

The Roles of Catenins in the Cadherin-mediated Cell Adhesion: Functional Analysis of E-Cadherin- α Catenin Fusion Molecules

Akira Nagafuchi,* Satoru Ishihara,*[†] and Shoichiro Tsukita*^{‡§}

*Laboratory of Cell Biology, Department of Information Physiology, National Institute for Physiological Sciences, Myodaiji-cho, Okazaki 444; [†]Department of Physiological Sciences, School of Life Sciences, The Graduate University of Advanced Studies, Myodaiji-cho, Okazaki 444; and [§]Department of Medical Chemistry, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Abstract. The carboxyl terminus-truncated cadherin (nonfunctional cadherin) has no cell adhesion activity probably because of its failure to associate with cytoplasmic proteins called α and β catenin. To rescue this nonfunctional cadherin as adhesion molecules, we constructed three cDNAs for fusion proteins between nonfunctional E-cadherin and α catenin, nE α , nE α N, and nE α C, where the intact, amino-terminal and carboxy-terminal half of α catenin, respectively, were directly linked to the nonfunctional E-cadherin, and introduced them into mouse L cells. The subcellular distribution and cell adhesion activity of nE α and nE α C molecules was similar to those of intact E-cadherin transfectants: they bound to cytoskeletons, were concentrated at cell-cell adhesion sites and showed strong cell adhesion activity. nE α N molecules, which also bound to cytoskeletons, showed very poor cell adhesion activity. Taken together, we conclude that

in the formation of the cadherin-catenin complex, the mechanical association of α catenin, especially its carboxy-terminal half, with E-cadherin is a key step for the cadherin-mediated cell adhesion. Close comparison revealed that the behavior of nE α molecules during cytokinesis was quite different from that of intact E-cadherin, and that the intercellular motility, i.e., the cell movement in a confluent sheet, was significantly suppressed in nE α transfectants although it was facilitated in E-cadherin transfectants. Considering that nE α was not associated with endogenous β catenin in transfectants, the difference in the nature of cell adhesion between nE α and intact E-cadherin transfectants may be explained by the function of β catenin. The possible functions of β catenin are discussed with a special reference to its role as a negative regulator for the cadherin-mediated cell adhesion system.

CADHERINS are a family of integral membrane proteins responsible for Ca²⁺-dependent cell-cell adhesion. They include many subtypes such as E-cadherin/uvomorulin, N-cadherin/A-CAM, P-cadherin, L-CAM, R-cadherin, etc. and are thought to be important regulators for various morphogenetic cell behaviors (Takeichi, 1991). Many novel observations have been made on the structures and functions of cadherins (Nagafuchi et al., 1993). One characteristic aspect of cadherins is that the cytoplasmic domain is the most highly conserved in amino acid sequences among the family members (Hatta et al., 1988; Suzuki et al., 1991), and that at least two cytoplasmic proteins called α and β catenins are tightly associated with this domain (Ozawa et al., 1989). When the carboxy-terminal region of the cytoplasmic domains of cadherins are deleted, these truncated cadherins can neither work as adhesion molecules (Nagafuchi and Takeichi, 1988) nor bind to α/β catenins (Naga-

fuchi and Takeichi, 1989; Ozawa et al., 1989, 1990). The cadherin molecules localizing at the cell-cell adhesion sites are associated with actin-based cytoskeletons (Hirano et al., 1987), while these nonfunctional truncated cadherins are by no means bound to cytoskeletons (Nagafuchi and Takeichi, 1988; Ozawa et al., 1989). These observations lead to the speculation that cadherins interact with actin-based cytoskeletons via catenins, and that this interaction is essential for them to act as cell adhesion receptors.

The first step for the evaluation of this speculation is to isolate cDNAs encoding catenins and to clarify their structures. The cDNAs encoding α catenin and its neural isotype, α N catenin, were cloned and sequenced (Herrenknecht et al., 1991; Nagafuchi et al., 1991; Hirano et al., 1992). The deduced amino acid sequence revealed that α catenin has a similarity to vinculin, one of the major constituents of adherens junctions (AJ)¹ (Geiger, 1979; Coutu and Craig,

Address all correspondence to Akira Nagafuchi, Dept. of Information Physiology, National Institute for Physiological Sciences, Myodaiji-cho, Okazaki 444, Japan. Tel.: 81 564 55 7851. Fax: 81 564 55 7853.

1. *Abbreviations used in this paper:* AJ, adherens junctions; DAPI, 4',6'-diamidino-2-phenylindole dihydrochloride.

1988). AJ is characterized by a well-developed plaque structure through which actin filaments are densely associated (Tsukita et al., 1992). AJ is subclassified into two types, cell-to-cell and cell-to-substrate, where cadherins and integrins act as adhesion molecules, respectively. In the AJ plaque, vinculin reportedly interacts with cytoskeletal components such as talin (Burridge and Mangeat, 1984), α -actinin (Wachsstock et al., 1987), paxillin (Turner et al., 1990), and vinculin itself (Otto, 1983) to play a key role in linking cadherins or integrins to actin-based cytoskeletons (Otto, 1990). The sequence similarity between α catenin and vinculin suggests that α catenin is also directly involved in the molecular linkage between cadherins and actin-based cytoskeletons. Recently, using human lung cancer cells, PC9, the expression of α catenin was shown to be required for cadherins to work as adhesion molecules (Hirano et al., 1992; Shimoyama et al., 1992; Watabe et al., 1994).

Molecular cloning and sequence analysis of β catenin cDNA clarified its similarity to the gene product of *armadillo*, one of the *Drosophila* segment polarity genes (Wieschaus and Riggelman, 1987), and to plakoglobin (McCrea et al., 1991). Most recently, using the *Xenopus* embryogenesis system, the antibody to β catenin was reported to induce a secondary body axis (McCrea et al., 1993). These findings suggest that β catenin is involved in the embryogenesis.

The cadherin-catenin complex reportedly occurs not only in vertebrates but also in *Drosophila* (Oda et al., 1993; Peifer, 1993). This suggests that this complex formation is somehow essential for the cadherin functions in general. Since cDNAs encoding cadherins and catenins are now available, we should apply gene engineering techniques to the clarification of the functions of the cadherin-catenin complex in the cadherin-mediated cell adhesion.

In the present study, we constructed three cDNAs encoding the fusion proteins between nonfunctional E-cadherin and α catenin, nE α , nE α N, and nE α C, where intact and amino- and carboxy-terminal halves of α catenin, respectively, were directly linked to the cytoplasmic end of the nonfunctional cadherin lacking its catenin-binding site. These fusion molecules were expressed in mouse L fibroblasts and the behavior and functions of these fusion molecules were compared with those of intact E-cadherins. The findings obtained here revealed that the mechanical association of α catenin, especially its carboxy-terminal half, with the cytoplasmic domain of E-cadherin is essential for the cadherin-mediated cell adhesion activity, and that β catenin may work as a kind of negative regulator of the cadherin-mediated cell adhesion. We believe that this study will lead to the better understanding of how catenins are involved in the molecular mechanism of the cadherin-mediated cell adhesion.

Materials and Methods

Cells

Mouse L cells (Earle et al., 1943) were grown in DMEM supplemented with 10% FCS. Their transfectants expressing E-cadherin (ELs8, Nagafuchi et al., 1987; EL β 1, Nose et al., 1988), nE α (nE α L2; see below), nE α N (nE α NL28; see below), or nE α C (nE α CL1; see below) were grown in the same medium containing 150 μ g/ml of G418.

Antibodies

The anti- α catenin monoclonal antibody (α 18) was obtained and characterized as described previously (Nagafuchi and Tsukita, 1994). Anti-E-cadherin monoclonal antibody (ECCD-2, concentrated by ammonium sulfate precipitation; Shirayoshi et al., 1986) was a generous gift from Dr. M. Takeichi (Kyoto University, Kyoto, Japan).

To obtain anti- β catenin mAbs, a cDNA fragment encoding amino acids 104–664 of mouse β catenin (Butz et al., 1992) was isolated using the PCR method. It was then inserted into the BamHI site of pMAL-cRI (New England BioLabs, Beverly, MA), and MBP- β catenin fusion protein was produced in *Escherichia coli*. Fusion protein was purified using preparative SDS-PAGE as described previously (Nagafuchi et al., 1991). Using the purified fusion protein as antigens, anti- β catenin mAb, 2D4, was obtained in rats according to the procedure described previously (Nagafuchi and Tsukita, 1994).

Constructs and Transfections

We constructed pBATE α , pBATE α N, and pBATE α C, expression vectors for nE α , nE α N, and nE α C molecules, respectively. For this purpose, we used two plasmids reported previously; (a) pBATEM2, a β actin promoter-based E-cadherin expression vector (Nose et al., 1988), and (b) pSK102B, which contains a 3.0-kb EcoRI fragment of the α catenin/CAP102 cDNA where the whole open reading frame (ORF) of α catenin is included and PstI-BglII adaptor is inserted into the PstI site just above the initiation methionine codon (Nagafuchi and Tsukita, 1994). For the production of pBATE α , the ClaI-XbaI fragment of pBATEM2 which encodes 71 aa catenin-binding domain of E-cadherin polypeptides was replaced with a 3.0-kb BglII-XbaI fragment of pSK102B including whole ORF. BglII and ClaI sites were blunt-ended before ligation. For the pBATE α N construction, pBATE α was digested with ClaI and XbaI, blunt-ended then religated. These rearrangements removed the ClaI-XbaI fragment which encodes the carboxy-terminal half of α catenin (aa 509–906). To construct pBATE α C, the ClaI-XbaI fragment of pBATEM2 was replaced with a 1.4-kb ClaI-XbaI fragment removed in the pBATE α N construct.

L cells (5×10^5 cells/3-cm plate) were cotransfected with 1 μ g of each expression vector and 0.05 μ g of pSTneoB (Katoh et al., 1987) for 48 h by lipofection method (Life Technologies, Inc., Grand Island, NY). The cells were then replated on three 9-cm dishes and cultured in the presence of 400 μ g/ml of G418 to select stable transfectants. Colonies of G418-resistant cells were isolated, recloned, and subsequently maintained in complete medium with 150 μ g/ml of G418. We isolated several stable clones for each transfection experiment. Since nE α L2, nE α NL28, and nE α CL1 clones expressed a relatively large amount of nE α , nE α N, and nE α C fusion molecule, respectively, we mainly used them for the demonstrations in this paper.

Gel Electrophoresis, Immunoblotting, and Total Protein Detection

SDS-PAGE was based on the discontinuous Tris-glycine system of Laemmli (1970). Cultured cells were washed in HEPES-buffered saline (HCMF) (Takeichi, 1977) supplemented with 1 mM CaCl₂ (HMF) then homogenized and immediately boiled in SDS-sample buffer (2% SDS, 0.125 M Tris-HCl, pH 6.8, 0.002% Bromophenol blue, 5% 2-mercaptoethanol, and 20% Glycerol). Immunoprecipitates were also dissolved and boiled in the same SDS-sample buffer. The lysate derived from 10^5 cultured cells or the immunoprecipitant derived from 5×10^6 cells were separated by SDS-PAGE and transferred to nitrocellulose sheets electrophoretically.

For immunoblotting, nitrocellulose transfers were incubated with mAbs (ECCD-2, α 18, or 2D4). Antibody detection was performed using an Amersham (Arlington Heights, IL) biotin-streptavidin system with biotinylated anti-rat Ig and NBT-BCIP system.

For the detection of total protein, the nitrocellulose transfer was incubated in phosphate-buffered saline supplemented with 0.3% Tween-20 (PBS-Tween) at 37°C for 30 min and washed three times in PBS-Tween at room temperature. Then the blots were washed with distilled water and incubated in AuroDye forte (Amersham Inc.) overnight.

Immunoprecipitation

Cells harvested from a confluent culture in eight 9-cm dishes were lysed in 16 ml of the extraction buffer (0.5% NP-40, 2 mM CaCl₂, 2 mM

phenylmethylsulfonyl fluoride and 20 $\mu\text{g/ml}$ leupeptin in HCMF), and centrifuged at 100,000 g for 30 min. The cell extract was preabsorbed twice with 100 μl anti-rat Ig agarose beads, then loaded on the column containing the same beads preincubated with 1/100 diluted ECCD-2 antibody. The column was washed extensively with the extraction buffer, followed by 2 ml of distilled water three times, then eluted with 500 μl of 1 M acetic acid two times. This acid eluate was lyophilized and resolved in a SDS lysis buffer to make the sample for electrophoresis.

Immunohistochemistry

Cells cultured on cover slips were fixed with 3.5% formaldehyde solution in HMF for 15 min at room temperature. For the NP-40 extraction, cells were extracted with 0.5% NP-40 in HMF for 15 min before fixation. After rinsing with TBS, the fixed cells were treated with 1% BSA in TBS for 30 min, and subsequently incubated with ECCD-2 antibodies for 30–60 min at room temperature. After extensive washing with TBS, the specimens were incubated with fluorescence-labeled second antibodies diluted with TBS containing 1% BSA for 30 min at room temperature. After washing thoroughly with TBS, the preparation was mounted with paraphenylenediamine to prevent bleaching. Samples were examined and photographed with a Zeiss Axiophot photomicroscope.

For the observation of cells in metaphase, cells were fixed with 2.5% glutaraldehyde in HMF for 30 min at room temperature. To visualize the chromosomes, 1 $\mu\text{g/ml}$ of 4'-diamidino-2-phenylindole dihydrochloride (DAPI) was mixed in the embedding solution.

Detergent Extraction of Cells

Cultured cells were extracted with 2.5% NP-40 in HMF, as described previously (Nagafuchi and Takeichi, 1988). For SDS-PAGE, 4×10^6 cells were lysed in 0.2 ml of the SDS-sample buffer.

Cell Dissociation Assay

Confluent cultured cells ($\sim 4 \times 10^6$ cells per 6-cm dish) were treated with 0.01% trypsin in HMF (TC treatment) or HCMF supplemented with 1 mM EGTA (pH 7.5) (TE treatment) for 30 min at 37°C and dissociated through 10 times pipettings. The extent of dissociation of cells was represented by the index N_{TC}/N_{TE} , where N_{TC} and N_{TE} are the total particle number after the TC and TE treatment, respectively.

Intercellular Motility Assay

Cells on a culture dish were labeled with DiI according to the method developed by Honig and Hume (1986), and dispersed by trypsin treatment. The 1×10^3 labeled cells were seeded on a 6-cm dish with a monolayer of 2×10^6 cells. After 48 h of culture, four sister cells which seemed to be derived from one seeded cell were examined by fluorescence microscopy. For the simplification of description, when the cell line A was seeded on a confluent culture of the cell line B, we called this type of experiment A/B analysis. For the quantification of intercellular motility, intercellular distances of all combinations of two cells (six sets) were measured and added up as Dc. As a control experiment, labeled cells were seeded on the same dish in the absence of a cell sheet. In this case, the intercellular distances were summed up as Dd. The degree of intercellular motility was represented as $(Dc - Dm)/(Dd - Dm)$, where Dm is the minimum value of Dc in tightly-packed four sister cells in nE α L/nE α L analysis. At least 24 independent samples were picked up to determine Dc or Dd for each transfectant cell line.

Results

E-cadherin- α Catenin Fusion Molecules and Their Interaction with Endogenous Catenins

E-cadherin reportedly forms the stable complex with α and β catenins in vivo through its carboxy-terminal region (catenin-binding domain; ~ 70 amino acid residues) (Fig. 1 a). The carboxyl terminus-truncated cadherin as shown in

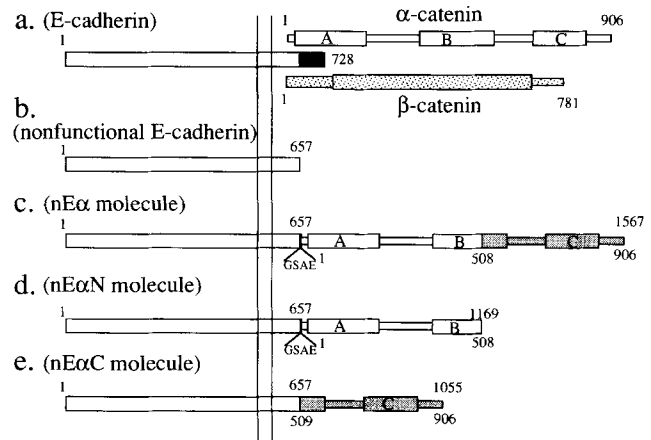


Figure 1. E-cadherin- α catenin fusion protein constructs. (a) Structures of E-cadherin, α and β catenin. The carboxy-terminal 70 amino acids of E-cadherin (closed box) is responsible for the catenin-binding. (b) Nonfunctional cadherin lacking the catenin-binding domain. (c–e) Fusion proteins of the nonfunctional E-cadherin with the full-length α catenin (nE α), the amino-terminal half α catenin (nE α N), or the carboxy-terminal half α catenin (nE α C). Both nE α and nE α N molecules contain an artificial amino acid sequence (GSAAE) at the fusion junction. Note that these fusion proteins lack the catenin-binding domain. Three domains of α catenin (A–C) are regions with similarity to vinculin molecules. A and C domain correspond to the talin binding domain and paxillin/vinculin binding domain in vinculin molecules, respectively.

Fig. 1 b has no ability to bind to either α or β catenin (Ozawa et al., 1989; Nagafuchi et al., 1991). Since this truncated cadherin loses its cell-binding function (Nagafuchi and Takeichi, 1988), it is called nonfunctional E-cadherin. To check whether the covalent association of α catenin molecule with the nonfunctional cadherin rescues the nonfunctional cadherin as adhesion molecules, we constructed three cDNAs for E-cadherin- α catenin fusion molecules, nE α , nE α N, and nE α C, where intact and amino- and carboxy-terminal halves of α catenin, respectively, were directly linked to the carboxy-terminal end of the nonfunctional E-cadherin molecules (Fig. 1, c–e).

The expression vectors for nE α , nE α N, and nE α C were introduced into mouse L cells, which have little endogenous cadherin activity. Cells stably expressing these molecules (nE α L, nE α NL, and nE α CL cells, respectively) were selected and examined by SDS-polyacrylamide gel electrophoresis followed by immunoblotting with anti-E-cadherin mAb, ECCD-2, specific for the extracellular domain of E-cadherin molecule (Fig. 2). The nE α fusion molecule migrated as a ~ 200 kD polypeptide, as would be expected from its construct. The nE α N and nE α C fusion molecules had the apparent molecular masses of ~ 155 and 145 kD, respectively. As Fig. 2 shows and as previously reported (Nagafuchi et al., 1987), no endogenous E-cadherin was detected in L cells (lane 1), while EL cells (lane 2), L cell transfectants with the full-length E-cadherin cDNA, expressed intact E-cadherin molecules with the molecular mass of 124 kD.

Next, we analyzed the interaction of intact E-cadherin, nE α , nE α N, or nE α C with endogenous α and β catenins in each transfectant. As reported previously, when we per-

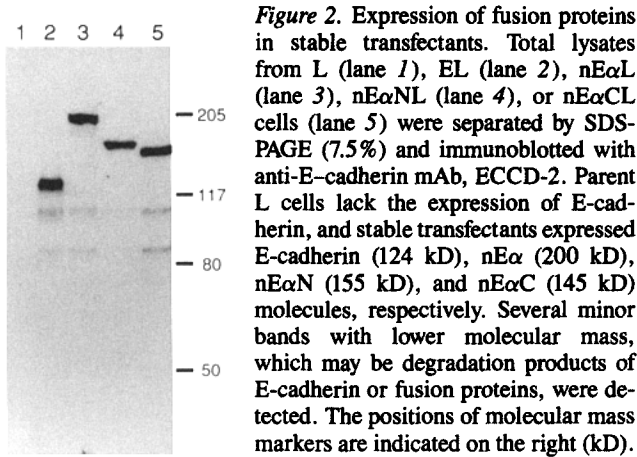


Figure 2. Expression of fusion proteins in stable transfectants. Total lysates from L (lane 1), EL (lane 2), nE α L (lane 3), nE α NL (lane 4), or nE α CL cells (lane 5) were separated by SDS-PAGE (7.5%) and immunoblotted with anti-E-cadherin mAb, ECCD-2. Parent L cells lack the expression of E-cadherin, and stable transfectants expressed E-cadherin (124 kD), nE α (200 kD), nE α N (155 kD), and nE α C (145 kD) molecules, respectively. Several minor bands with lower molecular mass, which may be degradation products of E-cadherin or fusion proteins, were detected. The positions of molecular mass markers are indicated on the right (kD).

formed the immunoprecipitation from EL cells with anti-E-cadherin mAb, ECCD-2, both endogenous α and β catenins were coimmunoprecipitated with intact E-cadherin molecules (Fig. 3, lane 1) (Nagafuchi et al., 1991). In contrast, under the same immunoprecipitation conditions from nE α L, nE α NL, or nE α CL cells, E-cadherin- α catenin fusion proteins were immunoprecipitated showing no association either with α or β catenins (Fig. 3, lanes 2-4). As reported previously (Ozawa et al., 1989; Nagafuchi et al., 1991), plakoglobin, another well-characterized cadherin-associated 83-kD protein (Knudsen and Wheelock, 1992; Peifer et al., 1992; Piepenhagen and Nelson, 1993), was hardly detected in immunoprecipitates from L cell transfectants expressing E-cadherin (data not shown).

In the previous study using anti- α catenin pAb, we showed that the α catenin protein expression is induced by the introduction of intact E-cadherins into L cells, but not by non-functional E-cadherin (Nagafuchi et al., 1991). The immunoblotting using anti- α catenin mAb, α 18, confirmed this induction (Fig. 4 A, lanes 1 and 2). This induction was not detected when nE α N or nE α C molecules were expressed in L cells (Fig. 4 A, lanes 4 and 5). This induction appeared to occur in nE α L cells (Fig. 4 A, lane 3), but whether the 102-kD mAb α 18-positive band is really endogenous α catenin or a degradation product of nE α remains to be elucidated. In immunocytochemical analysis using α 18 antibody, no concentration of α catenin was observed in nE α CL cells

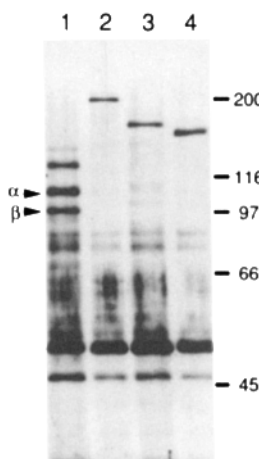


Figure 3. Total protein profiles of the immunoprecipitates with anti-E-cadherin mAb, ECCD-2. For each immunoprecipitation, extracts from 5×10^6 EL (lane 1), nE α L (lane 2), nE α NL (lane 3), or nE α CL (lane 4) cells were used. Only from EL cells were both α and β catenins (β) coimmunoprecipitated with E-cadherin, in the immunoprecipitates from the other transfectants neither α nor β catenins were detected. Most of the lower molecular mass bands are derived from immunoglobulin.

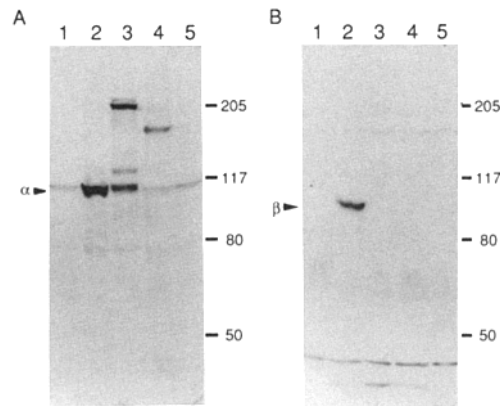


Figure 4. α and β catenin proteins in whole cell lysates. Immunoblots of L (lane 1), EL (lane 2), nE α L (lane 3), nE α NL (lane 4), or nE α CL (lane 5) cells with anti- α catenin mAb, α 18 (A) and anti- β catenin mAb, 2D4 (B). In A, since the epitope for mAb α 18 is located at the amino-terminal half of α catenin, not only α catenin (α) but also the fusion proteins containing the amino-terminal half of α catenin (nE α and nE α N in lanes 3 and 4) were recognized. Both α and β catenin expression was markedly induced by the exogenous intact E-cadherin expression (A and B, lane 2), whereas this induction was not observed in cells expressing fusion proteins. Only in the case of nE α L cells, did the α catenin expression appear to be significantly induced (A, lane 3), but it is not clear whether this 102-kD band is really endogenous α catenin or a degradation product of nE α molecules.

(data not shown). Interestingly, immunoblotting with anti- β catenin mAb, 2D4, revealed that in L cells the expression of β catenin was hardly detected, and the expression level of β catenin was significantly elevated only in EL cells, but not in either nE α L, nE α NL, or nE α CL cells (Fig. 4 B). Considering that among the nonfunctional cadherin, intact cadherin, nE α , nE α N, and nE α C, only intact cadherin molecules have the site for catenin binding, the introduction of the catenin-binding sites into L cells may be required for the induction of the expression of not only α but also β catenin proteins.

The immunoprecipitation experiments gave us no information about cadherins and their fusion proteins at cell-cell contacts which were resistant to the NP-40 extraction. However, considering that in nE α L, nE α NL, and nE α CL cells the expression level of α and β catenin remained very low without significant induction, we can conclude that the NP-40-resistant fusion molecules at cell-cell contacts were not associated with endogenous α and β catenin.

Subcellular Distribution and Cell Adhesion Activity of E-cadherin- α Catenin Fusion Molecules

As we previously reported (Nagafuchi et al., 1987, 1988), immunostaining with anti-E-cadherin mAb, ECCD-2, revealed that in the colony of EL cells, intact E-cadherin molecules were concentrated at the cell-cell adhesion sites in a linear fashion (Fig. 5, a and b), and these concentrated molecules were resistant to the NP-40 treatment showing their cytoskeleton binding (Fig. 5 c). As Fig. 5, d-f show, the behavior of nE α molecules in nE α L cells is similar to that of intact E-cadherin; their linear concentration and cytoskeleton binding. Furthermore, in these characteristics, the be-

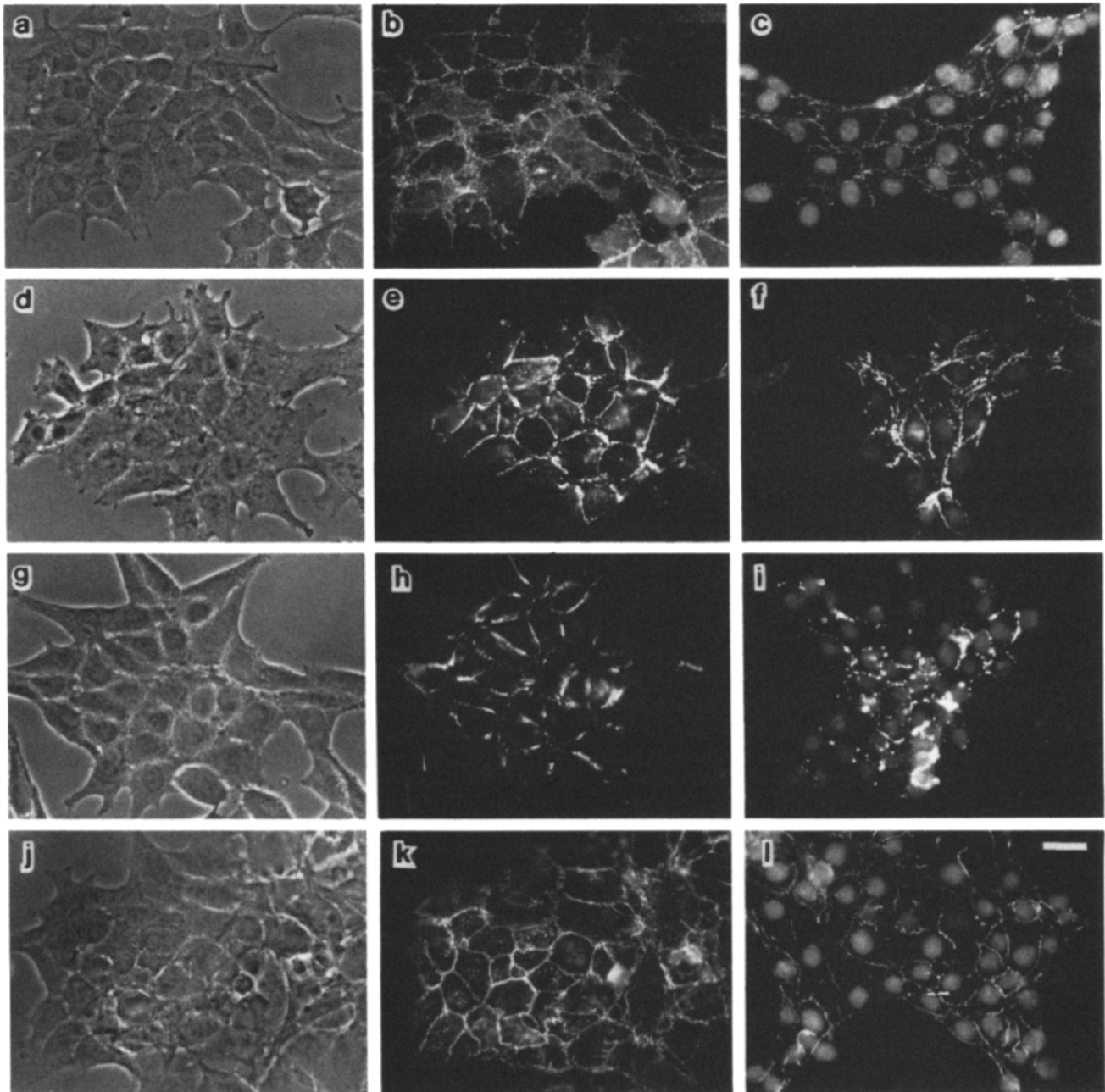


Figure 5. Subcellular distribution of E-cadherin and E-cadherin- α catenin fusion proteins in EL (*a-c*), nE α L (*d-f*), nE α NL (*g-i*), and nE α CL (*j-l*) cells. Phase contrast images of each cell line (*a*, *d*, *g*, and *j*), and accompanying immunofluorescence images of nonpermeabilized cells with anti-E-cadherin mAb, ECCD-2 (*b*, *e*, *h*, and *k*). In *c*, *f*, *i*, and *l*, cells were treated with NP-40 before the ECCD-2 immunostaining. Note that the distribution pattern of nE α N molecules in NP-40-treated cells (*i*) is clearly different from that of other molecules. Bar, 50 μ m.

havior of nE α C molecules was also indistinguishable from that of intact E-cadherins (Fig. 5, *j-l*). In sharp contrast, nE α N molecules, which appeared to be concentrated at the cell-cell borders (Fig. 5 *h*), aggregated as large dots after the NP-40 treatment (Fig. 5 *i*). The immunoblot analysis showed that about half of the intact E-cadherin and E-cadherin- α catenin fusion proteins was not extractable by NP-40 from their transfectants (Fig. 6). It was repetitively reported that most of the nonfunctional E-cadherin molecules with lack of catenin binding site could be extracted with this detergent (Nagafuchi and Takeichi, 1988, 1989; Ozawa et al., 1989).

To quantitatively compare the cell adhesion activity of E-cadherin- α catenin fusion molecules with that of intact E-cadherin, we performed a cell dissociation assay (Fig. 7). In this assay, for each transfectant, cells were treated with trypsin in the presence or absence of Ca^{2+} (TC or TE treatment, respectively) (Takeichi, 1977), dissociated by several times of pipetting, and then the number of particles, whether they are cell clusters or single cells, were counted (N_{TC} or N_{TE} , respectively). Since cadherin-mediated adhesion is reserved after TC but not after TE treatment, the value of N_{TC} is expected to be smaller than that of N_{TE} when cells

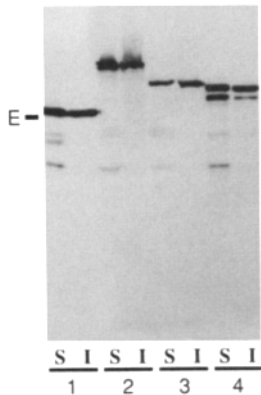


Figure 6. Detergent extraction of E-cadherin or E-cadherin- α catenin fusion proteins. Soluble (*S*) and insoluble (*I*) fractions derived from 2×10^5 EL (lane 1), nE α L (lane 2), nE α NL (lane 3), or nE α CL (lane 4) cells were separated by SDS-PAGE (7.5%) and immunoblotted with anti-E-cadherin mAb, ECCD-2. About half of the intact E-cadherin (*E*) and fusion proteins was detected in the insoluble fractions. Bands with smaller molecular size detected in some lanes are probably products of degradation.

have cadherin activity. In the parent L cells with no cadherin activity, both in the presence and absence of Ca^{2+} , cells were completely dissociated, so that the index $N_{\text{TC}}/N_{\text{TE}}$ was almost 1.0 ($N_{\text{TE}} = N_{\text{TC}}$) (Fig. 7 *a*). This means that in L cell transfectants this index reflects the adhesion activity of E-cadherin or E-cadherin- α catenin fusion molecules; the stronger their adhesion is, the smaller this index becomes. As Fig. 7, *b* and *c* show, nE α molecules had strong cell adhesion activity, which is resistant to trypsin digestion only in the presence of Ca^{2+} to the same extent as did intact E-cadherin molecules on EL cells. For EL and nE α L cells, the above indexes were 0.12 and 0.08, respectively. Interestingly, nE α CL cells also showed strong cell adhesion (index = 0.05; Fig. 7 *e*), whereas the nE α NL cells were easily dissociated even in the presence of Ca^{2+} (index = 0.76; Fig. 7 *d*).

In summary, considering their subcellular distribution, the cytoskeleton-binding ability and the cell adhesion activity, both nE α and nE α C molecules were indistinguishable from intact E-cadherin. Only nE α N molecules did not work well as adhesion receptors and showed peculiar subcellular distribution.

Cell-Cell Adhesion of EL, nE α L, and nE α CL Cells during Cytokinesis

Intact E-cadherin forms a stable complex with α and β catenins, while nE α and nE α C molecules were not associated with endogenous β catenin (see Figs. 1 and 3). However, as far as we had examined, the behavior and functions of nE α and nE α C molecules appeared to be indistinguishable from those of intact E-cadherin. Given that β catenin plays some important role in cadherin-mediated cell adhesion, the nature of cell adhesion of EL cells should be distinguished from that of nE α L and nE α CL cells in some respects. A close comparison between EL cells and nE α L (or nE α CL) cells revealed two clear differences in their cell adhesion behavior.

The first difference was found on their cell adhesion behavior during cytokinesis. Prior to cytokinesis, EL cells were rounded up, so that at a low magnification by phase contrast microscopy, the cells in metaphase were easily identified. In contrast, in the confluent cell sheets of nE α L and nE α CL cells, the cells in metaphase were hardly identified at a low magnification, although the density of dividing cells in these cell sheets was shown by DAPI staining to be similar to that in EL cells (data not shown). Thus, the dividing cells from each transfectant were observed at higher magnification by phase contrast microscopy (Fig. 8). In metaphase, EL cells were rounded up, and their cell adhesion to the surrounding cells appeared to be mostly disrupted, leaving very fine cellular processes in the newly developed intercellular gaps. In contrast, nE α L and nE α CL cells in metaphase were rather flattened, and appeared to fairly retain cell adhesion with surrounding cells. nE α and nE α C molecules were highly concentrated at these retained cell adhesion sites (data not shown).

Intercellular Motility of Transfectants

The second difference between EL and nE α L cells was in their stability of cell-cell adhesion. Time-lapse videomi-

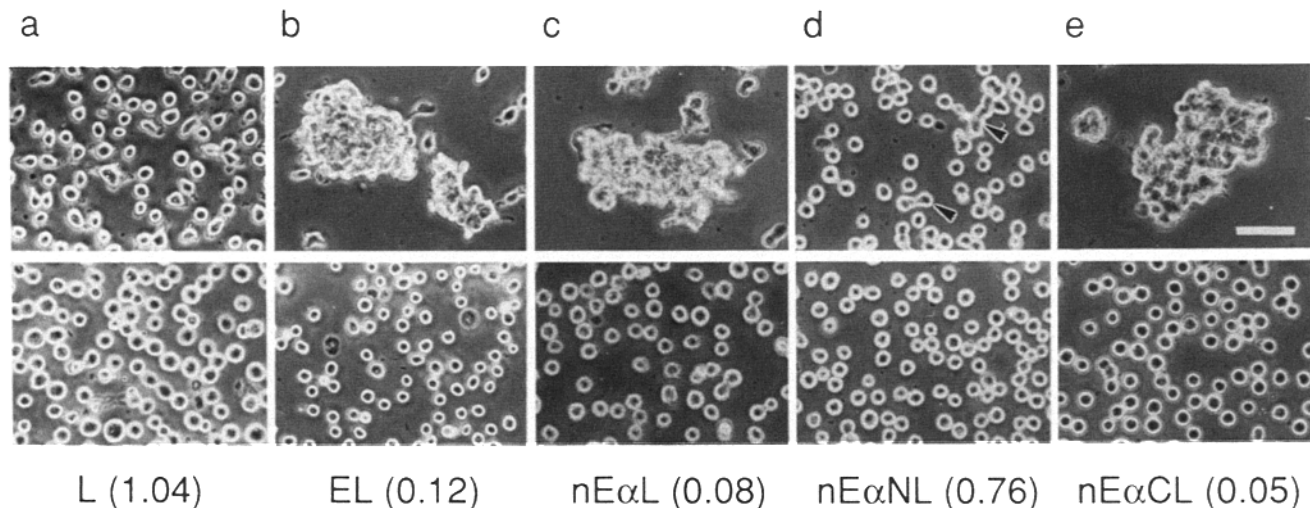


Figure 7. Cell dissociation assay of L (*a*), EL (*b*), nE α L (*c*), nE α NL (*d*), and nE α CL (*e*) cells. The confluent cell sheet of each cell line was treated with 0.01% trypsin in the presence (TC treatment; *top*) or absence (TE treatment; *bottom*) of Ca^{2+} , then dissociated by several times of pipetting. The cell dissociation index ($N_{\text{TC}}/N_{\text{TE}}$; see Materials and Methods in detail) is shown in parentheses. The lower the value of this index, the higher the activity of cell adhesion. nE α NL cells (*d*) were easily dissociated even in the presence of Ca^{2+} , although some small clusters of cells were observed (*arrow head*). Bar, 100 μm .

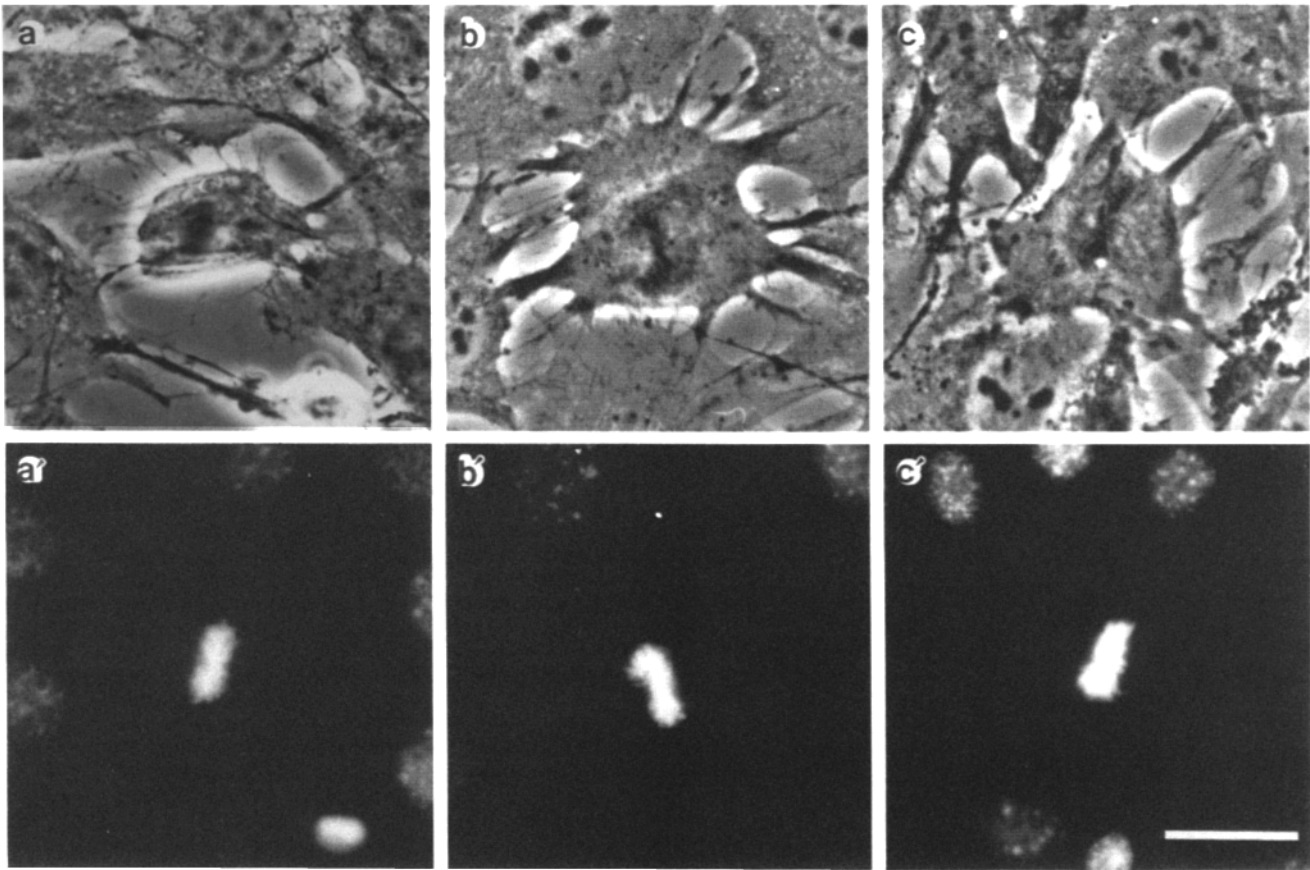


Figure 8. EL (*a* and *a'*), nE α L (*b* and *b'*), and nE α CL (*c* and *c'*) cells at the mitotic phase. Cells were fixed with glutaraldehyde and observed by phase contrast microscopy (*a–c*), and the localization of chromosomes in each cell was presented by the DAPI staining (*a'–c'*). In metaphase, EL cells were rounded up and their cell adhesion with the surrounding cells was mostly disrupted (*a*). By contrast, nE α L (*b*) and nE α CL cells (*c*) in metaphase were rather flat, retaining cell adhesion with surrounding cells. Bar, 50 μ m.

scopy revealed that EL cells repeatedly attach to and detach from the neighboring EL cells when they move actively especially after cytokinesis. By contrast, in nE α L cells, the cell–cell adhesion once established was hardly disrupted even after cytokinesis. To quantitatively describe this difference, we analyzed the intercellular motility of each cell line, i.e., the cell mobility ability in a confluent sheet: when the cell migration ability on the plastic dish is constant, the intercellular motility is thought to be influenced by the nature of cell adhesion. Here we developed a simple assay system for the quantification of the intercellular motility of each transfectant (Fig. 9 *A*).

In this system, a single cell labeled with a fluorescent dye (DiI) was seeded on a confluent culture of nonlabeled cells, and after a 48-h culture (two times the doubling time) the cell scatter property of four labeled sister cells was analyzed measuring the mass distance among these four cells. For the simplification of description, when the cell line *A* was seeded on a confluent culture of the cell line *B*, we called this type of experiment *A/B* analysis. First we compared the intercellular motility between the EL/EL and L/L analysis (Fig. 9 *B*). In the EL/EL analysis, four sister cells were fairly scattered (Fig. 9 *B*, *a*). This indicated that the reposition of EL cells actually occurred even within a confluent cell sheet. Interestingly, in the L/L analysis, the intercellular motility was significantly smaller than that in the EL/EL analysis (Fig. 9

B, *c*). Taking into consideration that EL and L cells showed a similar scattering property on a plastic dish in the absence of a cell sheet (Fig. 9 *B*, *b* and *d*), we have concluded that E-cadherin molecules expressing on the cell surface facilitate the intercellular motility (see Fig. 11).

Next, we compared the intercellular motility between EL, nE α L, and nE α CL cells, all of which showed strong cell adhesion activity (see Fig. 7). In sharp contrast to the elevated intercellular motility in the EL/EL analysis (Fig. 10, *a* and *b*), both the nE α L/nE α L and nE α CL/nE α CL analyses revealed that four labeled sister cells failed to scatter, forming a densely packed colony in a cell sheet (Fig. 10, *d*, *e*, *g*, and *h*). Considering that both EL, nE α L, and nE α CL cells exhibited the same scattering property on a plastic dish (Fig. 10, *c*, *f*, and *i*), we concluded that nE α and nE α C molecules suppressed the intercellular motility, while intact cadherin molecules facilitate it (see Fig. 11). Fig. 11 summarizes the quantitative results of the intercellular motility assay, where the index was calculated as described in Materials and Methods.

Discussion

Cadherins tightly interact with at least two cytoplasmic proteins called α and β catenins. This cadherin–catenin complex is now speculated to be a functional unit for Ca²⁺-depen-

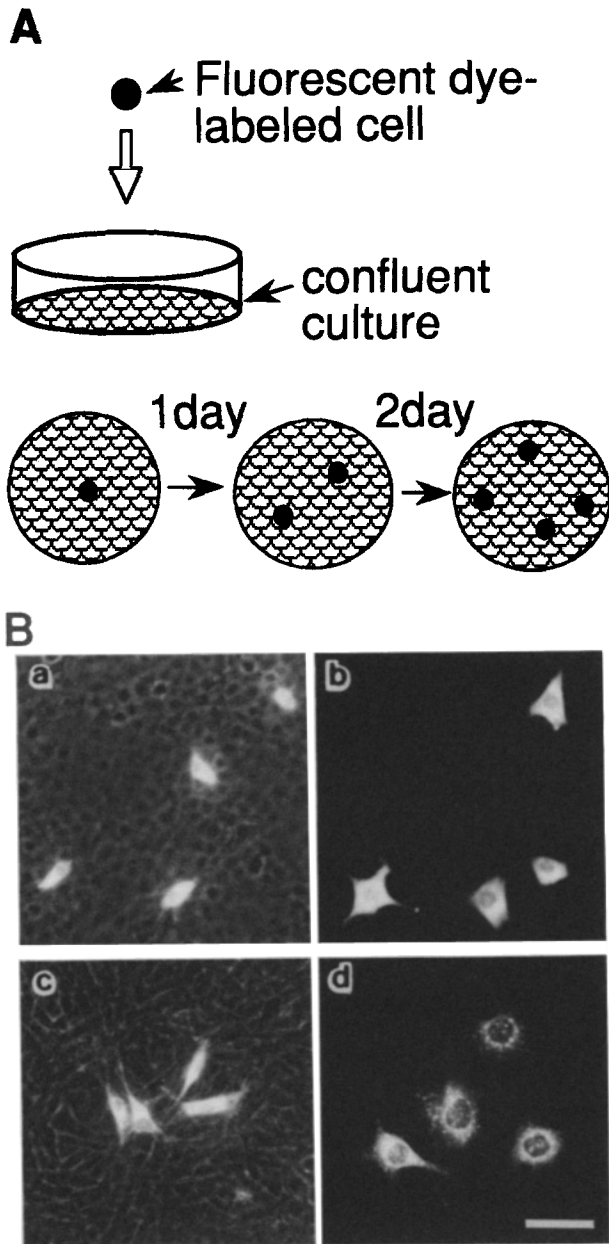


Figure 9. Intercellular motility of L and EL cells. (A) An in vitro assay system for intercellular motility. A single fluorescent dye-labeled cell (closed circle) is put on a confluent sheet of nonlabeled cells. During 2 d, this cell divides twice to produce four labeled sister cells, and the degree of scattering of these four cells is analyzed by fluorescence microscopy. When the cell line A was labeled and put on the sheet of the cell line B, this assay is called A/B analysis. (B) Scattered four sister cells of EL/EL analysis (a) and L/L analysis (c). In a and c, the fluorescence microscopic image of 4 sister cells (in white) were superimposed on the phase contrast image of a confluent cell sheet. Note that the degree of intercellular motility of EL cells is significantly larger than that of the L cells. EL and L cells show a similar scattering property on a plastic dish in the absence of a cell sheet (b and d, respectively). Bar, 100 μm .

dent cell-cell adhesion, because E-cadherin lacking its carboxy-terminal 70 amino acids has no cell adhesion activity or catenin-binding activity (nonfunctional cadherin) (Nagafuchi et al., 1993). If the catenin-binding is really re-

quired for the cell adhesion ability for E-cadherin, the cell adhesion function should be recovered by the covalent association of some parts of catenins with nonfunctional cadherin. Along this line, in the present study, we constructed cDNAs encoding E-cadherin- α catenin fusion molecules and introduced them into mouse L cells.

Functions of α Catenin

The function of α catenin was recently analyzed by the use of the human lung cancer cell line, PC9. PC9 cells showed very poor cell-cell adhesion activity, although they expressed a large amount of E-cadherin on their surface. Immunoprecipitation and immunoblotting revealed that these cells expressed β catenin normally, but lacked the expression of α catenin (Shimoyama et al., 1992). When α or αN catenin, the neural isotype of α catenin, was introduced into these cells, transfectants aggregate in very tight fashion (Hirano et al., 1992; Watabe et al., 1994). These observations indicate that the expression of α catenin is indispensable for the cell adhesion activity of E-cadherin molecules, although its molecular mechanism remained unclear.

The present analysis with E-cadherin- α catenin fusion proteins gave some insights into this molecular mechanism. We have found that the nonfunctional cadherin can be rescued as adhesion molecules by the covalent association of the full length of the α catenin molecule with its carboxy-terminal end. Considering that this fusion molecule, $\text{nE}\alpha$, has the α catenin sequence within itself and has no ability to bind to the endogenous catenins, we can conclude that β catenin is not involved in this rescue. Actually, in PC9 cells lacking the α catenin expression, E-cadherins are associated with β catenin and this E-cadherin/ β catenin complex does not work well as adhesion molecules (Shimoyama et al., 1992). Therefore, we can conclude that in the complex formation between E-cadherin, α and β catenins, the mechanical association of E-cadherin with α catenin is a key step for cadherin-mediated cell adhesion activity.

The nonfunctional cadherin was easily extracted by the NP-40 treatment (Nagafuchi and Takeichi, 1988; Ozawa et al., 1989), while $\text{nE}\alpha$ concentrated at cell-cell adhesion sites was resistant to this treatment, indicating that α catenin molecule has an ability to bind to cytoskeletons. The α catenin molecule has a similarity in amino acid sequence to vinculin. Vinculin reportedly binds to several distinct cytoskeletal proteins such as talin (Burrige and Mangeat, 1984), α -actinin (Wachsstock et al., 1987), paxillin (Turner et al., 1990), and vinculin itself (Otto, 1983). What kind of cytoskeleton is directly associated with α catenin remains to be elucidated.

The analysis of $\text{nE}\alpha\text{N}$ and $\text{nE}\alpha\text{C}$ molecules, where nonfunctional E-cadherin was covalently connected with the amino-terminal and carboxy-terminal half of α catenin revealed more details of α catenin functional domains. Judging from the sequence similarity between α catenin and vinculin (Nagafuchi et al., 1991), the amino- and carboxy-terminal half domain of α catenin correspond to the talin-binding domain (Jones et al., 1989) and paxillin/vinculin-binding domain in vinculin molecules (Molony and Burrige, 1985; Turner et al., 1990), respectively (see Fig. 1). Actually, both $\text{nE}\alpha\text{N}$ and $\text{nE}\alpha\text{C}$ were resistant to the NP-40 treatment, although the subcellular distribution of their NP-40-insoluble

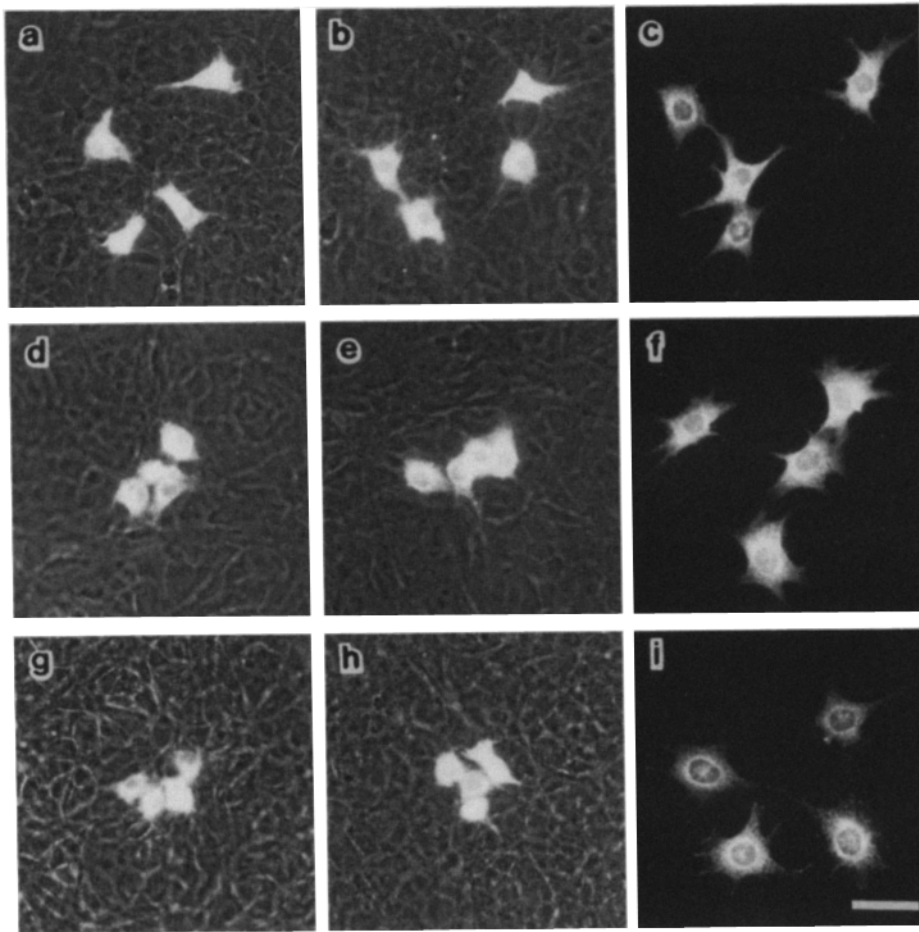


Figure 10. Intercellular motility in EL/EL (a and b), nE α L/nE α L (d and e) and nE α CL/nE α CL (g and h) analyses. In a, b, d, e, g, and h, the fluorescence microscopic image of four sister cells (in white) were superimposed on the phase contrast image of a confluent cell sheet. As compared to the EL/EL analysis, the intercellular motility in nE α L/nE α L and nE α CL/nE α CL analyses was mostly suppressed. These cell lines have a similar scatter property on a plastic dish in the absence of a cell sheet (c, f, and i, respectively). Bar, 100 μ m.

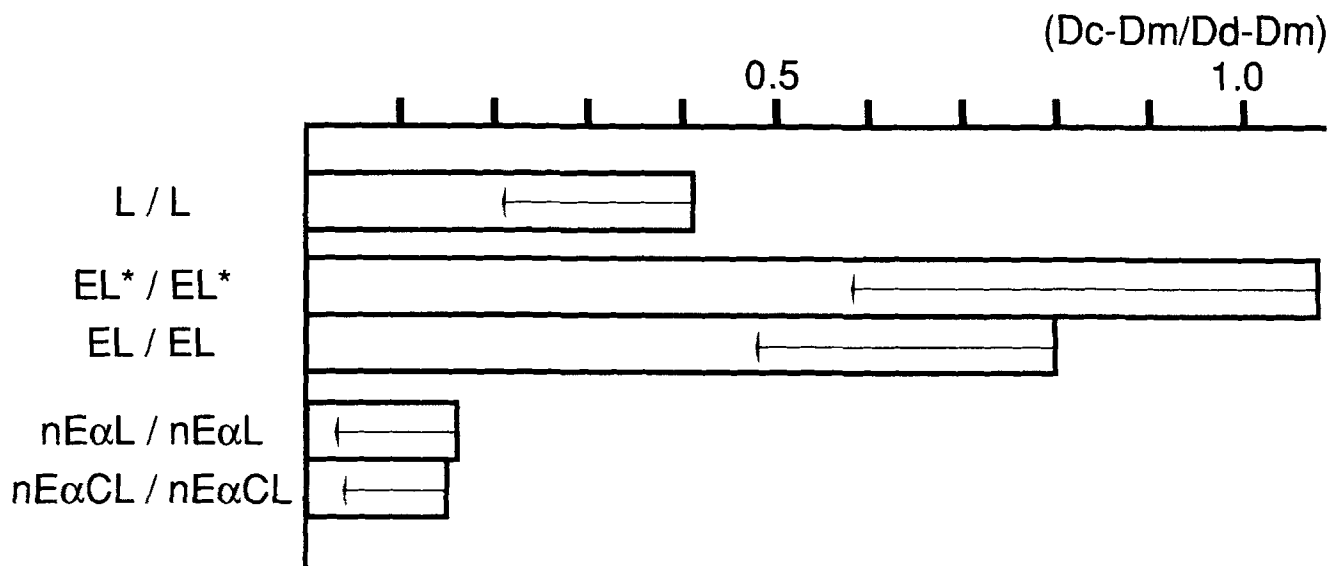


Figure 11. The degree of intercellular motility of L cell transfectants. The higher value in abscissa (intercellular motility index; [Dc-Dm]/[Dd-Dm]; see Materials and Methods in detail) represents the higher degree of intercellular motility. At least 24 independent samples were picked up to determine the intercellular motility index for each analysis. The degree of intercellular motility in L/L analysis is significantly smaller than that of EL/EL analysis and greater than that of nE α L/nE α L or nE α CL/nE α CL analysis ($P < 0.001$). EL*; another clone of the EL cell line (ELs8).

forms was completely different. Considering that nE α C, but not nE α N, showed strong cell adhesion activity typical to cadherin-mediated cell adhesion system, we can conclude that; (a) α catenin interacts with cytoskeletons at least in two distinct manners, at its amino- and carboxy-terminal half domains, and (b) the mechanical association of E-cadherin with the carboxy-terminal half of α catenin is indispensable to cell adhesion activity characteristic to cadherin-mediated adhesion system.

Possible Functions of β Catenin

To our knowledge, nE α and nE α C molecules are the first functional cadherins which do not associate with β catenin. Therefore, the comparison between intact E-cadherin and nE α or nE α C is expected to provide some information on the functions of β catenin in the cadherin-mediated adhesion system.

The difference between nE α L and EL cells was clearly and quantitatively shown in their ability in intercellular motility. The comparison of EL/EL with L/L analysis revealed that intact E-cadherin facilitated the intercellular motility. Time-lapse videomicroscopy revealed that the cell adhesion of EL cells was very dynamic and that they repeatedly attach to and detach from the neighboring EL cells when cells have high mobile activity especially after cytokinesis (data not shown). This repetitive attachment and detachment may allow EL cells to move around in the EL cell sheet. In contrast, the nE α L/nE α L analysis revealed that the scattering of four sister cells in the cell sheet was completely suppressed. Time-lapse videomicroscopy showed that the cell adhesion of nE α L cells was very stable and that the cell adhesion once established appeared to be rarely disrupted (data not shown). This may be the reason why nE α L cells hardly move around inside the nE α L cell sheet. Therefore, in the normal E-cadherin- α/β catenin complex, there seems to be an inside-to-outside regulation mechanism not only to support but also to suppress the cadherin cell adhesion function, and the nE α molecule may lack the latter mechanism. Considering that the nE α molecule has no ability to bind to β catenin, it is reasonable to speculate that β catenin is directly involved in this downregulation mechanism. In this line, it should be noted that the degree of dysfunction of E-cadherin has recently been reported to have a good correlation with the level of tyrosine phosphorylation of β catenin (Matsuyoshi et al., 1992; Behrens et al., 1993; Hamaguchi et al., 1993). The molecular mechanism by which β catenin regulates the cadherin function remains to be elucidated.

We also found that in metaphase the EL cells disrupted their cell-cell adhesion with neighboring cells and that the nE α L cells retained it. This can be rationalized as follows. When cells tend to round-up in metaphase, the dynamic cell adhesion of EL cells was disrupted and allow cells to round-up. By contrast, the stable cell adhesion of nE α L cells is hardly disrupted, which prevents the cells from rounding up.

What is the physiological role of the dynamic aspect of cell adhesion in which β catenin may be involved? The intercellular motility was observed in vivo in the process of morphogenetic rearrangement of cells in embryonic tissues. For example, during gastrulation in early *Xenopus* embryo, cells expressing cadherins are able to change their relative positions when they intercalate during epiboly and convergent extension (Gumbiner, 1992; Schneider et al., 1993). There-

fore, we speculate that β catenin plays an important role in the embryogenesis by endowing cadherins with the dynamic aspect.

β catenin is thought to be a vertebrate homologue of the *Drosophila armadillo* product (McCrea, 1991). The *armadillo* product plays a pivotal role in the determination of the segment polarity, and resides on the *wingless* signaling pathway (Wieschaus and Riggelman, 1987; Perrimon, 1994). Furthermore, in *Xenopus*, the antibody to β catenin was reported to induce a secondary body axis (McCrea et al., 1993). It is not clear whether or not these morphogenetic functions of β catenin are related to cadherins, although expression of Wnt-1, the vertebrate homologue of *wingless* product, has been reported to modulate the cadherin-mediated cell-cell adhesion (Bradley et al., 1993; Hinck et al., 1994). Recently, it was also reported that β catenin directly binds to the tumor suppressor APC protein (Rubinfeld et al., 1993; Su et al., 1993). The whole picture on the function of β catenin remains to be elucidated.

It was reported that plakoglobin, a homologue of β catenin, also associates with cadherin (Knudsen and Wheelock, 1992; Peifer et al., 1992; Piepenhagen and Nelson, 1993) and that β catenin and plakoglobin form mutually exclusive complexes with cadherin competing for the catenin-binding domain (McCrea and Gumbiner, 1991; Hinck et al., 1994). Since plakoglobin was hardly detected in experiments using L cell-based cadherin transfectants (Ozawa et al., 1989; Nagafuchi et al., 1991) and our fusion protein constructs lack the catenin-binding domain, we did not discuss its possible function here. Furthermore, at the cell-cell contacts, various proteins other than catenins or plakoglobin are thought to associate with the cadherin to make the cadherin-catenin complex resistant to the NP-40 extraction (Tsukita et al., 1992). Therefore, we should keep it in mind that the discussion on the possible function of β catenin here may be somehow oversimplified.

In summary, the data presented in this study favor the idea that β catenin works as a kind of negative regulator for the cell adhesion activity mainly exhibited by the mechanically associated E-cadherin and α catenin. If this is the case, many questions remain to be solved. What type of cytoskeletal proteins are directly associated with α catenin, especially with its carboxy-terminal half? How can β catenin regulate the cell adhesion function of the cadherin- α catenin system? Studies are being conducted in our laboratory to answer these questions.

We thank Professor M. Takeichi (Kyoto University, Kyoto, Japan) for the supply of ECCD-2 hybridoma cells and helpful discussions. We are also grateful to Dr. H. Okamoto (Keio University, Tokyo, Japan) for the suggestion about Dil staining and to Ms. T. Yamamoto (National Institute for Physiological Sciences, Okazaki, Japan) for technical assistance for antibody production.

This study was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan, and from the Ministry of Health and Welfare of Japan (to S. Tsukita and A. Nagafuchi).

Received for publication 19 April 1994 and in revised form 21 June 1994.

References

- Behrens, J., L. Vakaet, R. Friis, E. Winterhager, F. V. Roy, M. M. Mareel, and W. Birchmeier. 1993. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/ β -catenin complex in cells transformed with a temperature-sensitive v-SRC

- gene. *J. Cell Biol.* 120:757-766.
- Bradley, R. S., P. Cowin, and A. M. Brown. 1993. Expression of Wnt-1 in PC12 cells results in modulation of plakoglobin and E-cadherin and increased cellular adhesion. *J. Cell Biol.* 123:1857-1865.
- Burridge, K., and P. Mangeat. 1984. An interaction between vinculin and talin. *Nature (Lond.)* 308:744-746.
- Butz, S., J. Stappert, H. Weissig, and R. Kemler. 1992. Plakoglobin and β -catenin: distinct but closely related. *Science (Wash. DC)* 257:1142-1143.
- Coutu, M. D., and S. W. Craig. 1988. cDNA-derived sequence of chicken embryo vinculin. *Proc. Natl. Acad. Sci. USA* 85:8535-8539.
- Earle, W. R. 1943. Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. *J. Natl. Cancer. Inst.* 4:165-212.
- Geiger, B. 1979. A 130K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells. *Cell* 18:193-205.
- Gumbiner, B. M. 1992. Epithelial morphogenesis. *Cell* 69:385-387.
- Hamaguchi, M., N. Matsuyoshi, Y. Ohnishi, B. Gotoh, M. Takeichi, and Y. Nagai. 1993. p60^{V-SRC} causes tyrosine phosphorylation and inactivation of the N-cadherin-catenin cell adhesion system. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:307-314.
- Hatta, K., A. Nose, A. Nagafuchi, and M. Takeichi. 1988. Cloning and expression of cDNA encoding a neural calcium-dependent cell adhesion molecule: its identity in the cadherin gene family. *J. Cell Biol.* 106:873-881.
- Herrenknecht, K., M. Ozawa, C. Eckerskorn, F. Lottspeich, M. Lenter, and R. Kemler. 1991. The uvomorulin-anchorage protein α catenin is a vinculin homologue. *Proc. Natl. Acad. Sci. USA* 88:9156-9160.
- Hinck, L., W. J. Nelson, and J. Papkoff. 1994. Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing β -catenin binding to the cell adhesion protein cadherin. *J. Cell Biol.* 124:729-741.
- Hirano, S., N. Kimoto, Y. Shimoyama, S. Hirohashi, and M. Takeichi. 1992. Identification of a neural α -catenin as a key regulator of cadherin function and multicellular organization. *Cell* 70:293-301.
- Hirano, S., A. Nose, K. Hatta, A. Kawakami, and M. Takeichi. 1987. Calcium-dependent cell-cell adhesion molecules (cadherins): subclass specificities and possible involvement of actin bundles. *J. Cell Biol.* 105:2501-2510.
- Honig, M. B., and R. I. Hume. 1986. Fluorescent carbocyanine dyes allow living neurons of identified origin to be studied in long-term cultures. *J. Cell Biol.* 103:171-187.
- Jones, P., P. Jackson, G. J. Price, B. Patel, V. Ohanion, A. L. Lear, and D. R. Critchley. 1989. Identification of a talin binding site in the cytoskeletal protein vinculin. *J. Cell Biol.* 109:2917-2927.
- Kato, K., Y. Takahashi, S. Hayashi, and H. Kondoh. 1987. Improved mammalian vectors for high expression of G418 resistance. *Cell Struct. Funct.* 12:575-580.
- Kemler, R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.* 9:317-321.
- Knudsen, K. A., and M. J. Wheelock. 1992. Plakoglobin, or an 83-kD homologue distinct from β -catenin, interacts with E-cadherin and N-cadherin. *J. Cell Biol.* 118:671-679.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680-685.
- Matsuyoshi, N., M. Hamaguchi, S. Taniguchi, A. Nagafuchi, S. Tsukita, and M. Takeichi. 1992. Cadherin-mediated cell-cell adhesion is perturbed by v-src tyrosine phosphorylation in metastatic fibroblasts. *J. Cell Biol.* 118:703-714.
- McCrea, P. D., W. M. Briehar, and M. Gumbiner. 1993. Induction of a secondary body axis in *Xenopus* by antibodies to β -catenin. *J. Cell Biol.* 123:477-484.
- McCrea, P. D., C. W. Turck, and B. Gumbiner. 1991. A homolog of the armadillo protein *Drosophila* (Plakoglobin) associated with E-cadherin. *Science (Wash. DC)* 254:1359-1361.
- Molony, L., and K. Burridge. 1985. Molecular shape and self-association of vinculin and metavinculin. *J. Cell. Biochem.* 29:31-36.
- Nagafuchi, A., Y. Shirayoshi, K. Okazaki, K. Yasuda, and M. Takeichi. 1987. Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. *Nature (Lond.)* 329:341-343.
- Nagafuchi, A., and M. Takeichi. 1988. Cell binding function of E-cadherin is regulated by the cytoplasmic domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3679-3684.
- Nagafuchi, A., and M. Takeichi. 1989. Transmembrane control of cadherin-mediated cell adhesion: a 94kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regul.* 1:37-44.
- Nagafuchi, A., M. Takeichi, and S. Tsukita. 1991. The 102 kDa cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. *Cell* 65:849-857.
- Nagafuchi, A., S. Tsukita, and M. Takeichi. 1993. Transmembrane control of cadherin-mediated cell-cell adhesion. *Semin. Cell Biol.* 4:175-181.
- Nagafuchi, A., and S. Tsukita. 1994. The loss of the expression of α catenin, the 102kD cadherin-associated protein, in central nervous tissues during development. *Dev. Growth & Differ.* 36:59-71.
- Nose, A., A. Nagafuchi, and M. Takeichi. 1988. Expressed recombinant cadherins mediate cell sorting in model systems. *Cell* 54:993-1001.
- Oda, H., T. Uemura, K. Shiomi, A. Nagafuchi, S. Tsukita, and M. Takeichi. 1993. Identification of a *Drosophila* homologue of α -catenin and its association with the armadillo protein. *J. Cell Biol.* 121:1133-1140.
- Otto, J. J. 1983. Detection of vinculin-binding proteins with an ¹²⁵I-vinculin gel overlay technique. *J. Cell Biol.* 97:1283-1287.
- Otto, J. J. 1990. Vinculin. *Cell Motil. Cytoskeleton.* 16:1-6.
- Ozawa, M., H. Baribault, and R. Kemler. 1989. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1711-1717.
- Ozawa, M., M. Ringwald, and R. Kemler. 1990. Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proc. Natl. Acad. Sci. USA* 87:4246-4250.
- Peifer, M. 1993. The product of the *Drosophila* segment polarity gene *armadillo* is part of a multi-protein complex similar to the vertebrate adherens junction. *J. Cell Sci.* 105:993-1000.
- Peifer, M., P. D. McCrea, K. J. Green, E. Wieschaus, and B. M. Gumbiner. 1992. The vertebrate adhesive junction proteins β -catenin and plakoglobin and *Drosophila* segment polarity gene *armadillo* form a multigene family with similar properties. *J. Cell Biol.* 118:681-691.
- Perrimon, N. 1994. The genetic basis of patterned baldness in *Drosophila*. *Cell* 76:781-784.
- Piepenhagen, P. A., and W. J. Nelson. 1993. Defining E-cadherin-associated protein complexes in epithelial cells: plakoglobin, β -catenin and γ -catenin are distinct components. *J. Cell Sci.* 104:751-762.
- Rubinfeld, B., B. Souza, I. Albert, O. Muller, S. H. Chamberlain, F. R. Masiarz, S. Munemitsu, and P. Polakis. 1993. Association of the APC gene product with β -catenin. *Science (Wash. DC)* 262:1731-1734.
- Schneider, S., K. Herrenknecht, S. Butz, R. Kemler, and P. Hausen. 1993. Catenins in *Xenopus* embryogenesis and their relation to the cadherin-mediated cell-cell adhesion system. *Development* 118:629-640.
- Shimoyama, Y., A. Nagafuchi, S. Fujita, M. Gotoh, M. Takeichi, S. Tsukita, and S. Hirohashi. 1992. Cadherin dysfunction in a human cancer cell line: possible involvement of loss of α catenin expression in reduced cell-cell adhesiveness. *Cancer Res.* 52:5770-5774.
- Shirayoshi, Y., A. Nose, K. Iwasaki, and M. Takeichi. 1986. N-linked oligosaccharides are not involved in the function of a cell-cell binding glycoprotein E-cadherin. *Cell Struct. Funct.* 11:245-252.
- Su, L. K., B. Vogelstein, and K. W. Kinzler. 1993. Association of the APC tumor suppressor protein with catenins. *Science (Wash. DC)* 262:1734-1737.
- Suzuki, S., K. Sano, and H. Tanihara. 1991. Diversity of the cadherin family: evidence for eight new cadherins in nervous tissue. *Cell Regul.* 2:261-270.
- Takeichi, M. 1977. Functional correlation between cell adhesive properties and some cell surface proteins. *J. Cell Biol.* 75:464-474.
- Takeichi, M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science (Wash. DC)* 251:1451-1455.
- Tsukita, S., S. Tsukita, A. Nagafuchi, and S. Yonemura. 1992. Molecular linkage between cadherins and actin filaments in cell-to-cell adherens junctions. *Curr. Opin. Cell Biol.* 4:834-839.
- Turner, C. E., J. R. Glenney, and K. Burridge. 1990. Paxillin: a new vinculin-binding protein present in focal adhesion. *J. Cell Biol.* 111:1059-1068.
- Wachsstock, D. H., J. A. Wilkins, and S. Lin. 1987. Specific interaction of vinculin with α -actinin. *Biochem. Biophys. Res. Commun.* 146:554-560.
- Watabe, M., A. Nagafuchi, S. Tsukita, and M. Takeichi. 1994. Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line. *J. Cell Biol.* 127:247-256.
- Wieschaus, E., and R. Riggelman. 1987. Autonomous requirements for the segment polarity gene *armadillo* during *Drosophila* embryogenesis. *Cell* 49:177-184.