

Cell binding function of E-cadherin is regulated by the cytoplasmic domain

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Communicated by B. Geiger

Cadherins are a family of transmembrane glycoproteins responsible for Ca^{2+} -dependent cell–cell adhesion. Their amino acid sequences are highly conserved in the cytoplasmic domain. To study the role of the cytoplasmic domain in the function of cadherins, we constructed expression vectors with cDNAs encoding the deletion mutants of E-cadherin polypeptides, in which the carboxy terminus was truncated at various lengths. These vectors were introduced into L cells by transfection, and cell lines expressing the mutant E-cadherin molecules were isolated. In all transfectants obtained, the extracellular domain of the mutant E-cadherins was exposed on the cell surface, and had normal Ca^{2+} -sensitivity and molecular size. However, these cells did not show any Ca^{2+} -dependent aggregation, indicating that the mutant molecules cannot mediate cell–cell binding. The mutant E-cadherin molecules could be released from cells by nonionic detergents, whereas a fraction of normal E-cadherin molecules could not be extracted with the detergent and appeared to be anchored to the cytoskeleton at cell–cell junctions. These results suggest that the cytoplasmic domain regulates the cell–cell binding function of the extracellular domain of E-cadherin, possibly through interaction with some cytoskeletal components.

Key words: cadherin/cell adhesion molecule/cytoskeleton/cDNA transfection/transmembrane control

Introduction

Cadherins are a family of integral membrane glycoproteins responsible for Ca^{2+} -dependent cell–cell adhesion, and are divided into subclasses (Takeichi, 1988). So far, four members of the cadherin family, E-, P-, and N-cadherin and L-CAM, have been characterized on the basis of primary structure (Gallin *et al.*, 1987; Nagafuchi *et al.*, 1987; Nose *et al.*, 1987; Ringwald *et al.*, 1987; Hatta *et al.*, 1988). They are composed of 723–747 amino acids in the mature form, with similar primary structures. They have a single putative transmembrane domain, their amino terminus being located extracellularly and the carboxy-terminal side constituting the cytoplasmic domain, with 151–160 amino acids (Hatta *et al.*, 1988). Comparison of the amino acid sequences of different cadherins showed that ~50% of the amino acids are conserved (Hatta *et al.*, 1988). The degree of conservation, however, differs with the region of molecules, the highest conservation being in the cytoplasmic domain; 69–89% of amino acids were conserved in this region

among the cadherins identified (Hatta *et al.*, 1988). Recent studies suggested that cadherins interact with each other in a homophilic manner for binding cells (Nose *et al.*, 1988).

Indirect evidence suggests that cadherins are anchored to cytoskeletal components. Boller *et al.* (1985) found that uvomorulin (E-cadherin) is localized in the adherens junctions, which are known to be associated with the cortical actin belts in adult mouse intestine. Volk *et al.* (1986a,b) also found that A-CAM, presumably identical to N-cadherin, is associated with the adherens junctions of various cell types. Hirano *et al.* (1987) demonstrated that all subclasses of cadherins so far identified coincide with the cortical actin bundles localized at the boundaries between cells. They also showed that a fraction of cadherins cannot be extracted with nonionic detergents from cells. These observations suggest that cadherins are directly or indirectly associated with cytoskeletal components, including actin bundles.

Because of the high conservation in the amino acid sequence in the cytoplasmic domain, it is assumed that this region must play an indispensable role in the function of cadherins. For example, the function of cadherins might be regulated by cytoskeletal components through their cytoplasmic domain. The present study was designed to verify this possibility. We constructed expression vectors with cDNAs which encode E-cadherin polypeptides with deletions of varying lengths at the carboxy terminus. L cells were then transfected with these vectors, and their cadherin-mediated aggregating activity was assayed. The results showed that the deletion mutants of E-cadherin cannot exert cell-binding activity, even though they are expressed on the cell surface and have the intact extracellular domain. Furthermore, these deletion mutants had no ability to bind to detergent-insoluble cytoskeletal components. These results suggest that the cytoplasmic domain of cadherins is essential for their function, providing the first direct evidence that the function of a cell-adhesion molecule is regulated by the cytoplasmic region.

Results

Isolation and characterization of cell lines with mutant E-cadherin polypeptides

We constructed three kinds of cDNA which encode the deletion mutants of E-cadherin, EM21, EM22 and EM24. In E-cadherin polypeptides encoded by these cDNAs, different quantities of amino acids are assumed to be deleted from the carboxy terminus; 37 residues in EM21, 71 residues in EM22 and 135 residues in EM24 (Figure 1). These cDNAs were connected with the β -actin promoter and introduced into L cells, which have little endogenous cadherin activity, by co-transfection with a plasmid for neomycin resistance. Out of many G418-resistant clones obtained, cell lines which reacted positively with antibodies to E-cadherin were isolated, and three lines were chosen for further analysis. EL β 21, EL β 22 and EL β 24 represent

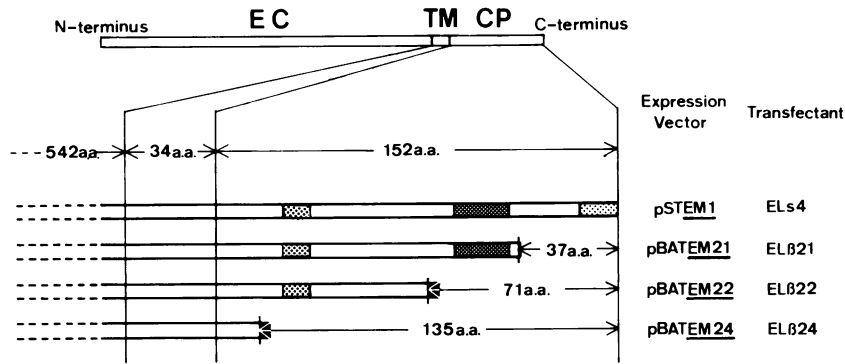


Fig. 1. Structure of E-cadherin polypeptides with carboxy-terminal deletions. pSTEM1 is an expression vector for E-cadherin polypeptide of normal size (Nagafuchi *et al.*, 1987). pBATEM21, pBATEM22 and pBATEM24 encode mutant E-cadherin proteins with deletions of 37, 71 and 135 amino acids at the carboxy terminus respectively. In these plasmids, the mutant E-cadherin cDNAs, EM21, EM22 and EM24, were connected with the β -actin-HSVtk tandem promoter. Three shadowed boxes indicate the regions with the highest conservation of amino acids (90–100%) among cadherin subclasses (Hatta *et al.*, 1988). Solid boxes are 1–4 amino acid residues added to the mutant carboxy termini as a result of mutant constructions. The names of the cell lines transfected with each plasmid are also shown. EC, TM and CP represent the extracellular domain, the transmembrane domain and the cytoplasmic domain of intact E-cadherin molecule respectively.

cell lines transfected with pBATEM21, pBATEM22 and pBATEM24 respectively (Figure 1). As a control, ELs4, which is an L cell line transfected with the full length cDNA for E-cadherin, was used. This cell line, formerly called EL4, was isolated in our previous study and shown to express functional E-cadherin polypeptides (Nagafuchi *et al.*, 1987).

Immunoblot analysis showed that each transfectant line expressed recombinant E-cadherin polypeptides with a size roughly proportional to the putative length of deletion (Figure 2A, lanes 1). The amount of E-cadherin polypeptides expressed in these lines was almost identical, as estimated by counting radioactivity bound to the E-cadherin bands on the immunoblot filters; those expressed in ELs4, EL β 21, EL β 22 and EL β 24 were in the ratio 1.00:1.03:1.21:0.85, the values representing the mean of repeated experiments. To test whether these molecules are exposed on the cell surface, we performed two kinds of assay. First, we examined whether they can be removed by treating live cells with trypsin. It is known that native cadherins present on the cell surface are degraded by treatment of cells with trypsin, in the absence of Ca^{2+} . Figure 2A (lanes 3) shows that all mutant as well as normal E-cadherin polypeptides were removed by the trypsin-EGTA treatment. Assuming that trypsin does not penetrate into cells, this result suggests that even the mutant molecules are exposed on the cell surface. Secondly, we stained live cells with antibodies to E-cadherin at a low temperature (Figure 3). All cell lines reacted with the antibodies, again indicating that all forms of E-cadherin molecules tested are exposed on the cell surface.

Properties of the extracellular domain of mutant E-cadherin polypeptides

We next examined whether or not the extracellular domain of the mutant E-cadherin polypeptides has a normal structure. This was tested under the two criteria, the Ca^{2+} -sensitivity and the molecular size. The extracellular domain of normal E-cadherin is Ca^{2+} -sensitive. E-cadherin is degraded into smaller fragments by treatment of cells with trypsin in the absence of Ca^{2+} , as mentioned above. However, this molecule becomes resistant to trypsin in the presence of Ca^{2+} (Takeichi, 1977). Figure 2A (lanes 2) shows that all mutant molecules expressed on transfected L cells show this

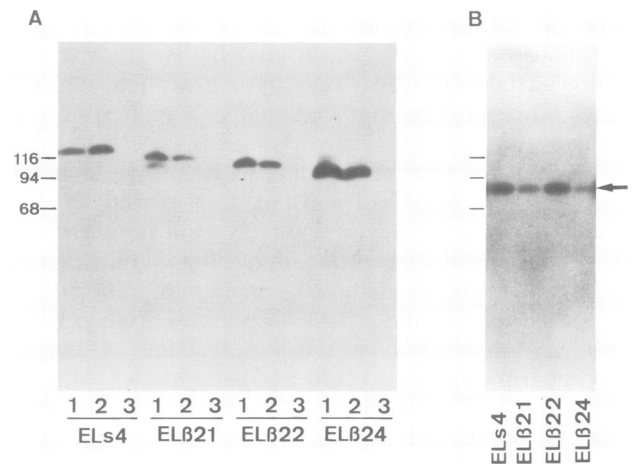


Fig. 2. Immunoblot analysis of E-cadherin polypeptides in different transfectants. (A) E-cadherin polypeptides in the cell lines transfected with different plasmids. Samples were obtained from non-trypsinized cells (lane 1), from cells treated with trypsin in the presence of Ca^{2+} (lane 2) and from cells treated with trypsin in the absence of Ca^{2+} (lane 3). A band with smaller molecular size in lane 1 for EL β 21 is probably a degradation product. Samples on each lane were derived from 2×10^5 cells, except that the samples of EL β 24 were derived from 6×10^5 cells. (B) Fragments of E-cadherin polypeptides released by treatment of the membrane fraction of cells with trypsin in the presence of Ca^{2+} . Samples on each lane were derived from 4×10^5 cells. Positions of mol. wt markers are shown. Arrow indicates the 84-kD tryptic fragment.

type of Ca^{2+} -sensitivity; that is, they are not degraded by trypsin treatment if Ca^{2+} is present. The second criterion was tested as follows. The extracellular domain of normal E-cadherin is cleaved into a 84-kD fragment which contains the amino terminus of the mature form of this protein, when the membrane fraction of cells is treated with trypsin in the presence of Ca^{2+} (Hyafil *et al.*, 1980; Shirayoshi *et al.*, 1986). The cleavage site for producing this tryptic fragment is thought to be located at the proximal region of the extracellular domain of E-cadherin, as estimated from its size, indicating that this fragment covers most of the extracellular domain of E-cadherin. When the membrane fraction of transfectants with the deletion mutants was treated with trypsin- Ca^{2+} , 84-kD fragments of E-cadherin were

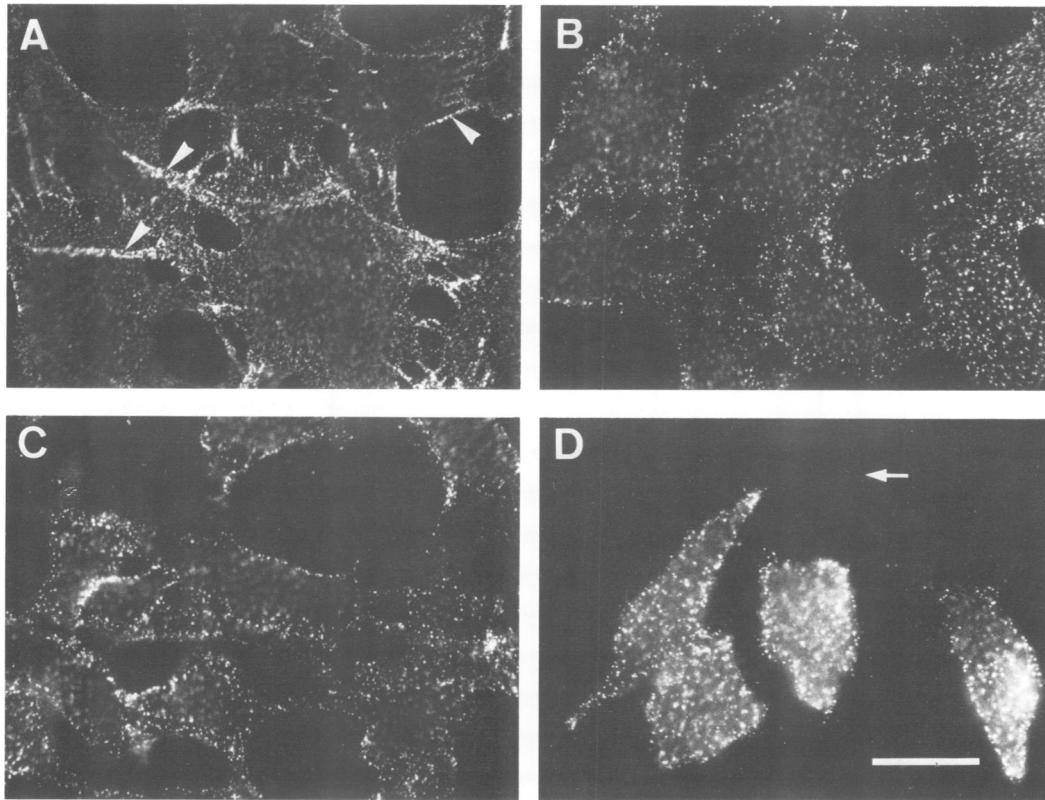


Fig. 3. Immunolocalization of E-cadherin polypeptides on the cell surface. (A) ELs4. (B) ELβ21. (C) ELβ22. (D) ELβ24. Arrowheads in (A) indicate filopodia attaching to the neighboring cells, on which E-cadherin molecules are accumulated. Note that such filopodia can be seen only in the ELs4 line (A). Arrow in (D) indicates a cell not expressing E-cadherin polypeptides, which is present in the ELβ24 line. Bar, 20 μm.

obtained from all samples (Figure 2B). These fragments were further degraded if treated with trypsin in the absence of Ca²⁺ (data not shown). These results suggest strongly that the extracellular domain of all mutant E-cadherin polypeptides has the same molecular structure as the native protein.

Immunolocalization of E-cadherins on the transfectants

During the course of the immunostaining experiments, we found three unique features in cells expressing the mutant proteins. (i) Functional E-cadherin is generally concentrated at the cell-cell contact sites (Hirano *et al.*, 1987). This was found in cultures of ELs4 cells with normal E-cadherin (Figure 3A). These cells attach to each other with filopodia, which were more intensely stained with antibodies to E-cadherin than other areas of the cell surface. In contrast, the mutant E-cadherins were not accumulated at the contact sites between cells (Figure 3B-D). (ii) E-cadherin showed a dot-like distribution on the cell surface in all the transfectant lines under the present staining conditions, probably resulting from crosslinking of antigens with antibodies. The size of these dots was smaller in ELs4 cells with normal E-cadherin than cells with the mutant molecules (Figure 3). This may suggest that the mutant molecules are more freely mobile in the cell membrane than the normal counterparts. (iii) All cells in cultures of the transfectant lines expressed E-cadherin equally, except in ELβ24 with the largest deletion. Cultures of this line always contained two cell populations, one strongly positive and the other completely negative in the expression of E-cadherin (Figure 3D). This tendency was

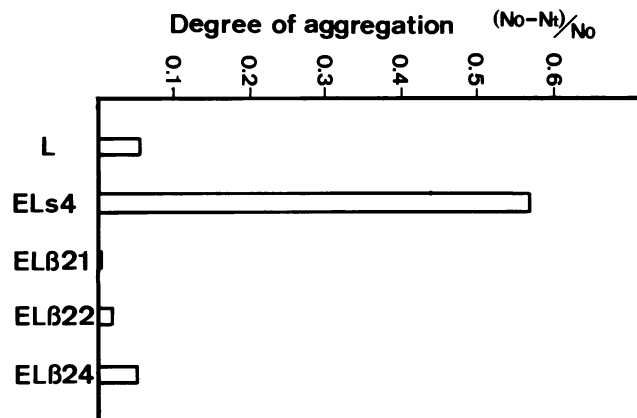


Fig. 4. Aggregation of cells with different E-cadherin polypeptides. Cells dissociated by TC-treatment were allowed to aggregate for 30 min in the presence of 1 mM Ca²⁺. The higher value in abscissa represents the higher degree of aggregation.

observed even after recloning of cells, and also found in all other L cell lines transfected with the same vector. Therefore, the expression of E-cadherin with this deletion may be unstable, for unknown reasons.

Aggregation of cells with mutant E-cadherin polypeptides

Generally, cells with cadherins aggregate in a Ca²⁺-dependent manner if they are dispersed by treatment with trypsin in the presence of Ca²⁺ (Takeichi, 1977). ELs4 cells with normal E-cadherin showed this type of

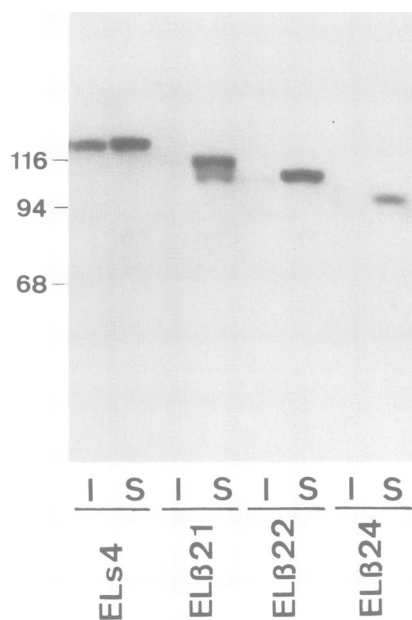


Fig. 5. Detergent extraction of E-cadherin polypeptides from different transfectants. I, insoluble fraction; S, soluble fraction. Samples on each lane were derived from 4×10^5 cells. Bands with smaller molecular size detected in some lanes are probably products of degradation.

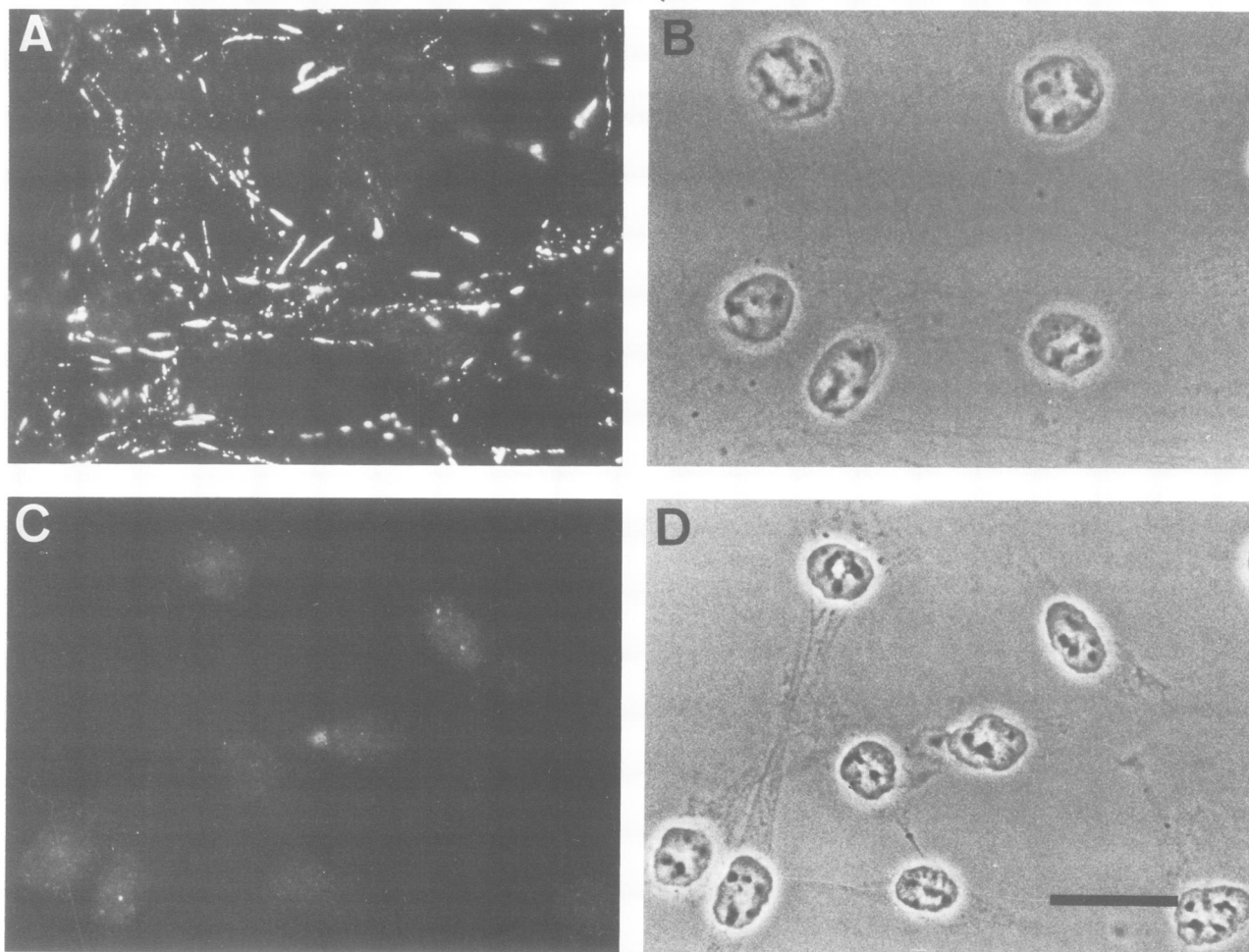


Fig. 6. Immunofluorescent detection of E-cadherin on cells treated with NP-40. (A) and (B) ELs4 cells. (C) and (D) ELβ22 cells. Each set of panels represents an immunofluorescent and a phase-contrast photomicrograph of the same field. Note the positive staining on filopodia at cell-cell boundaries in (A) and the negative staining in (B). Bar, 20 μ m.

aggregation. Although the amount of polypeptides expressed in cell lines with the mutant E-cadherin was almost equivalent to that in ELs4 cells as described above, these cells showed no aggregating activity under any condition tested (Figure 4).

Structural relations of mutant E-cadherins with cytoskeletons

Some fraction of native E-cadherin molecules cannot be extracted with nonionic detergents such as NP-40 (Hirano *et al.*, 1987). The immunoblot analysis showed that ~40% of the total E-cadherin molecules in a cell were not extracted with NP-40 in the case of ELs4 cells (Figure 5). When the cells with mutant E-cadherin polypeptides were subjected to this analysis, most of the molecules were released from cells by the NP-40 extraction (Figure 5). Immunostaining analysis revealed that E-cadherin molecules which cannot be extracted with NP-40 in ELs4 cells were localized only at cell-cell contact sites, but not on the free surface (Figure 6A). No staining for E-cadherin could be detected on NP-40-treated cells with the mutant E-cadherin. These results suggest that normal E-cadherin molecules are anchored to the cytoskeleton at cell-cell contact sites, and that this property is lost in the deletion mutants.

Discussion

The present studies demonstrate that E-cadherin molecules

cannot function when their carboxy terminus located in the cytoplasm is truncated at various lengths. They also provide evidence that the extracellular domain of these non-functional deletion mutants has normal properties with regards to the Ca^{2+} -sensitivity and the molecular size.

Wheelock *et al.* (1987) showed that the 80-kd tryptic fragments of the extracellular domain of cell-CAM 120/80, presumably the human homologue of E-cadherin, can inhibit cell–cell adhesion, suggesting that cadherins without the cytoplasmic domain are able to interact with intact molecules. It is therefore likely that the extracellular domain of the deletion mutants used in the present study also retain the inherent nature to interact with other cadherin molecules. In fact, all properties of the extracellular domain of mutant molecules tested were found to be the same as those of native molecules. Then, one would ask why cells with the mutant cadherins are unable to adhere to each other.

An important finding, related to the above question, is that the mutant molecules are all extractable from cells with NP-40, whereas normal molecules are not. We have demonstrated previously that cadherins coincide with the actin bundles at cell–cell contact sites (Hirano *et al.*, 1987). Others have shown that cadherins are localized in the adherens junctions, the structure associated with the cortical actin belts (Boller *et al.*, 1985; Volk *et al.*, 1986a,b). The present study has indicated that normal E-cadherins not extractable with NP-40 are located at cell–cell contact sites. These observations suggest that the anchoring of cadherins to the cytoskeleton plays a role in the cell–cell binding action of these proteins.

We can postulate two mechanisms to interpret the present findings. First, it is possible that the lateral movement of cadherin molecules in the cell membrane is controlled by the cytoskeleton, and the transport of these molecules into intercellular contact sites might be essential for establishing cell–cell adhesion. The mutant cadherins, unable to bind to the cytoskeleton, cannot be transported to these sites, and thus are unable to mediate the adhesion of cells.

Second, the interaction between cadherin and the cytoskeleton affects molecularly the functional state of the extracellular domain of cadherin polypeptides. Cadherin-mediated cell–cell adhesion may have multiple steps. The first step would be an interaction in the extracellular domains of cadherin molecules on apposed cell surfaces; this interaction may not lead directly to cell–cell connection. (Such an interaction could occur without the cytoplasmic domain, as suggested by the finding that the 80-kd fragments of cell-CAM 120/80 can compete with native molecules.) In the next step, the initial interaction with the extracellular domain instructs the cytoplasmic domain to bind to cytoskeletal components. The binding of the cytoplasmic domain to the cytoskeleton may then send a signal back to the extracellular domain, instructing this domain to make a stable connection with cadherin molecules on neighboring cells.

It is probable that cadherin-mediated cell–cell adhesion, in fact, has multiple steps. We have observed that the initial step of cadherin-mediated adhesion is strictly dependent on Ca^{2+} but the cadherin-mediated cell junctions, once formed, are not necessarily destroyed by removal of Ca^{2+} . For example, the adhesion between L cell transfectants expressing normal cadherin molecules cannot be dissociated easily with Ca^{2+} depletion, although they have no major

cell–cell adhesion molecules other than the exogenously introduced cadherins (unpublished data). We have also found that cadherins accumulated at the cell–cell junctions are not removed totally by EGTA treatment of cells (Hirano *et al.*, 1987).

It would be premature to conclude which of the mechanisms proposed above is actually operating. Since parts of the amino acid sequences deleted in the present study are highly conserved among cadherins (Hatta *et al.*, 1988), they must have common ligands. Identifying the molecules directly associated with the cytoplasmic domain of cadherins, if present, should help to further the understanding of the regulatory mechanism underlying intercellular adhesion.

Materials and methods

Plasmid construction

Constructions were carried out using standard methodology as described in Maniatis *et al.* (1982).

pBATEM21, pBATEM22 and pBATEM24, which encode the mutant E-cadherin polypeptides with the carboxy-terminal deletions, were derived from pBATEM2 (Nose *et al.*, 1988), which contains the E-cadherin cDNA covering the whole coding region, joined with the β -actin and HSVtk tandem promoter (McKnight, 1980; Fregien and Davidson, 1986). The four restriction sites, *Bst*EII, *Sma*I, *Cla*I and *Sac*I, in the open reading frame of the E-cadherin cDNA sequence were used to produce frame-shift mutants. The *Bst*EII site is located in the region encoding the extracellular domain of E-cadherin. The *Sma*I, *Cla*I and *Sac*I sites are located in the coding region for the cytoplasmic domain; these sites correspond to the 135th, 71st and 37th amino acid residues upstream from the carboxy terminus respectively. pBATEM21 and pBATEM24 were constructed by replacing the *Bst*EII–*Cla*I fragment with the *Bst*EII–*Sac*I fragment and with the *Bst*EII–*Sma*I fragment respectively. In each case, the *Cla*I or *Sac*I site was blunt-ended before the replacement. To construct pBATEM22, pBATEM2 was digested with *Cla*I, blunt-ended then re-ligated. These rearrangements caused frame shifts and produced new stop codons downstream from the *Sma*I, *Cla*I or *Sac*I site.

cDNA transfection

For the isolation of L cells stably transformed with plasmids pBATEM21, pBATEM22 or pBATEM24, cells were co-transfected with a plasmid for neomycin resistance and selected with G418 as described previously (Nagafuchi *et al.*, 1987). Cells were grown in Dulbecco's modified Eagle's MEM supplemented with 10% fetal bovine serum.

Immunocytochemistry

For staining E-cadherin localized on the cell surface, cell cultures were incubated with heat-inactivated rabbit antiserum raised against E-cadherin (Nagafuchi *et al.*, 1987) diluted one to 500 with the culture medium, for 20 min at 4°C. The cultures were then fixed by incubation with 3.5% paraformaldehyde in 10 mM Hepes-buffered saline containing 1 mM CaCl_2 (HMF) for 15 min at 4°C, then in 100% methanol for 15 min at –20°C. The samples were incubated with biotinylated anti-rabbit Ig diluted one to 100 with HMF for 30 min, followed by another incubation with fluorescein-streptavidin diluted one to 100 with HMF for 30 min. Finally, the preparations were mounted, examined and photographed with a Zeiss fluorescence microscope.

Cells treated with detergents were stained as follows. Cultures were incubated with 0.5% Nonidet P-40 (NP-40) in HMF for 30 min at room temperature. After removing NP-40, cells were fixed with paraformaldehyde and methanol, as above, and incubated with the rabbit anti-E-cadherin. Binding antibodies were visualized, as above. In these immunohistochemical experiments, all solutions for the immunoreactions contained 5% skim milk, and samples were washed with HMF five times after each incubation.

Immunoblot analysis

Samples were separated by SDS–PAGE using 7.5% polyacrylamide gels. After electrophoresis, proteins were transferred to nitrocellulose. Nitrocellulose transfers were incubated with ECCD-2, followed by incubation with ^{125}I -labeled anti-rat Ig (Amersham). The binding labeled antibodies were visualized by subjecting the transfers to autoradiography for a few days with an intensifying screen.

Trypsin treatment and aggregation of cells

Cells were trypsinized by two different treatments for the differential removal of E-cadherin, as described by Takeichi (1977). Briefly, cells were treated with 0.01% trypsin in the presence of 10 mM CaCl₂ (TC-treatment) or 1 mM EGTA (TE-treatment) at 37°C for 20 min. Generally, cadherins are left intact after the TC-treatment, but are digested by the TE-treatment.

For the cell aggregation assay, 2×10^5 cells dispersed by TC-treatment were placed on each well of a Nunclon 24-well plate with 0.5 ml HMF and allowed to aggregate for 30 min at 37°C, as described previously (Nagafuchi *et al.*, 1987). The extent of cell aggregation was represented by the index $(N_0 - N_t)/N_0$ where N_t is the total particle number after the incubation time t and N_0 is the total particle number at the initiation of incubation.

Trypsin treatment of membrane fraction

Cells (10^7) were washed five times with HMF, collected by scraping, disrupted by freezing and thawing, and then centrifuged at 100 000 g for 30 min. The precipitate, regarded as the membrane fraction, was incubated with 120 μ l of 0.01% trypsin in HMF supplemented with 10 mM CaCl₂ for 30 min at 37°C. After incubation, 5 μ l of 0.5% soybean trypsin inhibitor and 125 μ l of $2 \times$ SDS sample buffer containing 0.25 M Tris-HCl, pH 6.8, 4% SDS, 40% glycerol, 0.004% bromophenol blue and 2 mM phenylmethylsulfonylfluoride was added, and then subjected to electrophoretic analysis.

Detergent extraction of cells

Cultured cells were washed three times by HMF, collected into an Eppendorf microtube by scraping with a rubber policeman and then centrifuged at 14 000 r.p.m. for 5 min. After removing supernatant, 100 μ l of 2.5% NP-40 in HMF was added to the pellet of 10^7 cells. Samples were incubated for 10 min with mild pipetting, and then centrifuged at 100 000 r.p.m. for 30 min. To the supernatant, $2 \times$ SDS sample buffer was added to make the total volume 250 μ l and used as the detergent-soluble fraction. On the other hand, the pellet fraction was dissolved in 250 μ l of $1 \times$ SDS sample buffer and used as the detergent-insoluble fraction.

Acknowledgements

We thank Dr K.Okazaki for the β -actin promoter plasmid and Dr N.Duzgunes (University of California, San Francisco) for his critical reading of the manuscript. This work was supported by research grants from the Ministry of Education, Science and Culture of Japan. A.N. is a recipient of a Fellowship of the Japan Society for the Promotion of Science for Japanese Junior Scientists.

References

- Boller,K., Vestweber,D. and Kemler,R. (1985) *J. Cell Biol.*, **100**, 327–332.
Fregien,N. and Davidson,N. (1986) *Gene*, **48**, 1–11.
Gallin,W.J., Sorkin,B.C., Edelman,G.M. and Cunningham,B.A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 2808–2812.
Hatta,K., Nose,A., Nagafuchi,A. and Takeichi,M. (1988) *J. Cell Biol.*, **106**, 873–881.
Hyafil,F., Morello,D., Babinet,C. and Jacob,F. (1980) *Cell*, **21**, 927–934.
Hirano,S., Nose,A., Hatta,K., Kawakami,A. and Takeichi,M. (1987) *J. Cell Biol.*, **105**, 2501–2510.
McKnight,S.L. (1980) *Nucleic Acids Res.*, **8**, 5948–5964.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Nagafuchi,A., Shirayoshi,Y., Okazaki,K., Yasuda,K. and Takeichi,M. (1987) *Nature*, **329**, 341–343.
Nose,A., Nagafuchi,A. and Takeichi,M. (1987) *EMBO J.*, **6**, 3655–3661.
Nose,A., Nagafuchi,A. and Takeichi,M. (1988) *Cell*, in press.
Ringwald,M., Schuh,R., Vestweber,D., Eistetter,H., Lottspeich,F., Engel,J., Dolz,R., Jahniz,F., Epplen,J., Mayer,S., Muller,C. and Kemler,R. (1987) *EMBO J.*, **6**, 3647–3653.
Shirayoshi,Y., Hatta,K., Hosoda,M., Tsunasawa,S., Sakiyama,F. and Takeichi,M. (1986) *EMBO J.*, **5**, 2485–2488.
Takeichi,M. (1977) *J. Cell Biol.*, **75**, 464–474.
Takeichi,M. (1988) *Development*, **102**, 639–655.
Volk,T. and Geiger,B. (1986a) *J. Cell Biol.*, **103**, 1441–1450.
Volk,T. and Geiger,B. (1986b) *J. Cell Biol.*, **103**, 1451–1464.
Wheelock,M.J., Buck,C.A., Bechtol,K.B. and Damsky,C.H. (1987) *J. Cell. Biochem.*, **34**, 187–202.

Received on July 12, 1988; revised on September 5, 1988