

A Novel Cadherin Cell Adhesion Molecule: Its Expression Patterns Associated with Implantation and Organogenesis of Mouse Embryos

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Abstract. The Ca^{2+} -dependent cell adhesion molecules, termed cadherins, were previously divided into two subclasses, E- and N-types, with different adhesive specificity. In this study, we identified a novel class of cadherin, termed P-cadherin, using a visceral endoderm cell line PSA5-E. This cadherin was a 118,000-D glycoprotein and distinct from E- and N-cadherins in immunological specificity and molecular mass. In accord with these findings, cells with P-cadherin did not cross-adhere with cells with E-cadherin. P-Cadherin first appeared in developing mouse embryos in the extraembryonic ectoderm and the visceral endoderm at the egg cylinder stage and later was expressed in various tissues. The placenta and the uterine decidua most abundantly expressed this cadherin. The expression of P-cadherin was tran-

sient in many tissues, and its permanent expression was limited to certain tissues such as the epidermis, the mesothelium, and the corneal endothelium. When the tissue distribution of P-cadherin was compared with that of E-cadherin, we found that: (a) each cadherin displayed a unique spatio-temporal pattern of expression; (b) P-cadherin was co-expressed with E-cadherin in local regions of various tissues; and (c) onset or termination of expression of P-cadherin was closely associated with connection or segregation of cell layers, as found with other cadherins. These results suggested that differential expression of multiple classes of cadherins play a role in implantation and morphogenesis of embryos by providing cells with heterogenous adhesive specificity.

THE animal body is not a random collection of various cell types, but a structure with organized arrangements of cells. It has been suggested that the arrangement of cells is governed by selective affinity of cells; that is, cells seem to have the capability to recognize particular cell types in their adhesion (Townes and Holtfreter, 1955). Recent progress in the identification of cell adhesion molecules is making it possible to verify this hypothesis at the molecular level.

The Ca^{2+} -dependent cell-cell adhesion system, defined by its characteristic Ca^{2+} - and protease-sensitivity (Takeichi, 1977; Urushihara et al., 1979), shows interesting properties for elucidating selective cell adhesion mechanisms (Takeichi et al., 1985). We have identified molecules functional in this adhesion system in various cell types, terming them cadherins, and found that different cell types express different cadherins. For example, epithelial cells of many tissues expressed a cadherin with a molecular mass of 124,000 D, termed E-cadherin (Ogou et al., 1983; Yoshida-Noro et al., 1984), which is also called uvomorulin (Peyrieras et al., 1983), L-CAM (Gallin et al., 1983), and cell-CAM 120/80 (Damsky et al., 1983), and nervous tissues expressed a cadherin with molecular mass of 127,000 D, termed N-cadherin (Hatta et al., 1985; Hatta and Takeichi, 1986). These

different cadherins appear not to interact with each other, and may thus provide cells with adhesive specificity (Takeichi et al., 1981; Takeichi et al., 1985). Based on these observations, cadherins were divided into subclasses with different specificity. To date, two subclasses, E- and N-types, have been identified.

Recent studies on the tissue distribution of cadherins in chicken embryos (Thiery et al., 1984) demonstrated that all cells in early embryos initially express L-CAM, a chicken equivalent of E-cadherin, but during development many tissues lose it and instead begin to express N-cadherin (Hatta and Takeichi, 1986). Such a transition in expression of cadherins from E- to N-type was found in the mesoderm migrating through the primitive streak, in the invaginating neural plate and in the invaginating lens vesicle. In all these cases, the transition in cadherin expression was associated with the segregation of cell layers. We suggested, therefore, that regulation of expression of cadherins may be an important process in animal morphogenesis.

During the course of these studies, we found that certain cell types have neither E- nor N-cadherin, suggesting the presence of other classes of cadherins (Hatta et al., 1985). The mouse visceral endodermal cell line PSA5-E was one of them. In this study, we describe the properties of a cadherin

detected in this cell line. This cadherin, termed P-cadherin, is distinct from other cadherins in molecular mass, binding specificity, and tissue distribution, and is most abundant in the placenta. Studies of its expression pattern, comparing it with that of E-cadherin in mouse embryos, show that these two cadherins are differentially expressed during embryogenesis and their expression patterns are closely associated with various morphogenetic events.

Materials and Methods

Cells and Cultures

Mouse endoderm cell line PSA5-E (Adamson et al., 1977), mouse embryonal carcinoma F9 (Bernstine et al., 1973), mouse glioma G26-20 (Sundarraj et al., 1975), mouse fibroblast STO (Martin and Evans, 1975), and myeloma P3-X63-Ag8-U1 were used. For primary culture of the uterine decidua cells, the deciduas were isolated from the uterus of pregnant mice at 6.5 d of gestation. The tissues were fragmented into small pieces using scissors, and incubated in 0.25% trypsin (Difco Laboratories Inc., Detroit, MI, 1:250) and 0.5% collagenase (Wako Chemicals, Kyoto) in Ca^{2+} - and Mg^{2+} -free saline buffered with 10 mM Hepes (pH 7.4) (HCMF)¹ for 20 min. After the solution was removed, the tissues were rinsed with 1 mM EDTA and further incubated in 0.25% trypsin in HCMF for 15 min at 37°C. The treated deciduas were dissociated into single cells or small cell clusters by pipetting, and cultured for 16 h before use.

Cell cultures were carried out using a 1:1 mixture of DME and Ham's F12 (DH medium), supplemented with 10% FCS (DH10F).

Trypsin Dispersion and Aggregation of Cells

Suspensions of cells with active cadherins were prepared as described (Urushihara et al., 1979). Briefly, cells in monolayer cultures were treated with 0.01% trypsin in the presence of 1–5 mM CaCl_2 (TC-treatment) for 20 min at 37°C and washed with HCMF supplemented with 2 mM CaCl_2 (HMF). When necessary to obtain single cells, they were rinsed with cold HCMF and flushed through a pipette. To prepare cells without cadherins, cells were treated with 0.01% trypsin in the presence of 1 mM EGTA (TE-treatment) under the same conditions as for TC-treatment. Assay of aggregation of these cells was carried out as described (Urushihara et al., 1979).

Production of Hybridomas and Monoclonal Antibodies

A Donryu rat was immunized by several intraperitoneal injections of PSA5-E cells (1.5×10^8 cells/injection) homogenized with Freund's adjuvant. 3 d after the last injection, spleen cells were removed and fused with mouse myeloma P3-X63-Ag-U1 cells according to the method of Kohler and Milstein (1975). Screening of antibodies in culture supernatant of hybridomas was performed in two steps. The first step was to detect antibodies that bind to the surface of PSA5-E cells but not to that of F9 cells, which were cultured in 96-well plates. These cells were incubated with hybridoma supernatant for 45 min at 37°C. After rinsing them with HMF, bound antibodies were detected by ELISA (Posner et al., 1982) using a peroxidase-conjugated rabbit Ig to rat Ig (DAKOPATTS, Copenhagen). Antibodies reacting specifically with PSA5-E cells were thus selected, and were then screened for activity in binding to TC-treated but not to TE-treated PSA5-E cells by indirect immunofluorescence cytochemistry using FITC-conjugated rabbit Ig to rat Ig (DAKOPATTS) as the second agent.

Culture supernatant of hybridoma selected as above was collected from high density cultures and pooled. To concentrate antibodies, ammonium sulfate was added to the culture supernatant to 50% saturation and the resultant precipitate was dissolved in a small volume of HCMF and dialyzed against this solution. To purify antibodies, hybridomas were cultured in a serum-free DH medium. Precipitates with 50% saturated ammonium sul-

1. *Abbreviations used in this paper:* HCMF, Ca^{2+} - and Mg^{2+} -free saline buffered with 10 mM Hepes (pH 7.4); HMF, HCMF supplemented with 2 mM CaCl_2 ; TBS, Tris-buffered saline (pH 7.5) supplemented with 1 mM CaCl_2 ; TC-treatment, treatment with 0.01% trypsin in the presence of 1–5 mM CaCl_2 ; TE-treatment, treatment with 0.01% trypsin in the presence of 1 mM EGTA.

fate from this culture supernatant contained almost pure monoclonal antibodies.

Production of the monoclonal antibody ECCD-2 has been described elsewhere (Shirayoshi et al., 1986).

Immunoblot Analysis

Intact cells, tissue fragments, or their membrane fractions were dissolved in a buffer containing 2% SDS as described previously (Yoshida and Takeichi, 1982). After boiling for 3–4 min in the presence of 5% 2-mercaptoethanol, the samples were fractionated by SDS PAGE in 7.5% acrylamide gels according to the method of Laemmli (1970) with slight modifications. The fractionated materials in the gels were then electrophoretically transferred to nitrocellulose sheets according to the method of Towbin et al., (1979). The nitrocellulose sheets were coated with 5% skim milk (Difco Laboratories, Inc.) to avoid nonspecific binding of antibodies. The sheets were then incubated with the 10-fold concentrated supernatant of hybridoma followed by incubation with ^{125}I -labeled anti-rat Ig (Amersham Corp., Arlington Heights, IL). Antigen bands reacting with the antibodies were visualized by autoradiography using Fuji New RX X-ray films.

Immunohistochemical Procedures

Embryos were fixed in 2% paraformaldehyde in HMF for 1 h at 4°C. After being washed, the specimens were incubated in a graded series of sucrose (12–18%) in HMF at 4°C. They were then embedded in the OCT compound (Miles Scientific, Naperville, IL) and frozen in liquid nitrogen. Cryostat sections (10 μm thick) were picked up on slides previously coated with 1% gelatin and 0.1% chromium sulfate, and air dried.

Sections were incubated in the following solutions successively with several washings at each interval in Tris-buffered saline (pH 7.5) supplemented with 1 mM CaCl_2 (TBS): (a) in 5% skim milk in TBS for 20 min, (b) in the supernatant of hybridoma concentrated five to eight times, supplemented with 5% skim milk for 45 min, (c) in biotinylated anti-rat Ig (Amersham Corp.) diluted 1:50 with TBS containing 5% skin milk for 30 min, and (d) in fluorescein-streptavidin (Amersham Corp.) diluted 1:50 with TBS containing 5% skim milk for 15 min. After several washes in TBS the sections were mounted in 90% glycerol–10% TBS (pH 8.0) containing 0.1% paraphenylenediamine, to prevent bleaching (Johnson and Nogueira Araujo, 1981). Photographs were taken on Tri-X films (Kodak) by a Zeiss 18 FL microscope.

Results

Characterization of Cadherins in PSA5-E Cells

We screened monoclonal antibodies raised against PSA5-E cells to obtain antibodies specific to their putative cadherins. One antibody, designated as PCD-1, showed a unique binding property in that it bound to the surface of PSA5-E cells treated with trypsin in the presence of Ca^{2+} (TC-treatment), but not to cells treated with the same concentration of trypsin in the presence of EGTA (TE-treatment). Immunoblot analysis showed that this antibody reacts with a protein band with molecular mass of 118,000 D (Fig. 1). This band was detected in PSA5-E cells that were or were not trypsinized in the presence of Ca^{2+} , but not in those trypsinized with EGTA (Fig. 1, A and B). This trypsin sensitivity of the 118-kD molecule was consistent with the defined property of cadherins (Takeichi, 1977).

Immunoblot analysis showed that the PCD-1 does not react with any component in teratocarcinoma F9 cells expressing E-cadherin (Yoshida-Noro et al., 1984), glioma G26-20 cells expressing N-cadherin (Hatta et al., 1985), and fibroblast STO cells with cadherin activity (Fig. 1 B) The monoclonal antibody ECCD-2 specific to E-cadherin (data not shown) and the NCD-1 antibody specific to N-cadherin did not react with surface components of PSA5-E cells (Hatta and Takeichi, 1985). Taken together, the 118-kD molecule is dis-

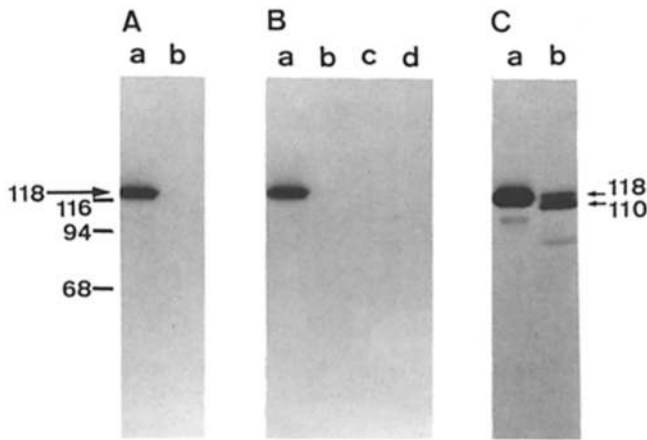


Figure 1. Immunoblot analysis of antigens to the monoclonal antibody PCD-1. (A) Lane a, TC-treated PSA5-E cells; lane b, TE-treated PSA5-E cells. (B) Lane a, PSA5-E cells; lane b, F9 cells; lane c, G26-20 cells; lane d, STO cells. (C) Effect of tunicamycin. Lane a, control PSA5-E cells; lane b, PSA5-E cells cultured in the presence of 0.1 $\mu\text{g/ml}$ of tunicamycin for 17 h.

tinct in immunological specificity from cadherins previously identified.

To determine whether the 118-kD molecule is a glycoprotein, we cultured PSA5-E cells in the presence of tunicamycin and used these cells for immunoblot analysis. After treatment of cells with tunicamycin, the PCD-1 antibody recognized two proteins, one of 118 kD and a lower molecular mass protein of 110 kD (Fig. 1 C, lane b). This change in molecular mass is consistent with that associated with glycoprotein with oligosaccharides. Moreover, this result suggested that PCD-1 recognizes a protein moiety of the 118-kD molecule.

To know whether PCD-1 can block the function of cadherins, we studied the effect of this antibody on the cadherin-mediated cell aggregation, using cells of primary cultures of the uterine decidua of pregnant mice that were strongly positive with PCD-1 as described below. The Ca^{2+} -dependent aggregation of the decidual cells dissociated by the TC-treatment was strongly inhibited by PCD-1 (Fig. 2). Antibodies recognizing E- and N-cadherins had no effect on aggregation of these cells.

All these results suggested that PCD-1 recognizes a novel class of cadherins and inhibits their function. We thus term

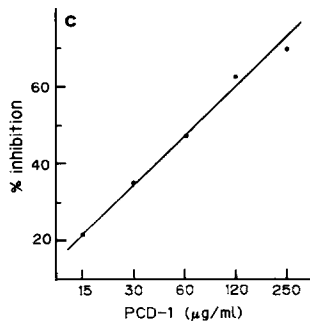


Figure 2. Effect of PCD-1 on Ca^{2+} -dependent aggregation of decidual cells. Decidual cells dissociated by TC-treatment were allowed to aggregate for 60 min in HMF in the presence of various concentrations of PCD-1 antibody. After measuring the rate of cell aggregation by a Coulter counter, the extent of inhibition of aggregation by the antibody was calculated as described (Urushihara et al., 1979).

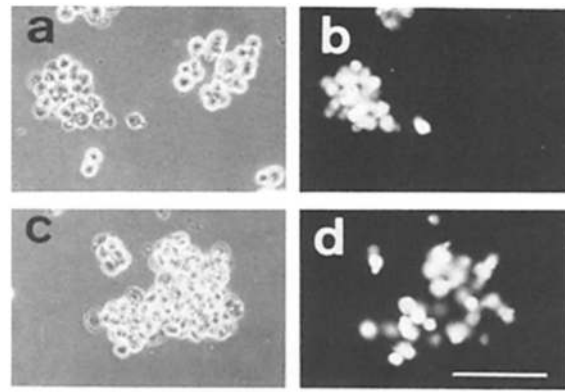


Figure 3. Selective aggregation of PSA5-E and F9 cells. PSA5-E cells were labeled with 5(-6)carboxyfluorescein diacetate succinimidyl ester as described by Bronner-Fraser (1985), and dissociated by TC-treatment. They were mixed with unlabeled F9 cells that were also dissociated by TC-treatment, and incubated for 20 min on a gyratory shaker. A control sample was prepared by mixing the labeled and unlabeled PSA5-E cells. (a and b) Experimental combination. (c and d) Control combination. (a and c) Phase-contrast micrographs. (b and d) Fluorescence micrographs of the same fields as in a and c, respectively. Bar, 100 μm .

the 118-kD molecule recognized by PCD-1 the placental cadherin (P-cadherin), because this molecule is most abundant in the placenta (see below).

Adhesive Specificity of Cells with P-Cadherin

To determine the functional specificity of P-cadherin, we examined whether PSA5-E cells with this adhesion molecule can cross-adhere with F9 cells expressing E-cadherin. These cells were dispersed by TC-treatment, leaving cadherins intact but destroying the Ca^{2+} -independent cell adhesion systems (Urushihara et al., 1979). The cells were then mixed in a 1:1 ratio and allowed to aggregate. To distinguish between the two cell types in the suspension, cells of one type were stained with a fluorescent dye before the dispersion treatment, and mixed with unlabeled cells of the same type or another type. The samples of cell aggregates were examined by fluorescence microscopy. Fig. 3 shows that PSA5-E cells and F9 cells tended to aggregate independently. This result suggested that these cells preferentially adhere to their own types, thus P- and E-cadherins probably have distinct binding specificities, although other adhesion molecules could also be involved in the observed selective aggregation.

Distribution of P-Cadherin in Mouse Embryos

To find out which tissues of mouse embryos express P-cadherin, we performed immunoblot analysis using embryos at 14–15 d of gestation. Fig. 4 A shows that the placenta contains an abundance of the 118-kD protein, whereas some other tissues, such as skin, intestine, heart and lung, only weakly express this protein. Brain and liver were essentially negative for P-cadherin. PCD-1 reacted with a band with a higher molecular mass than that of the 118-kD P-cadherin in skin; its relationship to the 118-kD band, however, remains to be determined.

We then examined the uterus before and after implantation of embryos (Fig. 4 B). Expression of the 118-kD protein be-

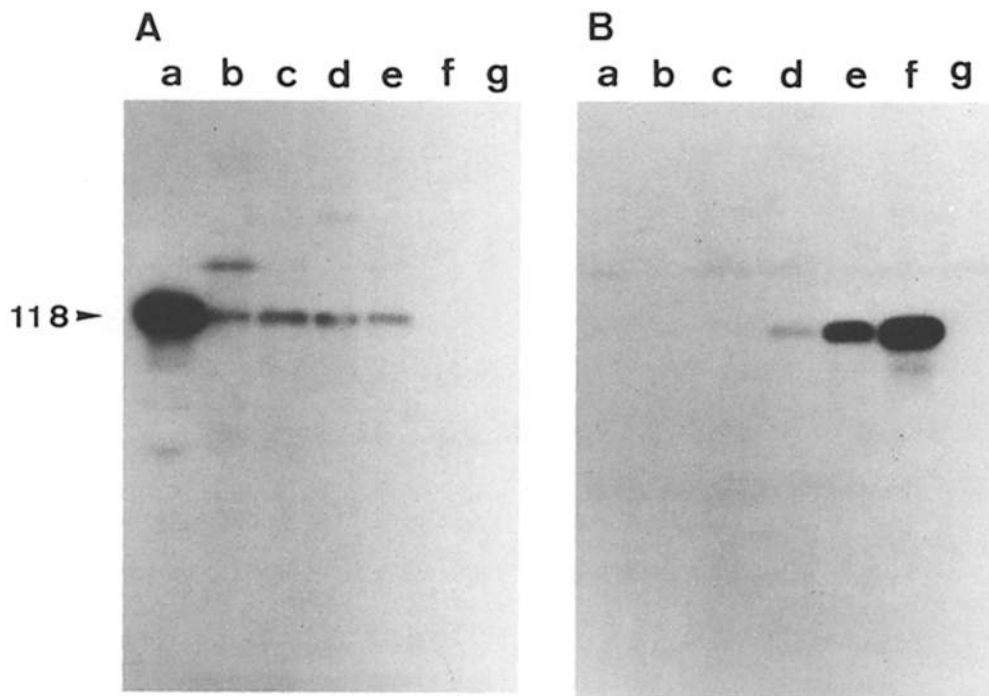


Figure 4. Immunoblot analysis of P-cadherin in various tissues. (A) Lane *a*, placenta; lane *b*, skin; lane *c*, intestine; lane *d*, heart; lane *e*, lung; lane *f*, liver; lane *g*, brain. These tissues were collected from the 14-d mice. (B) Uterus at varying pregnancy stages, from which embryos were removed. Lane *a*, non-pregnant; lane *b*, 3.5 d of gestation; lane *c*, 4.5 d of gestation; lane *d*, 5.5 d of gestation; lane *e*, 6.5 d of gestation; lane *f*, the decidua isolated from the uterus at the same stage as in lane *e*; lane *g*, the uterus without the decidua at the same stage as in lane *e*. In preparing these samples, tissues were weighed, and similar amounts of tissues dissolved in SDS were loaded on each lane of the gels.

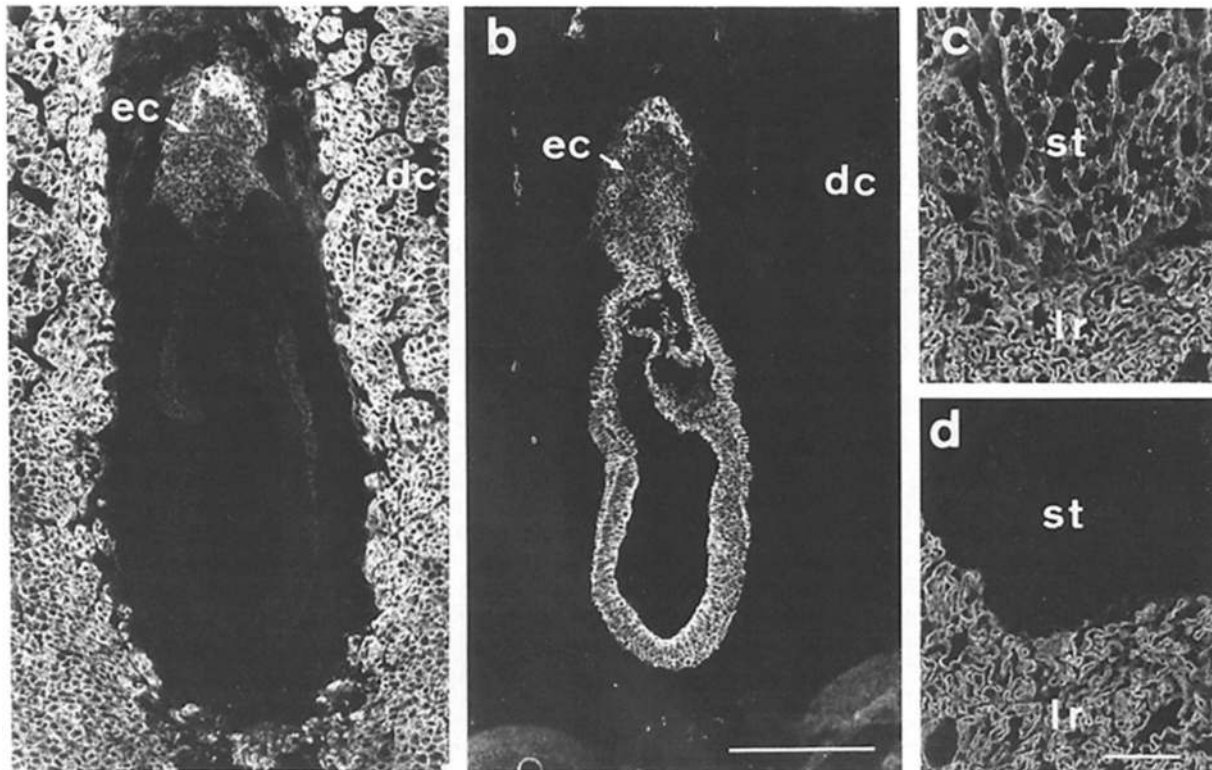


Figure 5. Expression of P- and E-cadherin in embryos at the egg cylinder stage and in the placenta. (a) Immunofluorescence stain for P-cadherin on a sagittal section of an embryo and surrounding uterine tissues at 7 d of gestation. (b) Stain for E-cadherin on a section adjacent to *a*. (c) Stain for P-cadherin on the placenta at 14 d of gestation. (d) Stain for E-cadherin on a section adjacent to *c*. Note the sharp boundary between the E-cadherin-positive region (labyrinth) and the negative region (spongiotrophoblast) of the placenta. *ec*, Ectoplacental cone; *dc*, decidua; *st*, spongiotrophoblast; *lr*, labyrinth. Bars: (a and b) 100 μ m; (c and d) 150 μ m.

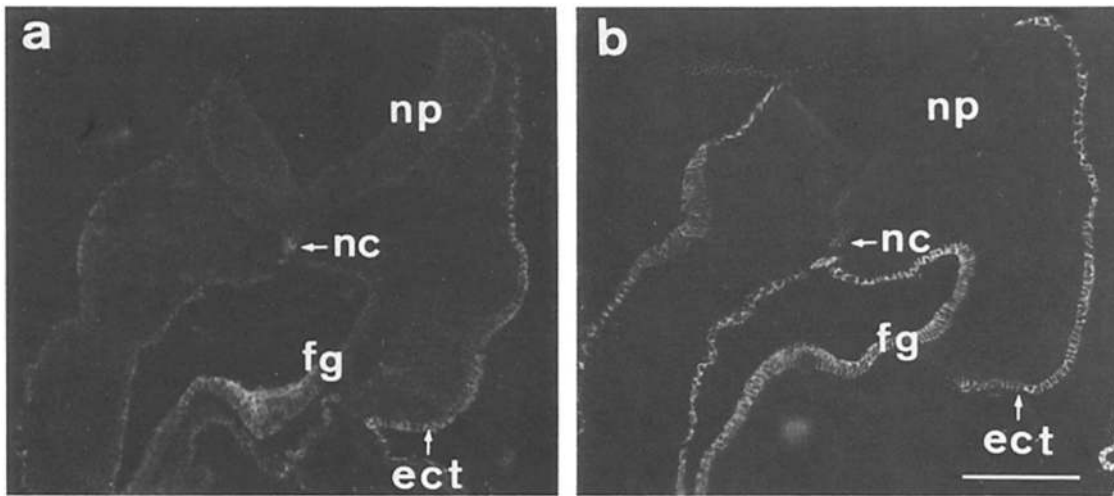


Figure 6. Expression of P- and E-cadherin during the neurulation. (a) Immunofluorescence stain for P-cadherin on a transverse section of an 8.5-d embryo. (b) Stain for E-cadherin on a section adjacent to a. np, Neural plate; nc, notochord; fg, foregut; ect, ectoderm. Bar, 200 μ m.

gan around 5 d of gestation, which corresponds to the beginning of decidual growth. When the decidua was separated from other parts of the uterus, the decidua only had P-cadherin (Fig. 4 B, lanes f and g). Strong expression in the uterus continued during the pregnancy.

Expression at the Egg Cylinder and Neurula Stage

To determine more precisely the localization of P-cadherin in the embryos and the uterus, we performed immunohistochemical analysis using embryos or fetuses at the egg cylinder stage to new-born stages. We also stained these materials with the ECCD-2 antibody recognizing E-cadherin to compare tissue distribution of P- and E-cadherin.

In embryonic tissues at 6–7 d of gestation, P-cadherin was expressed in the visceral endoderm and the extraembryonic ectoderm, but not in the embryonic ectoderm (Fig. 5 a). The ectoplacental cone particularly showed strong expression of P-cadherin. The mesoderm and the definitive endoderm formed by gastrulation was not stained for P-cadherin (Fig. 5 a). P-Cadherin was thus expressed only in the extraembryonic regions at the egg cylinder stage (until 7.5 d of gestation). In the maternal tissues, the uterine decidua showed strong expression of P-cadherin. The distribution of P-cadherin was therefore continuous from the embryonic to maternal regions of the future placenta.

Expression of E-cadherin in embryos at the above stages was detected in all embryonic and extraembryonic cells except for the parietal endoderm (Fig. 5 b), although its expression was suppressed in the mesoderm which appeared after gastrulation. The maternal tissues surrounding embryos did not express E-cadherin except in the uterine epithelium. Thus, P-cadherin was expressed on both sides of embryonic and maternal tissues of the early placenta, whereas E-cadherin was expressed only in the embryonic side. This distinct expression pattern of the two cadherins in the placenta lasted throughout the pregnancy. In the matured placenta, E-cadherin was detected in the labyrinth but not in the spongiotrophoblast and the decidua, whereas P-cadherin was

detected in all these tissues (Fig. 5, c and d). These immunohistochemical observations on the expression of P-cadherin in the uterus are consistent with the results of the immunoblot analysis (Fig. 4).

In embryos at 8.5–9 d of gestation, P-cadherin was detected in the embryonic ectoderm and in the embryonic endoderm undergoing formation of the gut (Fig. 6 a), whereas by this time it had disappeared from the visceral endoderm (visceral yolk sac). When differentiation of the mesoderm occurred, the lateral plate and the notochord (Fig. 6 a), but not other mesodermal derivatives, began to express P-cadherin. The mesothelium underlying the visceral yolk sac endoderm also expressed P-cadherin. Distribution of E-cadherin in the 8-d embryos was basically the same as that found at the earlier stages. It was detected in most of the ectodermal and endodermal tissues (Fig. 6 b) but not in the mesoderm. However, it disappeared from the neural plate when its invagination proceeded (Fig. 6 b). P-Cadherin was also not expressed in the neural plate (Fig. 6 a).

Expression during Ectodermal Organogenesis

The ectoderm expressed both P- and E-cadherin as described above. In investigating the expression pattern of P- and E-cadherin in tissues derived from the ectoderm of embryos at more advanced developmental stages, we focused the immunohistochemical analysis on the three morphogenetic events: the formation of eye, inner ear, and epidermis, as described below.

When the lens placode began to invaginate, this particular region of the ectoderm ceased to express P-cadherin although the other part of the ectoderm continued to express the two cadherins; thus, the invaginating lens vesicle contained only E-cadherin (Fig. 7, a and b). When the lens vesicle differentiated into the epithelium and lens fibers, the fibers ceased to express E-cadherin, although the epithelium continued to do so (Fig. 7, d and f). The primordium of retina, the optic cup, did not contain either P- or E-cadherin at the early stage (Fig. 7, a and b). As it differentiated into the neural retina and the pigmented retina, the pigmented layer began to

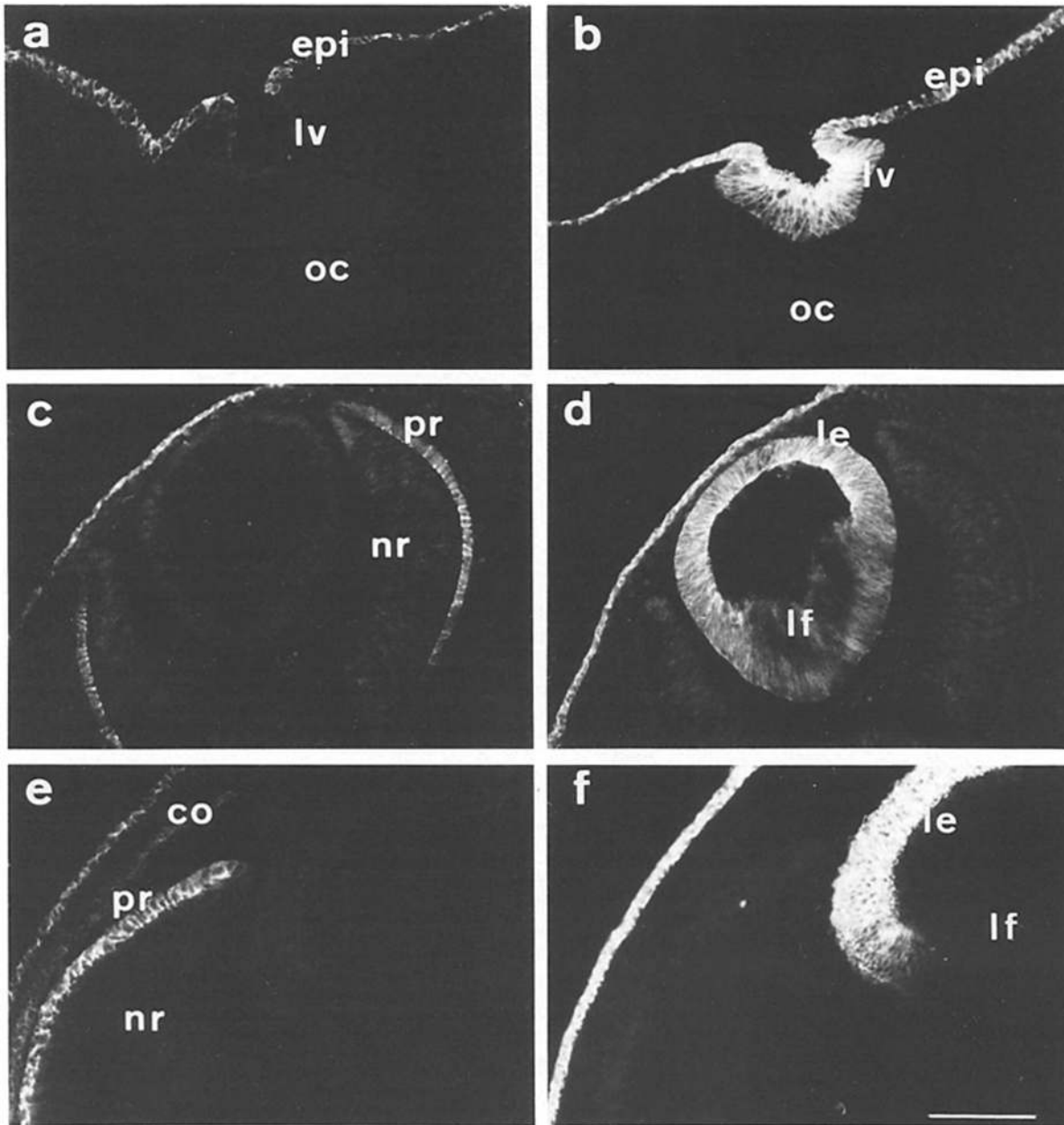


Figure 7. Expression of P- and E-cadherin during the eye formation. (*Left column*) Stain for P-cadherin. (*Right column*) Stain for E-cadherin. (*a and b*) Invaginating lens vesicles in a 10-d embryo. (*c and d*) Eye primordia in an 11-d embryo. (*e and f*) Eyes in a 14-d embryo. *epi*, Epidermis; *lv*, lens vesicle; *oc*, optic cup; *pr*, pigmented retina; *nr*, neural retina; *le*, lens epithelium; *lf*, lens fiber; *co*, corneal endothelium. Bar, 100 μ m.

express P-cadherin (Fig. 7, *c* and *e*). E-Cadherin was never expressed in the retina. The endothelium of cornea also expressed P-cadherin (Fig. 7 *e*).

The primordium of the inner ear, the otic placode, invaginates from the ectoderm to give rise to the otic vesicle. Fig. 8, *a* and *b*, show that both P- and E-cadherins were uniformly distributed on the otic vesicle at the early stage. After folding and branching of the vesicle, the distributions of P- and E-cadherin were no longer uniform, each displaying a distinct pattern (Fig. 8, *c* and *d*). Expression of the two cadherins overlapped in some regions, but in other regions, only E-cadherin was detected. There were spots where neither was detected. The border between these different re-

gions was generally sharp, although such borders cannot be detected morphologically on these cell layers without staining for cadherins.

The ectoderm covering the body surface differentiates into the stratified epidermis consisting of three layers: the stratum germinativum (the basal germinative layer), the stratum granulosum (the intermediate layer), and the stratum corneum (the fully keratinized layers). Fig. 8, *e* and *f*, shows sections of the skin of a new-born mouse stained for P- and E-cadherin. E-Cadherin (Fig. 8 *f*) was expressed in the stratum germinativum and the stratum granulosum, whereas P-cadherin (Fig. 8 *e*) was expressed only in the stratum germinativum. The stratum corneum did not contain either P- or E-cad-

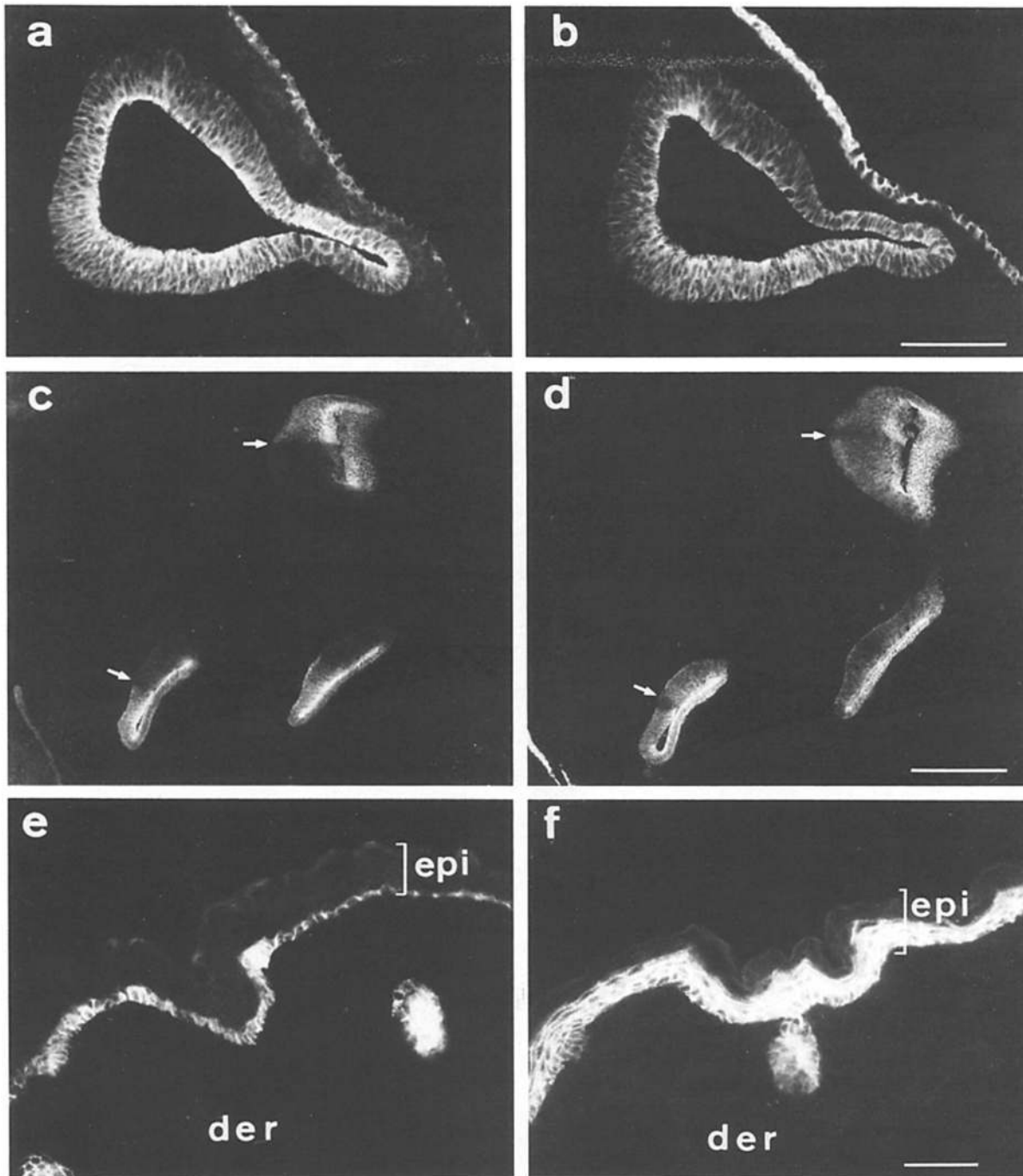


Figure 8. Expression of P- and E-cadherin on the otic vesicle and the epidermis. (*Left column*) Stain for P-cadherin. (*Right column*) Stain for E-cadherin. (*a*) Otic vesicle of a 10-d embryo; (*b*) a section adjacent to *a*. (*c*) Otic vesicle of a 14-d embryo; (*d*) a section adjacent to *c*. Arrows indicate regions that were neither stained for P-cadherin nor for E-cadherin. (*e* and *f*) Epidermis of a newborn mouse. *ep*, Epidermis; *der*, dermis. Bars: (*a* and *b*) 100 μ m; (*c* and *d*) 200 μ m; (*e* and *f*) 50 μ m.

herin. Thus, as cells of the basal layer move to the upper layer, they first lose P-cadherin and then E-cadherin in discrete steps.

Expression during Endodermal and Mesodermal Organogenesis

In the embryonic endoderm, expression of P-cadherin was first detected in the foregut at the neurulation stage, as described above. At later stages, P-cadherin was detected in the

lung epithelium (data not shown), but not in the pancreas, the intestinal epithelium (Fig. 9 *a*), and the liver (data not shown). In all endodermal organs, E-cadherin was always strongly expressed (Fig. 9 *b*).

The lateral plate mesoderm expressed P-cadherin, as described above. This expression continued in various derivatives of the lateral plate such as the epimyocardium (data not shown), the connective tissues of the blood vessels (data not shown) and intestine (Fig. 9 *a*), and the mesothelium lining

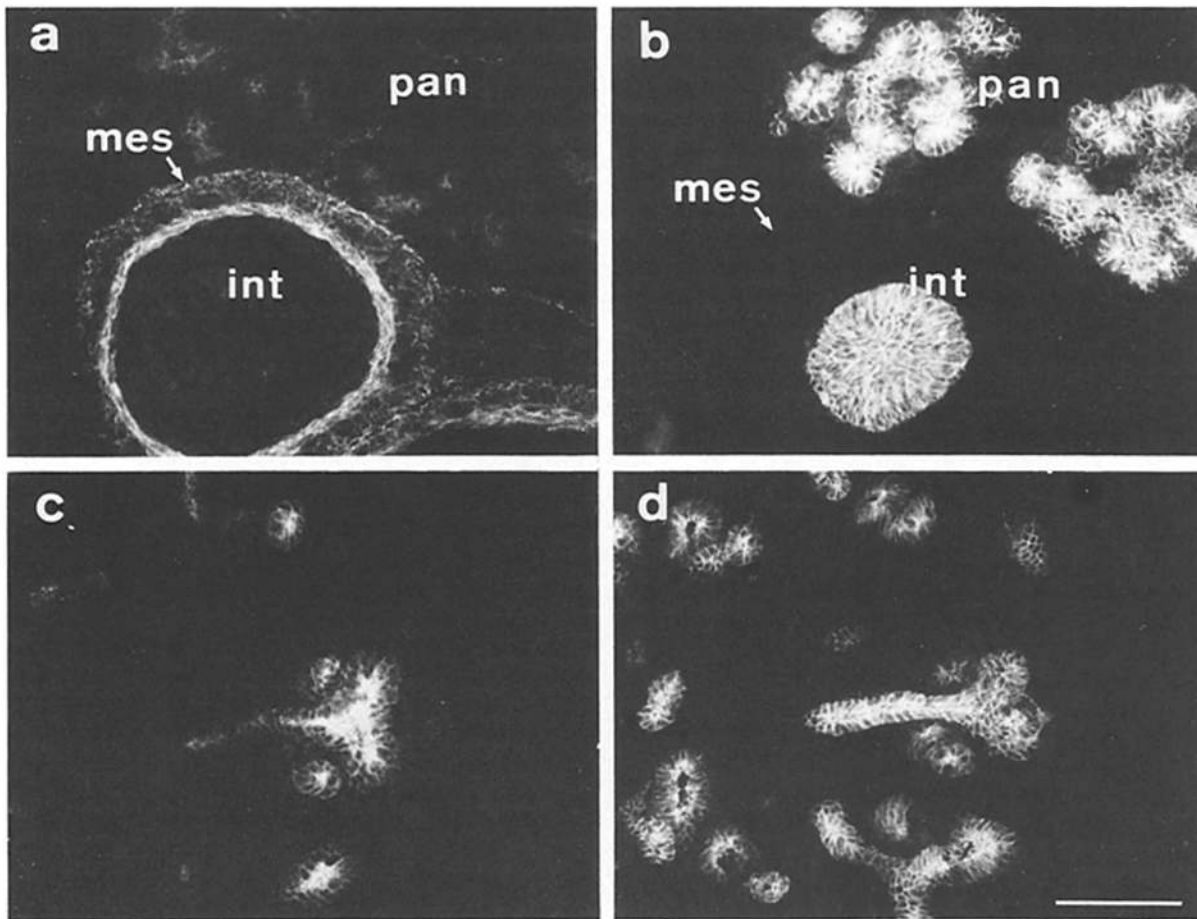


Figure 9. Expression of P- and E-cadherin in endodermal and mesodermal tissues. (*Left column*) Stain for P-cadherin. (*Right column*) Stain for E-cadherin. (*a*) A section of the intestine and surrounding tissues of a 14-d embryo. (*b*) A section adjacent to *a*. (*c*) A section of the metanephros. (*d*) A section adjacent to *c*. *mes*, Mesothelium; *pan*, pancreas; *int*, intestine. Bar, 100 μm .

of the peritoneal cavity (Fig. 9 *a*). P-Cadherin was, however, not seen in the vascular endothelium (data not shown). E-Cadherin was not detected in any of the lateral plate derivatives (Fig. 9 *b*).

The intermediate mesoderm was initially devoid of P- and E-cadherin. However, as these mesenchymal tissues were transformed into the epithelial structure of the urogenital system, such as the Wolffian duct, the mesonephric tubules and the metanephric tubules, both P- and E-cadherin appeared in these tissues although there were differences in their distributions. In the metanephros, all the metanephric tubules expressed E-cadherin (Fig. 9 *d*), whereas only a limited population of the tubules expressed P-cadherin (Fig. 9 *c*). The somite and its derivatives, such as muscle, cartilage, and dermis, expressed neither P- nor E-cadherin.

Discussion

The 118-kD glycoprotein identified in the present study showed a Ca^{2+} -sensitive property typical of cadherins (Takeichi, 1977), and it was similar in molecular mass to E- and N-cadherin (Yoshida-Noro et al., 1984; Hatta and Takeichi, 1986). Like E-cadherin (Shirayoshi et al., 1986), its molecular mass was slightly reduced in cells treated with tunicamycin. The PCD-1 antibody inhibited the cell aggregation

mediated by cadherins, as was found for antibodies to E- and N-cadherin in other cellular systems (Yoshida-Noro et al., 1984; Hatta et al., 1985; Hatta and Takeichi, 1986). The 118-kD molecule was, however, distinct from E- and N-cadherins in immunological specificity and tissue distribution. Aggregation experiments suggested that P-cadherin does not interact with E-cadherin to bind cells. Therefore, it is most likely that the 118-kD glycoprotein is a novel class of cadherin with a unique specificity.

While the patterns of tissue distribution for each type of cadherin were found to be unique, their distributions overlapped in many tissues. E- and P-Cadherins were both expressed in the ectoplacental cone, epidermis, some endodermal tissues and nephric tubules. Comparison of the present results with those of the distribution of N-cadherin (Hatta et al., 1985; Hatta et al., 1986) suggests that both P- and N-cadherins are expressed in each cell of the lateral plate mesoderm, corneal endothelium, and pigmented retina, and E- and N-cadherins are co-expressed in some regions of the epidermis. Occasionally all three cadherins may be co-expressed; for example, the otic vesicle, which was shown to express E- and P-cadherin in the present study, also expresses N-cadherin (Hatta et al., 1986). Adhesiveness of cells may thus be governed by differential expression of multiple types of cadherins. Since each type of cadherin seems to exhibit its

unique binding specificity, co-expression of heterotypic cadherins in differential combinations in a single cell must create a variety of heterogeneity in adhesive specificities of cells. Co-expression of Ca^{2+} -independent adhesion molecules, which are known to be present in many tissues (Urushihara et al., 1979), with cadherins should also modify the adhesive specificities of cells.

In exploring a role for the differential expression of cadherins in morphogenesis, we found many examples showing a clear correlation of expression of E- and P-cadherins with the association or segregation of cell groups. The most dramatic expression of P-cadherin was observed in the placenta both in the embryonic and maternal regions. The expression of P-cadherin in the uterus began with the appearance of the decidua, into which the extraembryonic cells expressing P-cadherin of implanted embryos invade for making the embryo-maternal connection. In contrast, E-cadherin was expressed only in the embryonic region of placenta with a sharp boundary to the maternal region. This may suggest complementary roles of the two cadherins, such that P-cadherin is required for association of embryonic and maternal tissues during the late implantation stage, while E-cadherin is essential in preventing the embryonic tissues from mixing with the maternal tissues. It should also be interesting to discover in future studies how these cadherins are involved in early stages of implantation of embryos, such as attachment of blastocysts to the uterine epithelium. The present results on the distribution of E-cadherin in extraembryonic regions are consistent with results obtained by Damjanov et al. (1986), who studied distribution of the cell-CAM 120/80 in early mouse embryos.

It is possible to explain the differential expression of P- and E-cadherins in the epidermis in a similar but reciprocal way. In this case, P-cadherin may be essential for segregation of the basal layer from the upper layers, while E-cadherin is important for connection of these layers. The differential expression of P- and E-cadherin observed in the otic vesicle may have a similar role.

The other type of correlation between segregation of cells and cadherin expression was observed in the process of lens formation, where complete separation of the lens vesicle from the overlying ectoderm occurs. During this process, expression of P-cadherin ceased in the invaginating lens vesicle, while expression of E-cadherin continued in this tissue. In other work (Hatta and Takeichi, 1985) we found that lens cells express N-cadherin. Therefore, expression of cadherins is probably switched from P- to N-type during lens vesicle formation. It is possible that this P to N transition in expression of cadherins is associated with the mechanism of separation of the lens vesicle from the overlying ectoderm. After formation of the lens, E-cadherin disappeared from the lens fibers, which is again correlated with the pattern of segregation of the lens epithelium and fibers. A similar correlation was observed between the expression pattern of P-cadherin and the segregation of pigmented retina and neural retina. Thiery et al. (1984) reported that the lens vesicle does not express L-CAM, which is thought to be the chicken equivalent of E-cadherin. This expression pattern of L-CAM is rather similar to that of P-cadherin in this particular case. There may be differences in the expression pattern of homologous adhesion molecules between species.

Patterns of expression of cadherins in embryos dynami-

cally changed during development in association with morphogenetic events; their expression was only transient in many embryonic tissues, but permanent in other tissues. This type of expression pattern was also observed with all other cell-cell adhesion molecules thus far identified (see Edelman, 1985, for review). Observations of such dynamic expression of cell adhesion molecules suggest that expression of each adhesion molecule is under a unique spatio-temporal control, but not tightly associated with expression of particular tissue-specific phenotypes of cells. The sharp boundaries between regions expressing and regions not expressing a particular class of cadherin observed in cell layers of the otic vesicle, which cannot be otherwise detected in a histological view, are reminiscent of the boundaries between the "compartments" found in *Drosophila* (Garcia-Bellido et al., 1979). There must be a genetic mechanism for controlling expression of cadherins at a higher order than that concerned with tissue-specific cell differentiation.

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