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CADHERINS: A MOLECULAR FAMILY IMPORTANT IN SELECTIVE CELL-CELL ADHESION

Masatoshi Takeichi

Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606, Japan

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CONTENTS

PERSPECTIVES AND SUMMARY

The animal body is a collective of heterogeneous cell types. The heterotypic cells are arranged in a precise order to form tissue structures in which the cells never intermix randomly. Generally, cells of the same phenotype are connected to each other and form a group. Groups of cells are demarcated from

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each other with sharp boundaries, especially between the cell groups expressing different phenotypes. Thus, tissues comprise compartments of different groups of cells. This is a general feature of tissue architecture, seen universally in the animal kingdom.

The formation of the highly ordered multicellular structures appears to be based at least in part upon some intrinsic properties of individual cells. It is well known that dissociated embryonic cells can self-assemble and reorganize tissuelike structures in vitro. The formation of parts of organs or tissues thus depends solely on the individual cell properties, but not on the overall body plan.

An important property that allows cells to form tissues is their own adhesiveness; multicellular organisms cannot exist without the association of cells. Another important property of cells is the ability to sort themselves from different cell types. The positional segregation of different cell types in the body is thought to be regulated by this cellular property to a large extent, although other factors might also be important. These two properties of cells are thus believed to be crucial for the construction of tissues. In this article, I present a summary of our recent knowledge on how animal cells recognize each other and adhere selectively to particular cell types, focusing on a group of cell-cell adhesion molecules called the cadherins.

SELECTIVE CELL ADHESION

The ability for dissociated cells to auto-assemble was first described in spongi (1), and it was in this system that the cell sorting phenomenon was first discovered. Sponge cells of different species segregate themselves from each other when mixed artificially (1, 2). Since these early discoveries, factors involved in spongi cell aggregation have been extensively studied, and characterized biochemically (e.g. 3). The molecular basis of how spongi cells recognize their own or another species, however, is still obscure, nor is it clear whether spongi share homologous mechanisms for cell adhesion and recognition with other animal species.

In the vertebrate system, Holtfreter was a pioneer in the study of self-assembling properties of cells. He and his colleagues discovered that dissociated embryonic cells of amphibia can reaggregate and reconstitute tissuelike structures (4). The basic process underlying this phenomenon was the positional segregation of different cell types in aggregates; that is, cells seek the same types and establish stable adhesion with them, resulting in the segregation of different cell types. Similar phenomena were later found in the homeotherms (5–8) and also in *Drosophila* (9). The cell-type-specific association of cells, therefore, generally occurs throughout the animal kingdom.

Various models have been proposed to explain how selective cell adhesion occurs. Some groups claimed the existence of tissue-specific intercellular adhesion molecules, while others emphasized the importance of nonchemical factors in cell segregation (10). To determine which mechanism is correct, however, we have had to wait until the molecular basis of cell adhesion was understood. To date, many classes of cell adhesion molecules have been identified, and with this knowledge it is now possible to discuss the mechanism of cell adhesion in molecular terms.

CELL-CELL ADHESION MOLECULES

Cell-cell adhesion is a complex system, in which various mechanisms and factors are involved. Functionally two distinct mechanisms, a Ca^{2+}-dependent and a Ca^{2+}-independent mechanism, cooperate in connecting cells together (11–22). A number of cell surface glycoproteins have been identified as intercellular adhesion molecules, and these have been classified into at least three major molecular families, the immunoglobulin (Ig) superfamily, the integrin superfamily, and the cadherin family.

NCAM is the most well characterized member of the Ig superfamily (23). This molecule is widely distributed in various tissues, although the alternative splicing products of the NCAM gene are distributed differentially in different tissues. Aggregation of cells mediated by NCAM is Ca^{2+}-independent. Liposomes carrying NCAM coaggregate in the NCAM-dependent manner, and they also attach to the surface of cells expressing NCAM. From the results of these studies, it is believed that NCAM interacts with itself in a homophilic manner. It had been suggested that other members of the Ig superfamily may exhibit similar cell-binding properties.

Integrins have been identified as a family of cell surface receptors that recognize extracellular matrices (24). Some members of this family, however, can act as intercellular adhesion molecules if their ligands are present on the surface of other cells; for example, LFA-1, a member of the integrin superfamily distributed in lymphocytes, binds to ICAM localized on various other cells.

Cadherins are a molecular family that is essential for the Ca^{2+}-dependent process of cell-cell adhesion (22). The family is divided into subclasses that show different tissue distribution patterns. This molecular family binds cells by means of homophilic interactions. Other molecular families have also been identified; for example, the LEC-CAM family has been implicated in lymphocyte homing (25).

Studies of these molecular families indicate that cell-cell adhesion is controlled by two distinct types of molecular interaction, homophilic and heterophilic, and all these interactions occur only among specific molecules.

Since the distribution of most of these molecules is not ubiquitous, any of them could serve as a factor responsible for the specific connections between limited cell types. However, it is unlikely that all the molecular families play an equivalent role in specific cell adhesion, since they are completely different from each other in molecular structure. It is likely that each family plays a distinct role in cellular interactions. One should then ask the question, whether any of these families is important for the cell sorting phenomena classically observed. The studies described below suggest that the cadherin family plays a crucial role in the sorting of different cell types in vertebrates.

CADHERINS

Ca^{2+} is an essential ion for animal cells to maintain intercellular contacts. If Ca^{2+} is removed from the extracellular environment, the cell-cell connection generally becomes loose, and, in extreme cases, multicellular systems are destroyed. Cadherins are the cell surface molecules involved in this Ca^{2+}-dependent adhesion machinery in vertebrates (22). Antibodies to cadherins can mimic the effect of Ca^{2+}-depletion. For example, if the antibodies are added to monolayer cultures of epithelial cells, the cell-to-cell cohesion tends to be disrupted; consequently, cells become unable to maintain epithelial sheets and their morphology changes to the one with a fibroblastic appearance (26–31). If the antibodies are added to embryonic tissues, their structures are severely distorted and in some cases they are dissociated into small cell clusters or even into single cells (32–38). Cadherins are thus fundamental for establishing and maintaining multicellular structures, especially in embryonic stages of vertebrate development.

Cadherins were originally identified as cell surface proteins with characteristic Ca^{2+}- and protease-sensitivities; that is, they are protected by Ca^{2+} against proteolysis but readily degraded if Ca^{2+} is absent (11, 39–44). Cells can display Ca^{2+}-dependent aggregation only when the molecules with these properties are present on their surface. Later on, antibodies were raised to such proteins, and found to inhibit Ca^{2+}-dependent cell aggregation. These molecules were then designated cadherins. Cadherins were identified independently in different laboratories, and called by different names such as L-CAM (45–48), uvomorulin (49–51), or cell-CAM 120/80 (27, 52). The term "cadherins" is used as a family name throughout this article.

Antibodies raised to cadherins identified on a particular cell type do not react with all the cells of the body, although cadherin activity is detected on all cells forming solid tissues. It was assumed that immunologically distinct cadherins were present in different cell types. In fact, three types of cadherins have been identified in mammals. These have properties in common, such as Ca^{2+}- and trypsin-sensitivity, but are distinct in antigenic specificity and

tissue distribution. These molecules were designated E-, N-, and P-cadherin (E-cadherin is identical to uvomorulin) (41, 53–56). A similar molecule was found in the chicken, L-CAM, whose tissue distribution resembles that of E-cadherin in mammals (45–48, 57). The tissue distribution of these molecules is summarized in my previous review (22).

Analyses of protein and cDNA sequences revealed that different cadherins are similar in their overall primary structure; their mature forms consist of 723 to 748 amino acids, and have a single transmembrane domain that divides the molecules into the amino-terminal extracellular and the carboxy-terminal cytoplasmic domain (Figure 1) (58–65). Amino acids are conserved among the cadherins in a range of 43 to 58%. The extent of the conservation differs with the region of the molecules; the most highly conserved region is the cytoplasmic domain, and the second most conserved region is around the amino-terminal portion.

The extracellular domain has internal repeats. Computer analyses revealed the presence of at least two major repeats. A close examination of the amino acid sequences demonstrates that various characteristic sequences consisting of three to five amino acids are repeated three to four times in the extracellular domain. These repeated sequences are well conserved among all three types of cadherins. The proximal region of the extracellular domain, adjacent to the transmembrane domain, has four cysteines, whose positions are conserved among the cadherins. The outline of these features of cadherins is shown in Figure 1. Putative N-linked glycosylation sites are detected throughout the extracellular domain, but the positions of these sites are not necessarily conserved among the cadherins.

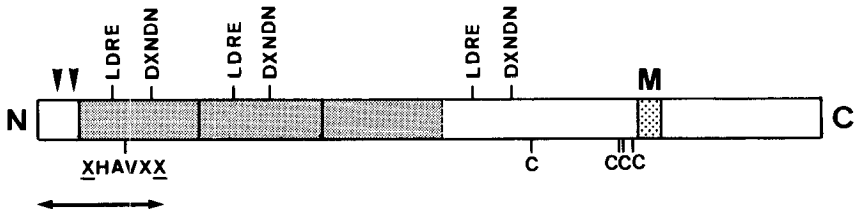

Figure 1 The putative primary structure of cadherins. All members of the cadherin family thus far identified in vertebrates exhibit a similar structure as shown here. Shaded regions represent internally repeated domains, of which the proximal third one has a weaker similarity to others. Two classes of major repeated sequences are shown; these sequences have also been detected in *Drosophila* (P. Mahoney and C. Goodman, personal communication). Bipolar arrow shows the amino-terminal 113-amino-acid region involved in determining binding specificities. The sequence containing HAV located in this region plays an important role such that the substitution of underlined amino acid residues can partially alter the specificity of E-cadherin. Arrowheads indicate the sites recognized by monoclonal antibodies that can inhibit cadherin function. N, amino terminus; M, membrane-spanning region; C, carboxy terminus. Smaller c represents cysteine residue.

Since cadherins function in a Ca^{2+}-dependent manner, it is thought that these molecules have binding sites for this ion. These sites are assumed to be located in the extracellular domain, since this domain, which can be isolated by trypsin cleavage from the rest of the molecule, has the ability to bind Ca^{2+} (62). However, the known consensus sequence for Ca^{2+}-binding is not detected. The idea that some of the repeated amino acid sequences play a role in Ca^{2+}-binding has been proposed (62).

cDNA TRANSFECTION STUDIES ON CADHERIN FUNCTION

In order to investigate the function of cadherins, cells transfected with cadherin cDNAs have been found to serve as a useful experimental system. Cells without cadherins, e.g. L or Neuro 2a cells, generally do no adhere strongly to each other. These cells, however, can acquire Ca^{2+}-dependent cohesiveness when transfected with cadherin cDNAs attached to an appropriate eukaryotic promoter (60, 63, 66–68). The morphology of the colonies of the transfected cell lines is generally converted from the dispersive type to the compact epithelial type by exogenous cadherin expression (60, 68). The intercellular adhesiveness of the transfected cells is proportional to the amount of cadherin molecules expressed. These results present solid evidence that cadherins play a crucial role in the cell-to-cell connection between animal cells.

The above observations also provide us with a clue on how cadherins bind cells. As mentioned, L cells transfected with cadherin cDNAs acquire cadherin-mediated cohesiveness. However, parent L cells without cadherin activity cannot adhere to any of the L cells expressing recombinant cadherins, suggesting that those cells do not have receptors or ligands for cadherins (69). Therefore, it is a reasonable conclusion that cadherins interact with cadherins only in a homophilic manner.

BINDING SPECIFICITIES OF CADHERINS

It was observed that when cells expressing one type of cadherin are mixed with cells expressing another type of cadherin and cultured in suspension, they tend to aggregate separately (70–72). These observations suggest that each cadherin type might have a binding specificity. This possibility has been tested by using L cells transfected with cDNA encoding E-, P-, or N-cadherin of the mouse. These cells were singly dispersed, mixed with each other, and cultured in suspension to test if they are intermixed or segregated when forming aggregates. The results clearly showed that cells tend to adhere preferentially to cells expressing the identical cadherin type (69, 73). This

adherence preference indicates that cadherins have a binding preference for their own type over another types.

It was then asked whether cadherins are involved in determining adhesive specificity of embryonic cells. The following experiments were designed to test this possibility (69). The lung of mouse embryos consists of two major cell types, epithelial and mesenchymal. The epithelial components express E- and P-cadherin, while the mesenchymal cells express N-cadherin. Lung cells dissociated with trypsin can reaggregate and reconstitute an original tissuelike architecture, in which the two cell types are segregated, as expected from the classical observations. To examine whether cadherins participate in the segregation of these cells, L cells expressing recombinant E-cadherin were added to lung cell suspensions and their adhesive behavior was followed.

Untransfected L cells were incorporated into the mesenchymal regions of lung cell aggregates. In contrast, the E-cadherin-expressing L cells were associated with the epithelial tubules also expressing E-cadherin. Thus, the preference of L cells was originally for mesenchymal cells, but this was altered for epithelial cells by acquiring E-cadherin expression. In this example, the type-specificity of the individual cadherins obviously was involved in the segregation of different cells.

Another line of evidence supporting the above notion has been obtained using a neurite outgrowth system (74). Retinal optic axons migrate through the optic stalk to the tectum in order to establish synaptic connections. In this pathway, the growth cones of the axons attach to and migrate on the surface of neuroepithelial cells. With regard to cadherin expression, both the optic axons and the neuroepithelial cells express N-cadherin. The possibility that N-cadherin mediates the connection between these two cell types was therefore raised and tested as follows.

Neuro 2a cells do not exhibit endogenous cadherin activity as do L cells, but those transfected with N-cadherin cDNA come to express this adhesion molecule. Small fragments of chicken embryonic neural retina were explanted on monolayer cultures of the Neuro 2a and the transfected cells, and neurite extension from the retina was examined after a few days of incubation. In the untransfected cell cultures, essentially no outgrowth of neurites was observed. In contrast, a vigorous extension of optic axons took place on the N-cadherin-transfected cells. The growth cones of the axons could attach only to the surfaces of the transfected cells, but neither to the parent Neuro 2a cells nor to the culture dish. Clearly, N-cadherin mediates the attachment of neurites to cells on the substratum. It was also demonstrated that the migration of neurites on astrocytes of myotubes is inhibited by antibodies to N-cadherin (75, 76).

The effect of the expression of E-cadherin on the substratum cells was then tested using a similar system. Preliminary results suggest that only N-cadherin

was effective in promoting the extension of retinal axons (74). The results showed that in this system as well, cadherin specificities were involved in the selective attachment of embryonic cells.

MOLECULAR BASIS FOR CADHERIN-MEDIATED SELECTIVE ADHESION

How does the preferential binding interaction occur between the identical types of cadherin molecules? Figure 2 illustrates a simple model, which proposes that the maximum affinity is achieved between molecules that have identical conformations.

In order to determine which portions of the cadherin molecules are responsible for their specific binding properties, chimeric molecules of mouse P- and E-cadherin were constructed. The amino-terminal regions of E-cadherin were replaced by those of P-cadherin in varying lengths, and the binding specificities of these chimeric cadherins were examined by using L cells transfected with the cDNA encoding these molecules. The results showed that the amino-terminal 113-amino-acid region of the extracellular domain was important for the specificity of these cadherins; that is, if this region of E-cadherin

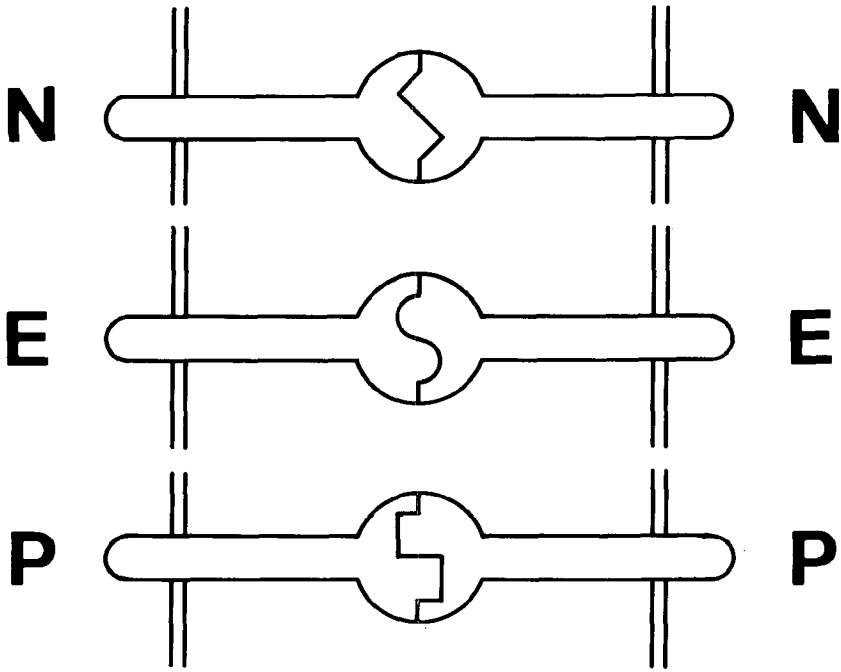

Figure 2 A model to explain how identical cadherins recognize each other.

was replaced by the corresponding region of P-cadherin, this chimeric molecule displayed P-cadherin specificity (A. Nose and M. Takeichi, unpublished). In this region, about 65% amino acids are identical between E- and P-cadherins, and the conserved and nonconserved amino acid sequences are scattered throughout the region. The nonconserved sequences are thus candidates for the sites that determine specificity.

The site-directed mutagenesis experiments, then, revealed that the replacement of amino acids at some specific positions that are adjacent to a conserved HAV sequence located in the above 113-amino-acid region (see Figure 1) partially converted the specificity of E-cadherin to P-type, but that other replacements had no effect. This indicates that these amino acids are of a primary importance in determining the type-specificities of cadherins. The alteration of the specificity due to these amino acid substitutions, however, was partial, and therefore, other sites in the amino-terminal 113-amino-acid region must cooperate in order to realize the full set of type-specific properties of cadherins.

The binding sites for monoclonal antibodies to P- and N-cadherin that are known to inhibit the cell binding function of these molecules have also been mapped. These sites are located at the 31st amino acid residue from the amino terminus of the mature form of the cadherin, and this position is identical for P- and N-cadherin (see Figure 1). The binding site of a monoclonal antibody inhibiting E-cadherin is also located at the 16th residue. These results suggest that the amino-terminal 113-amino-acid region of cadherins is essential not only for their specificity but also for their binding function. Interestingly, synthetic peptides with an amino acid sequence corresponding to that of the specificity-determining site containing HAV sequence as described above can inhibit the cadherin-mediated cell-cell interations (O. W. Blashuck and also C-H. Siu, personal communications). This region, therefore, might be important not only for the expression of the specificity of cadherins but also for their binding function. Therefore, this HAV region of the cadherins could be a center for homophilic interactions.

SPECIES-SPECIFICITIES OF CADHERINS

Cadherins have been identified on a molecular level in mammals (see 64, 65, for human), avians, and amphibia (77–80). Possibly, this molecular family is found in all vertebrates. Recently, in *Drosophila,* molecules with significant identities in amino acid sequence to vertebrate cadherins have been found (P. Mahoney and C. Goodman, personal communication), suggesting the possibility that this molecular family is distributed and essential in invertebrates as well.

Early observations by Moscona and his colleagues (81–84) demonstrated that mouse and chicken cells derived from the same tissues can intermix, but

do not segregate, in aggregation in vitro. Comparison of amino acid sequences of mouse and chicken N-cadherin shows that they were 92% identical. Using L cells transfected with the mouse and chicken N-cadherin, it has been found that these molecules can cross-react (73). Therefore, if the cells expressing N-cadherin were used in the experiments for testing the cross-adhesiveness between mouse and chicken cells, they could intermix in aggregates.

On the other hand, there have been arguments against the generality of Moscona's findings (85, 86). For example, it was reported that, although neural retinal cells of the mouse and the chicken can cross-adhere, hepatocytes cannot (87, 88). Interestingly, E-cadherin, a major cadherin in hepatocytes, is not so highly conserved between these two species. E-cadherin shows only 65% identity in the overall amino acid sequence to L-CAM, which is a major chicken hepatocyte cadherin. This difference might be related to the failure of mouse and chicken hepatocytes to cross-adhere. It then becomes intriguing to know why the differential diversification occurred between different cadherin types.

TRANSMEMBRANE CONTROL OF CADHERIN FUNCTION

Cadherins are transmembrane proteins, and their cytoplasmic domain is highly conserved among different members of this molecular family. In order to understand the role of the cytoplasmic domain in cadherin function, various regions of this domain of E-cadherin were deleted, and the deletion mutant molecules were introduced into L cells by the cDNA transfection method, and then their cell binding ability was assayed (89–91). The molecules with deletions in the carboxy-half portion of the cytoplasmic domain were unable to function in cell adhesion, although their extracellular domain was kept completely intact and exposed on the cell surface. Deletions on other regions of the cytoplasmic domain had no effect on the cell binding function of this molecule, although these regions are also conserved in the cadherin family.

The above results suggest that the carboxy-half region of the cytoplasmic domain plays a key role in cadherin function. How does the intracellular domain control the function of the extracellular domain? The following observations might provide us with a clue.

Cadherins are concentrated at cell-cell junctions on the cell surface, although they are also distributed on other parts of the cell surface that are not engaged in cell-cell connections (92). The cadherins located in the junctions coincide with cortical actin bundles, though not with stress fibers, as revealed by double-staining for cadherins and F-actin (92). Extraction of cells with non-ionic detergents does not remove the junctional cadherins or the cortical

actin bundles, nor destroy their colocalization. A considerable proportion of the total cadherins in a cell is thus insoluble in non-ionic detergents. As the distribution pattern of the insoluble cadherins perfectly coincides with that of cortical actin bundles, it appears that the two components are structurally associated. In fact, cadherins are known to be a transmembrane component of *zonula adherens*, a major intercellular junctional structure that contains cortical actin bundles (93–98).

After the carboxy-terminal deletion, cadherins lose not only their cell-cell binding function but also their ability to associate with cytoskeletons. Cytoplasmic deletions that do not cause the inactivation of cadherins also do not affect their ability to associate with cytoskeletons. These observations imply that the association of cadherins with actin-based cytoskeletons is essential for their cell binding action. However, cadherins seem to be not totally inactive without their cytoplasmic domain, since it has been demonstrated that the extracellular domain of E-cadherin isolated by tryptic cleavage can interfere with cell-cell adhesion in culture (99). Therefore, at the molecular level, the extracellular domain itself appears to have some activity for interacting with other extracellular domains. Formation of dimers or polymers of the isolated extracellular domain of cadherin, however, has never been observed.

It is possible that there are components to mediate the molecular interactions between cadherins and actin. It is known that some intracellular molecules, such as vinculin and α-actinin, are localized in *zonula adherens* junctions (100). There is no evidence, however, that these molecules are directly associated with cadherins. Recent work has indicated that there are some molecules tightly associated with the cytoplasmic domain of cadherin. When the soluble form of cadherins extracted with non-ionic detergents was subjected to immunoprecipitation, two to three other components coprecipitated, a major one of which is a 94-kd protein; these were not immunologically related to the cadherins (101–103). These molecules did not coprecipitate, however, with the carboxy-terminal deletion mutants of E-cadherin that lost cell binding activity (90, 91). These proteins are thus associated only with the functional form of cadherins.

The cell binding activity of cadherins, therefore, depends on their association with some specific proteins and actin-based cytoskeletons (Figure 3). These molecules may form a structural and functional network, which may be essential for the transmembrane control of cadherin-mediated cell adhesion. A reasonable question, therefore, would be what kinds of signals are transmitted beyond the cell membrane. One possibility is that the initial interactions between the extracellular domains of cadherin at apposed cell surfaces may cause a signal to be sent into the cytoplasm. This signal may activate some cytoplasmic components, inducing the binding of cadherins with actin-based cytoskeletons. The association of cadherins with cytoskeletons may somehow

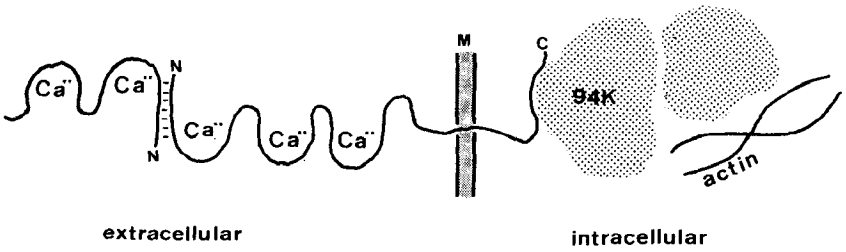

Figure 3 Schematic model for cadherin-cadherin and cadherin-cytoskeletal associations. Actual number of calcium ions binding to a cadherin molecule is not known. The pattern of folding of the molecule is also not determined. N, amino terminus: M, membrane-spanning region; C, carboxy terminus.

stabilize the interaction of the extracellular domains. In this way, intercellular adhesion might be established.

We thus believe that cadherin-mediated intercellular adhesion does not simply depend on pure molecular binding interactions, such as interactions between antigens and antibodies or lectins and carbohydrates, but that it is probably regulated by intracellular machinery. This idea agrees with the observations that cadherin-mediated cell aggregation is temperature-dependent (11). To elucidate how intracellular events regulate extracellular events in cadherin-mediated cell adhesion is a most intriguing subject for future studies.

The above-mentioned properties of cadherins are in contrast to those of Ig superfamily adhesion molecules. It should be stressed that cadherin-mediated adhesion is temperature-dependent, but Ig superfamily-mediated adhesion is not. The latter system can operate even in liposomes into which the molecules are inserted, indicating that no cytoplasmic machinery is required for its action. Although both cadherins and Ig superfamily adhesion molecules are grouped as molecules necessary for cell adhesion, the differences in their properties strongly suggest that they play distinct roles in cellular interactions. Therefore, the functions of these molecular families should be discussed separately. While several lines of evidence suggest that cadherins are crucial for maintaining multicellular architecture, equivalent evidence for the Ig superfamily has not been found to date. The latter may function in more regulatory processes in intercellular adhesion and recognition.

ROLE OF CADHERINS IN MORPHOGENESIS

Different members of the cadherin family are distributed in different spatio-temporal patterns in embryos. During development, cadherin types expressed

in a given cell population dynamically change as the cells differentiate. For example, epiblast cells of chicken embryos at the blastula stage express only L-CAM, the chicken epithelial cadherin (22, 57, 104). However, when gastrulation begins, mesodermal cells separating from the epiblast lose L-CAM and instead begin to express N-cadherin (22, 105, 106). In the process of neural tube formation, the neural plate that originally expresses L-CAM gradually loses it but acquires N-cadherin during separation from the overlying ectoderm, which continues the expression of L-CAM. Neural crest cells appearing between the overlying ectoderm and the neural tube also lose L-CAM, and migrate out of the dorsal region without expressing any known cadherins. Similar differential expression of cadherins occurs in the corresponding processes of mammalian development (52, 55, 103, 107).

The pattern of differential expression of cadherins in embryos, as seen in the above examples, can be generalized as follows (22). When a population of cells is to be separated from a parent cell layer, those acquire a new type of cadherin and/or lose the originally expressed cadherin type. On the other hand, when cells derived from different lineages are to be connected, they express the same types of cadherins. Thus, there is a correlation between the separation or association of cell layers and the differential expression of different cadherins. These observations suggest that the adhesive specificities of cells as a result of the cadherins play an important role in morphogenesis in vivo. It has also has been suggested that the decreased expression of cadherins is involved in invasion and metastasis of tumor cells. (108–110).

Generally, one or two types of cadherins are expressed in a cell, and their combination differs with cell type. Since other unidentified types of cadherins may exist, it is possible that each cell might express more complex combinations of different cadherins than presently known. Such a combination of different cadherins could create a wider variety of adhesive specificities between cells.

Townes & Holtfreter (4) presented a hypothesis to explain how the neural tube separates from the overlying ectoderm. According to their hypothesis, undifferentiated ectodermal cells are connected with a class of cell-cell adhesion molecules. During neural plate differentiation, these molecules are replaced by two distinct novel molecules. One is expressed in the differentiating neural tube and the other in the remaining part of the ectoderm. The authors proposed that these adhesion molecules interact specifically with identical molecules in a homophilic manner. The neural plate and the overlying ectoderm are thus separated from each other owing to the loss of their mutual affinity. The basic idea in this early model agrees surprisingly well with the actual findings on the differential expression of cadherin molecules with distinct binding specificities.

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