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Topical Review

Epithelial Cell Adhesion Mechanisms

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Introduction

Epithelia are cohesive tissues lining or covering the surfaces and cavities of the body. They are divided in simple epithelia, which consist of one layer of highly specialized cells and stratified epithelia, which are composed of several layers of epithelial cells. Simple and stratified epithelia adhere to the underlying tissue by their basal surface and have a free apical pole facing a luminal space. Basically, the distinction between the apical and basal surfaces of epithelia defines the apico-basolateral polarity, which has a major functional importance. For example, in the simple absorptive epithelium of the small intestine, absorbed molecules are transported through the cytoplasm from the apical pole to the basal surface, where they are delivered. In stratified epithelia (like epidermis), the polarity is not confined to the individual cells but is rather distributed throughout the whole tissue: in the basal layer of cells proliferation takes place, whereas in the apical layers, which do not divide, cells have mainly to maintain a high cohesiveness in order to resist the mechanical external stresses.

Functional polarity is accompanied by structural polarity, which means that the plasma membrane is divided into an apical and a basolateral domain each of which is structurally specialized; the specific segregation of some membrane proteins to one of these domains serves as a marker event of the structural polarity. The establishment and the maintenance of structural polarity, which represent most likely a prerequisite to the establishment of functional polarity, depend primarily on the existence of cell-cell interactions and cell-substrate interactions, mediated by cell adhesion molecules. Figure 1 summarizes the epithelial adhesion mechanisms that can be observed at the electron microscopy level. Although molecules belonging to more than one class of these adhesion structures can be identified, three kinds of adhesion mechanisms can be arbitrarily distinguished: specialized junctions, cell adhesion molecules and cell-substrate adhesion sites.

Epithelial cells are interconnected by specialized junctions: tight junctions, desmosomes, intermediate (or adhering) junctions, and gap junctions. Since tight junctions, which serve primarily as an occluding barrier, are established after adhesion between cells has taken place and help to seal intercellular spaces between adjacent cells, it can be postulated that this type of junction is not concerned with the first steps of intercellular recognition leading to adhesion. Gap junctions are by necessity involved in cell-to-cell adhesion, but this is a secondary function since their main role is to establish communication sites between adjacent cells that allow the passage of molecules. Thus, our review will focus on desmosomes and intermediate junctions, which are good candidates for providing true adhesion mechanisms. Although desmosomes have not yet been shown to possess cell adhesion molecules, it can be hypothesized that some proteins belonging to these structures or to adhering junctions must provide the mechanisms by which adjacent cells can recognize each other and adhere to one another; these molecules are named junction adhesion molecules (JAMs).

In addition, adhesion between epithelial cells is established and maintained through independent molecular sites of recognition and adhesion between cells, namely cell adhesion molecules (CAMs) expressed by adjacent epithelial cells. CAMs play, most likely, a key role in the first steps of recognition and adhesion between cells. Moreover, they may participate in the formation of adhering junctions.

The cell-substratum or cell-basal lamina adhesion mechanisms are various and can be identified

Key Words epithelium · cell adhesion molecules · intercellular junctions

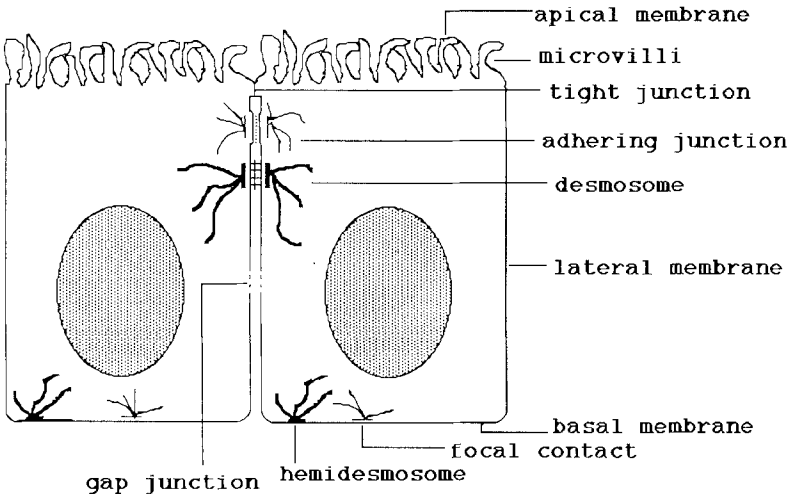

Fig. 1. Schematic representation of adhesion structures found in simple epithelia. Intercellular junctions (*tight junctions*, *adhering junctions*, *desmosomes* and *gap junctions*) are established between adjacent cells and are present on lateral cell membranes, while cell-substrate adhesion sites (*hemidesmosomes* and *focal contacts*) are observed at the basal pole of epithelial cells and connect the cells to the underlying basal lamina. The putative location of cell adhesion molecules (CAMs) has not been indicated in this diagram

as microscopically observable structures (hemidesmosomes or focal contacts) or they may be mediated by the interaction between specific membrane receptors and extracellular matrix components (e.g., collagens, fibronectin, laminin), which have been shown to be mediated by independent molecular sites at the basal surface. It should be stressed, however, that relationships exist between organized contact structures and specific receptors for the extracellular matrix: it has been clearly shown, for example, that fibronectin receptors are immobilized within focal contacts in stationary fibroblasts (Duband et al., 1988*a*).

It is the purpose of this review not to discuss in detail the cell-substrate adhesion mechanisms, which have received much attention in the past years (for review, Edelman, Thiery & Cunningham, 1989), but to consider the cell-cell adhesion mechanisms, mediated either by independent molecular sites (CAMs) or junction dependent molecules (JAMs).

CAMs: Introductory Remarks

Characterization

It has been long recognized that cells of dissociated animal tissue can assemble autonomously and reform the original tissue-like structures ("sorting-out") (Moscona & Moscona, 1952; Townes & Holtfreter, 1955). An important property of cells related to their morphogenetic capacity is their ability to recognize identical or different cell types, adhering preferentially to their own cell 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, especially for those comprising multiple cell types. It has been thus postulated that the information for establishing and maintaining cohesion among cells must lie in the expression and organization of molecules on the cell surface. Experimental efforts in several laboratories have resulted in the discovery of a class of cell surface molecules that seem to be involved in recognition events influencing the intercellular organization of cells in developing and regenerating tissues (for review, Edelman, 1986). They are known as cell adhesion molecules and the combined work during the last few years has resulted in a dramatic increase in the knowledge about these molecules and the processes they are involved in. The role of CAMs in embryogenesis and organogenesis, as well as in the maintenance of the histoarchitecture of tissues has been the subject of several recent reviews (Edelman, 1983; Edelman & Thiery, 1985).

Most CAMs have been defined using an immunological approach of generating antibodies which perturb cell-cell contacts. This approach was first successfully used by Brackenbury and co-workers (1977) and led to the characterization of N-CAM, now the best studied cell adhesion molecule. Since the number of CAMs identified in this way is limited, it was proposed that a relatively small number of these molecules, which are temporally and structurally regulated during development, are sufficient to regulate all necessary cell interaction events (Edelman, 1983). It should be pointed out, however, that most CAMs have been identified using a similar cell aggregation assay in combination with aggregation-perturbing antibodies. The similarity in the experimental approaches used could in fact limit the detection of additional CAMs. It seems thus reasonable to expect that the molecular genetic approach developed recently will allow the descrip-

tion of new CAMs. Little is known about the mechanism of action of these molecules, particularly whether the mechanism of binding is homophilic (identical CAMs expressed on different cells can bind one to the other) or heterophilic (one type of CAM can recognize and bind another type of CAM). However, it has been demonstrated that the mechanism of binding of N-CAM is homophilic.

One Subclass of CAMs is Calcium Dependent

The studies of Takeichi have led to the division of CAMs into two systems, the Ca^{2+}-dependent and the Ca^{2+}-independent systems (Takeichi, 1988). These two systems coexist on individual cells and can be easily differentiated by trypsin treatments. Calcium-dependent CAMs, or cadherins, are highly sensitive to trypsin, but can be protected by calcium against trypsin-mediated proteolysis. They exhibit, moreover, their adhesive function only in the presence of calcium. In contrast, the Ca^{2+}-independent CAMs are inactivated only with high concentrations of trypsin and the proteolytic degradation cannot be prevented by Ca^{2+} ions. They can function as adhesive molecules even in the absence of calcium ions. These two systems are entirely independent: trypsin-calcium dissociated cells cannot adhere to trypsin-EDTA treated cells of the same type (Takeichi et al., 1979; Gibralter & Turner, 1985). Moreover, the genes encoding cadherins have no sequence identity with the family of genes encoding the Ca^{2+}-independent CAMs. Cadherin genes share high percent identity of their nucleotide sequences, particularly in the portion encoding the intracytoplasmic region. Among the members of the Ca^{2+}-independent CAM gene family that belongs to the immunoglobulin superfamily, there is no striking sequence identity.

In epithelia, Ca^{2+}-dependent CAMs (cadherins) have been described: these cadherins are L-CAM (or E-cadherin), A-CAM, and N-cadherin.

Molecular Cloning and Primary Structure

cDNAs encoding the chicken L-CAM and the mouse E-cadherin and N-cadherin have been cloned and sequenced (Gallin et al., 1985, 1987; Schuh et al., 1986; Nagafuchi et al., 1987; Hatta et al., 1988). Ringwald et al. (1987) reported the amino acid sequence of the mouse uvomorulin, which was found to be identical to that of E-cadherin, leading to the definitive evidence that these molecules are identical.

Figure 2 represents the primary amino acid

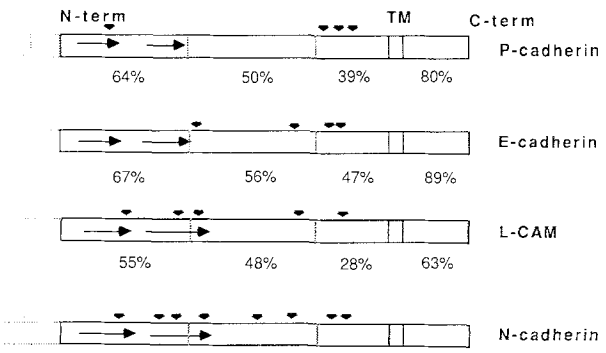

Fig. 2. Primary structures of cadherins deduced from the nucleotide sequences of their cDNAs. The putative extracellular domain is comprised between the N-terminal end of mature protein (*N-term*) and the putative transmembrane region (*TM*). It is divided in three different regions with dotted lines. Percent similarities in amino acid sequences between cadherins is shown for each extracytoplasmic region and for the putative cytoplasmic domain, limited by the C-terminal end of the mature protein (*C-term*). Untranslated regions are indicated by dotted lines at the N-terminus; potential glycosylated sites are shown with arrowheads and the major internal repeats with arrows (reprinted with permission from Takeichi, 1988)

structures of these molecules as deduced from their nucleotide sequences. It shows that the molecules have a similar primary structure of 723-748 amino acid length. The deduced amino acid sequence of each molecule contains a putative signal sequence, an untranslated region and a highly hydrophobic region. This hydrophobic region represents most likely the transmembrane region, suggesting that these molecules are integral membrane proteins. Most interestingly, all of these molecules are very similar to each other in their amino acid sequences as well as in their structural topology (Fig. 2).

The cytoplasmic domain is very conserved among all the members of the cadherin/Ca^{2+}-dependent CAM family, suggesting the importance of this region for the cadherin function. Nagafuchi and Takeichi (1988) suggested that the cytoplasmic domain could regulate the intercellular adhesion mediated by the extracellular domain, possibly through its interaction with some elements of the cytoskeleton.

The extracellular domain is conserved among the members of the cadherin family but to a lesser extent than the cytoplasmic domain. This domain is characterized by the presence of internal repeats, which are conserved among the subclasses of the cadherin family. Average similarity in amino acid sequences deduced from the nucleotide sequences between the mouse E-cadherin and P-cadherin is 58%, that between mouse N-cadherin and chicken L-CAM is 50%,whereas the similarity between E-cadherin and L-CAM is only 65%. Since mouse E-

cadherin and chicken L-CAM are believed to be interspecies homologues, this weak similarity could result from an extended divergence of this gene between the different species. Nevertheless, there is no other protein which shares similarity with the cadherin/L-CAM family (E-cadherin, L-CAM, but also P-cadherin and N-cadherin), that represents a new gene family, entirely independent from that of Ca^{2+}-independent CAMs.

L-CAM

Characterization

Several groups have succeeded in characterizing this type of Ca^{2+}-dependent cell adhesion molecule. Kemler and co-workers (1977) found that a rabbit antiserum raised against teratocarcinoma cells inhibited compaction of early mouse embryo. An 84-kD peptide, which was released from a membrane preparation of teratocarcinoma cells by trypsin treatment in the presence of calcium ions, was found to neutralize the decompacting effect of the antisera (Hyafil et al., 1980). The native form of the 84-kD peptide was identified as a 120-kD glycoprotein (Peyrieras et al., 1983, 1985). This molecule was called uvomorulin.

Takeichi's group used the "Fab strategy" to identify what was then termed E-cadherin. Fab preparations of a rabbit antiserum raised against teratocarcinoma F9 cells were found to inhibit the Ca^{2+}-dependent aggregation of these cells (Takeichi et al., 1981). This inhibitory activity was fully absorbed with trypsin-calcium dissociated F9 cells, but not with trypsin-EDTA dissociated F9 cells, suggesting that the inhibition of aggregation with this antibody was due to a direct block of Ca^{2+}-dependent molecules. In an attempt to identify the target molecules for this antibody, Yoshida and Takeichi (1982) detected a 34-kD polypeptide able to absorb the inhibitory effect of the Fab fragments in the supernatant of trypsin-calcium dissociated F9 cells. The native form of this 34-kD fragment was identified as a 124-kD glycoprotein, present in trypsin-calcium but not in trypsin-EDTA treated F9 cells.

Comparison of amino acid sequences between uvomorulin and E-cadherin has provided definitive evidence that they are identical molecules (Nagafuchi et al., 1987; Ringwald et al., 1987).

Other approaches have led to the characterization of molecules with similar properties. Human mammary carcinoma cells spontaneously release an 80-kD peptide into the serum-free culture medium. Antibodies that induce disruption of the target cells and also decompaction of mouse embryos (Damsky et al., 1981, 1983) detected a 120-kD glycoprotein, termed cell-CAM 120/80, present in cell membranes. The properties of this molecule are, thus, identical to those of E-cadherin or uvomorulin. Behrens and co-workers (1985) found Arc-1 molecules with properties similar to those of E-cadherin, uvomorulin and CAM 120/80, on canine epithelial cells.

Using chicken hepatocytes, Edelman's group identified a 124-kD glycoprotein, termed L-CAM. Specific antibodies against this molecular species inhibited the Ca^{2+}-dependent aggregation of these cells (Bertoletti et al., 1980; Gallin et al., 1983; Cunningham et al., 1984). This molecule could be cleaved into an 81-kD peptide with trypsin in a Ca^{2+}-dependent manner.

In summary, all molecules described here show similar properties. (i) Their molecular weight is similar. (ii) They are sensitive to Ca^{2+}. In the presence of Ca^{2+}, they are not degraded even if live cells are treated with trypsin. However, when a membrane fraction of cells is treated with trypsin, even in the presence of Ca^{2+}, the molecules are degraded into 80-84 kD peptides. In the absence of Ca^{2+}, these peptides are further digested into smaller fragments (Yoshida & Takeichi, 1982; Vestweber & Kemler, 1985; Shirayoshi et al., 1986). (iii) They show a similar tissue distribution pattern: immunological studies revealed that all of these molecules are present in epithelial cells found in a variety of embryonic and adult tissues (Edelman et al., 1983; Ogou et al., 1983; Vestweber & Kemler, 1984; Hatta et al., 1985; Nose & Takeichi, 1986). All these observations suggest strongly that these molecules are either identical or interspecies homologues.

Spatial Organization

While the most prominent features of the primary sequence of L-CAM and related cell adhesion molecules have been well characterized, little is known about the three-dimensional organization of these molecules. A recent work by Becker and colleagues (1989) has provided insights on the information about the spatial structure of CAMs. They found by electron microscopy of rotary shadowed extracellular domain of L-CAM obtained as a proteolytic fragment that the molecule has a rod-shaped structure that contains a hinge region which is apparently flexible. Its extracellular binding domain does not seem to form aggregates in solution. Moreover, they observed the same structure for N-CAM, which is a calcium-independent cell adhesion molecule that does not share amino acid sequence identity with L-CAM. These results suggest therefore

that the general configuration of cell adhesion molecules and the presence of a flexible hinge could represent essential elements in assuring effective and specific cell-cell adhesion.

Transfection Experiments

To address the issue as to whether E-cadherin or L-CAM are key elements in the recognition events between epithelial cells leading to the establishment and maintenance of epithelial sheets, full-length cDNAs have been introduced into cells that have very little endogenous E-cadherin or L-CAM activity. Nagafuchi and co-workers (1987) successfully transfected E-cadherin in L fibroblasts deficient in E-cadherin activity. Most of the L-cell transformants expressing E-cadherin acquired high Ca^{2+}-dependent aggregation activity and exhibited an alteration in their morphology: whereas L cells formed monolayers of dispersed cells that lack intercellular contacts, transfected L cells were able to form compact colonies of tightly packed cells. Furthermore, transfection of either E-cadherin or P-cadherin into L cells demonstrated that transfected cells preferentially adhered to cells expressing the same cadherin subclass. Similarly, transfection of L-CAM into mouse sarcoma S180 cells led to the expression of this gene in several clones, which acquired an epithelioid morphology. Moreover, in contrast to the untransfected S180 cells, transfected clones had large increases in adhaerens junctions and gap junctions, suggesting that the expression of epithelium-specific CAMs could be a necessary event for the extensive expression of junctional structures (Mege et al., 1988).

L-CAM Expression is Modulated during the Epithelium-to-Mesenchyme Transition in Embryo

Several studies in chicken embryo as well as in mouse embryo have shown that L-CAM appears very early during development, at the cleavage stage (Ogou et al., 1982; Thiery et al., 1984; Crossin et al., 1985; Vestweber & Kemler, 1985) and plays a key role in the blastomere compaction (Hyafil et al., 1980; Damsky et al., 1983). At the implantation stage, L-CAM is expressed in all cells. However, as cells differentiate into various types, L-CAM disappears from some cell layers. The most striking example is given by the modulation of L-CAM during gastrulation and neurulation: during gastrulation, the molecule remains present on ectoderm but is not detected on mesodermal and definitive endodermal cells. During neurulation, L-CAM disappears from the neural ectoderm (Fig. 3; *see also* Thiery et

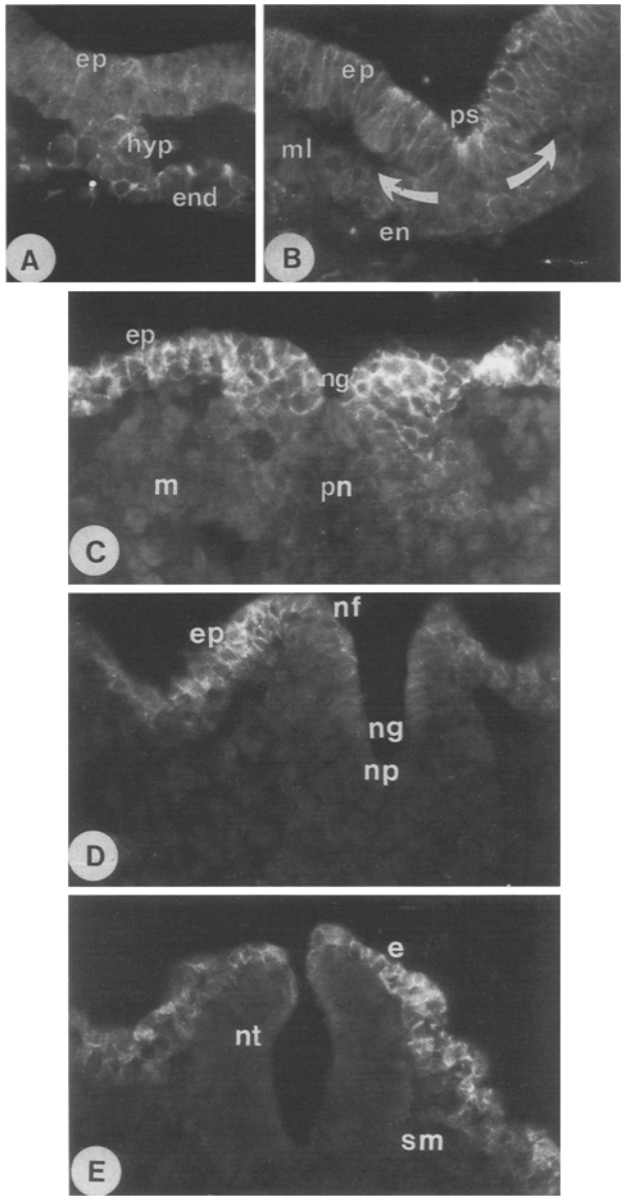

Fig. 3. Modulation of L-CAM during gastrulation and neurulation in chick embryo. (*A*) The three primitive germ layers (*ep*: epiblast, *hyp*: hypoblast and *end*: endophyll) are stained with a rabbit anti-L-CAM antibody. (*B*) At the primitive streak (ps) stage, in the anterior part of the primitive streak, the mass of ingressing middle layer cells (*ml*) and the definitive endoderm (*en*) are not stained with anti-L-CAM, while the most superficial cells remain labeled. (*C*) Formation of the neural plate: at the anterior part of the embryo, the epiblast (*ep*) is uniformly stained, including at the neural groove level (*ng*). The mesodermal cells (*m*) and the presumptive notochord (*pn*) are negative for L-CAM staining. (*D*) More rostrally, the neural groove (*ng*) is more pronounced. L-CAM is present in the epiblast (*ep*) and in the neural fold (*nf*) but disappears from the ventral part of the neural plate (*np*). (*E*) Slightly more rostrally, the neural tube (*nt*) is almost completely closed. The only L-CAM positive cells are ectodermal cells (*e*). The neural tube and the somatic mesenchyme (*sm*) do not react with anti-L-CAM

al., 1984). In contrast, other regions of the ectoderm and all endodermal cells maintain the expression of L-CAM, as long as they differentiate into epithelial cells. In older embryos, essentially all epithelial cells derived from the ectoderm and the endoderm express L-CAM, with some exceptions (lens cells, keratinized epithelial cells). Neural and mesodermal cells do not have L-CAM. Epithelial components of the urogenital system derived from the mesoderm (i.e., mesenchyme), such as mesonephric and metanephric tubules express L-CAM after their induction into epithelial cells (Thiery et al., 1984; Vestweber & Kemler, 1984). In such a case, the appearance of L-CAM does not correlate with the time of induction, but rather with the stage when the first induced cells begin to aggregate. The expression of L-CAM is thus correlated with the epithelial state, whereas modulation of L-CAM arises when mesenchyme is produced from epithelial tissues.

L-CAM Expression Is Involved in the Establishment of Epithelial Cell Polarity

The relationships between cell adhesion and cell junction formation have not been clearly elucidated. One prominent hypothesis is that cell adhesion mediated by CAMs is an early recognition event that is a prerequisite to the formation of specialized intercellular junctions and to the subsequent establishment of epithelial cell polarity (Takeichi, 1987). In fact, the distinction between CAMs and intercellular junctional molecules has become less clear: for example, in some epithelial cell types L-CAM has been found to be highly concentrated in adhaerens junctions (Boller et al., 1985; Vestweber & Kemler, 1985) and A-CAM, which was first described as a component of adhaerens junctions, belongs to the cadherin family (*see below*). Transfection experiments with full-length cDNA encoding L-CAM into mouse S180 sarcoma cells have shown that the expression of transfected L-CAM is accompanied by the appearance of gap junctions (Mege et al., 1988). On the other hand, Gumbiner and Simons (1986) found that L-CAM was critically involved in the rapid resealing of tight junctions between confluent Madin-Darby canine kidney (MDCK) cells and in the formation of apicobasolateral polarity. Moreover, Gumbiner and coworkers (1988) reported that specific inhibition of L-CAM function inhibited the formation of all forms of intercellular junctions including tight junctions, adhering junctions and desmosomes in MDCK cells. From these preliminary experiments one could infer that L-CAM function is a prerequisite to the cascade of events leading to the formation of specialized junctions.

A-CAM and N-Cadherin

In an attempt to isolate adhaerens junction specific proteins, Volk and Geiger (1984) isolated a 135-kD protein named A-CAM which was first described as localized along cardiac muscle intercaled discs. They later gave evidence that this molecule is a membrane-bound glycoprotein localized predominantly, if not exclusively, in adhering junctions of some epithelial cells (lens cells, cardiac muscle intercalated discs, nervous tissues); however, not all adhering junctions contain this protein. For example, intestinal epithelium, liver hepatocytes, kidney tubules, and many other epithelia contain adhering junctions while they are apparently devoid of A-CAM (Volk et al., 1987).

A-CAM Is a Calcium-Dependent Molecule

When cultured chicken lens cells were incubated in the absence of Ca^{2+} ions, A-CAM became exposed to exogenously added antibodies or to proteolytic enzymes. It is noteworthy that adhering junctions were split into two halves with A-CAM exposed on their surfaces and that the actin-containing, junction-associated belt of microfilaments was detached from the plasma membrane and displaced towards the perinuclear cytoplasm within a comparable time frame (Volk & Geiger, 1986*a*). Whereas addition of Ca^{2+} to EGTA-treated cells resulted in the rapid recovery of adhering junctions including the reorganization of A-CAM, incubation of EGTA-treated cells during the Ca^{2+} recovery phase with Fab fragments of anti-A-CAM specifically inhibited the reformation of adhering junctions (Volk & Geiger, 1986*a*). This inhibition was accompanied by striking changes in microfilament organization, which formed fragmented actin bundles throughout the cytoplasm. These results strongly suggest that A-CAM participates in intercellular adhesion present at the level of adhering junctions and is involved in actin filament assembly.

Upon addition of trypsin to EGTA-treated cells, A-CAM was cleaved into three major cell-bound antigenic peptides with apparent molecular weights of 78, 60 and 46 kD, suggesting that the extracellular domain of A-CAM has a size $\geq$ 90 kD (Volk & Geiger, 1986*b*).

Molecular Properties of A-CAM

By detergent partitioning assay using Triton X-114 biphasic system, it has been demonstrated that A-CAM is an integral membrane protein, with a large extracytoplasmic domain (Volk & Geiger, 1986*b*).

Furthermore, it has been shown that A-CAM is a Con A-binding protein, bearing carbohydrate moieties all along the extracytoplasmic domain.

A-CAM Distribution during Chicken Embryonic Development

Immunolocalization of A-CAM in early chick embryo revealed an extensive labeling of ectodermal placodes as well as a prominent but transient expression in several mesoderm-derived epithelia. Most interesting are the cases of A-CAM modulation during epithelial remodeling in neurulation and during epithelial-mesenchymal transition in somite formation (*see* Fig. 4; *see also* Duband et al., 1987). In these morphogenetic processes, A-CAM apparently contributes to intercellular adhesion leading to the assembly of a complex adhering junction characterized by extensive interactions with the cytoskeleton.

N-Cadherin, N-cal-CAM and A-CAM Share Similar Properties

Using the already defined strategy to isolate cadherins, Takeichi and co-workers (1985), obtained a monoclonal antibody against the Ca^{2+}-dependent cell adhesion system of brain cells that disrupted contacts between adjacent brain cells of mouse embryos. The antigen was called N-cadherin (for neural-cadherin). The distribution pattern of the antigen recognized by the monoclonal antibody was similar to that found for A-CAM: it was found in neurohypophysis, lens epithelium, cardiac muscle, myotubes of skeletal muscles and was absent from several epithelial tissues, such as epidermis, mammary gland, liver, pancreas, stomach, salivary gland, thyroid, and adenohypophysis. Although there is no definitive evidence that A-CAM and N-cadherin are identical, tissue distribution and Ca^{2+}-sensitivity favor this hypothesis. When N-cadherin cDNA was transfected in L fibroblasts, the transformants acquired the N-cadherin-mediated aggregation property, indicating that the cloned cDNA contained all the information necessary for the cell-cell binding action of this molecule (Hatta et al., 1988).

Lilien and his colleagues identified cell surface proteins on chicken neural retina that were protected by Ca^{2+} ions against proteolysis (Grunwald et al., 1981; Cook & Lilien, 1982) and termed them N-Cal-CAM (Bixby et al., 1987). Antibodies against N-Cal-CAM inhibit the Ca^{2+}-dependent system of the neural retina, as do anti-N-cadherin antibodies, suggesting that these molecules belong to the same cadherin subclass, if they are not identical.

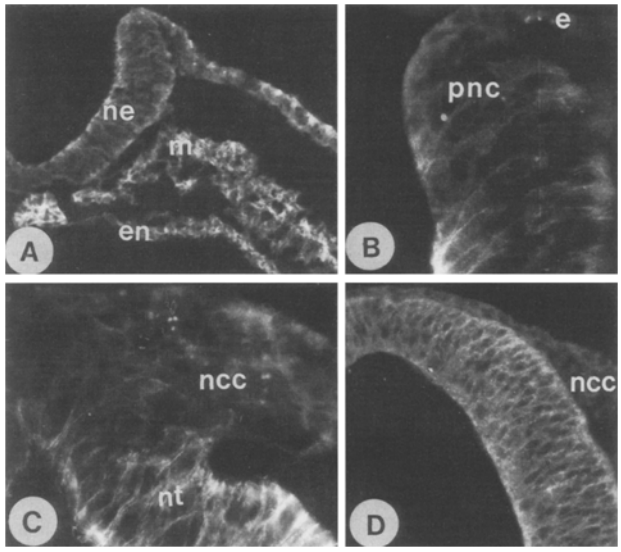

Fig. 4. A-CAM modulation during mesencephalic neural crest cell migration. (*A* and *B*) Mesencephalon prior to neural crest cell migration: neural epithelial cells (*ne*), mesenchyme (*m*) and endoderm (*en*) are A-CAM positive, whereas premigratory neural crest cells (*pnc*) located in the folds are only weakly labeled. The apparent staining of the ectoderm (*e*) is due to autofluorescence. (*C*) Neural crest cells have undergone their migration. The neural tube (*nt*) is almost completely closed. A-CAM staining is nearly absent from neural crest cells (*ncc*) while it is expanding to the median part of the neural tube. (*D*) After their departure from the neural tube, neural crest cells (*ncc*) are A-CAM negative. In contrast, the neural tube is strongly stained with anti-A-CAM (reprinted with permission from Duband et al., 1988*b*)

Cell-CAM 105

Characterization

This membrane glycoprotein has been described essentially by Öbrink's group, who found that cell-CAM 105 is involved in reaggregation of isolated hepatocytes in vitro (Ocklind & Öbrink, 1982); an immunological functional assay demonstrated that it was the molecule able to neutralize the inhibition of hepatocyte reaggregation exerted by monovalent polyspecific rabbit antibodies raised against plasma membranes.

Molecular and Functional Properties

The protein was purified to homogeneity from rat liver membranes. Biochemical analyses demonstrated that the protein consisted of two structurally similar, N-glycosylated polypeptide chains with apparent molecular weights of 105 and 110 kD, respec-

tively. Complete deglycosylation of the molecule shifted the molecular weight to 54 and 58 kD, respectively. The molecule could be phosphorylated on serine residues exclusively. When the molecule was integrated into liposomes, cell-CAM containing liposomes were able to bind to hepatocytes. The binding was specifically inhibited by Fab fragments of monospecific antibodies against cell-CAM 105. Self-aggregation of cell CAM-bearing liposomes was observed when high amounts of the protein were incorporated into liposomes (Öbrink et al., 1986). These results strongly suggested that the protein was a cell adhesion molecule, and that the mechanism of binding was homophilic.

Tissue Distribution in Adult and Embryonic Tissues

The distribution of cell-CAM 105 in adult tissues is not restricted to epithelia, since vessel endothelia and peripheral blood cells also express the molecule (Odin & Öbrink, 1987). In mouse fetal tissues, cell-CAM 105 is first detected at the blastocyst stage, where it becomes expressed in the trophoblast cells of the trophectoderm. It is absent from the 12-day-old embryo, and is re-expressed on liver megacaryocytes at day 13 of gestation. At day 16, it is found on parenchymal liver cells, on epithelial cells of both the proximal kidney tubule and of the small intestine mucosa. Modulation of cell-CAM 105 has been studied in regenerating liver: the most dramatic changes of both the concentration and the cell surface location of cell-CAM 105 occur at the time when the hepatocyte proliferation is initiated, whereas the amount and membrane location of cell-CAM 105 come down to control levels when the growth of liver has ceased (Ogawa et al., 1979). The cell surface distribution of cell-CAM 105 is somewhat indicative of the putative role of the molecule in cell adhesion: in reaggregating freshly dissociated hepatocytes cell-CAM 105 is first distributed all around the cells and becomes localized at the cell contacts after prolongated aggregation time. In vivo, in simple epithelia (including liver) and some endothelial cells, cell-CAM 105 is located at the apical surface. In intestinal cells, for example, it is concentrated at the contact points between adjacent microvilli of the brush border, indicating that in these cells cell-CAM could contribute both to cell-cell interactions and to interactions between microvilli of individual cells that may regulate the structure and function of the apical brush-border regions (Hansson et al., 1989). In stratified epithelia, cell-CAM 105 is located at the cell-cell borders of the suprabasal layers. This distribution is consistent with the role of cell-CAM 105 in cell-cell adhesion. Finally, in blood cells cell-CAM 105 is localized primarily in intracellular compartments, which may serve as a storage pool of the molecule that could be released at the cell surface when the cells are activated (for example, the surface of aggregating platelets is strongly cell-CAM positive). Taken together with the observation that several forms of the molecule seem to exist in different cells and tissues, these results suggest that cell-CAM 105 is a multifunctional protein, whose primary role is in membrane-membrane binding at the cell surface.

Specialized Junctions

As mentioned before, among the specialized junctions postulated or demonstrated to provide cells with adhesion systems, are desmosomes and adhering junctions.

Desmosomes

Desmosomes are punctate adhesive intercellular junctions, that occur in most types of epithelial cells (Cowin & Garrod, 1983; Moll et al., 1986). They are absent, however, from pigmented retinal epithelial cells (Docherty et al., 1984) and lens epithelium. Desmosomes are not exclusively epithelium-specific molecules, since they are also present in a number of nonepithelial cell types, like the intercalated discs of heart muscle (Franke et al., 1982), and the arachnoid and pia mater of the meninges. They are absent from skeletal and connective tissues, nervous tissue and blood cells. Desmosomes may be considered as adhesive intercellular links between the intermediate filament cytoskeletons of adjacent cells (Overton, 1974; Garrod, 1985); intermediate filaments attach in bundles to the cytoplasmic plaques of desmosomes and either terminate at the nuclear envelope or anchor in other desmosomes. In epithelia, desmosomes therefore act as structural links that maintain cytoskeletal continuity throughout the cell sheet or tissue.

Biochemical Studies

The structure and composition of desmosomes has been investigated in numerous studies (for review, Cowin et al., 1985; Franke et al., 1986), and biochemical analyses have allowed the identification of at least seven major polypeptides in desmosomes of stratified epithelia. There are four nonglycosylated proteins, desmoplakin I (M_r 250 kD), desmoplakin II (M_r 215 kD), plakoglobin (M_r 83 kD) and a basic polypeptide of M_r 75 kD (band 6 protein). In addition, three glycoproteins have been identified; des-

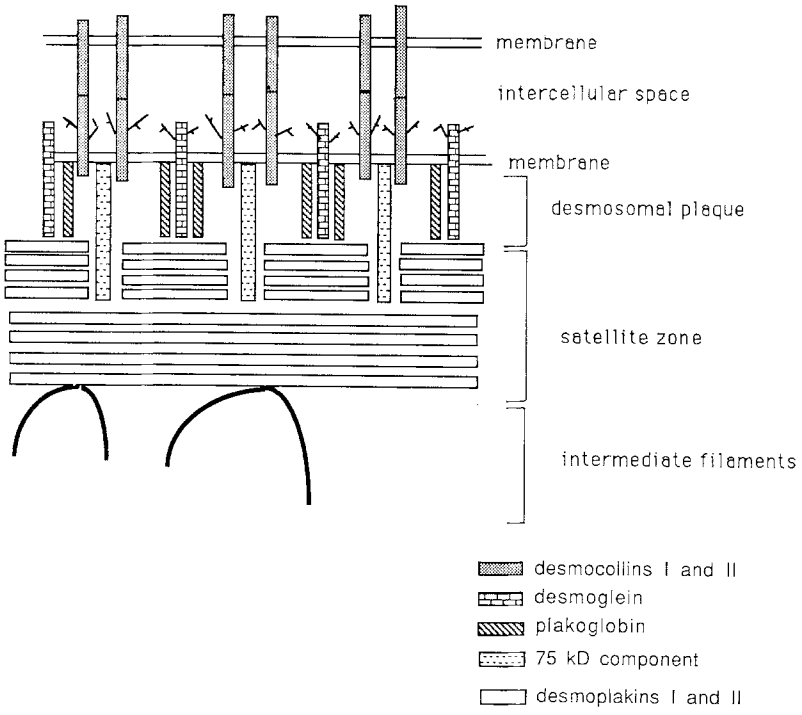

Fig. 5. Putative location of the major desmosomal proteins. Desmoglein and desmocollins are glycoproteins extending an extracytoplasmic region into the intercellular space. Putative adhesion between desmocollins is shown. Desmosomal plaque is formed by the assembly of desmoplakins, plakoglobin and 75-kD component. Desmoplakins are found also in the satellite zone to which intermediate filaments are anchored (reprinted with permission from Garrod et al., 1989)

moglein (M_r 165 kD) and desmocollins I (M_r 130 kD) and II (M_r 115 kD). Some other proteins participate in the formation of desmosomes: desmocalmin, a high molecular weight calmodulin which binds cytokeratins (Tsukita & Tsukita, 1985) and a glycoprotein of M_r 140 kD (Jones et al., 1986). Desmoplakins I and II are biochemically and antigenically related to each other, as are desmogleins I and II, while the remaining proteins are distinct (Cohen et al., 1983; Mueller & Franke, 1983; Kapprell et al., 1985). Miller and colleagues (1987) have used specific antibodies on ultrathin frozen sections to immunolocalize the major protein and glycoprotein components within desmosomes of bovine snout epidermis. Desmoplakins I and II are located predominantly in a region between the desmosomal plaque and the intermediate filaments. Plakoglobin is located within the desmosomal plaque, as well as the 75-kD component. Desmoglein is present both in the extracellular space and in the desmosomal plaque. Desmocollins I and II are situated primarily in the intercellular space with a short cytoplasmic extension, a distribution consistent with the postulated role of these molecules in desmosomal adhesion (Cowin et al., 1984; Garrod & Cowin, 1986). A diagrammatic model of desmosome structure is shown in Fig. 5.

Desmosome Stability is Ca^{2+} Dependent

In the last decade the problem of assembly and disassembly of desmosomes and desmosomal plaques has been approached primarily by calcium switch experiments (Hennings et al., 1980; Kartenbeck et al., 1982; Mattey & Garrod 1986*a*,*b*; Pasdar & Nelson, 1988), since in cells cultured in low calcium medium, desmosomes are split and internalized as half desmosomes. They are reformed when calcium concentration is brought back to normal level. Despite the fact that the mechanism by which Ca^{2+} ions act to stabilize desmosome structures is not yet known, Ca^{2+} switch experiments have allowed the study of the rate of synthesis and turn-over of some desmosomal proteins in normal and in low-calcium medium. From these studies, it has been inferred that desmocollins and desmoglein could be the limiting factors to desmosome assembly (Penn et al., 1987), thus suggesting that the initiating event in desmosome formation might be the adhesive recognition between desmocollins I and II expressed on the membrane of adjacent cells, followed by the patching of these molecules and by the subsequent recruitment of other desmosomal components from the cytoplasmic pools (Garrod et al., 1989).

Desmosomal Cell-Adhesion Molecules

While there is no definitive evidence that any of the desmosomal proteins could be considered as authentic Ca^{2+}-dependent CAMs, some preliminary results (Cowin et al., 1984; Watt et al., 1984; Mattey & Garrod, 1986*b*) suggest that desmocollins might play the role of adhesive molecules. This conclusion is based on the following arguments: after re-

moval of Ca^{2+} ions from the cell culture, desmocollins I and II are evenly redistributed on the cell surface, whereas the other desmosomal components are internalized. The redistribution of cadherins on the cell surface depending on the cell state and on the culture conditions has been noticed several times (Gumbiner & Simons, 1986; Öbrink et al., 1989). Furthermore, Fab' fragments against desmocollins are able to inhibit desmosome formation (Cowin et al., 1984). If these molecules are Ca^{2+}-dependent CAMs, their sequencing should show amino acid sequence identities characteristic of all the members of the cadherin family. Furthermore, if they are cadherins, it could be postulated that antibodies directed against the highly conserved cytoplasmic region of cadherins will also recognize desmocollins.

Adhering Junctions

This group of cell contacts is characterized by their association with actin-containing microfilaments (Farquar & Palade, 1963; Geiger et al., 1983) and by the presence of vinculin-rich "plaques" along their cytoplasmic surfaces. The plaques also contain plakoglobin, which is a protein shared in common by desmosomes and adhering junctions (Cowin et al., 1986).

Adhering Junctions are Ca^{2+} Dependent

Adhering junction stability is dependent on the presence of Ca^{2+} ions: if calcium is removed from the cell culture medium, adhering junctions are split and the complex formed by vinculin-rich plaques and actin bundles is detached from the plasma membrane and is displaced towards the perinuclear region (Volberg et al., 1986). Calcium switch experiments have allowed the study of the process of adhering junction assembly which occurs upon addition of calcium in cultures previously maintained in low-calcium medium. They have led to the concept of junction assembly as a polar process, the cell-cell contact triggering the assembly of vinculin-rich plaque and leading consequently to the assembly of the actin-containing filament system (Avnur et al., 1983; Geiger et al., 1984).

Adhering Junction Cadherins

As already mentioned, A-CAM was first described as a major component of adhering junctions that possessed cell-adhesion properties (Volk & Geiger, 1986*a*,*b*). On the other hand, Boller and colleagues (1985) have found that L-CAM is preferentially located in adhering junctions of hepatocytes, and Behrens and co-workers (1985) have described L-CAM enriched adhering junctions in cultured kidney epithelial cells. Since the tissue distributions of L-CAM and A-CAM in adult and embryonic tissues are different and roughly complementary, it can thus be postulated that the cell-adhesion molecule present in adhering junctions is either L-CAM or A-CAM depending on the cell type. Since some studies had suggested that the mechanism of binding of L-CAM or A-CAM was homophilic (L-CAM binding only to L-CAM and A-CAM to A-CAM), the distribution of these two cadherins in adhering junctions of different cell types would suggest that homophilic binding could be a sorting process during embryogenesis and adult tissue regeneration allowing different cell types not to be mixed. However, a contradictory result has been published by Volk et al. (1987), who found that heterotypic adhering junctions could be formed between L-CAM-containing liver cells and A-CAM-containing lens cells. Further experiments are thus needed to examine the spatiotemporally coordinated interactions of these molecules during development and adult life.

Conclusion

Without the establishment and maintenance of specialized junctions, the specific association of which characterizes epithelial cells, the epithelium-specific polarity would not exist. The major goal of this review has been to develop the idea that relationships between CAMs and junctions do occur. Although CAMs were first described as morphoregulatory molecules essential for the normal development of the embryo, it has become evident that they may also have a role in epithelial cohesiveness as specialized junctions do. The arguments that suggest relationships between Ca^{2+}-dependent CAMs (cadherins) and junctions are the following: (i) Some junction-specific molecules and cadherins are identical. (ii) In some cases, the expression of specific cadherins at the cell surface leads to the establishment of specialized junctions. (iii) The inhibition of cadherin-mediated adhesion blocks the establishment of specialized junctions. (iv) Junctions and cadherins are equally sensitive to Ca^{2+} ions. Moreover, chelation of external Ca^{2+} ions dissociates epithelial cells.

Despite the existing evidence suggesting that the expression of cadherins could regulate the assembly of junctions, the hierarchy of events leading to epithelial cohesiveness is largely unknown. Furthermore, it is not yet clear whether the mechanisms of epithelial adhesion are regulated at the

transcriptional, translational or posttranslational level. Moreover, further studies will have to shed new light on the biological factors involved in the creation and maintenance of epithelial cohesiveness. But the intensive effort of several laboratories will probably be fruitful in the near future and will give further insights into the mechanisms that regulate the assembly/disassembly of junctions and the modulation of cadherins in physiological as well as pathological situations.

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