Review

Looking for cell-cell adhesion molecules: a cadherin story

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(Contributed by Masatoshi Takeichi, M. J. A.)

Abstract: Animal cells have the intrinsic ability to adhere to each other and to form multicellular structures. Classic studies suggested that cells have divalent cation-dependent mechanisms for holding themselves together. Cadherins were identified as Ca^{2+}-sensitive cell surface molecules; and extensive studies revealed that this family of molecules plays critical roles in cell-to-cell adhesion and tissue organization, and even in synapse formation. Further studies showed that molecules belonging to the cadherin superfamily have more diverse functions, such as those to regulate planar cell polarity. Thus, we have been able to disclose parts of the molecular mechanisms of how animal cells can assemble and organize themselves into complex multicellular structures.

Key words: Cadherin; catenin; cell adhesion; morphogenesis; neuron; synapse.

All forms of life can be roughly categorized as either unicellular or multicellular organisms. The former group includes bacteria, yeast and protista, while the latter encompasses nearly every form of life visible to the human eye. Single-celled organisms are autonomous individual cells that do not need to assemble in groups to live, but multicellular life, as the name implies, have bodies formed by aggregations of many cells. There can be many different types that take on diverse functions within the body; groups of cells of similar type may also assemble into tissues, and assemblages of tissues may work with other tissues to form organs, such as lungs, kidneys, heart or brain. Only after all the cells, tissue and organs are established and in place is the multicellular organism capable of independent life; its individual cells cannot live on their own.

But what is it about cells in multicellular organisms that, unlike in unicellular life forms, causes them to assemble into groups without detaching? This is a mystery that researchers have been trying to solve for many years. I should mention at this point that the ways

that animals and plants develop are very different, so I'll be limiting the rest of this discussion to animal development. Adhering to another is actually an essential property of animal cells. For example, it is possible to forcibly separate living animal cells by using any of various methods, but if these cells are then cultured, they will naturally begin to re-adhere with each other and reconstitute a multicellular state. Even more surprisingly, they are able to distinguish between different types of partners when adhere. If you remove cartilage and epithelial cells form the body and mix them together, the two different types of cells will assemble into separate groups, and the resultant assemblies of cells will even return to their original tissue organizations. This ability, called self-assembly, can be seen at maximum effect when our own bodies have been injured and cells near the site of the injury assemble to repair the wound.

I became interested in what molecules enable animal cells to adhere to each other and how cells recognize partners, and began to study these questions in my research. Up until the 1970s, many excellent scientists had also been interested in these problems and various theories had been proposed and fiercely debated, but the questions remained unresolved. I decided that the problem of cell adhesion was too big to be tackled in one
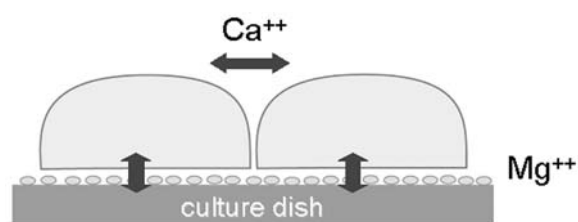
Fig. 1. Differential requirement of divalent cations for cell-cell and cell-substrate adhesion.

piece, and resolved to break it down and try to solve each individual process and mechanism instead of trying to solve the whole thing at once.

There are actually two types of cell adhesion. In one, cells adhere to other cells, while in the other, cells adhere to something other than a cell. The latter of the two, in which cells bind to matter that fills the spaces between cells, called the extracellular matrix, corresponds to the mechanism by which cells adhere to a glass or plastic dish when they are being proliferated in culture. Essentially, the two forms of adhesion are cell-cell adhesion and cell-extracellular matrix adhesion. I began to think that the two different type of adhesion might rely on different mechanisms from differences in "divalent cation dependency." Body fluids contain large amounts of divalent calcium and magnesium ions. I observed that cell-extracellular matrix adhesion depended on magnesium, while calcium was important to cell-cell adhesion (Fig. 1).[1),2)] From this, it was relatively simple to hypothesize that two different mechanisms were at work. At that point, my research focused only on cell-cell adhesion, but it was later shown that the phenomenon involving magnesium was a result of integrin activity.

Next, the mechanisms of cell-cell adhesion were even further subdivided into those that required calcium (calcium-dependent) and those that did not (calcium-independent) (Fig. 2).[3)] I was certain that if we looked at each of these types of mechanisms separately, we'd be able to get to the true nature of cell-cell adhesion. When we searched for the molecules functioning in these mechanisms, we found that in both cases, proteins on the surface membranes of cells were involved. If either of the mechanisms functioned, cells would adhere and assemble together. But there was a fundamental difference between the two. It appeared that the cell's physiologic activity was essential to the function of the molecule working in the calcium-dependent

process.[3)] For example, it completely failed at low temperatures. The calcium-independent mechanism, however, functioned regardless of the cell's physiologic activity. It seemed to be a simple molecular reaction. Comparing the two, I decided that the calcium-dependent mechanism must be more important and decided to study that more deeply.

The research did not always go smoothly, but after much trial and error, I finally succeeded in identifying the protein involved in the calcium-dependent mechanism, and named it cadherin.[4),5)] The cadherin molecule is what's known as a "membrane protein." This protein passes through the cell membrane, extending its amino terminal into the extracellular space, where it binds to a cadherin on the surface of another cell.[6)] The result is that the cells adhere to each other. Many experiments proved that cadherins are essential to cell-cell adhesion. For example, if the function of cadherins is blocked by antibodies, cell aggregates break up more readily (Fig. 3), and the structures of previously highly-ordered tissues degrade (Fig. 4).[7)-9)] The same effect is seen when the cadherin gene is deleted.[10)] On the other hand, if cadherin cDNA is expressed in cadherin-deficient cells, these cells became adhesive to each other (Fig. 5).

Interestingly, there are multiple types of cadherins, each of which works in different types of cells (Fig. 6).[6)] For example, the type known as E-cadherin functions in a kind of cell called an epithelial cell, while N-cadherins work in neurons and heart cells, and VE-cadherins are used by blood vessel cells. What's more, each type of cadherin binds most strongly with other cadherins of the same type (homophilic interaction).[11),12)] Due to these properties of cadherins, cells of similar types are able to selectively adhere to each other (Fig. 7). I believe that these findings have helped answer, at least partially, the question of how cells are able to identify their correct partners, even when different types of cells have been mixed together.

A look at the distribution of cadherins in the body reveals something interesting. Cells that adhere to each other always have the same cadherins; a principle that applies even beyond the borders of organs.[13)] For example, the skin and the lining of the esophageal epithelium and the mucosal linings of the stomach and small and large intestines can be seen to comprise a single continuous layer of cells, and all of these adhere to each other by E-cadherins. Endothelial cells in blood vessels also form a continuous lining of the entire vascular system, and all use VE-cadherins in their adhesion. Importantly, epithelial cells expressing E-cadherins,
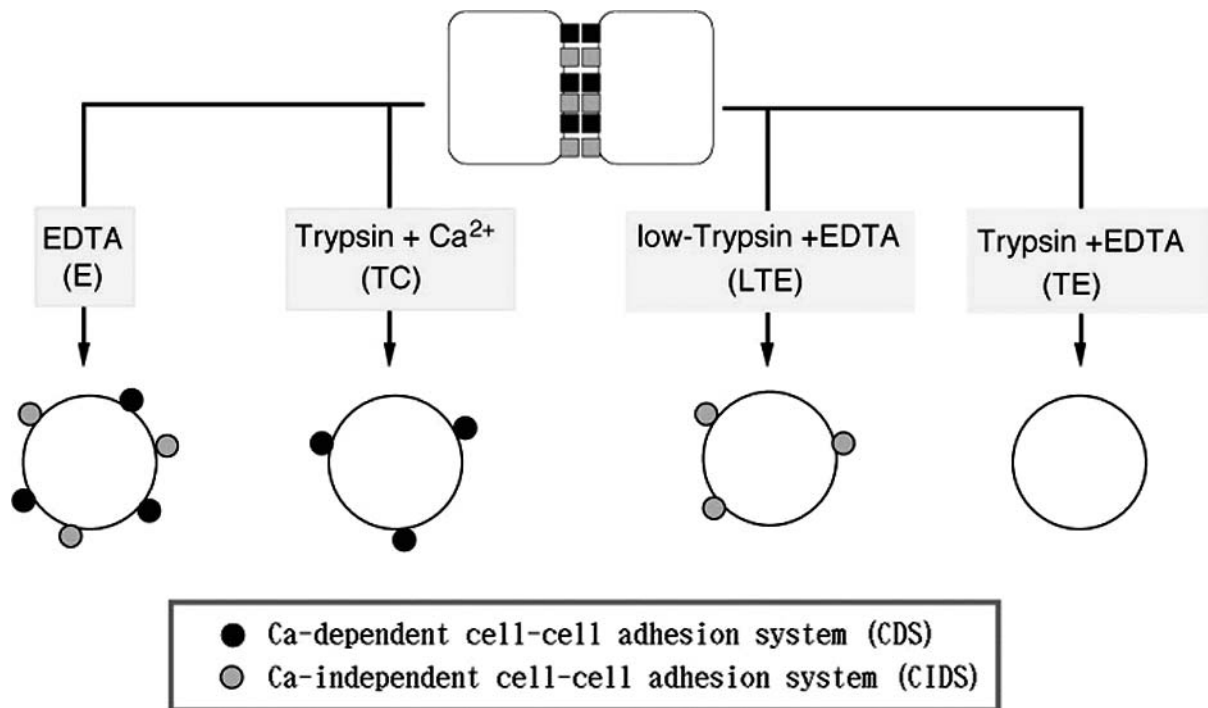
Fig. 2. Presence of two distinct cell-cell adhesion systems, Ca^{2+}-dependent and -independent ones. These two are different in their sensitivities to trypsin and Ca^{2+}; and one can prepare cells with only one of these systems or neither of them.
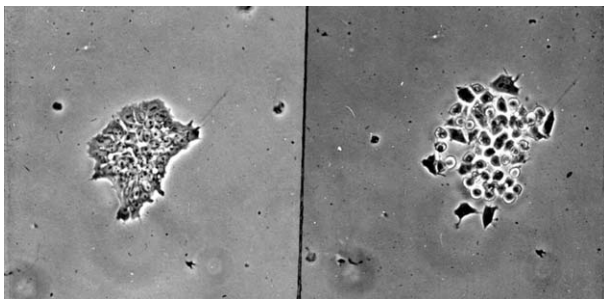

Fig. 3. Cadherin-blocking antibodies can disperse cells. A colony of teratocarcinoma cells (left) were incubated with an anti-E-cadherin monoclonal antibody for several hours (right).

endothelial cells expressing VE-cadherins and the group of cell types expressing N-cadherins, do not come into contact with or adhere to each other, with few exceptions. (Even in cases where these cells border each other closely, they are separated by the basement membrane or extracellular matrix.) This suggests that the specificity of cadherin-based cell-cell adhesion may be involved in the establishment of the continuity and independence of tissues in the body. We now know that there are about 20 different types of cadherins in any

vertebrate species; their distribution patterns are complex and individual cells frequently express more than one type of cadherin. This means that the role of the expression of different cadherins in the body may not be quite so straightforward or simple in reality, but cadherins seem to be nonetheless important to the development of the animal body.

All cadherins have similar structures (Fig. 6). The region that extends outside of the cell is divided into five units, and calcium ions bind to the junctions between these units.[14] Observed under an electron microscope, the cadherin molecule appears to be cylindrical, but a transformation occurs in the absence of calcium. It seems that calcium ions stabilize the cadherin's three-dimensional structure. It also appears that cadherins form dimers and that these bind with dimers on the surface of another cell, causing the cells to adhere. In this situation, cadherins of the same type have the strongest affinity for each other.

As research progressed, we began to resolve the mystery of why calcium-dependent adhesion, which is to say adhesion based on cadherin activity, was dependent on cellular physiologic activity. The intracellular region of the cadherin molecule binds to proteins called
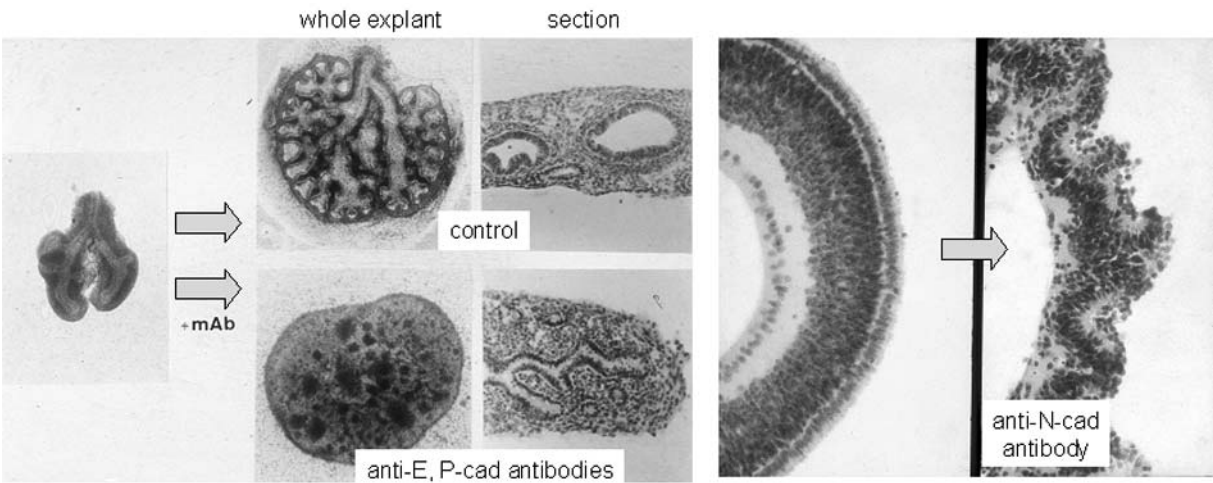
Fig. 4. Cadherin-blocking antibodies disrupt tissue organization. Left, lung primordia were cultured in the absence (control) or presence of antibodies to E- and P-cadherin expressed by these tissues. Right, embryonic neural retinas were incubated with anti-N-cadherin antibodies.
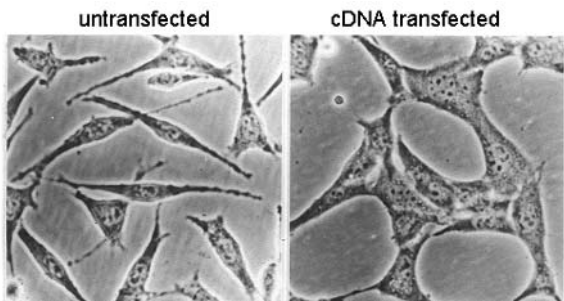

Fig. 5. Cadherin cDNA transfection induces cell-cell contacts. L cells do not have any cadherins, displaying a disperse morphology. Transfection of them with E-cadherin cDNA results in their close associations.
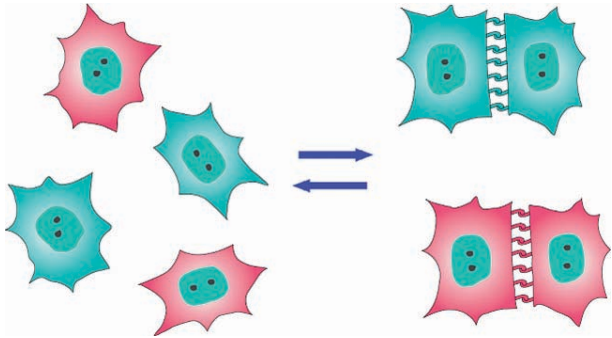

Fig. 7. Each cadherin molecule preferentially binds to the same type of cadherin by homophilic interactions.
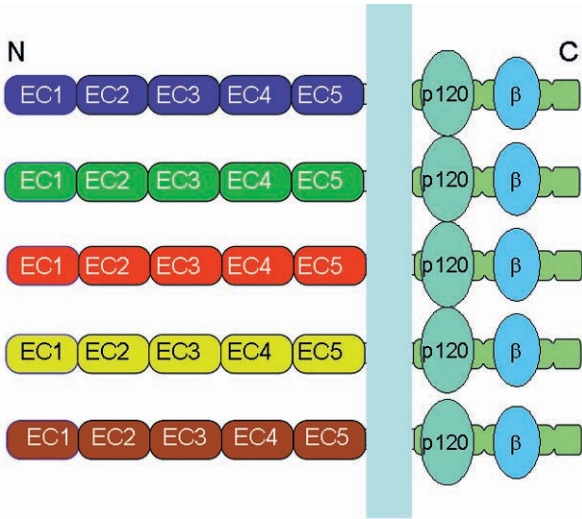

Fig. 6. Members of the classic cadherin family. They share a similar domain organization, binding to p120- and β-catenin at the conserved cytoplasmic domain. Each cadherin is named, such as E-cadherin and N-cadherin.

catenins, which are also able to bind to contractile proteins such as actin. If catenin is lost from a cell, the effect is a weakening of cell adhesion (Fig. 8),[15)-17)] almost as if the cell lacked cadherins. It seems that cadherin function in some way depends on these contractile proteins, which system relies on biological energy, meaning that it is not unusual for cell adhesion to be dependent on physiologic energy as well. Cell adhesion should not be thought of as a simple sticking-together; in fact, it's not inappropriate to call it a "living" phenomenon. Cells use machinery that we can refer to as the adhesion apparatus to achieve different objectives, sometimes binding stably to other cells, other times widening the gaps between cells, and in extreme cases, even detaching altogether. These kinds of problems remain the subject of intensive research, particularly in the contexts of cancer invasion
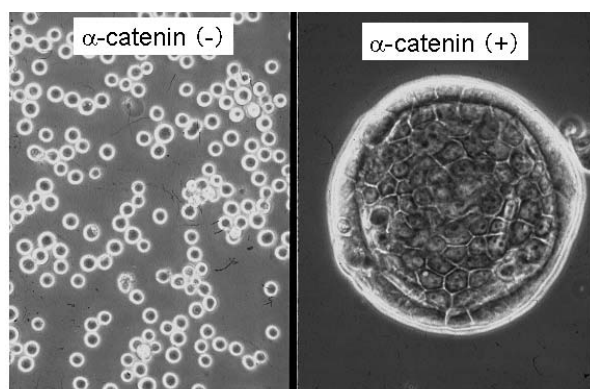
Fig. 8. Catenins are required for the adhesive function of cadherins. α-Catenin-deficient PC12 cells are disperse (left), but transfection of them with α-catenin cDNA induces their epithelioid organization (right).
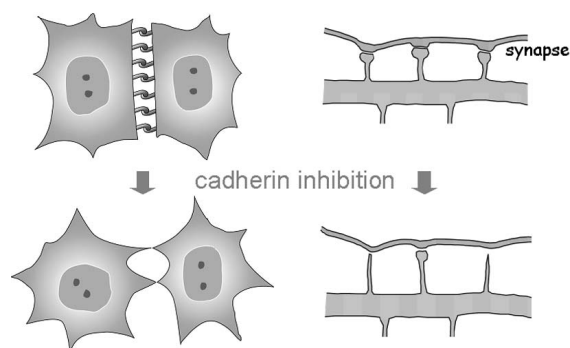

Fig. 9. Cadherins are required for synaptic junction formation. Without cadherin activities, synaptic contacts become unstable.
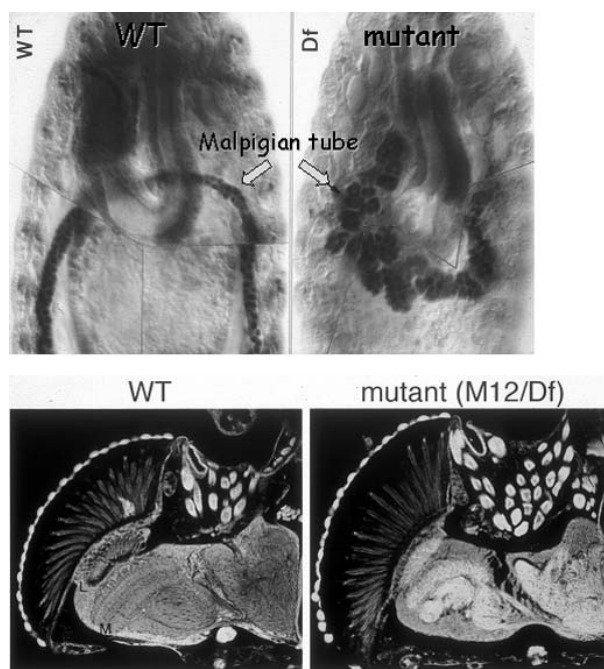

Fig. 10. Cadherin mutations in Drosophila cause disruption of tissue architecture. Upper, a DE-cadherin mutant showing disorganized Malpigian tubes. Lower, a DN-cadherin mutant with disorganized intra-brain structures.

and metastasis. This is of interest because if the cadherin machinery is disturbed in cancer cells, it may weaken adhesion with other cells and make it easier for these cells to invade.[18] And in fact, may abnormalities in cadherins have been observed in cancer cells.[19],[20] Continued research in this field will be of particular importance.

Recently, we have also found that the function of cadherins is important in neuronal synapses as well. Synapses are points of contact between neurons that allow for the transduction of neural signals, and are one form of cell-cell junction. Cadherins also accumulate here.[21] Excitatory synapses, which transduce neural excitatory states, form at the heads of small projections called spines found on dendrites, and if a spine's cadherin function is interfered with, the spine's shape changes and abnormalities in synapse formation occur (Fig. 9).[22] As

described above, cadherin activity is supported by catenins, and if a catenin called αN-catenin is removed from a cell, the structure of the spines that form synapses become extremely unstable.[23],[24] Furthermore, mice that lack a certain cadherin or αN-catenin show a range of aberrant neural activity. For example, mice lacking cadherin-11 seem to experience lower than normal anxiety.[25] Now that we know that cadherins function at these specialized forms of neuronal cell junction known as synapses, it will be important to consider how cadherins and catenins might work as regulatory factors in normal neural activity. Such studies may help to identify causes of a range of neurological disorders.

Cadherins have been discovered in invertebrates as well, and it seems likely that they are present in all multicellular animals.[26],[27] Cadherin mutations in flies cause various defects in cell adhesion similar to those seen in vertebrates (Fig. 10),[28]-[30] showing that these molecules are important for tissue formation in all species. However, the degree of that importance does vary between species; for example, in a nematode species, epithelial cadherin is not essential for cell
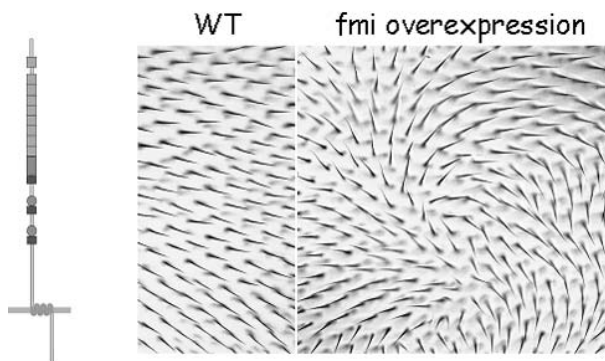
Fig. 11. Flamingo cadherin (*fmi*) is important for planar epithelial cell polarity. Left, molecular organization of the Flamingo protein. Right, wing hair polarity is disorganized by *fmi* over-expression.
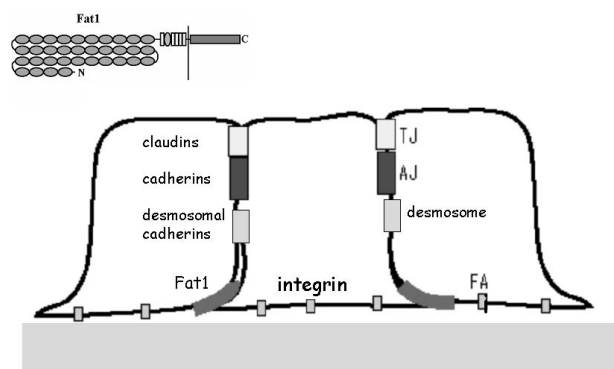

Fig. 12. Fat cadherin functions at the basal portion of epithelial cells, contrasted with classic cadherins that are more important for apical junction organization in this cell type.

adhesion but rather important for cell shape regulation and cytoskeletal organization. There appears to be some diversity in cell-cell adhesion mechanisms between taxa.

We also now know that there are many molecules that resemble the cadherins. Each of these has a slightly different structure than the cadherin structure described above, and the group is categorized collectively as the cadherin superfamily. The biological roles of these molecules remain unclear, but they retain the cadherin's homophilic binding, and it seems that their roles have become diversified and specialized. In this group, the molecules Flamingo and Celsr are seven-pass transmembrane proteins that interestingly seem to function in determining epithelial cell polarity (Fig. 11) and the control of dendrite formation.[31),32] Another molecule, Fat1, is the largest member of the cadherin superfamily and functions both in cell-cell adhesion in the basal region (Fig. 12) and as a regulator of actin remodeling.[33] Cadherin-23 is a component of the structural links between hair cells in the inner ear, which are necessary for hearing, and the loss of this cadherin results in deafness. The cadherin superfamily shows great functional diversity, and it is exciting to think about what future research will reveal. Cadherin superfamily genes represent a much higher proportion of the total number of genes in vertebrates than in invertebrates. This seems to indicate that a variety of cadherins and related molecules are necessary for the development of the complex bodies of higher-order animals.

I have been discussing the importance of cadherins in cell-cell adhesion. Here I should stress that the cell-cell adhesion phenomena are complex, and cells connect with each other by coordinating the activity of other mechanisms and other types of adhesion molecules as well. For example, in epithelial cells, molecules known as claudin and occludin are essential components of tight intercellular junctions. Without these, epithelial cells are unable to bind together into a tightly sealed layer of cells specific to epithelium. The localization of cadherin to the adhesion zone is also promoted by an immunoglobulin-like molecule called nectin. And, as I mentioned previously, molecules such as the catenins and actin support cadherin activity in the cell's interior. Thus, for complete understanding of cell adhesion mechanisms, it is clear that we need more works. Cells that have been dissociated move about without direction, but once they contact other cells, the adhesion apparatus begins to function, making it safe to say that cells become controlled by all the mechanisms underlying multicellular processes. Many mysteries remain unresolved, but by untangling the inner workings of this control system, we may one day gain a clearer understanding of the evolution from unicellular to multicellular life.

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Profile

Dr. Masatoshi Takeichi is director of the RIKEN Center for Developmental Biology (CDB) in Kobe, Japan as well as director of the Cell Adhesion and Tissue Patterning research group at the same institute. He completed the B. Sc. and M. S. programs in biology at Nagoya University before receiving a doctorate in biophysics from Kyoto University in 1973. After attaining his Ph.D., Dr. Takeichi took a research fellowship at the Carnegie Institute Department of Embryology under Dr. Richard Pagano. He then returned to Kyoto University, attaining a full professorship in the Department of Biophysics (1986-1999), before becoming professor in the Department of Cell and Developmental Biology in the Graduate School of Biostudies at the same university. He assumed his current positions at the CDB in 2000. Dr. Takeichi is best known for his discovery of cadherins, which are fundamental in the mechanisms of cell-cell adhesion. He was the first to recognize that intercellular adhesion involves two distinct mechanisms – calcium-dependent and calcium-independent – and to identify the molecular bases for each. He named the molecule responsible for calcium-dependent adhesion 'cadherin,' and went on to identify a group of related molecules, now known as the cadherin family. These molecules are differentially expressed according to tissue type and developmental stage, and function by allowing cells with compatible cadherins to recognize and bind to each other. Dr. Takeichi's studies of the molecular mechanisms involved in these processes led him to identify the α-catenins as a critical family of proteins bound to the cadherin cytoplasmic domain. His recent work focuses on the role of cadherins in synapse and neural network formation, as well as cadherin/catenin-mediated controls of morphogenetic cell behavior. Through these achievements, Dr. Takeichi received a number of awards and honors, including Asahi Award (1994), Uehara Prize (1996), Japan Academy Prize (1996), Ross Harrison Prize (2001), Keio Medical Science Prize (2001), Person of Cultural Merits (2004), and Japan Prize (2005). He is also a member or honorary member of the Japan Academy, American Academy of Arts and Sciences, the American Association of Anatomists, and European Academy of Sciences.