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The cadherins: cell–cell adhesion molecules controlling animal morphogenesis

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Summary

Cadherins are a family of glycoproteins involved in the Ca^{2+}-dependent cell–cell adhesion mechanism which is detected in most kinds of tissues. Inhibition of the cadherin activity with antibodies induces dissociation of cell layers, indicating a fundamental importance of these molecules in maintaining the multicellular structure. Cadherins are divided into subclasses, including E-, N- and P-cadherins. While all subclasses are similar in molecular weight, Ca^{2+}- and protease-sensitivity, each subclass is characterized by a unique tissue distribution pattern and immunological specificity. Analysis of amino acid sequences deduced from cDNA encoding these molecules showed that they are integral membrane proteins of 723–748 amino acids long and share common sequences; similarity in the sequences between subclasses is in a range of 50–60 % when compared within a single animal species.

L cells, with very little endogenous cadherin activity, transfected with the cadherin cDNA acquired high cadherin-mediated aggregating activity. Their colony morphology was altered by the ectopic expression of cadherins from the dispersed type to the compact type, providing direct evidence for a key role of cadherins in cell–cell adhesion. It has been suggested that cadherins bind cells by their homophilic interactions at the extracellular domain and are associated with actin bundles at the cytoplasmic domain.

It appears that each cadherin subclass has binding specificity and this molecular family is involved in selective cell–cell adhesion. In development, the expression of each cadherin subclass is spatiotemporally regulated and associated with a variety of morphogenetic events; e.g. the termination or initiation of expression of a cadherin subclass in a given cell collective is correlated with its segregation from or connection with other cell collectives. Antibodies to cadherins were shown to perturb the morphogenesis of some embryonic organs *in vitro*. These observations suggest that cadherins play a crucial role in construction of tissues and the whole animal body.

Key words: cadherins, cell adhesion molecule, CAM, calcium, protein, antibody.

Introduction

Cells of dissociated animal tissues can assemble autonomously and reform the original tissue-like structures (Moscona & Moscona, 1952; Townes & Holtfreter, 1955; Weiss & Taylor, 1960). In some animal species, dispersed embryonic cells can even reconstruct the complete embryonic body (Guidice, 1962; Spiegel & Spiegel, 1975; Dan-Sohkawa *et al.* 1986). The construction of tissues, thus, seems to depend at least partly upon the intrinsic morphogenetic capacity of individual cells. An important property of cells associated with their morphogenetic capacity is their ability to recognize identical or different cell types, adhering preferentially to their own type when mixed with others (e.g. Roth & Weston, 1967). Such selectivity in cell–cell adhesion probably has a key role in the organization of tissues comprising multiple cell types. Therefore, it is important to elucidate the molecular basis of selective cell adhesiveness in order to understand tissue construction mechanisms.

To this end, a variety of cell adhesion molecules have been identified (see review by Damsky *et al.* 1984). Various models have also been proposed to explain the mechanism of selective cell adhesion (see review by Curtis, 1967). In this essay, I focus on a particular class of cell–cell adhesion molecules, termed 'cadherins', and discuss their role in animal morphogenesis. They display properties which can be

implicated in a variety of morphogenetic behaviours of cells including selective adhesion.

Two distinct cell–cell adhesion mechanisms

Cell–cell adhesion is a complex system in both its structural and functional aspects. Ultrastructural studies revealed that cells are connected with multiple types of junction, such as tight junctions, adherens junctions, gap junctions and desmosomes. Takeichi (1977) found that cell–cell adhesion mechanisms are functionally divided into two systems, the Ca^{2+}-dependent and the Ca^{2+}-independent systems. These two systems coexist on single cells, and can be differentially removed by trypsin treatments. The Ca^{2+}-dependent system (CADS) is highly sensitive to trypsin, but can be protected by Ca^{2+} against proteolysis. In contrast, the Ca^{2+}-independent system (CIDS) is inactivated only with high concentrations of trypsin and the proteolytic degradation cannot be protected by Ca^{2+}. Therefore, if cells are treated with a high concentration of trypsin in the presence of Ca^{2+} (TC-treatment), CADS is left intact but CIDS is removed. If cells are treated with a low concentration of trypsin in the absence of Ca^{2+} (LTE-treatment), CIDS is left intact but CADS is inactivated (Urushihara *et al.* 1979). Reaggregation of these treated cells thus can be mediated only by CADS or CIDS.

Treatment of cells with a high concentration of trypsin in the absence of Ca^{2+} (TE-treatment) causes disappearance of both CADS and CIDS from cell surfaces, rendering cells completely nonadhesive to each other.

Protection against proteolysis of a cell aggregation mechanism by Ca^{2+} was first observed by Steinberg *et al.* (1973) and the above findings were confirmed using different cell types (Urushihara *et al.* 1977; Ueda *et al.* 1980; Grunwald *et al.* 1980; Brackenbury *et al.* 1981; Magnani *et al.* 1981; Thomas & Steinberg, 1981; Thomas *et al.* 1981; Gibralter & Turner, 1985; Knudsen, 1985; Nomura *et al.* 1986). CADS and CIDS are entirely independent systems; TC-treated cells (with CADS only) of a given type cannot adhere to LTE-treated cells (with CIDS only) of the same type (Takeichi *et al.* 1979; Gibralter & Turner, 1985). They are immunologically distinguished (Urushihara *et al.* 1979) and have physiologically distinct properties; e.g. activity of CADS is temperature-dependent while that of CIDS is not (Takeichi, 1977). Generally, cells establish tighter connections with CADS than with CIDS (Atsumi & Takeichi, 1980).

Cell–cell adhesion molecules thus far identified can be classified into either CADS or CIDS. For example, the 125×10^3 M_r (125K) glycoprotein (Urushihara & Takeichi, 1980), N-CAM (see review by Rutishauser, 1984), Ng-CAM (Grumet *et al.* 1984), L1 (Rathjien & Schachner, 1984), G4 (Rathjien *et al.* 1987) belong to the CIDS group, as they do not require Ca^{2+}. Some molecules that can be classified into CIDS require Mg^{2+}; e.g. LFA-1, a member of the integrin superfamily involved in leukocyte cell–cell adhesion, requires Mg^{2+} (Rothlein *et al.* 1986). Determination of the amino acid sequence for each molecule will allow further classification of CIDS; N-CAM is now classified as a member of the immunoglobulin superfamily (Cunningham *et al.* 1987). Cadherins are the major component of CADS, as described in detail below.

Identification of Ca^{2+}-dependent cell–cell adhesion molecules

Definition of the Ca^{2+}-dependent cell–cell adhesion system (CADS)

Ca^{2+} is an essential ion for cell–cell adhesion in all animal species; generally, incubation of tissues in Ca^{2+}-free media facilitates their dissociation. Since Ca^{2+} could be involved in multiple processes of cell adhesion, we define 'CADS' as a mechanism whose components are exposed on cell surfaces, require Ca^{2+} for cell–cell binding action and are protected by Ca^{2+} against proteolytic cleavage. It was found that a large variety of cell lines and cells freshly collected from tissues display this type of aggregating property.

A key property of CADS is its resistance to trypsin treatment in the presence of Ca^{2+} (TC-treatment). This effect of Ca^{2+} on CADS was observed in resistance not only to trypsin but also to many kinds of proteolytic enzymes (Takeichi *et al.* 1981). This unique character has been utilized as a marker for CADS in its molecular identification. In principle, cell surface proteins present on TC-treated cells but not on LTE- or TE-treated cells are regarded as candidates for molecules of CADS. Surface proteins with such protease sensitivity have been, in fact, found using fibroblasts and teratocarcinoma cells (Takeichi, 1977; Takeichi *et al.* 1981).

Immunological identification of CADS molecules in teratocarcinoma

The 'Fab strategy' has often been used for the identification of cell adhesion molecules (e.g. Müller & Gerisch, 1978; Brackenbury *et al.* 1977). Antisera raised against whole cells or their cell membranes sometimes contain antibodies to cell adhesion molecules. Fab preparations of such antisera are expected to inhibit cell–cell adhesion. If antisera of this activity are obtained, it should be possible to identify molecules that can absorb the adhesion-inhibitory effect of the antibodies; these molecules are candidates for adhesion molecules.

Fab preparations of an antibody, obtained by injecting teratocarcinoma F9 cells into a rabbit,

inhibited the CADS mediated aggregation of these cells (Takeichi *et al.* 1981). This inhibitory activity of the antibody (anti-F9) was fully absorbed with TC-treated F9 cells but not with TE-treated F9 cells, suggesting that the inhibition of aggregation with this antibody is due to a direct block of CADS. Then, Yoshida & Takeichi (1982) attempted to identify molecules that can neutralize the aggregation-inhibitory effect of the anti-F9. It was assumed that fragments of hypothetical CADS molecules would be released from cell surfaces by trypsin treatment of cells in the absence of Ca^{2+} (TE-treatment). In fact, substances that absorb the aggregation-inhibitory effect of the anti-F9 Fab were detected in the supernatant of TE-treated cell suspensions. Fractionation and immunoblot analysis of the supernatant showed that a 34K molecule has the antibody-absorbing activity. In order to identify the native form of this 34K fragment, cell surface proteins that can compete with this fragment in immunoprecipitation with the anti-F9 were sought and a 124K glycoprotein was found to share an epitope with the 34K fragment. This 124K glycoprotein was detected in TC-treated F9 cells but not in TE-treated F9 cells. It was, therefore, concluded that this 124K glycoprotein is a component of CADS on teratocarcinoma F9 cells.

A more direct immunological method was later employed, that is use of monoclonal antibodies. A hybridoma line producing a monoclonal antibody that specifically blocks the activity of CADS on F9 cells was selected (Yoshida-Noro *et al.* 1984). This monoclonal antibody, ECCD-1, actively dissociates colonies of teratocarcinoma cells. Immunoblot analysis showed that ECCD-1 recognizes a 124K glycoprotein as its major antigen, whose size is exactly the same as that identified using the anti-F9. These results provided more conclusive evidence for involvement of the 124K glycoprotein in F9 CADS. This molecule was termed 'cadherin'.

Similar molecules identified by different approaches

Rabbit antisera raised against teratocarcinoma cells inhibit compaction of early mouse embryos (Kemler *et al.* 1977). In an effort to identify target molecules for antibodies with this effect, an 84K peptide, which is released from a membrane fraction of teratocarcinoma cells by trypsin treatment in the presence of Ca^{2+}, was found to neutralize the decompacting effect of the antisera (Hyafil *et al.* 1980). The native form of the 84K peptide was identified as a 120K glycoprotein (Peyrieras *et al.* 1983, 1985). This molecule, called 'uvomorulin', is similar to the teratocarcinoma cadherin in various ways. Comparison of amino acid sequences between these two molecules has now provided definitive evidence that they are identical (Nagafuchi *et al.* 1987; Ringwald *et al.* 1987).

Other approaches have reached a similar conclusion. Human mammary carcinoma cells spontaneously release an 80K peptide into the serum-free culture medium, antibodies to which induce disruption of mutual adhesion of the target cells and also decompaction of mouse embryos (Damsky *et al.* 1981, 1983). These antibodies detected a 120K glycoprotein, termed Cell-CAM120/80, from cell membranes; therefore, the 80K peptide is probably a degradation product of the 120K form. Properties of this molecule are, thus, similar to those of cadherin or uvomorulin. Similar molecules were also found on canine epithelial cells, called Arc-1 (Behrens *et al.* 1985).

Using chicken hepatocytes, Edelman's group identified a 124K glycoprotein, termed L-CAM, specific antibodies against which inhibited Ca^{2+}-dependent aggregation of these cells (Bertolotti *et al.* 1980; Gallin *et al.* 1983; Cunningham *et al.* 1984). This molecule can be cleaved into a 81K peptide with trypsin in a Ca^{2+}-dependent manner. Again, this molecule has properties similar to the mammalian molecules described above.

In summary, all molecules described in this section show similar properties as follows. (1) Their molecular mass is similar. (2) They are sensitive to Ca^{2+}. In the presence of Ca^{2+}, they are not degraded when live cells are treated with trypsin. However, when a membrane fraction of cells is treated with trypsin in the same ionic condition, the molecules are degraded into peptides with M_rs of 80–84K. In the absence of Ca^{2+}, these peptides are further degraded into smaller fragments, such as the 34K peptide (Yoshida & Takeichi, 1982; Vestweber & Kemler, 1985; Shirayoshi *et al.* 1986*a*). (3) They show a similar tissue distribution pattern, as described below. These observations strongly suggest that these molecules are identical or interspecies homologues (see Table 1).

Finding of cadherin subclasses

Immunological studies revealed that the Ca^{2+}-dependent adhesion molecules (teratocarcinoma cadherin, uvomorulin, Cell-CAM120/80, Arc-1 and L-CAM) described in the above section are present in epithelial cells found in a variety of embryonic and adult tissues (Ogou *et al.* 1983; Edelman *et al.* 1983; Vestweber & Kemler, 1984*a*; Hatta *et al.* 1985; Nose & Takeichi, 1986; Damjanov *et al.* 1986). However, there are many cell types in which this molecule is not detected, such as cells of neural tissues and fibroblasts, although these tissues show activity of CADS. In an effort to identify Ca^{2+}-dependent cell–cell adhesion molecules in these cells, Takeichi and his colleagues obtained monoclonal antibodies blocking CADS of mouse and chicken brain cells, designated

Table 1. *Cadherin subclasses and the related molecules*

Molecule	Animal	Tissue distribution	References
E-cadherin	mouse	*early embryo (at preimplantation)*	Yoshida & Takeichi, 1982
uvomorulin	mouse	blastomeres	Hyafil *et al.* 1980
Cell CAM120/80	human/mouse	inner cell mass	Damsky *et al.* 1983
Arc-1	dog	trophectoderm	Behrens *et al.* 1985
L-CAM	chicken	*early embryo (at postimplantation)*	Gallin *et al.* 1983
		ectoderm	
		endoderm	
		late embryo	
		most epithelial tissues	
N-cadherin	mouse/chicken	*early embryo*	Hatta & Takeichi, 1986
A-CAM	chicken	mesoderm	Volk & Geiger, 1984
N-Cal-CAM	chicken	notochord	Bixby *et al.* 1987
		late embryo	
		neural tissues	
		lens & some other epithelial tissues	
		cardiac & skeletal muscles	
		nephric primordia	
		some mesenchymal tissues	
		mesothelium	
		primordial germ cells	
P-cadherin	mouse	*early embryo*	Nose & Takeichi, 1986
		extraembryonic ectoderm	
		visceral endoderm	
		lateral plate mesoderm	
		notochord	
		late embryo	
		placenta	
		epidermis & some other epithelial tissues	
		pigmented retina	
		mesothelium	
gp140K	*Xenopus*	epithelial lines	Nomura *et al.* 1988

Molecules that have been shown or believed to be identical or the interspecies homologue are grouped. *Early and late embryo* roughly define embryos at the stage before and after neurulation, respectively. This table does not cover all tissues, especially those expressing N- and P-cadherin. As the tissue distribution pattern of each cadherin subclass changes during development, some of the tissues listed here express them only transiently.

NCD-1 (Hatta *et al.* 1985) and NCD-2 (Hatta & Takeichi, 1986), respectively, and also a monoclonal antibody blocking that of mouse PSA5-E cells (an extraembryonic cell line), designated PCD-1 (Nose & Takeichi, 1986). When antigens to these antibodies were identified and compared with the teratocarcinoma cadherin, striking similarities were found in their molecular weight, Ca^{2+}-sensitivity and protease cleavage pattern (Shirayoshi *et al.* 1986*a*). However, they were clearly distinct in immunological specificities and tissue distributions.

These findings suggested that molecules functional in CADS of different cell types are heterogeneous, being provided with similar but distinct structures. These immunologically distinct molecules were, thus, defined as the cadherin subclasses and termed E-cadherin (epithelial cadherin), N-cadherin (neural cadherin) and P-cadherin (placental cadherin), each corresponding to the antigen to monoclonal antibodies ECCD-1, NCD-1 and PCD-1, respectively (Hatta *et al.* 1985; Nose & Takeichi, 1986).

Tissue distribution of these cadherin subclasses was studied using chicken and mouse embryos. In contrast to the epithelial distribution of E-cadherin (= the teratocarcinoma cadherin or uvomorulin), N-cadherin was detected in many nonepithelial tissues such as neural tissues and muscles (Hatta *et al.* 1987). P-cadherin was detected in both epithelial and non-epithelial tissues, most abundantly in the placenta (Nose & Takeichi, 1986). Thus, each cadherin subclass has a unique tissue distribution pattern (see Table 1).

Lilien and his colleagues have identified cell surface proteins on chicken neural retina that are protected by Ca^{2+} against proteolysis (Grunwald *et al.* 1981, 1982; Cook & Lilien, 1982), and termed them N-CalCAM (Bixby *et al.* 1987). Antibody against N-Cal-

CAM inhibits CADS of the neural retina, suggesting that this molecule is identical to N-cadherin or another subclass of cadherins. Geiger and his colleague isolated a 135K protein from a membrane fraction enriched with the intercalated discs of chicken cardiac muscles (Volk & Geiger, 1984). Termed A-CAM, this molecule is associated with the intercellular adherens junctions whose formation is Ca^{2+}-dependent. Also, a monoclonal antibody to A-CAM binds to this molecule in a Ca^{2+}-dependent manner (Volk & Geiger, 1986*a*,*b*). The tissue distribution and the Ca^{2+}-sensitivity of this molecule resemble those of N-cadherin.

As to cadherin-like molecules in other animal species, only data on *Xenopus* are available. A monoclonal antibody which can disrupt cell–cell adhesion in *Xenopus* epithelial lines was obtained (Nomura *et al.* 1988). The 140K antigen to this antibody has properties very similar to cadherins.

Table 1 summarizes cadherin subclasses and the related molecules so far identified.

Molecular cloning of cadherin cDNAs and their primary structure

cDNAs encoding cadherins have been cloned and the complete or nearly complete nucleotide sequences of the chicken L-CAM, the mouse E-, the mouse P- and the chicken N-cadherin have been published (Gallin *et al.* 1985, 1987; Schuh *et al.* 1986; Nagafuchi *et al.* 1987; Nose *et al.* 1987; Hatta *et al.* 1988). Ringwald *et al.* (1987) also reported the amino acid sequence of the mouse uvomorulin, which turned out to be identical to that of E-cadherin.

It was found that all of these molecules have a similar primary structure of 723–748 amino acids long. The deduced amino acid sequence of each molecule contained a putative signal peptide, a putative precursor region and a highly hydrophobic region. This hydrophobic region is most likely to be a transmembrane region of the proteins, suggesting that these molecules are integral membrane proteins. Most interestingly, the four molecules are similar to each other in their amino acid sequences as well as in their structural topology (Fig. 1). Amino acid sequences of the four molecules are most conserved in the putative cytoplasmic domain and secondarily conserved in the *N*-terminal region of the extracellular domain, suggesting the general importance of these regions for the cadherin function. The proximal region of the extracellular domain was least conserved, although this region contained several cysteines whose positions are fully conserved. These molecules are also characterized by the presence of internal repeats of several unique sequences in the extracellular domain which are conserved among the subclasses. Average similarity in amino acid sequences between the mouse E- and the mouse P-cadherin is 58%, that between the chicken N-cadherin and the chicken L-CAM is 50%. Interestingly, similarity between the mouse E-cadherin and the

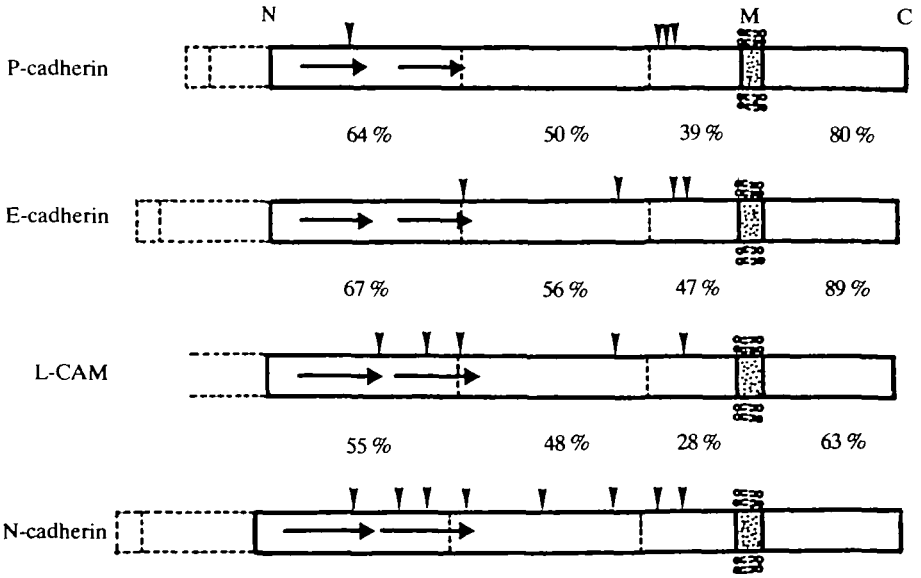

Fig. 1. The primary structures of cadherin subclasses, the mouse P- and E-cadherin, and the chicken L-CAM and N-cadherin. Percent similarities in amino acid sequence between subclasses in three different regions of the putative extracellular domain, divided with dotted lines, and in the putative cytoplasmic domain, are shown. The major internal repeats are shown with arrows. Potential *N*-linked glycosylation sites are shown with arrowheads. The putative precursor regions are drawn with dotted lines at the *N*-terminus. N, the putative *N*-terminus of mature proteins; M, the putative transmembrane region; C, the putative *C*-terminus.

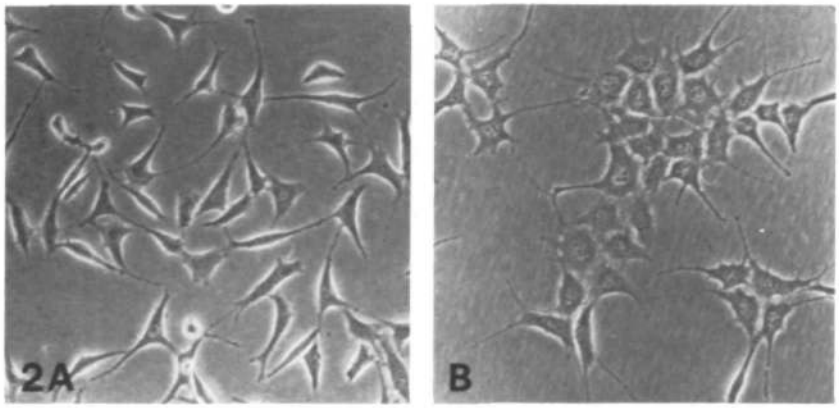

Fig. 2. Ectopic expression of E-cadherin in L cells. (A) Normal L cells. (B) A line of L cells transfected with the E-cadherin cDNA. Note the formation of clusters in culture of the transfected cells.

chicken L-CAM, which are believed to be the interspecies homologues, is only 65%. It is not clear whether this value implies that this cadherin subclass diverged to this extent between the species or that they are not interspecies homologues.

These results clearly demonstrated that cadherin subclasses and L-CAM are a group of molecules which are genetically related to each other. Southern blot analysis suggested that each molecule is encoded by independent genes. Probably they have a common ancestor gene, whose duplication and diversification might have resulted in the formation of a family of molecules with heterogeneous sequences. A computer search found no proteins with sequences that have significant similarity to those of cadherins. Therefore, cadherins/L-CAM constitute an entirely new gene family.

Transfection of cells with cadherin cDNAs

In order to test whether the cloned cadherin cDNAs contain all information necessary for the cadherin function, the full-length E-cadherin cDNA joined to a virus promoter was introduced into L cells which have very little endogenous cadherin activity (Nagafuchi *et al.* 1987). Many of the transfectants expressed E-cadherin derived from the introduced cDNA. Cell aggregation experiments demonstrated that these L cell transfectants acquired high Ca^{2+}-dependent aggregating activity. The extent of this aggregating activity was closely correlated with the amount of E-cadherin proteins expressed.

Interestingly, most of the L cell transfectants expressing E-cadherin were morphologically altered. Generally, L cells do not form tight intercellular connections in monolayer cultures, whereas the L cell transfectants were tightly associated with each other, forming compact colonies (Fig. 2). Adhesion behaviours of L cells were, thus, altered by exogenous introduction of E-cadherin cDNA. This type of experiment has also been successful using cDNA encoding P- and N-cadherin (Hatta *et al.* 1988). These results have provided the first direct evidence that cadherins are cell–cell adhesion molecules.

How do cadherins bind cells?

With regard to the mechanism of the cadherin-mediated cell–cell adhesion, one would ask the question whether cadherins interact with other identical cadherin molecules in a homophilic manner or interact with some receptor molecules. The results of cDNA transfection experiments favour the former possibility, since they demonstrate that expression of a single class of cadherin peptides is sufficient for L cells to acquire the activity of cadherins. These experiments, however, do not exclude the possibility that L cells inherit the receptors for cadherins. If this is the case, normal L cells should be able to adhere to L cell transfectants expressing exogenous cadherins. This, however, does not occur (A. Nose & M. Takeichi, unpublished data). Therefore, it is likely that cadherins interact with other cadherins in joining cells.

The above hypothesis is also supported by the following observation: cadherins are accumulated at the cell–cell boundary of homotypic cells. However, cadherins are not detected at the boundary between L cells without E-cadherin and other cells with E-cadherin, suggesting that this molecule is not involved in the adhesion between these heterotypic cells (Hirano *et al.* 1987).

What is the role of Ca^{2+} in the cadherin-mediated cell adhesion? Removal of Ca^{2+} from cell culture medium causes the immediate disruption of cadherin- or A-CAM-mediated cell–cell adhesions (Volk & Geiger, 1986*b*). This is usually accompanied by disappearance of cadherins from cell–cell boundaries; cadherins become rather uniformly distributed on cell surfaces in the absence of Ca^{2+} (Hirano *et al.* 1987). The cadherin localization at the cell–cell boundary, however, is reversibly restored within a short period after addition of Ca^{2+}.

There is evidence that Ca^{2+} directly reacts with the extracellular region of cadherin molecules and controls their activity. The 80–84K fragment of the extracellular region of cadherins, obtained by trypsin-Ca^{2+}-treatment of a cell membrane fraction as described above, is further degraded by trypsin treatment in the absence of Ca^{2+}, suggesting its Ca^{2+}-sensitive property (Hyafil *et al.* 1981; Gallin *et al.* 1983; Shirayoshi *et al.* 1986*a*). It was shown that some monoclonal antibodies to cadherins recognize the antigens only in the presence of Ca^{2+}, suggesting that cadherins undergo conformational changes by reacting with Ca^{2+} (Hyafil *et al.* 1981; Yoshida-Noro *et al.* 1984: Hatta *et al.* 1985). Recently, Ringwald *et al.* (1987) directly demonstrated that ^{45}Ca binds to the 84K fragment of uvomorulin/E-cadherin blotted on a nitrocellulose sheet. In analysis of amino acid sequences of cadherins, however, we found no known consensus sequence for the Ca^{2+}-binding site, such as the EF-hand. Some characteristic sequences such as D–X–N–D–N are conserved in all subclasses in the form of the internal repeats; they might have some function in the interaction with Ca^{2+} (Ringwald *et al.* 1987; Hatta *et al.* 1988).

It should be noted that monoclonal antibodies NCD-2 and PCD-1, which can block the function of N-cadherin and P-cadherin, respectively, recognize the *N*-terminal region of these molecules. This observation suggests the importance of this region for the cell–cell binding function of these proteins. Vestweber & Kemler (1985) identified a 26K proteolytic fragment of uvomorulin/E-cadherin as a functional site of this molecule. It would be interesting to know the location of this site on the determined amino acid sequence.

Cadherins are glycoproteins. Inhibition of *N*-linked glycosylation by culturing cells with tunicamycin did not affect the E-cadherin activity (Shirayoshi *et al.* 1986*b*), suggesting that the glycosylation is not necessary for the cell–cell binding function of this molecule. The same conclusion has been drawn by Vestweber & Kemler (1984*b*).

Possible association of cadherins with actin

Analysis of the primary structure of cadherins showed that amino acid sequences of the cytoplasmic domain are highly conserved among different subclasses. What is the role of the cytoplasmic domain?

It was found that uvomorulin/E-cadherin is localized at the intermediate junctions (*zonula adherens*) in the intestinal epithelium of adult mice (Boller *et al.* 1985) (see Fig. 3). A-CAM was also found to be associated with the intercellular adherens-type junctions (Volk & Geiger, 1986*a*,*b*). These findings strongly suggested that cadherins or the related

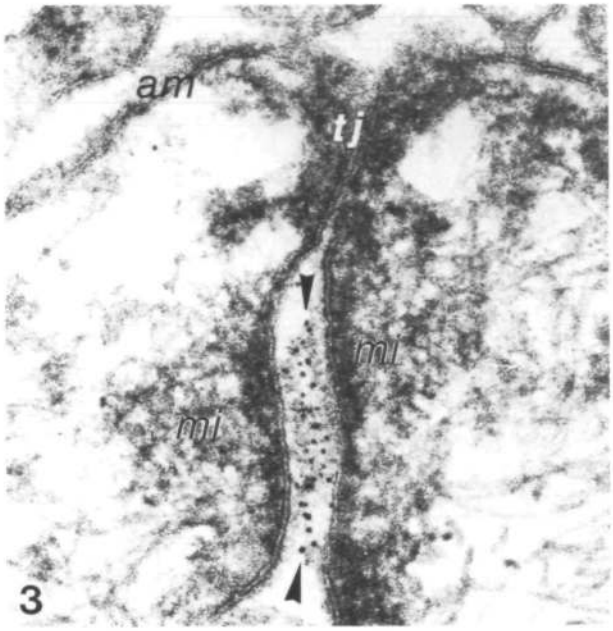

Fig. 3. Immunoelectron microscopy to detect E-cadherin in the intestinal epithelium of an adult mouse. Tissue was treated with the monoclonal antibody ECCD-2 and subsequently with a gold-conjugated second antibody. Note the specific localization of gold particles in the intercellular space of the *zonula adherens* junction, as shown with arrowheads. *mi*, microfilaments; *tj*, tight junction; *am*, apical surface membrane. The photograph was kindly taken by Dr Nobutaka Hirokawa, Tokyo University.

molecules are components of intercellular adherens junctions, such as *zonulae adherentes*, which are known to be associated with actin bundles.

Hirano *et al.* (1987) studied the localization of cadherins and actin bundles in various cultured cells by a double immunostaining method and found that cadherins present at the cell–cell boundary perfectly coincided with the cortical actin bundles, while they showed no coincidence with the actin stress fibres. Even when cell cultures were treated with cytochalasin D, the colocalization of these two molecules was not destroyed. It has also been shown that cadherins coincide with the actin bundles even after extraction of cells with nonionic detergents.

All these results suggest that cadherins are associated with the cortical actin bundles either in a direct or indirect manner. The intermediate junction or *zonula adherens* is probably a specialized form of cadherin distribution in cell membranes. In many embryonic cells, cadherin localization is not always associated with characteristic junctional structures.

What, then, is the function of the cadherin–actin association? We have no definitive answer to this question, but the following possibilities exist.

(1) The cortical actin bundles may regulate the function of cadherins directly or indirectly. Interaction of the cytoplasmic domains of cadherins with actin bundles may be essential for maintaining the active state of the extracellular domains. If this kind of mechanism were present, cells could actively regulate their adhesion; they could attach to or detach from other cells as necessary, using such a cytoplasmic machinery.

It is known that the activity of cadherins is strictly temperature-dependent (Takeichi, 1977). This suggests that the cadherin-mediated cell–cell adhesion does not depend upon a simple molecule–molecule interaction, but requires some physiological processes. This may be contrasted with the temperature-independent N-CAM-mediated cell adhesion, which has been demonstrated to occur by a pure adhesive interaction between N-CAM molecules (Rutishauser *et al.* 1982; Hoffman & Edelman, 1983).

(2) In contrast to the above model, the extracellular domains of cadherins may control some function of actin bundles. Conformation of the extracellular domains of cadherins may be altered as a result of cell–cell binding reactions. These changes may produce signals that are transducted through the cytoplasmic domains of the molecules into the actin.

This possibility is supported by some experimental results. Removal of Ca^{2+} from the extracellular environment, which causes inactivation of cadherins, induces the irreversible release of the plaque material of *zonula adherens* containing actin bundles and vinculin from the cell cortex (Volberg *et al.* 1986). Volk & Geiger (1986*b*) showed that binding of Fab fragments of antibodies to the extracellular region of A-CAM results in disorganization of the actin network.

It is thus possible that cadherins may actively control some function of the cortical actin bundles and their associated molecules. If so, this system could be involved in the contact-mediated regulation of cell motility, such as the 'contact inhibition of movement' (Abercrombie, 1967), since actin is the major component for cellular motile machineries.

(3) Cadherins may be associated with actin bundles merely for anchoring. The cortical actin belts associated with the *zonula adherens* are thought to be essential for the morphogenetic contraction of epithelial sheets (Baker & Schroeder, 1967; Wessels *et al.* 1971). By structural association of cadherins with the contractile actin belts, a contracting force produced by individual cells can be efficiently transducted into the whole cell sheet; without such an organization, cell sheets may not be able to contract in a proper direction.

In the first model, a signal is transducted from actin to cadherins; in the second model, a signal moves in the opposite direction; in the third model, no signal transduction is supposed. It is also possible that the signal pathways are reciprocal. Although these models are highly speculative, the association of cadherins with actin bundles must have an important function for various contact-mediated cell–cell interactions.

It should be noted that the fibronectin receptor and the related molecules (integrins) also have intracellular association with actin bundles, and the integrin-mediated adhesion sites and the *zonula adherens* share common components such as vinculin (Geiger, 1979). This may imply that the cadherin-mediated cell–cell adhesion and the integrin-mediated cell–substrate adhesion have in part a common regulatory mechanism.

Structural relations of cadherins with other junctions

Specialized junctional structures, such as tight, gap and desmosome, are arranged in stereotypic order at the cell–cell boundary. There might be some regulatory interactions among them for their systematic formation. Atsumi & Takeichi (1980) reported that the gap junctions are formed in TC-treated V79 cells but not in LTE-treated V79 cells in the early stage of their aggregation, suggesting that cadherins must be present for the genesis of the gap junctions. It was also found that treatment of teratocarcinoma cells with antibodies to block the E-cadherin activity suppresses the dye transfer between cells (Kanno *et al.* 1984). The possibility remains, however, that the effect of antibodies could be indirect, since inhibition of cadherin-mediated junctions sometimes induces contraction of cells, which may indirectly affect the maintenance of other junctions.

Gumbiner & Simons (1986) reported that the inhibition of a uvomorulin-like molecule with antibodies on MDBK cells results in disruption of the tight junction function. Again, it remains to be determined whether the effect of the antibody is direct or not. A desmosome fraction obtained from the epidermis usually contains E-cadherin. However, EM-immunohistochemistry shows that some antibodies to uvomorulin/E-cadherin do not stain the desmosome (Boller *et al.* 1985). Structural and functional relations of cadherins with various junctions are, thus, largely unknown.

Cadherins in selective cell–cell adhesion

Elucidating the mechanism of selective cell–cell adhesion is an important issue in developmental biology. The following observations suggest that cells

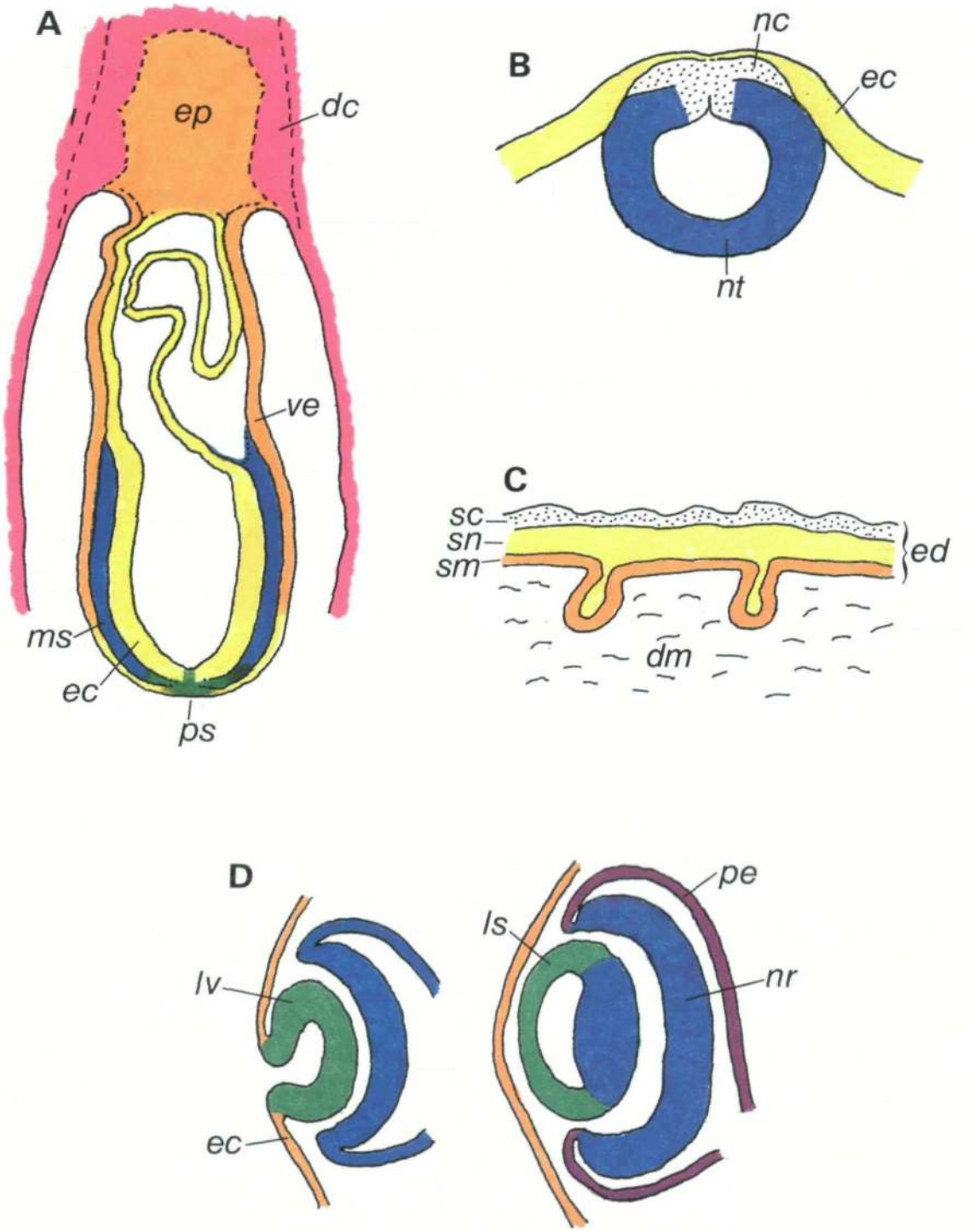

Fig. 4. Differential expression of cadherin subclasses in embryos. (A) Sagittal section of a mouse embryo at the primitive streak stage and surrounding uterine tissues. (B) Closing neural tube in chicken. (C) Embryonic skin in mouse. (D) Developing eyes at the two successive stages. In D, the distribution of E- and P-cadherin found in mouse was overlapped with that of N-cadherin detected in chicken. *Yellow*, E-cadherin (L-CAM); *pink*, P-cadherin *blue*, *N*-cadherin; *orange*, E- + P-cadherin; *green*, E- + N-cadherin; *purple*, P- + N-cadherin. Uncoloured regions have unidentified or no cadherin. *dc*, decidua; *dm*, dermis; *ec*, ectoderm; *ed*, epidermis; *ep*, ectoplacental corn; *ls*, lens; *lv*, lens vesicle; *ms*, mesoderm; *nc*, neural crest; *nr*, neural retina; *nt*, neural tube; *pe*, pigment epithelium; *ps*, primitive streak; *sc*, stratum corneum; *sm*, stratum germinativum; *sn*, stratum granulosum; *ve*, visceral endoderm.

owe their specific adhesiveness at least in part to specificities of cadherin subclasses.

It was found that teratocarcinoma and fibroblastic cells aggregate independently when mixed (Takeichi *et al.* 1981). In this experiment, cells were pretreated to remove all cell–cell adhesion molecules except cadherins by 'TC-treatment', suggesting that cadherins were responsible for the observed selective aggregation. We now know that teratocarcinoma and fibroblastic cells have distinct cadherin subclasses. Similar results have been obtained using different cell types; e.g. the segregation occurs in the mixture of teratocarcinoma cells (with E-cadherin) and glioma cells (with N-cadherin) (Takeichi *et al.* 1985) and in that of teratocarcinoma cells and PSA5-E cells (with P-cadherin) (Nose & Takeichi, 1986). Gibralter & Turner (1985) also showed that myoblasts do not crossadhere to fibroblasts when their aggregation is mediated with the cadherin-like activity, although it is not known whether these cells have different cadherin subclasses. These results suggested that cadherin subclasses have distinct cell–cell binding specificities and are responsible for preferential adhesion of identical cell types, although the possible involvement of other cell surface molecules present on 'TC-treated' cells in the observed phenomena is not ruled out.

Observations by Hirano *et al.* (1987) supported the hypothesis for the presence of specificities on cadherin subclasses. In his experiments, various cell types with different cadherin subclasses were mixed in cell cultures and stained immunofluorescently to localize cadherins. As described above, cadherins are concentrated at the cell–cell boundaries in cultures of homogenous cell populations. However, the boundaries between cells with different cadherin subclasses were only weakly stained or not stained at all with antibodies.

The above observations suggest that cadherins preferentially interact with the same subclasses. It was, however, sometimes observed that the boundaries between cells with different cadherin subclasses are stained with antibodies as strongly as those between homotypic cells (Hirano *et al.* 1987). Volk *et al.* (1987) suggested that lens cells with A-CAM and liver cells with L-CAM can form chimaeric junctions in their mixed cultures, showing that A-CAM and L-CAM coincide in the heterotypic cell boundaries. These observations can be explained in two ways. (1) Cadherins can interact with different subclasses, although the binding affinity between different subclasses might be weaker than that between identical subclasses. (2) Chimaeric junctions between heterotypic cells might have been formed by some unidentified cadherin subclasses present on the two cell types mixed in the experiments. These cadherins might induce accumulation of other cadherins into the junctions by some molecular interaction.

Molecular evidence that cadherin subclasses have distinct, as well as common, amino acid sequences supports the idea that each cadherin subclass has both a unique binding specificity and the capacity to interact with different subclasses. This idea accords with the general observations that different cell types can adhere nonspecifically to each other to form chimaeric aggregates but are eventually sorted out. Many of the early observations describing the segregation or selective aggregation of cells can be explained in terms of cadherin subclass specificities. For example, the famous experiments by Townes & Holtfreter (1955) showed that the epidermal precursor cells segregate from the neural plate cells when mixed. In this combination, the former expresses E-cadherin and the latter expresses N-cadherin. Many other observations described by these authors can be explained in a similar way. Roth & Weston (1967) found that neural retina cells and liver cells preferentially adhere to their own type when mixed; neural retina cells have N-cadherin and hepatocytes have E-cadherin. As described below, each cell type in tissues has a characteristic combination of cadherin subclasses which may determine its adhesive specificities.

To obtain crucial evidence for the cadherin subclass-specificities, L cells transfected with cadherin cDNAs provide an ideal experimental system, since the property of each cadherin subclass can be assayed under the common L cell background. Our recent experiments using such cells are providing evidence that cadherin subclasses are directly involved in selective cell–cell adhesions (A. Nose & M. Takeichi, unpublished data).

Differential expression of cadherins in embryogenesis

The tissue distribution of cadherins in developing embryos has been studied using both mouse and chicken (Edelman *et al.* 1983; Thiery *et al.* 1984; Crossin *et al.* 1985; Hatta *et al.* 1985; Damjanov *et al.* 1986; Nose & Takeichi, 1986; Hatta *et al.* 1987). Distribution of L-CAM in the chicken is similar to that of E-cadherin in the mouse although the earliest developmental stages of chicken embryos have not been studied because of technical difficulty. In the following description, unless otherwise noted, the term 'E-cadherin' is used also to represent its mammalian relatives and L-CAM for convenience.

Tissue distribution of N-cadherin was studied in greatest detail in the chicken (Hatta *et al.* 1987); that of the mouse N-cadherin was studied only by the complement-dependent cytotoxicity test (Hatta *et al.* 1985). Although more precise studies will be needed

for the mouse N-cadherin, the data so far available indicate that the pattern of tissue distribution of N-cadherin is similar in both species. It is not known whether the chicken has P-cadherin or not, since antibodies raised against mouse P-cadherin, which are the only ones available at present, do not react with chicken cells.

The pattern of expression of each cadherin subclass in development has been found to be correlated with morphogenesis of tissues and embryos (Fig. 4). These results are summarized for each cadherin subclass below.

E-cadherin

E-cadherin is expressed in blastomeres of mouse embryos at the cleavage stage, probably even at the 1-cell stage (Ogou *et al.* 1982) and plays an indispensable role in their compaction at the 8- to 16-cell stage (Hyafil *et al.* 1980; Damsky *et al.* 1983; Shirayoshi *et al.* 1983; Vestweber & Kemler, 1984*a*; Johnson *et al.* 1986). At the implantation stage, E-cadherin is expressed in all cells of embryos. However, as cells differentiate into various types, this molecule disappears from some cell layers. The prominent example is seen in the mesoderm. Mesodermal cells migrate through the primitive streak into the space between the ectoderm and the endoderm; these cells lose E-cadherin during the migration (Fig. 4A). When the neural plate invaginates, this region of the ectoderm also loses E-cadherin (Fig. 4B). Other regions of the ectoderm and all endodermal cells maintain the expression of E-cadherin, and this expression persists as long as they differentiate into epithelial cells. In older embryos, essentially all proliferating epithelial cells derived from the ectoderm and the endoderm express E-cadherin, although some terminally differentiated, nonproliferating epithelial cells such as lens fibre and keratinized epidermal cells, lose this molecule (Fig. 4C,D).

Neural and mesodermal tissues, apart from a few exceptions, do not have E-cadherin. Epithelial components of the urogenital system which are derived from the mesoderm, such as mesonephric and metanephric tubules, express E-cadherin after differentiation from mesenchymal cells. Some mesothelial layers also have this molecule (Damjanov *et al.* 1986). We recently found that some regions of the differentiating neural tube possess an epitope recognized by a polyclonal antibody to E-cadherin (Y. Hirai & M. Takeichi, unpublished data).

N-cadherin

N-cadherin is first detected upon gastrulation in some cells of the ectoderm (epiblast) located at the primitive streak, which are just about to invaginate. Invaginating cells initially have both E-cadherin and N-cadherin. However, cells differentiating into mesoderm soon lose E-cadherin (see above) and come to express only N-cadherin (Fig. 4A). Cells entering endoderm also express N-cadherin, although transiently, but do not lose E-cadherin. The N-cadherin expression in different cell layers at later developmental stages will be discussed separately.

N-cadherin in mesodermal development

As the mesoderm differentiates into various tissues, the expression of N-cadherin changes dynamically; many cells continue its expression but others lose it. Typical examples of the dynamic change in the N-cadherin expression are seen in the somite (Duband *et al.* 1987) (Fig. 5) and nephrotome differentiation as below.

When mesodermal cells in the segmental plate, the precursor of the somites, become organized into a cylindrical epithelial structure with core cells, N-cadherin distribution becomes weakly polarized onto the luminal side of the epithelium. As the epithelial cylinder is segmented into U-shaped units, the intensity of N-cadherin further increases at the luminal side. Upon completion of somite formation with closure of the U-shaped epithelium, the polarized expression of N-cadherin is maximized (Fig. 5). At the next stage, cells of the sclerotome region are converted into mesenchymal cells and begin their migration. Coincidentally, these cells lose N-cadherin, although other parts of the somite continue the strong expression of this molecule. With the onset of migration of the dermatome cells, N-cadherin is then reduced in this region. Cells of the myotome continue to express N-cadherin expression until the differentiation of skeletal muscles occurs.

During development of the mesonephros, the condensation of primordial cells is accompanied by increased N-cadherin expression. This strong expression persists until cells are organized into mesonephric tubules. After fusion of the mesonephric tubules with the Wolffian duct, N-cadherin is lost and replaced with E-cadherin (see above). A similar pattern of transient expression of N-cadherin is observed during metanephros development.

Many other tissues derived from mesoderm express N-cadherin transiently or permanently. The strongest permanent expression occurs in cardiac muscle.

N-cadherin in neural development

N-cadherin appears in the neural plate during its invagination (Fig. 4B). This appearance is coordinated with the disappearance of E-cadherin from this cell layer. After formation of the neural tube, N-cadherin becomes the major cadherin of this tissue. During differentiation of the central nervous system,

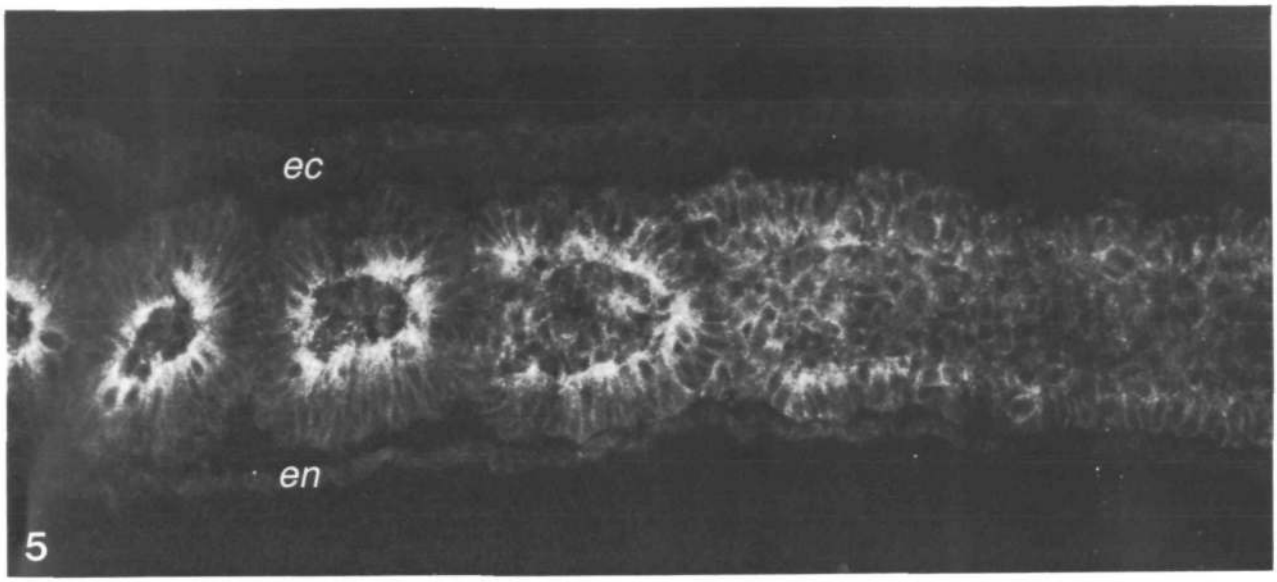

Fig. 5. Immunofluorescent staining for N-cadherin on a sagittal section at the somite level of a chicken embryo at the 16-somite stage. Left, anterior of the embryo. *ec*, ectoderm; *en*, endoderm. Note change in the distribution pattern of N-cadherin during somitogenesis. Undifferentiated mesodermal cells are out of field at the right side.

the amount of N-cadherin expressed becomes regionally different. For example, in the neural retina, all cells, including the optic nerves, express N-cadherin equally at the early developmental stages. However, N-cadherin is gradually lost from most of the retina and eventually remains only in the outer limiting membrane (M. Matsunaga & M. Takeichi, unpublished data). Similarly, in the cerebellum N-cadherin is distributed evenly at the undifferentiated stage, but becomes restricted in some layers during its maturation.

At the stage of closure of the neural plate, the neural crest becomes recognizable. The region of ectoderm giving rise to the neural crest ceases to express E-cadherin and only temporarily expresses N-cadherin. The neural crest cells beginning their migration express neither E- nor N-cadherin. They are thus demarcated from the overlying ectoderm (which expresses E-cadherin) and from the neural tube (which expresses N-cadherin) (Fig. 4B), although they have some cadherin activity (Aoyama *et al.* 1985). These cells maintain this situation during migration. However, when neural crest cells reach their destinations and differentiate, many of them express N-cadherin. For example, some neural crest cells terminate their migration at the lateral sides of the neural tube and form aggregates which differentiate into the dorsal root ganglia. This aggregation of neural crest cells is accompanied by expression of N-cadherin. Neurites extending from the dorsal root ganglia, which extend to the neural tube and to other target sites, also express N-cadherin. Most ganglionic cells derived from the neural crest seem to express N-cadherin at their early developmental stages, although this expression appears to be suppressed in many of them at the later stage.

N-cadherin in ectodermal and endodermal development

The major cadherin in epithelial cells is E-cadherin as described above. However, N-cadherin is coexpressed in some local regions of these epithelia. For example, their colocalization occurs in the primordia of many endodermal organs, the lens epithelium, and the ectodermal and endodermal region of the visceral cleft.

P-cadherin

This has been studied only using mice. P-cadherin is first detected in the extraembryonic layers of early embryos, such as the ectoplacental cone and the visceral endoderm, at the stage of implantation (Fig. 4A). The uterus of a nonpregnant mouse does not express P-cadherin. However, as decidual cells grow in the uterus in response to the attachment of embryos to the uterine wall, it was found that they strongly express P-cadherin (Fig. 4A). These decidual cells become associated with the extraembryonic layers of embryos also expressing P-cadherin after invasion of embryos through the uterine epithelium.

Therefore, the situation is established that P-cadherin is continuously distributed from the maternal tissues to the embryonic tissues giving rise to the placenta, suggesting that this molecule may serve to connect embryos to the uterus. In contrast, E-

cadherin is localized only in the embryo. P-cadherin is not expressed in the luminal uterine epithelium, suggesting that it is not involved in the initial process of implantation of embryos.

Around the neurula stage, various tissues, which derive from all three germ layers, begin to express P-cadherin. The overlying ectoderm differentiating into the epidermis expresses P-cadherin. In the fetal epidermis, P- and E-cadherin are differentially expressed; the germinative basal layer has both P- and E-cadherin, the middle layer has only E-cadherin and the keratinized top layer has neither of them (Fig. 4C). Some other ectodermal tissues, such as the inner ear primordium, also coexpress P- and E-cadherin in a regionally different pattern. Neural tissues usually do not have P-cadherin; however, some of the epithelial tissues derived from the neural tube, such as the pigmented retina, express P-cadherin at least at the embryonic stage (Fig. 4D).

As for mesodermal tissues, the lateral plate mesoderm and its derivatives express P-cadherin; in particular, the mesothelium covering many organs expresses this molecule up to the adult stage. Metanephric tubules also express P-cadherin together with E-cadherin. Some endodermal derivatives, such as the foregut and the lung, express P-cadherin; however, such expression seems to occur only at the embryonic stage.

Role for cadherins in embryonic morphogenesis

As described above, each cadherin subclass shows a unique spatiotemporal pattern of expression in developing embryos. These observations allow us to infer how cadherins are involved in animal morphogenesis, as summarized below.

(1) Cadherins so far identified are not 'tissue-specific' molecules. Each subclass is detected in a variety of tissues derived from all three germ layers. Other classes of cell adhesion molecule, such as N-CAM, are also detected in a broad spectrum of tissues. This implies that specific cell–cell adhesions that occur in embryogenesis do not necessarily depend upon strictly tissue-specific adhesion molecules, but are governed by the spatiotemporally regulated expression of a limited number of different classes of cell adhesion molecules, as hypothesized by Edelman (1984*a*); that is, the same molecules can be used for specific cell adhesions at different positions and different developmental stages of embryos.

(2) Many cell types express multiple cadherin subclasses simultaneously and their combination differs with cell type. For example, the lens epithelium and the visceral cleft have E- and N-cadherin, the epidermis and the inner ear primordium have E- and P-cadherin, and some mesodermal derivatives have P- and N-cadherin. These observations suggest that adhesion properties of individual cells are governed by varying combinations of multiple cadherins.

(3) Expression of cadherins is dynamic and switched from one subclass to another in relation to morphogenetic events. The pattern of cadherin expression can be subgrouped into the following three categories.

Cadherin expression and cell layer separation

It was observed that the switching in expression of cadherins from one subclass to another or the termination of expression of a certain cadherin subclass in a tissue is associated with its separation from its parent tissue, e.g. during the formation of the mesoderm, the neural tube, the lens vesicle, the neural crest and the sclerotome, and the differentiation of keratinocytes. These phenomena suggest that the subclass-specificities of cadherins play a role in separation of cell layers during embryogenesis, as found in *in vitro* systems.

Cadherin expression and cell layer recognition

Morphogenesis involves processes in which cells derived from different lineages or different positions are brought into contact and connected with each other. Heterotypic cells to be joined usually express identical cadherin subclasses. For example, when the uterine decidual cells are associated with the extra-embryonic cells of embryos to form the future placenta, both cell layers express P-cadherin. This type of cadherin expression strongly suggests that the subclass-specificities of cadherins are important for recognition between cells which are developmentally scheduled to be joined.

Cadherin expression and cell rearrangement

Mesenchymal cells are converted into epithelial cells or *vice versa* in development. Corresponding to such rearrangement of cells, the expression pattern of cadherins on *individual* cells is altered, as seen in somite development. The change in cadherin distribution on individual cells may be essential for arranging cells into a particular pattern.

If cadherins regulate morphogenesis in the ways discussed above, the timing for switching on or off the expression of each cadherin subclass must be precisely controlled during differentiation of cells. Although the control mechanism for cadherin expression is totally unknown, there must be a cell-type-specific regulation for expressing a particular set of cadherin subclasses in a given cell type, as suggested by the cell fusion experiment by Atsumi *et al.* (1983).

It should be noted that the expression of cadherins is coordinated with that of other classes of cell–cell adhesion molecules. For example, the spatiotemporal pattern of expression of N-cadherin is similar to that of N-CAM (Hatta *et al.* 1987) whose expression is also spatiotemporally controlled, for example its association with embryonic inductions (Edelman, 1984*b*). This suggests the presence of some regulatory mechanism to coordinate the expression of different classes of cell adhesion molecules.

Analytical approaches to the morphogenetic role of cadherins

The above discussion is based mostly upon phenomenological observations. Analytical studies are, therefore, necessary to confirm the ideas and hypotheses. One way to investigate the role of cadherins in morphogenesis is to examine the effect on morphogenetic phenomena of antibodies that block the cadherin-mediated cell–cell adhesion. Several reports have been made along this line.

Vestweber *et al.* (1985) studied the effects of an antibody to uvomorulin/E-cadherin on kidney differentiation and found that this antibody had no effect on the formation of metanephric tubules. It has been shown, however, that the developing kidney expresses the three cadherin subclasses in a differential pattern; therefore, this line of study must be reexamined using combinations of various antibodies. Gallin *et al.* (1986) tested the effect of an antibody to L-CAM on the feather development of the back skin of chick embryos *in vitro*. Interestingly, the pattern of the mesenchymal condensation in the dermis was severely perturbed by the antibody although L-CAM is expressed only in the epidermis, and a scale-like structure, rather than feathers, developed from the treated tissues. Bixby *et al.* (1987) tested the effect of an antibody to N-Cal-CAM on motor neurone migration *in vitro*. They did not find any effect of this antibody when it was added alone. However, it had a significant effect on the migration of motor neurones when added together with antibodies to N-CAM and an ECM receptor. Matsunaga & Takeichi (unpublished data) recently examined the effect of antibodies to N-cadherin on the morphogenesis of neural retina and found that these antibodies severely affect the alignment of cell layers, particularly at the photoreceptor layer.

These experiments clearly indicate that cadherins are in fact important for the morphogenesis of tissues. Using antibodies, however, is not always very successful in investigating the morphogenetic roles of cadherins. While this method is most useful in *in vitro* systems, it is not always easy to reproduce morphogenetic phenomena in cultures. The best approach toward the present aim should be to modify artificially the gene expression of cadherins in given morphogenetic systems and to see its effect. Hopefully, this kind of approach will become possible in the near future.

Future studies

The following points must be clarified in future studies.

(1) How many cadherin subclasses are present? Many cell types with cadherin activity do not react with any of the antibodies to cadherins available at present. Furthermore, cadherin activity of many cell types expressing identified cadherin subclasses is not completely inhibited with antibodies to these subclasses. These observations imply the presence of other subclasses. Studies on these points should be important in establishing whether selective cell adhesion is controlled by a small or large number of specific molecules.

(2) What is the molecular mechanism of cadherin–cadherin interaction? The molecular dissection of cadherin peptides combined with DNA transfection experiments should enable us to determine functions of different regions of cadherin molecules. Using the same method, we might be able to determine the molecular basis for subclass specificities. The role of the association of cadherins with actin bundles must also be clarified.

(3) Do invertebrates have cadherins? The answer to this question is important in considering the scope of the cadherin-dependent control of animal morphogenesis.

(4) Do cadherins mediate not only cell–cell adhesion but also regulate cell differentiation? The molecular basis of cell contact-dependent regulation of cell differentiation is totally unknown in vertebrates. Do cadherins have some role in it?

(5) What is the functional relation of cadherins to other cell adhesion molecules? Cells coexpress cadherins and other classes of cell–cell adhesion molecules. There must be differential roles for these molecules.

(6) Are cadherins involved in pathogenetic behaviours of cells, such as metastasis of cancers?

(7) What genes control cadherin expression? The differential expression of multiple cadherins in development must be under the strict control of regulatory genes. It is most important to identify such genes to understand the genetic mechanisms of morphogenesis.

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