhibit aggregation was examined. Induced cells incubated with the LI-cadherin–specific antiserum formed less numerous aggregates than cells treated with preimmune serum (Fig. 17), supporting the notion that LI-cadherin itself is a cell adhesion molecule. Since cadherin cell adhesion molecules have been shown to be Ca²⁺-dependent, the effect of Ca²⁺ depletion on LI-cadherin–mediated cell adhesion was examined. In the presence of EGTA the ability of induced S2/pRmHa-
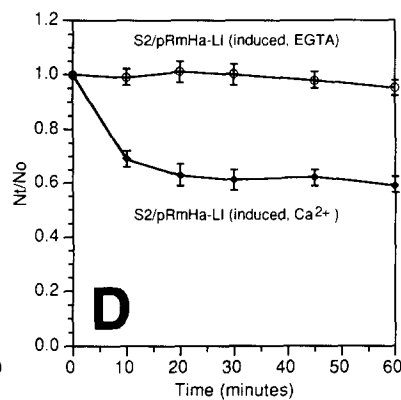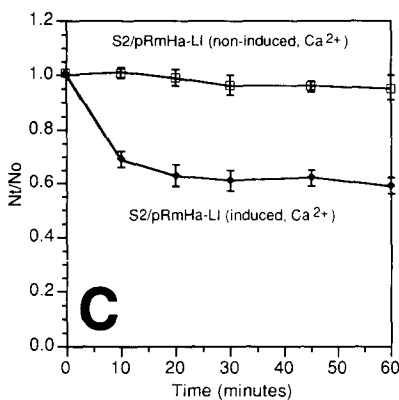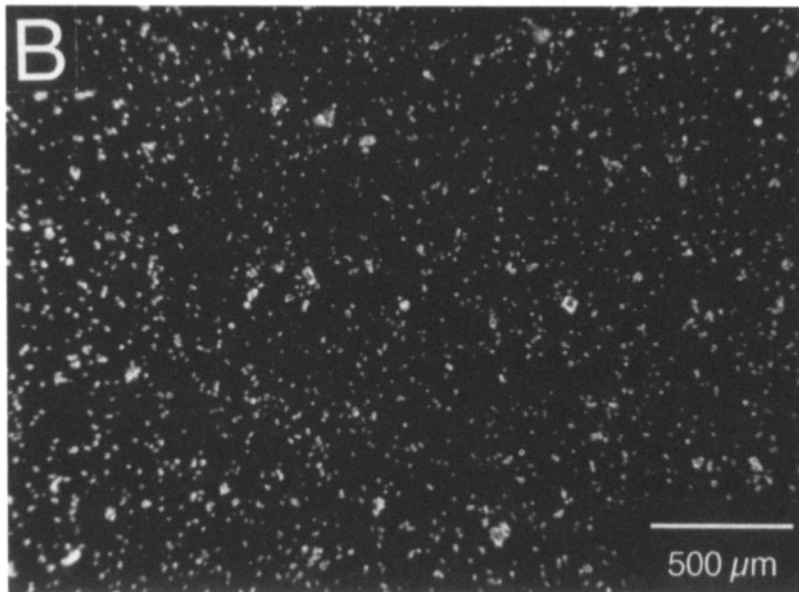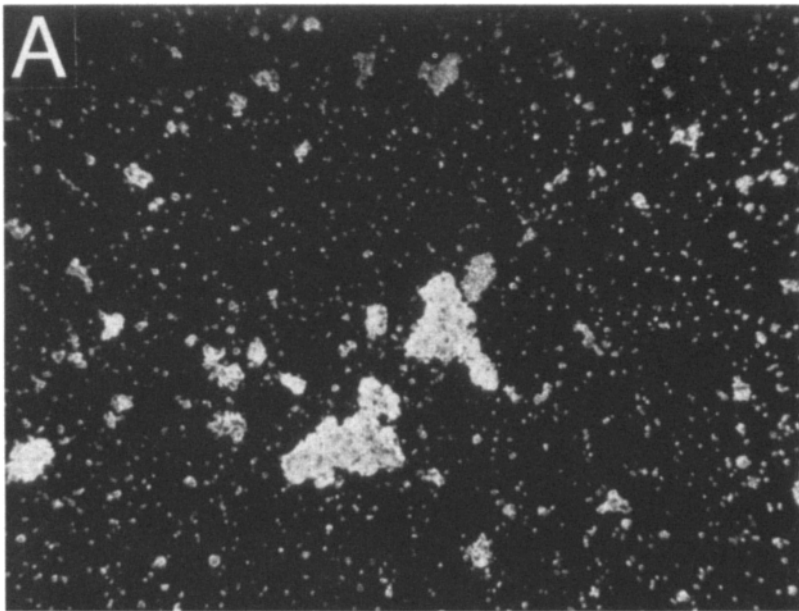
Figure 15. Aggregation of S2 cells transfected with LI-cadherin cDNA. S2/pRmHa-LI cells were cultured in the presence of 0.7 mM $CuSO_4$ to induce the expression of LI-cadherin, resuspended, and allowed to aggregate as described in Materials and Methods. (*A* and *B*) Phase-contrast photomicrographs of induced (*A*) and noninduced (*B*) S2-pRmHa-LI cells after agitation for 6 h. (*C*) Kinetics of intercellular aggregation. Induced and noninduced S2/pRmHa-LI cells were allowed to aggregate for the times indicated. Aliquots were removed and the number of particles was counted. The number of particles at time t (N_t) divided by the number of particles at time 0 (N_0) was plotted versus time. (*D*) Kinetics of cell aggregation of induced S2/pRmHa-LI cells was measured in the presence of either 5 mM Ca^{2+} or 10 mM EGTA as in (*C*).
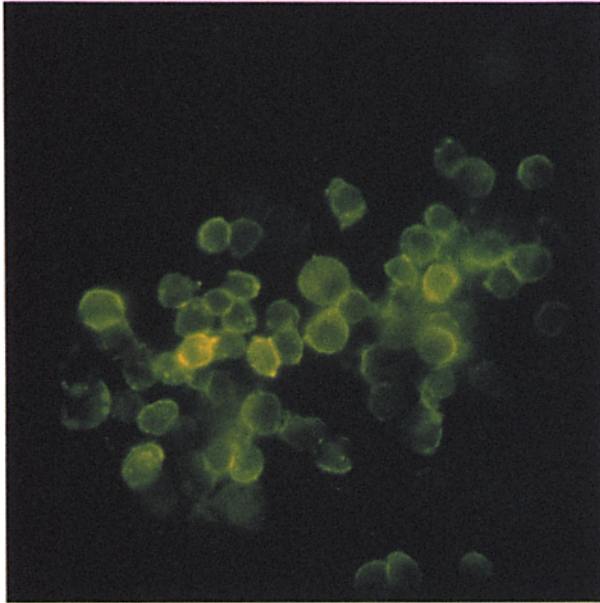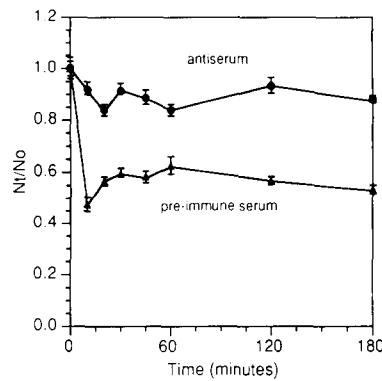
Figure 16. Immunofluorescence localization of LI-cadherin in transfected *Drosophila* S2 cells. Transfected S2 cells expressing LI-cadherin were labeled for indirect immunofluorescence with anti-hgp125 IgG and FITC-conjugated secondary antibody as described in Materials and Methods.

Figure 17. Inhibition of cell aggregation of S2/pRmHa-LI cells by LI-cadherin-specific anti-hgp125 antiserum. Aggregation of induced S2/pRmHa-LI cells was measured as described in the legend to Fig. 15 *C* in the presence of either anti-hgp125 antiserum or of preimmune serum in a final concentration of 1:100 each.

LI cells to form aggregates was completely inhibited (Fig. 15 *D*). In summary, these experiments strongly suggest that LI-cadherin itself mediates Ca²⁺-dependent cell adhesion.

Discussion

We have characterized a novel member of the cadherin family of cell adhesion molecules representing a new subtype within this multigene family.

Four major characteristics make this protein unique among the known cadherins. First, in addition to the four extracellular domains (EC1, EC2, EC3, and EC4) that are equivalent to the corresponding domains of the classical cadherins (Ringwald et al., 1987; Takeichi, 1991), the novel cadherin has two extra domains termed EC1a and EC2a. Similar to EC1, EC2, EC3, and EC4, EC2a is composed of ∼110 amino acids and contains modifications of the LDRE, DXNDN, and DXD motifs. EC1a has a similar length as the five repeated extracellular domains, but does neither contain one of the three conserved sequence motifs, nor shows significant homology with the repeated domains. Carboxy-terminal to EC4, LI-cadherin, as well as the classical cadherins, have another extracellular domain that differs from the preceding homologous domains with respect to both size and lack of the LDRE, DXNDN, and DXD motifs (Fig. 11). In the LI-cadherin, this premembrane domain contains three cysteine residues close to the transmembrane domain equal to the highly conserved three cysteine residues of the classical cadherins (Takeichi, 1991). When compared to classical cadherins from different species (Table II), EC1 is the best conserved domain among the different domains of LI-cadherin. This domain has been shown to be involved in the adhe-

sive function and binding specificities of E- and P-cadherin (Nose et al., 1990). In both E- and P-cadherin, the EC1 domain contains a conserved HAV peptide that together with its flanking amino acids seems to be responsible for the binding specificity observed between individual cadherin subtypes (Blaschuk et al., 1990). In the EC1 domain of LI-cadherin, the HAV motif is replaced by an AAL sequence, similar to M-cadherin having a FAL sequence instead of the HAV motif (Donalies et al., 1991). Similarly, in the case of the desmosomal cadherins, the HAV sequence has been changed to RAL or FAT (Goodwin et al., 1990; Koch et al., 1990, 1991, 1992). Other cadherins such as T-cadherin and cadherin-5 also lack the characteristic HAV sequence (Ranscht and Dours-Zimmermann, 1991; Suzuki et al., 1991). Moreover, LI-cadherin differs from the classical cadherins in its NH₂-terminal region because it lacks both the precursor segment and the endogenous protease cleavage site RXKR (Ozawa and Kemler, 1990) at the COOH-terminal end of this segment.

A second major difference concerns the cytoplasmic tail of LI-cadherin. Whereas among the classical cadherins this domain is highly conserved and consists of ∼150-160 amino acids, it has only 18 amino acids in the LI-cadherin. As has been demonstrated for uvomorulin/E-cadherin, the cytoplasmic domain may mediate binding to the cytoskeleton by interaction with catenins (Ozawa et al., 1989). At least two regions of the cytoplasmic domain are involved in this function. Besides a region within the COOH-terminal 72 amino acid residues responsible for the specific recognition of catenins (Nagafuchi and Takeichi, 1989; Ozawa et al., 1990), a second region of the cytoplasmic domain proximal to the transmembrane domain mediates protein interactions required for cadherin function (Kintner, 1992). The cytoplasmic domain of LI-cadherin exhibits a limited similarity with two regions within the cytoplasmic domain of classical cadherins (Fig. 12). The first four NH₂-terminal amino acids share homology with the region within the cytoplasmic portion of the classical cadherins that is proximal to the transmembrane domain. Furthermore, the last 14 COOH-terminal amino acids exhibit limited similarity to a region within the potential catenin-binding site described for the classical cadherins.

Third, LI-cadherin is also different from the recently described group of protocadherins (Sano et al., 1993) and from cadherin-5, another unconventional cadherin (Suzuki et al., 1991). In the extracellular domain, LI-cadherin contains the four highly conserved cysteine residues typical for classical cadherins, one between the LDRE and DXNDN motifs in the EC4 domain, the other three, which are not found in protocadherins, in the premembrane domain proximal to the transmembrane domain. Another difference concerns the premembrane domain that contains the DRE sequence in cadherin-5 (Suzuki et al., 1991), but not in LI-cadherin. Furthermore, the cytoplasmic domain of LI-cadherin is considerably shorter than that of protocadherins and cadherin-5. In addition, it shows limited similarity to two subdomains within the cytoplasmic tail of classical cadherins, whereas the cytoplasmic region of the protocadherins and cadherin-5 does not show any significant homology with those of known cadherins. Fourth, apart from these structural differences, LI-cadherin is also distinguished from other known cadherins by its tissue distribution. Analysis of mRNA using a 1.1-kb internal sequence of the LI-cadherin open reading frame as a probe, revealed the presence of a 3.9-kb transcript of LI-cadherin solely in the liver and intestine. In both tissues, LI-cadherin is expressed in epithelial cells, i.e., in hepatocytes, and in enterocytes and goblet cells, respectively, as shown by immunolabeling of semithin sections (Fig. 5). LI-cadherin could not be detected in hepatic endothelial cells, similar to uvomorulin/E-cadherin that could not be found in vascular endothelial cells (Heimark et al., 1990). By contrast to uvomorulin/E-cadherin, which is expressed in a wide range of epithelial cells (for review see Geiger and Ayalon, 1992), expression of LI-cadherin seems to be restricted to hepatocytes of liver, and enterocytes and goblet cells of intestine. In both, hepatocytes and enterocytes, LI-cadherin is located on the basolateral surface where adhesive functions predominate, but is absent from the apical cell surface (Fig. 5). On the basolateral surface of enterocytes, LI-cadherin is almost entirely restricted to areas of enterocyte–enterocyte contact. Within these areas, LI-cadherin appears to be uniformly distributed, with the exception of the junctional complex and desmosomes where LI-cadherin cannot be detected. In its extrajunctional localization, LI-cadherin differs from E-cadherin, which is concentrated in adherens junctions (Boller et al., 1985). On the other hand, the extrajunctional localization of LI-cadherin supports previous observations that cadherins are not always entirely restricted to these junctional sites, but may also be present in extrajunctional regions of the basolateral membrane (Gumbiner and Simons, 1987; Gumbiner et al., 1988; Salomon et al., 1992). On the basolateral surface of hepatocytes, LI-cadherin is also localized at sites facing the space of Disse. Presence on the sinusoidal surface of hepatocytes has also been shown for uvomorulin/E-cadherin (Vestweber and Kemler, 1984). The biological significance of the presence of both cadherins on the sinusoidal surface is unknown.

The assumption that LI-cadherin may function as an intercellular adhesion molecule is supported both by the results of the cell adhesion assay using MH 7777 cells that express LI-cadherin physiologically (Fig. 2), and by the transfection experiments in Drosophila S2 cells (Fig. 15). Adhesion of both MH 7777 cells and of transfected S2/pRmHa-LI cells was inhibited by LI-cadherin-specific antibodies, suggesting

that LI-cadherin is directly involved in cell adhesion. To test whether the cell adhesive function of LI-cadherin is Ca^{2+} dependent, the aggregation of LI-cadherin–transfected S2 cells was examined in the presence of EGTA. The same experimental system has been used to study the Ca^{2+} dependence of other cell adhesion molecules in transfected S2 cells (Snow et al., 1989; Elkins et al., 1990). Since aggregation of LI-cadherin transfected S2 cells was inhibited in the absence of Ca^{2+}, cell adhesion activity of LI-cadherin is likely to be Ca^{2+} dependent. Moreover, we have evidence that LI-cadherin has Ca^{2+}-binding activity and is protected from proteolytic cleavage in the presence of Ca^{2+} (Gessner, R., D. Berndorff, N. Lock, P. Bringmann, N. Schnoy, W. Reutter, and R. Tauber, manuscript in preparation).

Drosophila S2 cells proved to be a useful tool for the characterization of the adhesive function of LI-cadherin in this paper, and of other vertebrate cell adhesion molecules (Felsenfeld et al., 1994). The finding that the vertebrate LI-cadherin can mediate intercellular adhesion of invertebrate cells indicates that the adhesive function of LI-cadherin does not depend on the interaction with cytoplasmic cadherin-binding proteins essential for the adhesive function of classical vertebrate cadherins (Ozawa et al., 1990). On the other hand, the functional activity of LI-cadherin in transfected S2 cells might reflect that this vertebrate cadherin may interact with invertebrate catenin homologues that were recently described in Drosophila (Peifer et al., 1992; Oda et al., 1993). The interaction of LI-cadherin with cytoplasmic proteins in MH 7777 cells and Drosophila S2 cells is under current investigation.

In conclusion, our findings suggest that LI-cadherin represents a novel type of cell adhesion molecule within the cadherin multigene family, different from the classical cadherins such as E, P-, and N-cadherin, the GPI-anchored T-cadherin as well as the recently described group of protocadherins and cadherin-5. This novel cadherin is expressed in adult liver and intestine, and may have a role in the morphological organization of these two tissues. Moreover, with respect to the role of cadherin cell adhesion molecules in morphogenesis, LI-cadherin might be involved in the development of the liver and intestine during embryogenesis.

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