

Calcium-dependent Cell-Cell Adhesion Molecules (Cadherins): Subclass Specificities and Possible Involvement of Actin Bundles

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Abstract. Cadherins are a family of cell-cell adhesion molecules and are divided into subclasses with distinct adhesive specificities and tissue distribution. Here we examined the distribution of cadherins at contact sites between cells expressing the same or different cadherin subclasses. Each cadherin was concentrated at the boundary between cells expressing an identical cadherin subclass, irrespective of the cell types connected. However, such localization decreased or disappeared at the boundary between cells containing different cadhe-

rin subclasses. We also found that the localization of cadherins precisely coincided with that of actin bundles; both were detected at the apical region of cell sheets. This co-localization was retained even after cells were either treated with cytochalasin D or extracted with the detergent NP40. These results suggest that each cadherin subclass preferentially interacts with its own molecular type at intercellular boundaries, and that cadherin molecules may be associated with actin-based cytoskeletal elements.

CADHERINS are a family of glycoproteins which play a role in the Ca^{2+} -dependent cell-cell adhesion mechanism. They appear to have a major role in establishing and maintaining stable intercellular adhesion in most kinds of tissues (Takeichi et al., 1986). Cadherins are divided into subclasses, such as E-, N-, and P-types. In spite of their similar biochemical properties, different cadherins have distinct tissue distribution and distinct immunological and cell-binding specificities (Hatta et al., 1985; Hatta and Takeichi, 1986; Shirayoshi et al., 1986; Nose and Takeichi, 1986; Hatta et al., 1987). Our experiments have shown that cells with E-type do not adhere to cells with N- or P-type when they were mixed under the condition that only cadherins are active in linking cells (Takeichi et al., 1981, 1985; Nose and Takeichi, 1986). These results suggest that the specificity of each cadherin subclass plays an important role in preferential adhesion of cells with their own type.

Several laboratories have identified cadherin-like molecules independently, such as uvomorulin (Peyrieras et al., 1983), L-CAM (Gallin et al., 1983), cell-CAM 120/80 (Damsky et al., 1983), Arc-1 (Behrens et al., 1985), and A-CAM (Volk and Geiger 1986a, b). It is believed that the first four molecules are identical to E-cadherin although animal species used for their identification are not all the same. We have actually found that the partial amino acid sequence of L-CAM reported by Cunningham et al. (1984) is identical to that of cadherins (Shirayoshi et al., 1986). A-CAM is similar to N-cadherin in its tissue distribution and biochemical properties, as discussed by Volk and Geiger (1986b).

The molecular and structural basis of cadherin-dependent cell adhesion is unknown. Do cadherins bind cells in a homophilic or heterophilic manner? Are cadherins con-

trolled by cytoskeletal elements? Why do cells with different cadherin subclasses segregate? Studies on uvomorulin (Boller et al., 1985) showed that this molecule is concentrated in the adherens junction (the intermediate junction) in the intestinal epithelium. Volk and Geiger (1986a, b) showed that A-CAM is localized in the adherens-type junctions of heart and lens cells. Immunocytochemical studies of cadherins have also shown that they tend to be concentrated in the apical region of many epithelial tissues, where the intercellular adherens junctions are located (Hatta and Takeichi, 1986; Nose and Takeichi, 1986; Hatta et al., 1987). These observations suggest that cadherins are associated with specialized junctions such as the zonula adherens.

In the present study, we have examined the distribution of cadherins on cells which are in contact with homologous or heterologous cells. We have also examined whether cadherins are associated with actin bundles. The results suggest that each subclass of cadherins interacts preferentially with its own type, and they appear to be associated with actin bundles.

Materials and Methods

Cells

Cell lines of mouse teratocarcinoma F9 (Bernstein et al., 1973), mouse endoderm PSA5-E (Adamson et al., 1977), mouse glioma G26-20 (Sundarraj et al., 1975), mouse mammary tumor MTD-1A, which is a subclone isolated from the original MTD-1 line (Enami et al., 1984), human mammary carcinoma MCF-7 (Soule et al., 1973), and tk⁻ L (Murayama-Okabayashi et al., 1971) were used. These were cultured on round coverslips (25 mm in diameter) for the immunocytochemical analysis.

To obtain hepatocytes, livers were isolated from 14-d-old mouse em-

byros or 13-d-old chick embryos and incubated in 0.1% collagenase (Wako Chemicals, Kyoto, Japan) in HEPES-buffered Ca^{2+} - and Mg^{2+} -free saline (HCMF)¹ supplemented with 2 mM CaCl_2 for 30 min at 37°C. Lens epithelial cells were isolated from 13- to 14-d-old chicken embryos and dissociated as described above. To obtain glial cells, spinal cords were isolated from 11-d-old chicken embryos and fragmented into small pieces using a knife, and incubated in 0.25% trypsin (Difco Laboratories, Inc., Detroit, MI; 1:250) in HCMF for 15 min at 37°C. These enzyme-treated tissues were pipetted to obtain small cell clusters. They were then cultured on round coverslips precoated with collagen for 1-2 d before use. The uterine decidual cells were prepared according to Nose and Takeichi (1986).

Culture medium used was a 1:1 mixture of DME and Ham's F12 medium supplemented with 10% FCS.

Antibodies

Rat mAbs against mouse E-cadherin (ECCD-2), mouse P-cadherin (PCD-1), and chicken N-cadherin (NCD-2) were prepared as described (Hatta and Takeichi, 1986; Shirayoshi et al., 1986; Nose and Takeichi, 1986). Antibodies in hybridoma culture supernatant were precipitated by 50%-saturated ammonium sulfate, dissolved in HCMF supplemented with 1 mM CaCl_2 (HMF) and dialyzed against the same solution. The antibody solutions were diluted with HMF as much as possible in a range to give the optimal immunofluorescent staining. The second antibodies used were FITC-labeled or rhodamine-labeled anti-rat Ig (Dakopatts, Copenhagen). These antibodies were diluted 1-100 before use with HMF.

Immunofluorescent Cytochemistry

Cells cultured on coverslips were briefly rinsed with HMF and fixed with 3.5% paraformaldehyde in HMF for 30 min at 4°C. After rinsing with 50 mM TBS (pH 7.4) containing 1 mM CaCl_2 (TBS-Ca), the fixed cells were extracted with acetone at -20°C for 10 min, and rinsed again with TBS-Ca. Cells were then treated with 1% BSA in HMF for 30 min, and subsequently incubated with mAbs against cadherins in HMF for 60 min at room temperature. After extensive washing with TBS-Ca, the samples were finally incubated with fluorescence-labeled second antibodies diluted with HMF containing 1% BSA for 60 min at room temperature. After washing thoroughly with TBS-Ca and then briefly with distilled water, the preparations were mounted with 90% glycerol-10% TBS-Ca containing paraphenylenediamine to prevent bleaching (Johnson and Nofueira-Araujo, 1981). They were examined and photographed by a Zeiss fluorescence microscope using filter systems, No. 10 for FITC and No. 15 for rhodamine. All solutions used for preparing the samples contained 1 mM Ca^{2+} to protect cadherins against proteolysis.

Actin Staining

Actin bundles were stained with rhodamine-phalloidin (Molecular Probes, Inc., Junction City, OR) according to Wulf et al. (1979). For double-staining for cadherins and actin, the rhodamine-phalloidin solution was added to the FITC-labeled second antibody solution in a 1:20 ratio. This mixture was used in the step for immunofluorescent staining of cells with the second antibodies. Rhodamine and FITC were selectively visualized by the filter systems as described above.

Vital Cell Staining

To fluorescently label living cells, cultures were incubated with 30 μM 5-(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) in HMF for 1 h at room temperature with constant agitation (Bronner-Fraser 1985). The cells were washed, trypsinized, and plated on coverslips for use. Fluorescence, which is detected by the Zeiss No. 10 filter system but not by the No. 15 system, remained on cells for at least 24 h.

Results

Distribution of Cadherins on Cell Surfaces

We examined the distribution of E-, N-, and P-cadherin on

1. *Abbreviations used in this paper:* CFSE, 5-(6)-carboxyfluorescein diacetate succinimidyl ester; HCMF, HEPES-buffered Ca^{2+} / Mg^{2+} -free saline; TBS-Ca, Tris-buffered saline/ CaCl_2 .

Table I. Expression of Cadherin Subclasses on Various Cell Types Used

Cell type	Cadherin subclass expressed
Mouse teratocarcinoma F9	E
Mouse mammary tumor MTD-1A	E
Mouse hepatocytes	E
Chick hepatocytes	E
Human mammary tumor MCF-7	E
Mouse extraembryonic endoderm	
PSA5-E	P
Mouse uterine decidual	P
Mouse glioma G26-20	N
Chick spinal cord glia	N
Chick lens epithelium	N
Chick liver fibroblasts	N
Mouse L	-*

* L cells have very low expression of unidentified cadherin (Takeichi et al., 1981).

surfaces of cells listed in Table I by immunofluorescent cytochemistry. Table I also summarizes which cadherin subclass is expressed in these cells. Since mAbs to each cadherin subclass show some species-specificity in their immunohistochemical staining, we used mouse and human cells for staining of E-cadherin, mouse cells for that of P-cadherin, and chicken cells for that of N-cadherin.

We first studied the distribution of E-cadherin and found that all cells examined showed essentially a similar distribution pattern. E-cadherin was always concentrated at the intercellular boundaries as sharp lines (Fig. 1). The morphology of immunofluorescently stained intercellular boundaries varied with cell types. F9 cells tended to show straight lines (Fig. 1 *a*), while hepatocytes and MTD-1A cells had zigzag lines due to microvillous protrusions (Fig. 1, *d* and *e*). Such E-cadherin staining on cell-cell boundaries seemed to be accumulated in the apical region of cells, since more intense staining was detected when the microscope was focused on the apical and subapical planes of cell layers, as opposed to basal planes, particularly in a high cell density condition (Fig. 1, *b* and *c*).

The cell surface which is not in contact with other cells, such as the top surface of cells, was generally not stained intensely for E-cadherin, although irregular and patch-like staining patterns were sometimes observed. The edge of pseudopodia attaching to the dish was usually devoid of E-cadherin (Fig. 1). Some of hepatocytes located at the margin of a colony, however, showed an unusual pattern of E-cadherin distribution, in which the E-cadherin-positive line was detected not only at the intercellular boundary but also on the free cell surface, making a belt-like structure (Fig. 1 *e*). Such a staining pattern was not generally seen with cultures of established cell lines.

The pattern of distribution of N- and P-cadherin was essentially the same as that found for E-cadherin; they were always concentrated at the intercellular boundary (Fig. 6).

Removal of Ca^{2+} from the medium by the addition of EGTA caused disruption of cell-cell adhesion. Coincidentally, intense staining of cadherins originally seen at the intercellular boundary gradually disappeared, although it was sometimes still detected if cells retained their mutual connections even

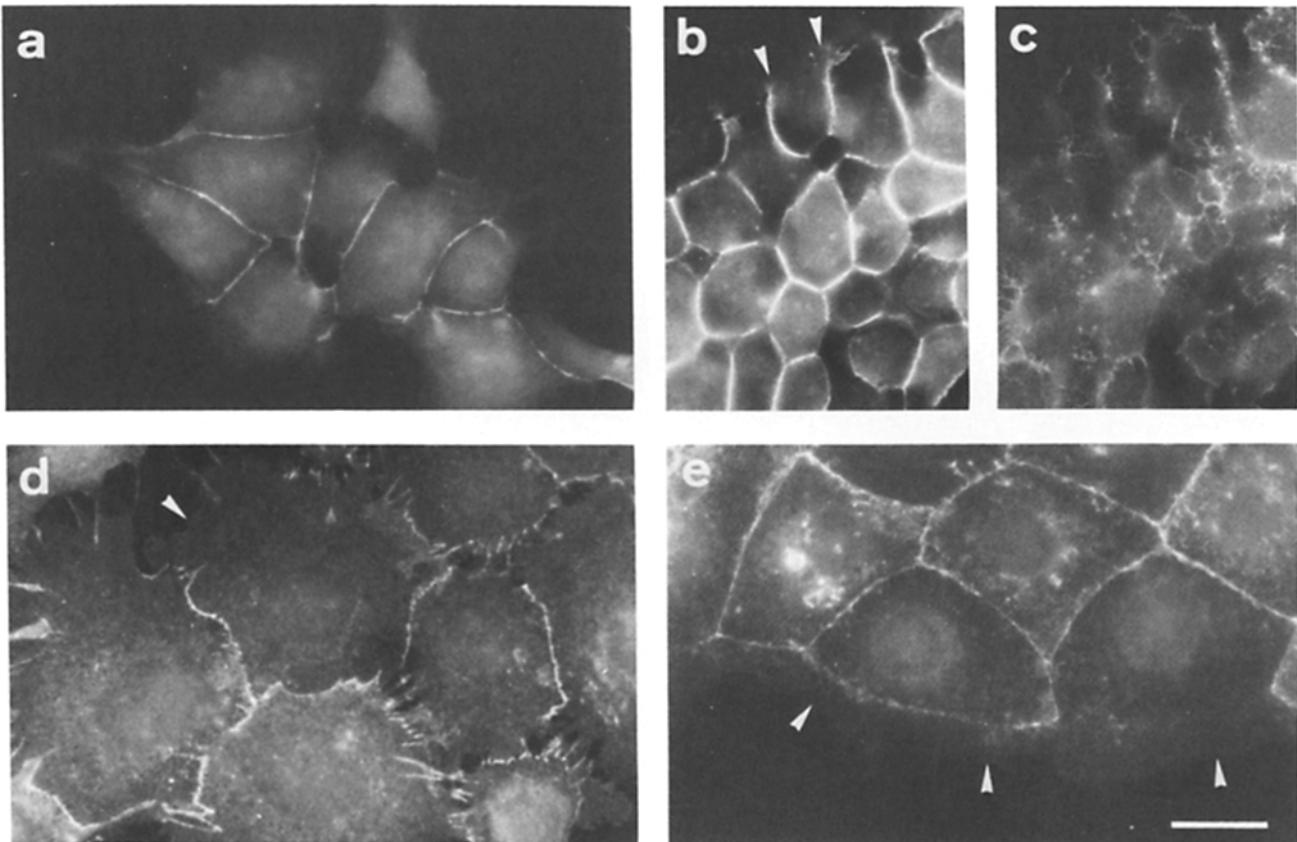


Figure 1. Immunofluorescent staining for E-cadherin. (a) F9 cells in low density. (b and c) F9 cells in high density focused at the subapical plane (b) and the basal plane (c). (d) MTD-1A cells. (e) Hepatocytes at the marginal zone of a colony. Note a "ring" of E-cadherin staining on a hepatocyte with free edge. Arrows indicate cell edges attaching to the substratum but not attaching to other cells, which show no E-cadherin staining. Bar, 20 μm .

in Ca^{2+} -free medium (Fig. 2). When Ca^{2+} was added to such cultures, cellular adhesion quickly recovered together with reacquisition of cadherin staining (data not shown). We did not find any accumulation of cadherins at the cell-substrate-adhesion sites.

Distribution of Cadherins in Heterotypic Cell-Cell Adhesion

The experiments described above examined only the adhesion between homotypic cells. We then studied distribution of cadherins on cells in contact with different cell types. Two

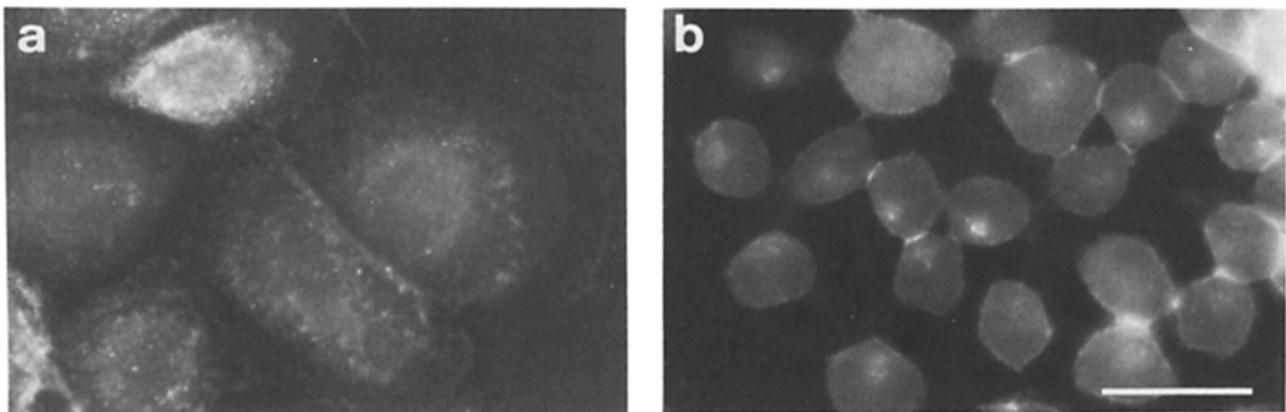


Figure 2. Effect of removing Ca^{2+} from the medium on the cadherin distribution in hepatocytes (a) and F9 cells (b). Cells were incubated in 0.1 mM EGTA and 1 mM MgCl_2 in HCMF for 30 min, and stained for E-cadherin. Note the disappearance of the intense cadherin staining from the cell periphery, except at some cell-cell contact sites. Bar, 20 μm .

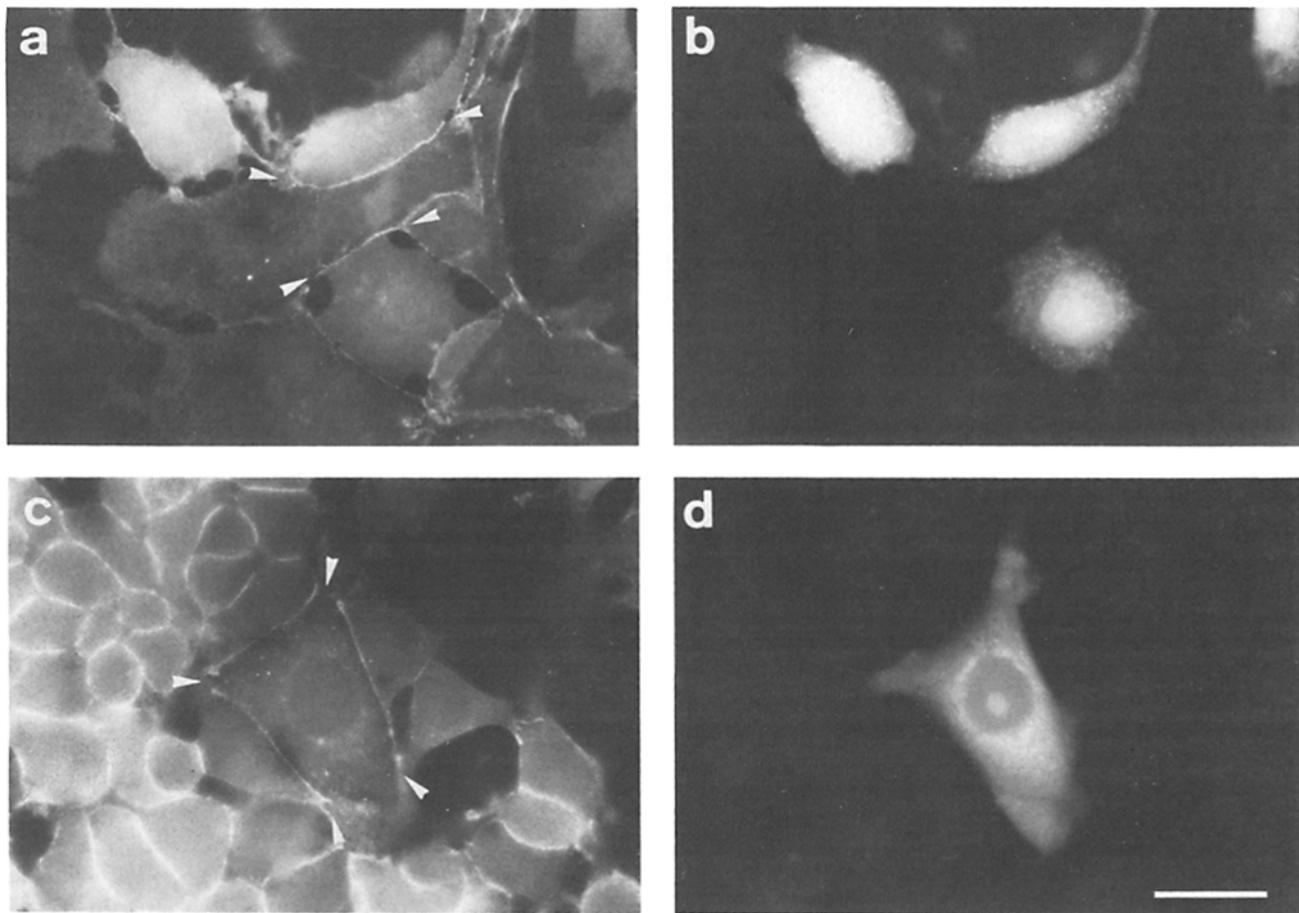


Figure 3. Distribution of cadherins in mixed cultures of F9 and hepatocytes (*a* and *b*) and F9 and MCF-7 (*c* and *d*), all expressing E-cadherin. (*a* and *c*) E-cadherin stained with the rhodamine-conjugated second antibody. In these cultures, F9 cells were prelabeled with CFSE as visualized in *b* and *d*, which are the same field as *a* and *c*, respectively. Note positive staining at the boundary between heterotypic cells, shown by arrows. Bar, 20 μ m.

kinds of heterotypic cell combinations were made: One was the combination of heterotypic cells having a homologous cadherin subclass, and the other was that of cells with heterologous cadherin subclasses. Two cell types, one of which had been labeled with the fluorescent dye, CFSE, were mixed, cultured overnight, and stained for cadherins with the rhodamine-labeled second antibody.

For the first series of combinations, we mixed F9 and mouse hepatocytes, or F9 and MCF-7, all of which expressed E-cadherin. These heterotypic cells adhered to each other in monolayer cultures. We then found intense staining for E-cadherin at the boundary between these heterotypic cells (Fig. 3). We also found a similar result in a mixture of chick lens epithelial cells and chick liver fibroblasts, both expressing N-cadherin, as described below.

For the second series, we mixed two cell types expressing different cadherin subclasses. Cell combinations for this series of experiments were divided into two groups, the mouse-mouse cell combination and the chick-chick cell combination. For the mouse-mouse cells, F9 and PSA5-E, F9 and G26-20, F9 and L, and PSA5-E and G26-20 were mixed (Fig. 4); and for the chick-chick cells, lens epithelial cells were mixed with hepatocytes (Fig. 5, *a* and *b*). In these

combinations, cells tended to cluster with their own type segregating from the other type. Under a proper cell density, however, contact or overlapping between heterotypic cells was established. In such cultures, we generally found that cadherins were either absent or not intensely concentrated at the boundary between heterotypic cells expressing different cadherin subclasses (Figs. 4 and 5).

The mixed cultures of chick lens epithelium and hepatocytes always had contamination of fibroblasts derived from the liver. We found that many of these fibroblasts express N-cadherin. When lens cells adhered with the N-cadherin-positive fibroblasts, the boundary between these cells always had an accumulation of N-cadherin (Fig. 5, *c* and *d*). Therefore, lens cells discriminate fibroblasts and hepatocytes in liver cell cultures in their adhesion. It should be also noted that N-cadherin was not detected at the boundary between the fibroblasts and hepatocytes, which is the natural cell combination in liver tissues (data not shown). Quantitative analysis of these observations is presented in Table II.

Co-localization of Cadherins with Actin

Cells were double-stained for cadherins and actin. We found that the localization of all subclasses of cadherins coincided

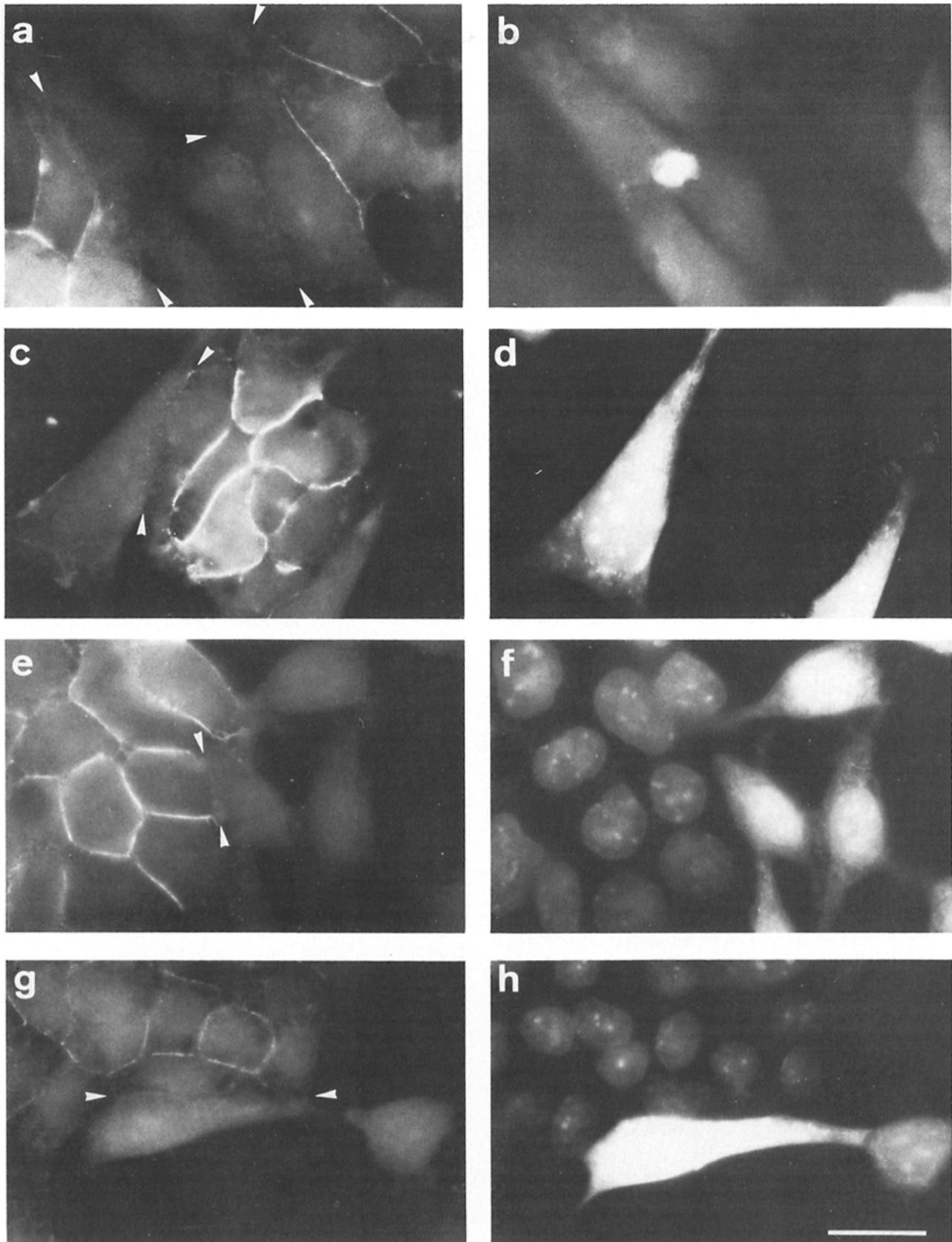


Figure 4. Distribution of cadherins in mixed cultures of different cell types expressing heterologous cadherins. (*a* and *b*) F9 and PSA5-E. (*c* and *d*) F9 and G26-20. (*e* and *f*) F9 and L. (*g* and *h*) PSA5-E and G26-20. (*a*, *c*, and *e*) Stained for E-cadherin; (*g*) stained for P-cadherin. In *b*, *d*, *f*, and *h*, which are the same fields as corresponding photographs of the left column, PSA5-E, G26-20, L cells, and G26-20 are labeled with CFSE, respectively. Note the absence of intense cadherin staining in the boundary between heterotypic cells. Arrows indicate the boundary between heterotypic cells. Bar, 20 μ m.

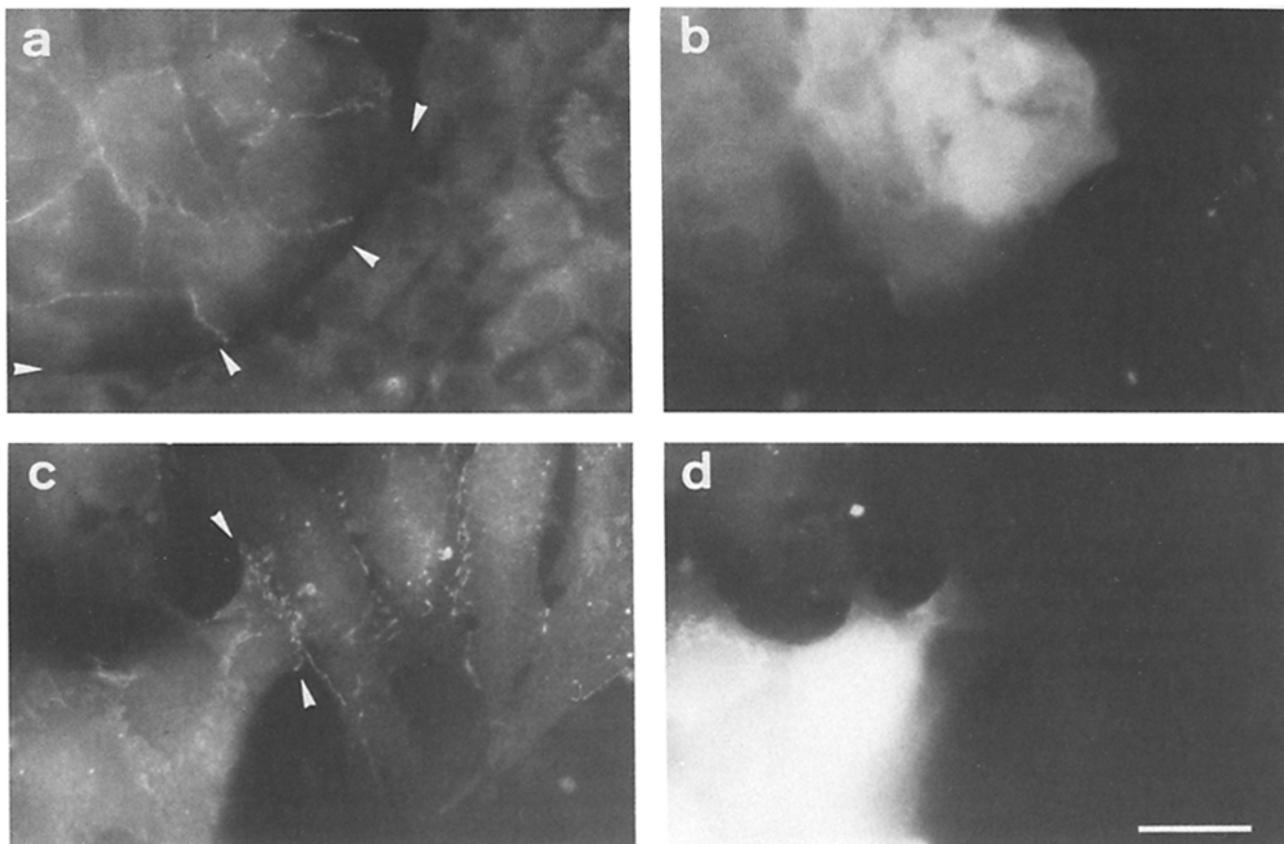


Figure 5. Distribution of N-cadherin in mixed cultures of lens and liver cells. (*a* and *b*) Contacts between lens and hepatocytes; (*c* and *d*) contacts between lens and fibroblasts. (*a* and *c*) Stain for N-cadherin; (*b* and *d*) the same fields as *a* and *c*, respectively, which visualize lens cells labeled with CFSE. Note the absence of intense N-cadherin staining in the boundary between lens and hepatocytes, but the positive stain is detected in the boundary between lens and fibroblasts as well as in the boundary between fibroblasts. Arrows indicate the boundary between heterotypic cells. Bar, 20 μ m.

with that of actin present at the lateral cell cortex (Fig. 6). Wherever cadherins were detected, actin was stained with exactly the same localization pattern. This was, however, not the case in the opposite sense; that is, all actin bundles were not co-localized with cadherins. Especially, actin stress fibers appeared to have no correlation with cadherin distribution.

The co-localization of cadherins and actin could be merely coincidental, since actin belts are known to be present at the cell cortex. However, the pattern of cadherin very accurately coincides with that of actin staining, as seen in the photographs shown in Fig. 6. Furthermore, actin bundles associated with cadherins were detected only in the apical region of cells but not in the ventral region (data not shown). The following experiments further suggest a structural association of cadherins with actin bundles.

We examined effects of cytochalasin D on the distribution of cadherins and actin. Although the morphology of cell-cell binding sites was altered by cytochalasin D treatment, cellular connections themselves were usually retained. When F9 cells were examined, the staining pattern of E-cadherin was not severely affected by this drug although cells were rounded up. In these cells, actin still coincided with E-cadherin as observed in non-treated cells (Fig. 7, *a* and *b*). The cytochalasin treatment induced aggregation of actin in the cytoplasm.

Such aggregates of actin were always associated with intercellular boundaries (Fig. 7 *b*).

In hepatocytes treated with cytochalasin D, their surface morphology was severely affected, but their cell-cell binding

Table II. Extent of Staining Intensity for Cadherins at the Cellular Boundary between Various Cell Types

Combinations	Percent cellular boundary stained		
	Intensely	Weakly	Not stained
F9-F9	100	0	0
MTD-1A-MTD-1A	77	15	8
F9-MTD-1A	75	19	6
F9-PSA5-E	6	46	48
F9-L	2	13	85
Lens-hepatocyte	3	25	72
Lens-fibroblast	68	22	10

Mouse cell combinations were stained for E-cadherin, and chicken cell combinations of lens, hepatocytes and liver fibroblasts were stained for N-cadherin. Approximately 50 pairs of cells, which are in contact with each other, were randomly selected. Immunofluorescent staining intensity at the cellular boundary in each combination was examined by fluorescent microscopy. Cellular boundaries stained as intensely as in homotypic combinations were scored as "stained intensely"; those showing no staining were scored "not stained"; and those stained with the intermediate intensity were scored "stained weakly."

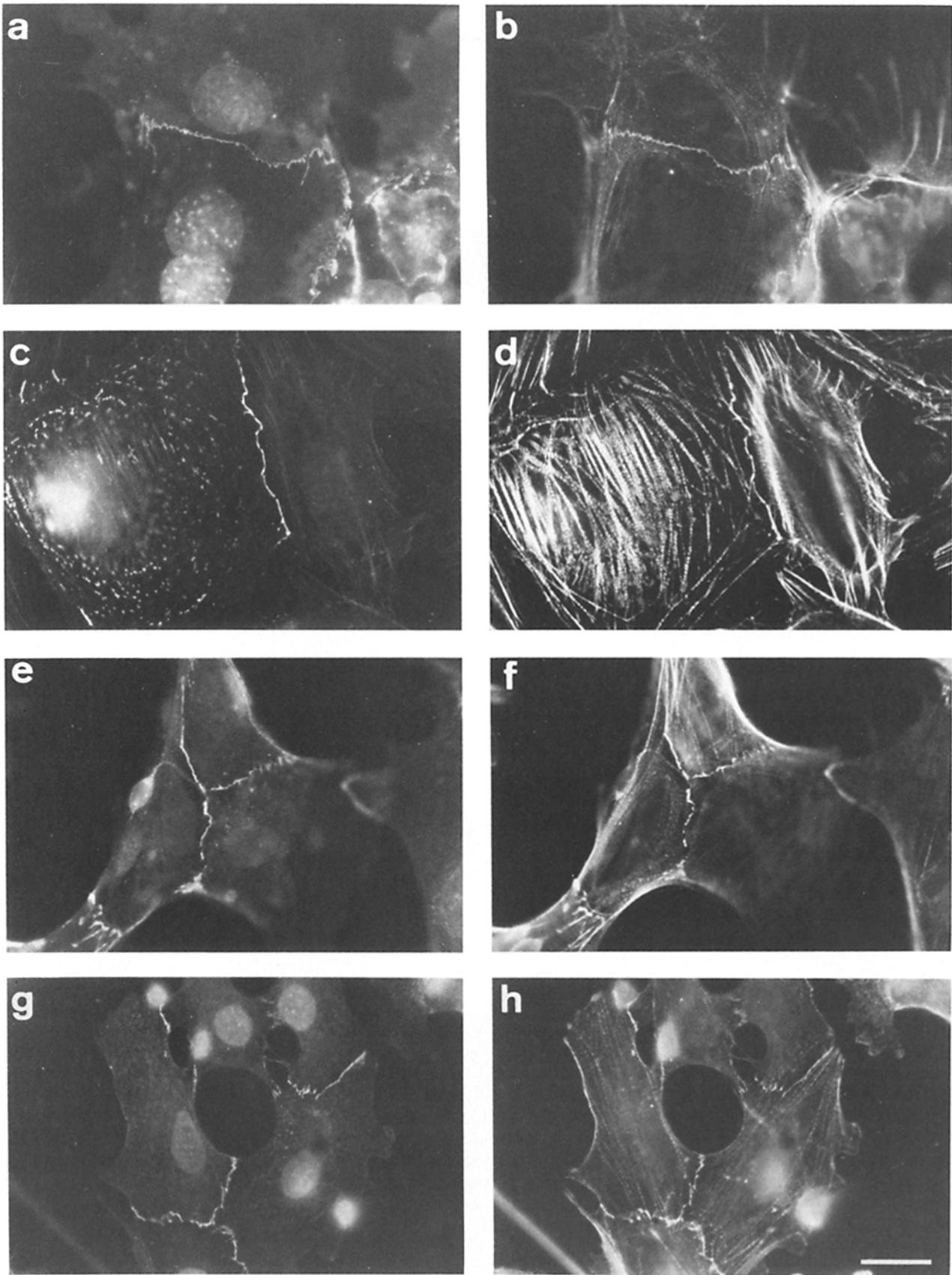


Figure 6. Co-localization of cadherins with actin, detected by double-staining method. (a and b) Hepatocytes. (c and d) Uterine decidua cells. (e, f, g, and h) Spinal cord cells. (Left column) Staining for cadherins; (right column) staining for actin in corresponding fields. Bar, 20 μ m.

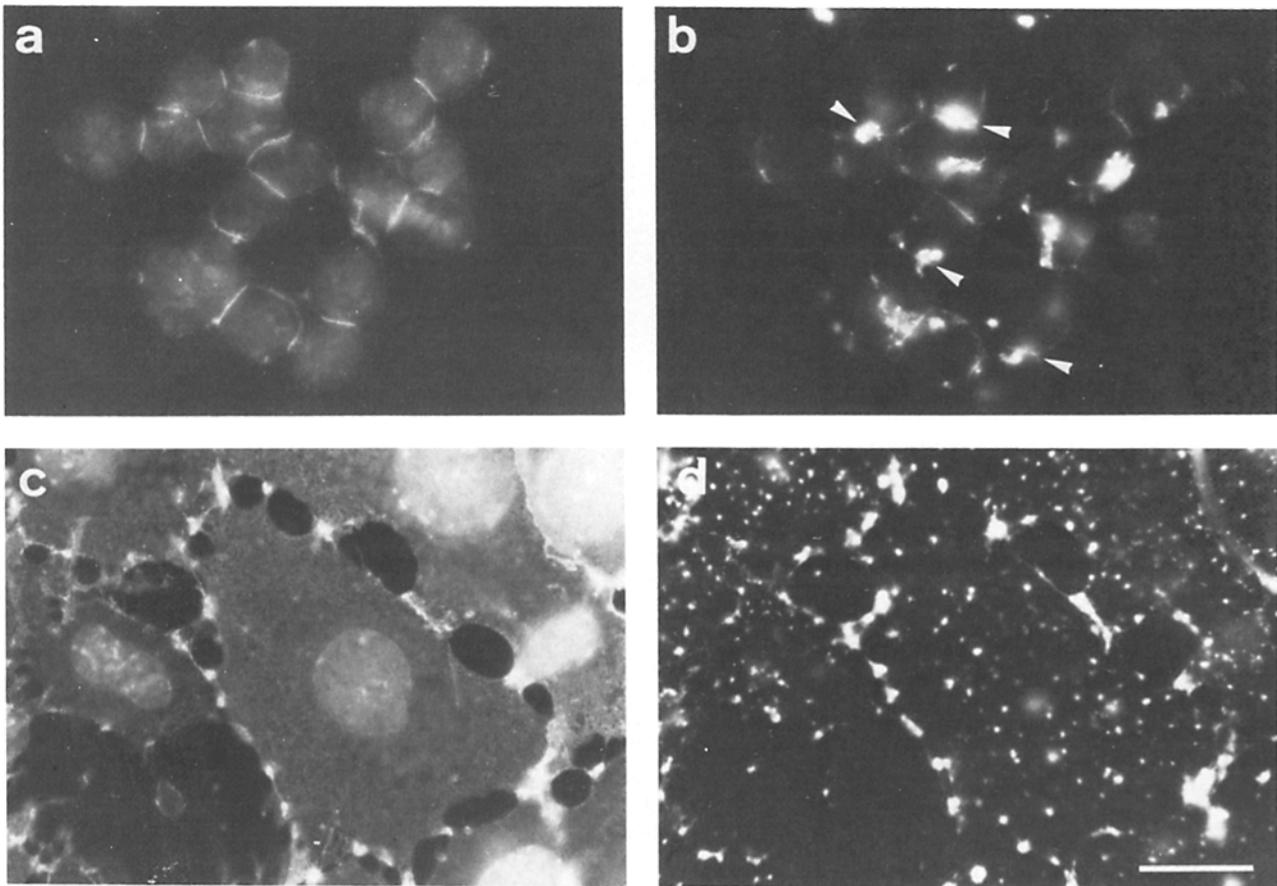


Figure 7. Effect of cytochalasin D on the localization of cadherins and actin. (a and b) F9 cells. (c and d) Hepatocytes. (Left column) Staining for E-cadherin; (right column) staining for actin in corresponding fields. Cells were cultured in the presence of 1 µg/ml cytochalasin D for 60 min. Arrows indicate some of actin aggregates induced by cytochalasin D associated with the cellular boundary. Bar, 20 µm.

sites were retained. E-cadherin staining was intensely concentrated at these sites (Fig. 7 c). Actin bundles were disrupted and aggregated in these cells. It was found, however, that all E-cadherin-stained sites coincided exactly with actin staining, although there were many actin aggregates not co-

localized with E-cadherin (Fig. 7 d). Similar results were obtained with spinal glial cells expressing N-cadherin and decidual cells expressing P-cadherin (data not shown). These experiments strongly suggest that cadherins are structurally associated with actin bundles.

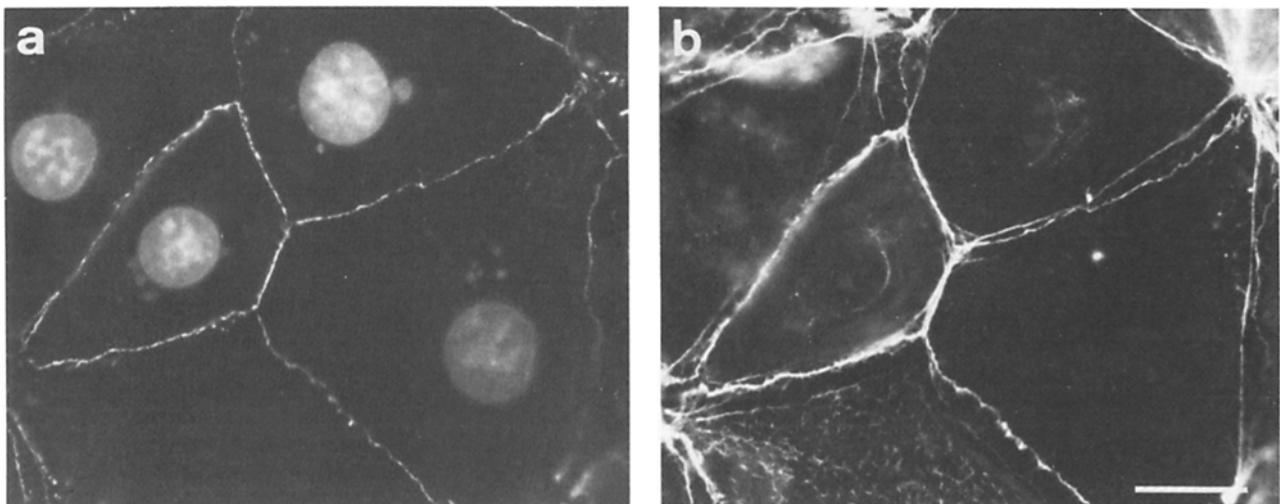


Figure 8. Effect of NP40 extraction on the distribution of E-cadherin and actin in MCF-7 cells. Cells were treated with 0.5% NP40 in HMF for 30 min at room temperature and then fixed. (a) Staining for E-cadherin; (b), staining for actin in corresponding field. Bar, 20 µm.

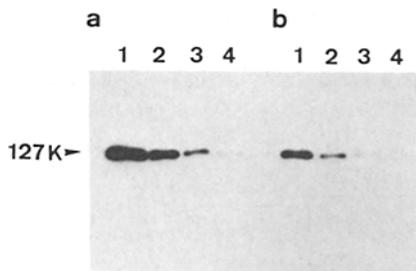


Figure 9. Immunoblot analysis of E-cadherins on F9 cells nontreated (a) and treated with NP40 (b). Cells (1×10^7) were treated with 4 ml of 0.5% NP40 in HMF for 30 min under a constant shaking. The sample was then centrifuged at 100,000 *g* for 30 min and the pellet was dissolved in 1 ml of an SDS buffer for the SDS-PAGE (Nose and Takeichi, 1986). As the control, nontreated whole cells were dissolved in the SDS buffer in the same ratio of cell number/buffer volume as for the NP40-treated cells. These samples were loaded on each lane for the SDS-PAGE after dilution of 1 \times (lane 1), 2 \times (lane 2), 4 \times (lane 3), and 8 \times (lane 4). The electrophoretic gel was then processed for immunoblot analysis to detect E-cadherin as described (Nose and Takeichi, 1986). As seen, $\sim 1/2$ as much of cadherins in cells is detected in the cell fraction that is not solubilized with NP40.

We further found that extracting cells with a nonionic detergent NP40 of cells did not remove either cadherins or actin bundles (Fig. 8). Co-localization of cadherins with actin bundles was also not affected by the detergent treatment. The immunoblot analysis showed that about half as much of the total E-cadherin in F9 cells is not extracted by NP40 (Fig. 9). Similar results were obtained for P- and N-cadherin using embryonic tissues (data not shown). These findings again suggest that cadherins are associated with the actin-based cytoskeletal element.

Discussion

The present study showed that all cadherin subclasses were concentrated at the intercellular boundary even in cell combinations derived from different tissues as long as cells have an identical cadherin subclass. This tendency, however, decreased or even disappeared at the boundary between heterotypic cells expressing different cadherin subclasses. This suggests that cadherins interact with each other in a homophilic way to link cells, and each subclass preferentially reacts with its own type. Probably, cadherin molecules can move laterally on cell membranes as most membrane proteins do, and those entering into the intercellular spaces might interact with homologous molecules present on the neighboring cells across the intercellular gap. The formation of intercellular bridges between cadherin molecules may immobilize themselves, resulting in their accumulation in the area of cell-cell adhesion. Different cadherin subclasses may interact with each other by lower affinities, thus resulting in weaker or no cadherin accumulation in the cell-cell boundary.

There is an alternative way to explain the present observations; e.g., to postulate the presence of receptors for cadherins specific to each cadherin subclass. To fulfill this model, each receptor must have the same cell type-specific distribution as that of the corresponding cadherin subclasses; otherwise, the cadherin subclass-specific adhesion cannot be ex-

plained. Although the simpler "homophilic model" is more likely, we need further studies to obtain conclusive evidence for this model.

A question is raised: how are cadherins driven into the cell-cell contact sites? The present observations strongly suggest that all subclasses of cadherin molecules are structurally associated with actin bundles located in the cell cortex. This conclusion is consistent with ultrastructural observations that uvomorulin (Boller et al., 1985) and A-CAM (Volk and Geiger, 1986a, b), both of which are cadherin-like molecules, are localized in the intercellular adherens (intermediate) junction in certain tissues, and this junction is known to be associated with actin belts (see Geiger et al., 1985b for review). Our previous immunocytochemical studies on histological sections of embryonic tissues also showed that cadherins were concentrated at the apical side of epithelial layers where the zonula adherens are located (Hatta and Takeichi, 1986; Nose and Takeichi, 1986; Hatta et al., 1987). Taken together, it is likely that cadherins are a component of intercellular junctions of the adherens-junction type, and their action and distribution are controlled through actin filaments. It should be noted, however, that cadherins are not always localized at the apical side of epithelial cell layers but distributed evenly around the entire intercellular boundary in many embryonic tissues (Nose and Takeichi, 1986). Thus, they might also be able to function in cellular binding without forming any specialized structures.

To prove the association of cadherins with actin, we have to carry out extensive ultrastructural and biochemical studies and to find mediators for this association, if present. Vinculin can be a candidate for such an action, since it is known to be a component of the adherens junction (Geiger et al., 1981; Geiger et al., 1985a). However, vinculin is present also in the focal cell-substratum-adhesion sites, in which cadherins are not present. The distribution of plakoglobin, which was recently described as a plaque component of various intercellular junctions (Cowin et al., 1986), is rather similar to that of cadherins. Therefore, it may be interesting to examine in future studies whether or not cadherins bind to this plaque component.

It remains to be solved whether or not activity of cadherins per se depend on their association with cytoskeletons. We observed in the present study that cytochalasin D did not disrupt cadherin-dependent adhesion, as we had found previously (Takeichi 1977). This drug also failed in destroying the cell-cell adhesion in other systems (Miranda et al., 1974). It may be, however, premature to conclude that actin filaments have no role in the cadherin action, since cytochalasin D did not destroy actin bundles associated with intercellular junctions although it did disrupt stress fibers.

If the functional links between cadherins and cytoskeletal elements were proven, we should be able to answer such questions relevant to the mechanism of cell-cell interaction as how the junctional complex is formed, how the polarity of epithelial cell sheets is established and how the morphology of cell sheets is altered after malignant transformation. Answering these questions is very important in elucidating mechanisms of organization of multicellular systems.

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