How cells assemble: A fundamental process in the formation of the body

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The world of living things can be broadly divided into single-celled and multicellular organisms. The former group includes bacteria, yeast, and protozoa, while the latter consists of nearly every life form visible to the naked eye. Unicellular organisms are individual cells that do not need to associate with other cells in order to live. In contrast, the body of a multicellular organism contains multiple cells, sometimes huge numbers of them. Each of these cells works only as a building block within some greater bodily structure and cannot function as an independent living entity. The different types of cells that make up a multicellular organism each have specific roles to play, which they can realize only by assembling into a larger functional unit.

A number of features distinguish multicellular organisms from single-celled ones. One of the most fundamental of these is the ability of the cells in a multicellular organism to adhere to each other. Although both plants and animals can be referred to as multicellular organisms, these two kingdoms differ greatly in terms of structure, and I will limit my talk today to a discussion of multicellular animals (or metazoans). Metazoan cells are able to adhere to one another. Tissues made up of such cells can be dissociated into individual living cells by various methods, and, when cultured under appropriate conditions, the cells spontaneously adhere to each other and reorganize into multicellular structures. What is perhaps even more surprising is that such cells demonstrate the ability to recognize specific adhesion partners. For example, when a tissue containing cartilage cells and epithelial cells is dissociated and the cells randomly mixed, cells of both types recognize and adhere preferentially to their compatible partners - cartilage to cartilage and epithelium to epithelium - enabling the reconstitution of a multicellular assembly by isolated individual cells. This shows that animal cells have an innate capacity for organizing into complex, tissue-specific structures. The importance of this ability is seen most clearly in wound healing, in which cells in the vicinity of the wound area associate with each other to reconstitute the damaged tissue.

Early in my career, I became interested in identifying molecules involved in cell-cell adhesion and, in particular, working out the means by which cells are able to recognize specific adhesion partners, questions that I continue to explore today. By the 1970s, many outstanding researchers had shown interest in these same questions, proposing a range of hypotheses and engaging in heated debates, but none had been able to solve the mystery of cell-cell adhesion. It struck me that the process must involve a set of complex mechanisms and that the best approach to solving the puzzle might be to break it down into its component parts and attack each question individually, rather than to try to arrive at a single universal explanation.

Cell adhesion takes two main forms: the adhesion of cells with other cells, and adhesion between cells and non-cellular material. In the latter process, cells adhere to a complex substrate known as the extracellular matrix, which fills the spaces between cells. A similar type of cell adhesion is also seen when cells proliferate on a glass or plastic culture dish, using its surface as a kind of scaffold. We refer to the two forms of cell adhesion as "cell-cell" and "cell-matrix" adhesion. I found that these two types of cell adhesion differ from each other in their dependency on divalent cations, and it occurred to me that both might be controlled by

different mechanisms. Calcium and magnesium are the two most important divalent ions present in body fluids, and I noted that each seemed to be necessary for a different form of adhesion to take place, with cell-matrix adhesion requiring magnesium ions and cell-cell adhesion dependent on calcium. My subsequent research has focused mainly on cell-cell adhesion, but it is now clear from others' work that magnesium-dependent cell-matrix adhesion is mediated by integrins.

I next found that cell-cell adhesion can itself be categorized into calcium-dependent and calcium-independent processes. I was sure that by studying these mechanisms separately, I would be able to develop a better understanding of the underlying principles. I began by searching for molecules playing central roles in the two processes and found that, in each case, cell-cell adhesion involved proteins present on the cell surface and that the function of either mechanism was sufficient to produce adhesion between cells. There is, however, a fundamental difference between the two mechanisms in that cellular activity seems to be necessary for calcium-dependent adhesion. At low temperatures, for example, the calcium dependent mechanism does not function at all, while the calcium-independent mechanism works even in the absence of cellular activity, suggesting that calcium-independent cell-cell adhesion is purely a molecular reaction. This led me to think that the calcium-dependent mechanism would be of greater significance in cellular functions and I decided to study the phenomenon in more detail.

Cadherins are transmembrane, cell-surface proteins that mediate cell-cell adhesion in a calcium-dependent manner. These molecules extend through the cell surface membrane, and the binding of extracellular domains of cadherin molecules present on neighboring cells results in cell-cell adhesion. Experiments have shown that cadherins are essential for cell-cell adhesion and, interestingly, that various types of cadherins exist, each of which functions in specific types of cells. For example, a form called E-cadherin is expressed and functions in epithelial cells, while another form, N-cadherin, is found in neural cells. Cells that express cadherins of a certain type adhere only to cells presenting the same type of cadherins. This may help to explain the phenomenon of selective adhesion (adhesion only to cells of a specific type), which has been observed in mixed populations of cells. Since the discovery of this selectivity, dozens of varieties of cadherins and related molecules have been identified. Many different types of cadherins seem to be required for the morphogenesis of complex body structures, and while cadherins were first discovered in vertebrates, they are currently thought to be present in all metazoan organisms.

Subsequent research has made it clear why the calcium-dependent mechanism of cadherin-mediated cell-cell adhesion requires cellular activity. The intracellular domain of the cadherin molecule binds to other proteins, called catenins, which themselves interact with contractile proteins, such as actin. These contractile proteins seem to be important to cadherin function, and since their activity requires biological energy sources, it is little wonder that cell-cell adhesion itself is dependent on the physiological activity of cells. It can be seen from this that cell adhesion is neither static nor a simple gluing together of cells, but is a vital, dynamic process. Cells utilize cell-cell adhesion machinery in different ways, as dictated by

specific functional demands. At times a group of cells might form tight and stable associations, while in a different setting the same cells might form looser junctions or, in extreme cases, detach from each other completely. Ongoing research into these mechanisms suggests that this cell adhesion machinery may be important to understanding the behavior of metastatic cancer cells, as indicated by the finding that metastasis tends to accelerate when aberrancies in cadherin function disrupt intercellular adhesion. A number of abnormalities in cadherin function have already been identified in cancer cells, and further investigations into the role of cadherins in cancer are strongly warranted.

It has also become clear that cadherins play an important role in the regulation of the functions of the specialized form of cell-cell junction known as synapses, which are the central points of communication in neural networks. Multiple defects in neuronal function have been demonstrated in experimental models of cadherin loss-of-function. Afflictions of the nervous system remain one of the great unresolved medical problems confronting mankind, and it is my hope that my work will make a contribution to determining pathological factors underlying neurological and psychiatric disorders.

From integrin-binding RGD peptides to vascular homing peptides

Dr. Erkki Ruoslahti

This Japan Prize recognizes the discovery of the RGD cell attachment sequence and of the cell surface receptors that recognize this sequence in extracellular matrix proteins. The origins of the RGD story go back to when I was a postdoctoral fellow at Caltech 1968-1970. Some of the Caltech researchers postulated the existence of a zipcode-like recognition system that would guide cell positioning during development and in the maintenance of tissue architecture. I was already at that time interested in cancer, and it seemed to me that if a recognition system guiding cell movements and positioning really existed, there would have to be something wrong with it in cancer because cancer cells do not follow positional rules. I decided that this is what I would work on having established my own laboratory.

Together with Dr. Antti Vaheri, who later on became Professor and Chairman of the Department of Virology at the University of Helsinki, we designed experiments to isolate cell surface proteins that might mediate cell recognition. These experiments resulted in the discovery of a protein that was present on the surface of normal fibroblasts, but not on retrovirally-transformed fibroblasts. We also found early on that the protein was present in normal plasma. Together with Dr. Deane Mosher, who went to work with Vaheri, we later named the protein fibronectin. It turned out that ours is not the only claim to the discovery of fibronectin, several other laboratories could make a that claim as the identity of fibronectin became clearer.

We spent the next few years characterizing the properties of fibronectin in normal and malignant cells, and, in 1977, Dr. Eva Engvall and I found that fibronectin binds to denatured collagen (gelatin), a discovery that made it possible to isolate essentially unlimited quantities of fibronectin from plasma. Armed with this ability, we set out to study the functionally active domains of fibronectin, soon focusing on its cell attachment domain. This work really picked up in speed when a talented postdoctoral fellow, Michael Pierschbacher, joined the laboratory. Using a monoclonal antibody he made, we were able to isolate a small fragment of fibronectin that promoted cell attachment and that upon sequencing turned out to contain 108 amino acids. We next tested synthetic peptides that covered this sequence, and by following the activity and making the peptides shorter and shorter, ended up with an active tetrapeptide. We also showed that fourth (C-terminal) amino acid could be varied, making the key sequence a tripeptide-arginine-glycine-aspartic acid, or RGD. This peptide has since been shown to be a key recognition sequence for cell attachment in a broad range of species ranging from Drosophila to human.

We proposed that fibrinogen and collagens could function as RGD-dependent cell attachment proteins and that viruses might use fibronectin mimicry to bind to mammalian cells. We also suggested that RGD peptides might be useful in blocking adhesion-dependent biological and disease processes such as platelet aggregation, and invasion and metastasis of malignant cells. These predictions turned out to be accurate.

At the time, we discovered the RGD sequence, the cellular receptors that mediate attachment to fibronectin and other adhesion proteins had not been identified. Having the RGD peptides from the cell attachment site of fibronectin at hand, Robert Pytela, an Austrian

postdoctoral fellow in my laboratory, succeeded in isolating two RGD-binding receptors, a fibronectin receptor and a vitronectin receptor. These receptors became founding members among a family of receptors now known as integrins.

The fibronectin and vitronectin receptors recognized different protein ligands, but in each case, the recognition was based on the RGD sequence. I found this extremely exiting, it was exactly what I had set out to look for in 1970: a cell surface recognition system that would resemble immune recognition. It took 15 years, but the mission was accomplished and we were able to put together the story in a well-cited review published in *Science* (Ruoslahti and Pierschbacher, 1987).

RGD and the RGD paradigm have generated drugs that are used to treat diseases. The availability of the various RGD-directed integrins allowed us to show that RGD peptides could be designed to be selective for individual RGD-directed integrins (Pierschbacher and Ruoslahti, 1987). Other integrins are similarly inhibited by peptides with sequences related to RGD (an aspartic acid residue, in particular, is shared by the various integrin ligands). Indeed, pharmaceutical companies have developed RGD-type compounds that are far more potent than our original RGD peptides and are highly specific for a single integrin. A modified RGD peptide and an RGD peptidomimetic that inhibit platelet aggregation are on the market for prevention of restenosis after angioplasty. Compounds that inhibit the $\alpha 4\beta 1$ integrin are in use for suppression of inflammatory reactions, and inhibitors of the $\alpha v\beta 3$ integrin show promise as anti-angiogenic agents. Other applications are likely to emerge.

I have continued working on the same paradigm that led me to fibronectin and RGD: how do cells find their appropriate place in the body and what goes wrong with malignant cells that metastasize? We were using peptide libraries displayed on phage to identify RGD-type peptides for individual integrins, and it occurred to me that we could use phage libraries in live mice to detect vascular specificities that might be involved in tumor metastasis. Indeed, we have shown that every tissue we have analyzed puts a specific signature on its vasculature and have identified a tumor molecule, metadherin, that binds to lung vasculature and is involved in metastasis. We have also used the *in vivo* phage screening method to isolate peptides that specifically home to tumors, and have shown that coupling of drugs and drug-like molecules to homing peptides can increase the potency of the drug and decrease its side effects. The RGD sequence and integrins have already had an impact on clinical medicine. I hope that these new peptides and their vascular receptors will also prove useful in the treatment of disease.