Journal of Cell Science, Supplement 17, 139-145 (1993)
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Epithelial cell adhesion and development of cell surface polarity: possible mechanisms for modulation of cadherin function, organization and distribution

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SUMMARY

Epithelial cell adhesion is principally regulated by calcium-dependent cell adhesion proteins, termed cadherins. Recent studies indicate that cadherin function is modulated by a class of proteins, termed catenins, that bind to the cytoplasmic domain of cadherin. Here we review the evidence that catenins regulate cadherin function in cell-cell adhesion, and discuss their role in inititiating cell surface polarity in epithelial cells.

Key words: epithelia, adhesion, cadherin, cytoskeleton, polarity

INTRODUCTION

Multicellular organisms are composed of heterogeneous cell types that are organized during development into distinct patterns to form tissues and organs. One of the important, primary processes involved in regulating the establishment and maintenance of these patterns is cell-cell adhesion. Pioneering studies by Holtfreter and colleagues, and Steinberg and Moscona and colleagues established the central principle that the interaction between cells within a heterogeneous cell population is based upon the specificity and extent of adhesion between those cells; cells of the same type tended to aggregate and, therefore, be sorted out from the other cells (for discussion, see Trinkaus, 1984). The molecular basis for cell adhesion has since been shown to be due to the cell surface expression of a family of glycoproteins that bind with high specificity to each other on adjacent cells (Kemler, 1992a). These proteins are expressed in distinct patterns during tissue and organ morphogenesis, suggesting that they play an important and direct role in the temporal and spatial regulation of cell interactions and cell sorting during tissue formation. Furthermore, loss of expression of these proteins correlates with loss of intercellular adhesion, which is an early event in the induction of metastatic disease (Vieminckx et al., 1991).

Two functionally distinct mechanisms of cell adhesion have been described: Ca^{2+}-dependent, and Ca^{2+}-independent adhesion (Takeichi, 1977). Both processes are mediated by cell surface glycoproteins that have been classified into three major families of cell adhesion molecules (CAMs): the immunoglobulin (Ig) superfamily, integrins and cadherins (Kemler, 1992b). N-CAM is a well characterized member of the Ig superfamily of CAMs; its interactions on adjacent cells are homotypic and Ca^{2+}-independent (Goridis and Brunet, 1992). Early studies on the function of N-CAM in proteoliposomes provided direct biochemical evidence of modulation of CAM avidity by CAM concentration; a 2-fold increase in the amount of N-CAM produced a 30-fold increase in its binding (Goridis and Brunet, 1992; Rutishauser et al., 1982). The integrin family of CAMs is primarily involved in cell interactions with the substratum (Sanchez-Madrid and Corbí, 1992). However, LFA-1, a member of the integrin family expressed on lymphocytes, interacts with I-CAM, a ligand on the surface of endothelial cells (Sanchez-Madrid and Corbí, 1992). The cadherin family of CAMs mediates Ca^{2+}-dependent cell-cell adhesion in a wide variety of cell types (Kemler, 1992a). Here we discuss possible mechanisms for modulating cadherin function.

CADHERIN FAMILY MEMBERS

Three basic classes of cadherins have been characterized, E-, P- and N-cadherin (Kemler, 1992a; Takeichi, 1977). Initial evidence that cadherins play a role in modulating cell interactions was obtained using antibodies raised against the extracellular domain of a given cadherin, which was released from cells by trypsin in the presence of Ca^{2+} (Gumbiner and Simons, 1986). These antibodies inhibit cell-cell adhesion and cause clusters of cells to disaggregate in tissue culture. Addition of specific cadherin antibodies to preimplantation embryos blocks compaction, and their addition to develop-

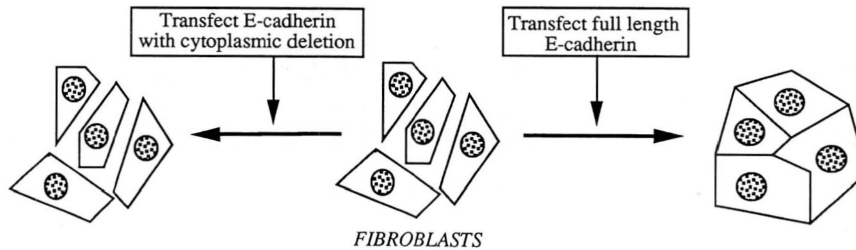

Fig. 1. Fibroblast expression system for analyzing cadherin functions. Fibroblasts do not express cadherins. However, transfection of cadherin full-length cDNA results in expression of the protein at the cell surface, interaction with endogenous catenins, and induction of Ca^{2+}-dependent cell-cell adhesion. Truncation of part of the C-terminal cytoplasmic domain of cadherins results in loss of catenin binding and cell-cell adhesion (for details, see text).

ing tissues in the embryo inhibits further development; similar results have been obtained with antibodies to other CAMs (reviewed by Kemler, 1992a).

Molecular analysis of cadherin structure has provided important insights into the function of different protein domains in homotypic binding between cadherins, and interactions between cadherins and cytoplasmic proteins (Fig. 1). Cadherins span the membrane once with their N termini located in the extracellular space and their C termini in the cytoplasm (Kemler, 1992a); one exception is T-cadherin, which lacks the transmembrane domain and the cytoplasmic tail and appears to be anchored to the membrane via a glycophosphotidylinositol moiety (Vestal and Ranscht, 1992). Different cadherins exhibit an overall sequence identity of 43-58% (Kemler, 1992a). The extracellular domain of different cadherins exhibits the least homology, while the cytoplasmic domain has the highest homology. The extracellular domain comprises three repeat domains (Fig. 1), each of which contains two putative Ca^{2+} binding sites (Ringwald et al., 1987). Mutagenesis of an amino acid in one of the Ca^{2+}-binding sites in the N-terminal repeat domain abolishes cell adhesion (Ozawa et al., 1990a). Recent evidence suggests that the N-terminal repeat contains a cell adhesion recognition motif (Nose et al., 1990). The N-terminal 113 amino acid domain contains a tripeptide, *HAV*, that is highly conserved between cadherin classes (Fig. 1). Antibodies raised against this domain inhibit cadherin function and block cell adhesion (Nose et al., 1990). Additionally, synthetic peptides containing the sequence *HAV* inhibit compaction of mouse preimplantation embryos, which normally occurs through E-cadherin-induced cell adhesion (Blaschuk et al., 1990). These results indicate that the N-terminal repeat domain of cadherins may be important for homotypic binding function. However, little is known about the affinity of these interactions, or whether the cytoplasmic domain plays a role in modulating the avidity of homotypic E-cadherin binding.

Definitive evidence that cadherins are involved in homotypic binding in cell-cell adhesion was obtained when cadherin cDNAs were expressed in fibroblasts (Nagafuchi et al., 1987). Fibroblasts do not exhibit significant Ca^{2+}-dependent cell adhesion properties. However, expression of cadherins in these cells results in Ca^{2+}-dependent cell-cell recognition and the formation of compact cell aggregates (colonies) similar to those formed by epithelial cells (Nagafuchi et al., 1987; see Fig. 1). Mixtures of cells expressing different classes of cadherins sort out from one another such that cells expressing the same cadherin class, or similar cell surface levels of cadherin, form separate aggregates (Nose et al., 1988). Through studies of cadherin function in this fibroblast expression system, evidence has also been obtained that the cytoplasmic domain of cadherins plays an important role in cell adhesion (Fig. 2). Deletions of the C-terminal half of the cytoplasmic domain result in the expression of a truncated protein at the cell surface with an intact extracellular domain (Ozawa et al., 1989). These cells do not form stable cell-cell contacts, or localize cadherin to cell-cell contacts, even when the cells are grown at high density (McNeill et al., 1990; Ozawa et al., 1989). The loss of cadherin function as a result of these cytoplasmic deletions appears to be due, at least in part, to loss of interactions with cytoplasmic proteins.

CADHERIN-ASSOCIATED CYTOPLASMIC PROTEINS

Indirect evidence that cadherins associate with cytoplasmic proteins has come from the observation that cadherins co-localize to sites of cell contact with actin-associated junctions (Hirano et al., 1987). In addition, a limited number of cytoplasmic proteins have been found to co-immunoprecipitate with cadherins solubilized from whole cells (Fig. 3). The most prominent of these cytoplasmic proteins have apparent molecular masses of 102,000, 92,000 and 84,000 Da and have been termed α-, β- and γ-catenin, respectively (Ozawa et al., 1989). Insight into the possible function of two of the catenins has been recently obtained.

α-Catenin

α-Catenin is approximately 30% identical to vinculin within three conserved domains (Herrenknecht et al., 1991; Nagafuchi et al., 1991). These domains include a region of vinculin that is known to bind talin as well as a region that is thought to be involved in the binding of vinculin molecules to one another (Jones et al., 1989; Molony and Burridge, 1985; Molony-Milam, 1985). Vinculin is found in cell-cell and cell-substratum adhesions and appears to be involved in linking membrane proteins to the cortical cytoskeleton. Similarly, α-catenin may link cadherins to the actin cytoskeleton that co-localizes with cadherin in adherens junctions. It has been demonstrated that the interaction of

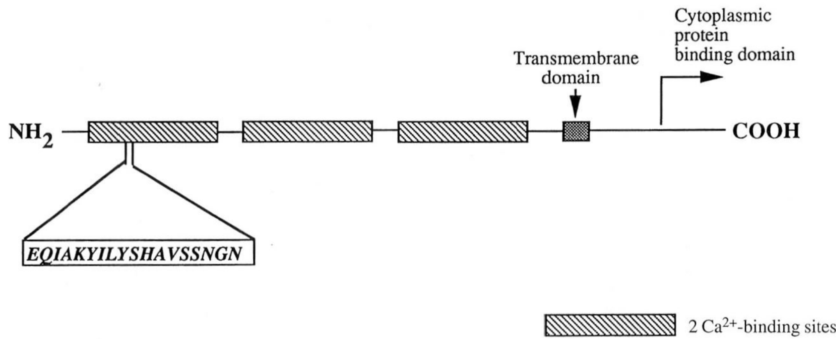

Fig. 2. E-cadherin functional domains (for details, see text).

cadherin complexes with DNAse I (presumably via actin) is dependent on the ability of the cadherin to interact with catenins (Ozawa et al., 1990b).

Cells lacking α-catenin are unable to form stable contacts despite high expression levels of E-cadherin and β-catenin. The ability of these cells to adhere to one another is increased dramatically by the introduction of a neuronal isoform of α-catenin which has been identified as an N-cadherin-associated protein and shows 82% similarity to α-catenin (Hirano et al., 1992). Similarly, cells expressing α-catenin and β-catenin, but lacking cadherins, can be induced to form an epithelioid phenotype by the introduction of N-cadherin or E-cadherin (Hirano et al., 1992). This shows that different cadherin subtypes can interact with different isoforms of α-catenin and vice versa.

β-Catenin/plakoglobin/armadillo family of proteins

The sequence of β-catenin shows ~65% similarity to armadillo, a segment polarity gene in *Drosophila* (McCrea et al., 1991). Additionally, β-catenin shows ~60% similarity to plakoglobin, a major component of desmosomes also found associated with cadherin complexes in adherens junctions. Since plakoglobin and armadillo also share 63% similarity, plakoglobin and β-catenin are considered to be the vertebrate homologues of armadillo (McCrea et al., 1991; Peifer et al., 1992). All three proteins contain internal repeats in the central part of the protein that may be involved in the formation of homo- or hetero-oligomers (Peifer et al., 1992). All members of this protein family are localized to the plasma membrane and the lateral regions of polarized cells, where they co-localize with the actin cytoskeleton and are associated with intercellular junctions (Peifer and Wiechaus, 1990). All three proteins have been detected in both cytoskeletal-associated and cytoplasmic pools, as judged by Triton X-100 extractability (Peifer et al., 1992; Peifer and Wieschaus, 1990; Riggleman et al., 1990).

γ-Catenin

The amino acid sequence for γ-catenin has not been identified. Its apparent molecular mass is identical to that of plakoglobin and the suggestion that these two proteins are identical has been made (Knudsen and Wheelock, 1992; Peifer et al., 1992). Two pieces of evidence seem to contradict this suggestion: in two-dimensional gels of E-cadherin immunoprecipitates, a protein with a much more acidic pI than plakoglobin can be resolved from plakoglobin; also, E-cadherin immunoprecipitates from which all plakoglobin has been removed still contain a protein that migrates with the same size as γ-catenin (Piepenhagen and Nelson, 1993). However, further study of this putative γ-catenin has

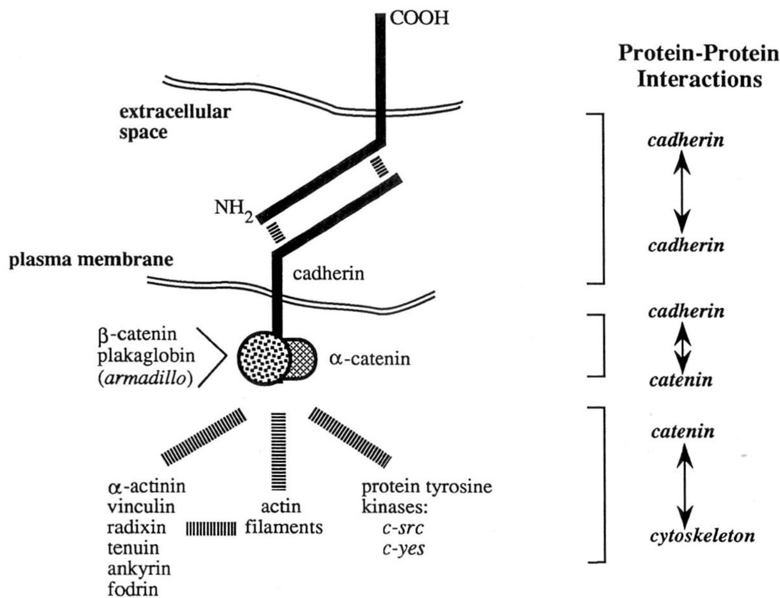

Fig. 3. Protein-protein interactions between the cytoplasmic domain of cadherins, and cytoplasmic proteins - evidence for a linkage between cadherins and the cytoskeleton (for details, see text).

revealed that it may be a proteolytic fragment of cadherin, thereby supporting the idea that plakoglobin is indeed γ-catenin (A. Barth and W. J. Nelson, unpublished observation).

The binding site(s) for catenins on the cytoplasmic domain of cadherins is poorly understood. However, none of the catenins, or membrane-cytoskeleton proteins, are associated with truncated cadherin containing a 72 amino acid deletion of the C-terminal cytoplasmic domain, called LΔC4-17, indicating that these cytoplasmic proteins bind to this region (Ozawa et al., 1989). Since cells expressing cadherin mutant LΔC4-17, lacking the binding site(s) for these cytoplasmic proteins, do not form cell-cell contacts, it is possible that this linkage may play a role in modulating cell adhesion, perhaps by clustering cadherins at the cell surface and increasing the avidity of binding. However, nothing is known about the mechanisms of interaction between cadherins and these cytoplasmic proteins, or their specific roles in modulating cell adhesion.

FROM CELL ADHESION TO CELLULAR REORGANIZATION: REGULATION OF CADHERIN FUNCTION

The results discussed above indicate that cadherins mediate cell-cell recognition and adhesion, and that a direct linkage between cadherins and cytoplasmic (cytoskeletal) proteins is important in cell adhesion and cellular morphogenesis. However, the mechanism(s) involving transduction of cell-cell adhesion, through cadherins, into the reorganization of cell structure and function is unknown. Several levels of complexity are possible. The mechanism could involve increased local concentrations of cadherins at sites of cell-cell contact that cause multivalent stabilization of weak interactions with cytoplasmic proteins through the cytoskeleton. Several examples of this type of mechanism have been cited, including the activation of complement by the binding of Clq to cell surface antigen-IgG complexes, and cellular degranulation triggered by extracellular cross-linking of IgE-receptor complexes (Labrecque et al., 1989; Peerschke and Ghebrehiwet, 1990).

Signal transduction could (also) be involved in mediating cell adhesion by cadherins. Occupancy of cadherin (homotypic binding) could result in the activation of a classical signal transduction response through second messengers (Ca^{2+}, inositol phosphates, protein kinase C). Evidence that second messengers may be involved in N-CAM-mediated adhesion has been reported by Schuch et al. (1989) (see also Doherty et al., 1991), who demonstrated that addition of antibodies to the extracellular domain of the cell adhesion proteins N-CAM or L1 to PC12 cells induced an increase in intracellular Ca^{2+} levels, and a reduction in inositol phosphates and intracellular pH.

EARLY EVENTS IN CELL-CELL INTERACTIONS: FORMATION OF TRANSIENT AND STABLE CELL-CELL CONTACTS

Recent studies have begun to analyze in detail the interaction between epithelial cells and the consequences of cell adhesion on the formation of structurally and functionally distinct membrane domains. These studies indicate that the interaction of cadherin molecules on the surface of neighboring cells is the initial event in a cascade of events that eventually lead to major morphological and biochemical changes within the cells participating in making contacts.

During the initial phases of cell contacts, cells extend a number of filopodia towards one another, transiently form cell contacts which are then retracted, and then establish a stable cell contact. After a variable period of time, the stable contact begins to spread along the apposed membranes by a zippering activity, rather than through the coalescence of multiple individual contacts along the apposed membranes (McNeill et al., 1993). This suggests that a nucleating event may be needed in order to form a stable contact. The direction of zippering does not appear to be biased towards previous sites of transient contacts.

Analysis of the Triton X-100 insolublity of E-cadherin, a preliminary assay for association with the cytoskeleton, revealed no detectable protein at the contact at the time of initiation of a stable cell contact or during the following 10 minutes. However, after ~10 minutes of stable cell contact, rapid acquisition of Triton X-100 insolubility was detected specifically at the contact site (McNeill et al., 1993). It is possible that the increase in the Triton X-100 insoluble pool of E-cadherin was due to accumulation of protein at the contact site. However, the total amount of E-cadherin at the contact remains unchanged during this time (McNeill et al., 1993).

One possible interpretation of the acquisition of Triton X-100 insolubility is that E-cadherin becomes associated with the cytoskeleton after 10 minutes of stable cell-cell contact. However, it is also possible that this is a reflection of another form of molecular reorganization of E-cadherin, like the lateral aggregation of many E-cadherin molecules. The increase in the Triton X-100 insoluble pool of E-cadherin did not correlate with changes in the distribution of actin or fodrin, suggesting that the acquisition of the Triton X-100 insolublity is due to changes in E-cadherin itself, or other closely associated proteins such as the catenins.

MODULATION OF CADHERIN FUNCTION AND DISTRIBUTION DURING THE FORMATION OF CELL-CELL CONTACTS

At present, we have little information on the mechanisms involved in the formation of transient or stable cell-cell contacts. The transient contacts may be mediated by low affinity interactions between randomly distributed cadherin molecules on the surface. This interaction may generate intracellular signals that cause the recruitment of cadherin molecules to the region of the initial contact to increase the local concentration of cadherins leading to the stabilization of cell contacts and the formation of stable adherens junctions.

Signal transduction and second messengers

The signals that could mediate the recruitment of cadherin to sites of cell contact include the activation of second mes-

senger pathways involving G-proteins and the activation of kinases and/or phosphatases. G-proteins have been described to play a role in the morphoregulatory activities of N-cadherin as well as the calcium-independent adhesion molecule N-CAM (Doherty et al., 1991; Schuch et al., 1989). These studies revealed that morphogenic changes mediated by N-cadherin and N-CAM could be inhibited by pertussis toxin, a known inhibitor of G-proteins. The G-proteins appeared (at least in part) to mediate their effect by opening calcium channels which in turn could lead to the activation of other intracellular factors that act on the proteins involved in adhesion.

Preliminary results also indicate that extracellular activation of E-cadherin may initiate signal transduction events. It is not clear, however, how, in the cases of these single membrane-spanning proteins, homotypic extracellular binding is transduced across the membrane. Classical studies of signal transduction by the EGF receptor show that binding of EGF induces dimerization of the receptor (Hurwitz et al., 1991) which results in activation of the receptor tyrosine kinase (Kashles et al., 1991). The dimerization is mediated by a region of the protein near the transmembrane domain. E-cadherin and the EGF receptor share amino acid homology in this region, suggesting that E-cadherin may also dimerize.

A change in the phosphorylation state of cadherins and associated proteins may cause a change in the ability of the complex to interact with cytoskeletal components or with other complexes. The cytoskeletal proteins adducin and ankyrin are modulated by phosphorylation, and their ability to crosslink spectrin networks depends on their phosphorylation (Bennett, 1990). E-cadherin and the catenins are phosphorylated in vivo but a correlation between their assembly into complexes with the cytoskeleton or their adhesive function and their phosphorylation state has not been established. However, the observation that tyrosine phosphorylation by v-*src* interferes with cadherin-mediated cell adhesion suggests a role for phosphorylation in this process (Matsuyoshi et al., 1992). It is possible that the phosphorylation of catenins and/or cadherins modulates the binding of cadherin-catenin complexes to the cytoskeleton or to each other. If each component of the complex regulated different interactions, the activation of kinases or phosphatases specific for each component would regulate different functions of the adhesion complex. This could provide a complex regulatory mechanism that can be finely tuned by the action of several different, possibly independently regulated components.

Regulation of complex formation with catenins

Adhesive function and distribution of cadherin at the cell surface may be modulated by regulation of the binding of cadherin with catenins. Both β-catenin and plakoglobin associate with cadherins immediately following synthesis (Ozawa and Kemler, 1992), indicating that binding is a constitutive process and does not occur in response to cell contact. Newly synthesized α-catenin, on the other hand, is not found in association with newly made cadherin until 30-60 minutes after synthesis (Ozawa and Kemler, 1992) (L. Hinck, J. Papkoff, W. J. Nelson and I. S. Näthke, in preparation); this is similar to the time required for newly synthesized cadherin to be transported to the cell surface (Siemers et al., in preparation). Similarly, β-catenin appears in sites of cell contact with the same kinetics as cadherin after cell contact is initiated, whereas α-catenin is not found at these sites until later times (Hinck et al., in preparation). A large portion of α-catenin is not associated with cadherin and α-catenin is more easily removed from cadherin than β-catenin (McCrea and Gumbiner, 1991; Ozawa and Kemler, 1992). These observations suggest that α-catenin may be able to exchange from one cadherin molecule at the cell surface to another, thus modulating cadherin function at the cell surface. The presence of a large non-cadherin associated pool of α-catenin further reveals that α-catenin may have functions unrelated to cadherin-mediated adhesion.

Based on its similarity to vinculin, it has been proposed that α-catenin links cadherins to the cytoskeleton (Nagafuchi et al., 1991). If indeed it only associates with cadherin on the cell surface it may modulate the association of cadherin-catenin complexes with the cytoskeleton in response to cadherin-mediated adhesion. The titration of α-catenin from a Triton X-100-soluble to a Triton X-100-insoluble pool within 2-6 hours after cell contact is consistent with the idea that its association with the cytoskeleton increases after cadherin-mediated cell contact (Hinck et al., in preparation). Cadherins also increase their association with the cytoskeleton after cell contact, but it has not been established that Triton X-100-insoluble cadherin molecules are always associated with α-catenin molecules.

We have recently shown a mutually exclusive association of E-cadherin with the armadillo family members, resulting in the formation of different pools of E-cadherin containing either β-catenin or plakoglobin (Hinck et al., in preparation). Based on the significant sequence similarity between β-catenin and plakoglobin, it is possible that they compete with one another for the same binding site on cadherins. The central domain containing the internal repeats, thought to be responsible for interaction with other proteins, is the most conserved part between these two proteins (and armadillo) and this region may be involved in their interactions with cadherin (Peifer et al., 1992; Peifer and Wiechaus, 1990). We believe the different complexes may have properties that allow them to perform separate functions including the propagation of different signals. A plakoglobin-containing complex may be able to interact with a different set of second messengers from a β-catenin-containing complex, so that stimulation of a cell surface cadherin bound to plakoglobin would initiate a different cascade of events to that initiated by a cadherin bound to β-catenin. The N- and C-terminal regions of plakoglobin and β-catenin display significant sequence disparity (McCrea et al., 1991) and these regions could be responsible for the interaction with different sets of second messenger proteins. Additionally, plakoglobin and β-catenin may be responsible for the ability of the cadherin complex to interact with different cytoskeletal components. It is also possible that interactions between cadherin and either β-catenin or plakoglobin are modulated differently by phosphorylation.

The fact that many vertebrate cells express both, plako-

globin and β-catenin, and that both proteins are associated with cadherin as soon as they are synthesized, raises the question of how the formation of the complex between cadherin and these proteins is regulated. One possibility is that the relative amounts of plakoglobin and β-catenin in the cell determine how many complexes of each type are formed. Alternatively, post-translational modification of either cadherin or plakoglobin and β-catenin may favor one association over the other. Another question that arises from these observations is whether a cadherin - β-catenin complex on one cell can interact only with a cadherin - β-catenin complex on the contacting cell. This would imply that a conformational difference between the two types of complexes is detectable in the extracellular domain of cadherin.

RECRUITMENT OF CADHERIN TO THE CONTACT SITE

Approximately 1 hour after the establishment of a stable contact there is an increase in the amount of E-cadherin at the contact site (McNeill et al., 1993). Recruitment of E-cadherin to the contact site could be due to diffusion of protein from adjacent non-contacting regions of the membrane to the site of cell contact, similar to ligand-induced capping of cell surface receptors. Most ligand receptors are multivalent, but it is possible that cadherin molecules dimerize to form multivalent receptors for each other. E-cadherin dimerization could potentially be mediated by a short amino acid sequence containing four conserved cysteine residues in the extracellular domain of cadherins, near the transmembrane region.

Another source for recruitment of cadherin to the contact site could be intracellular stores that release cadherins specifically at the site of cell contact. Intracellular compartments containing cadherin are observed by immunofluorescence and intracellular cadherin is rapidly recruited to the plasma membrane after removal of surface cadherin with trypsin (Shore et al., in preparation). The compartment that contains the intracellular cadherin stores does not appear to be an early endosome, as cadherin internalized into early endosomes from the surface is rapidly degraded with a half life of less than 3 hours, whereas the intracellular pool has half life of at least 5 hours.

LATE EVENTS IN CELL-CELL INTERACTIONS: REMODELLING OF THE CELL SURFACE

The long-term effects of cadherin-mediated cell adhesion include extensive reorganization of the membrane and cytoskeletal protein composition of the membrane (Rodriguez-Boulan and Nelson, 1989). This is especially important in polarized, transporting epithelial cells in which distinct cell surface domains are established at the contact site (part of the basal-lateral membrane domain), and at the non-contacting membrane (apical membrane domain).

Interactions between E-cadherin and cytoplasmic (cytoskeleton) proteins may be directly involved in generating structurally and functionally distinct membrane domains. E-cadherin-mediated cell-cell contacts in MDCK cells result in the accumulation of E-cadherin, Na^+,K^+-ATPase and the membrane-cytoskeleton at sites of cell-cell contact; furthermore, protein complexes containing membrane-cytoskeletal proteins and Na^+,K^+-ATPase and E-cadherin have been isolated from extracts of MDCK cells (Nelson and Hammerton, 1989). We have proposed that the reorganization of Na^+,K^+-ATPase is driven by assembly of the membrane-cytoskeleton that is induced by E-cadherin-mediated cell-cell adhesion. This hypothesis was tested by analyzing the distribution of these proteins in fibroblasts transfected with E-cadherin constructs (McNeill et al., 1990). Fibroblasts do not normally express E-cadherin, but constitutively express Na^+,K^+-ATPase and components of the membrane-cytoskeleton which are uniformly distributed at the surface of cells. Expression of E-cadherin in fibroblasts results in the redistribution of these proteins to sites of cell-cell contacts, in a distribution similar to that in polarized MDCK cells (McNeill et al., 1990). This result provides strong evidence that expression of E-cadherin induces a remodelling of the cell surface distribution of an unrelated membrane protein. That this process requires linkage of these two membrane proteins to cytoplasmic (cytoskeleton) proteins was shown by expressing E-cadherin with the deletion comprising the C-terminal 72 amino acids of the cytoplasmic domain (LΔC4-17). Under those conditions, Na^+,K^+-ATPase and the membrane-cytoskeleton did not localize to cell-cell contacts, presumably due to loss of the linkage between E-cadherin and Na^+,K^+-ATPase through the membrane-cytoskeleton as a result of the deletion of the cytoplasmic domain of E-cadherin.

At present we do not know the mechanism(s) involved in inducing assembly of the membrane-cytoskeleton at cell-cell contacts. The membrane-cytoskeleton comprises several well-characterized proteins including: ankyrin, fodrin (spectrin), adducin, protein 4.1 and actin (Bennett, 1990). Adducin, protein 4.1 and actin associate with cell-cell contacts in MDCK cells and are thought to function in the interaction of spectrin and actin filaments (Bennett, 1990). The interaction between these proteins and catenins (see above) is not known. It is noteworthy that protein interactions in the membrane-cytoskeleton are regulated by Ca^{2+} and phosphorylation; phosphorylation of protein 4.1 decreases its affinity for spectrin and its ability to mediate spectrin-actin interactions; adducin bundles actin filaments and is a substrate for phosphorylation by protein kinase C. These post-translational modifications may play an important role in modulating membrane-cytoskeleton assembly in response to E-cadherin-mediated cell-cell adhesion.

This work was supported by grants from the NIH (GM35527) and American Cancer Society to W. J. Nelson. Inke S. Näthke is the recipient of a postdoctoral fellowship from the Damon Runyan-Walter Winchell Cancer Resarch Fund, and Lindsay Hinck is the recipient of a Cancer Biology Predoctoral Fellowship. W. J. Nelson is an Established Investigator of the American Heart Association.

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