Cell Adhesion Molecules as Morphoregulators

A Short Survey

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I am extremely honored to have been chosen for the Karger Prize and I would like to express my sincere thanks to the Council of the Heinz Karger Foundation for their decision and particularly to Dr. Bernard Sordat for his very kind and perhaps too idealistic view of my scientific career. I am very much indebted to Karger Publishers for this generous prize which will be helpful for my laboratory.

The International Symposium on Cell to Cell Interactions, sponsored by Karger Publishers for their 100th anniversary, is one of the most important contributions to the medically oriented sciences as well as to fostering privileged exchange between fundamental scientists. It is certainly a recognition of a recently exploding field in the life sciences. The list of speakers is particularly impressive and it really demonstrates that cell interactions are of central importance in many disciplines. I am particularly pleased to see how these different disciplines are adopting very similar concepts.

Perhaps it is not a coincidence that nearly a hundred years ago, the field of cell recognition was born through the pioneering work of distinguished experimental embryologists. Wilson, in 1907, showed that mechanically dissociated cells from Red Sea sponges could reaggregate to form an organism resembling the original sponge. In 1925, Galtsoff did a crucial experiment by mixing cells of two different species of sponges with red and brown-yellow colors, respectively. These cells started to aggregate randomly, but soon the mosaic structure transformed into two clusters of cells of the two different colors. The same paradigm was applied to the case of amphibians. In the 1930s and 40s, Holtfreter did a series of very elegant experiments with amphibians to show that cells derived from different embryonic anlage would finally sort out in a pattern that resembled the organization of embryonic tissues. These results led Holtfreter to propose
that cells express selective affinities; however, it was impossible to elaborate this concept further at the time. By the end of the 1960s, major efforts had been made to provide a molecular explanation for this very important biological property. Nevertheless, our understanding still remained very limited. In addition there were two prevailing hypotheses which were not very encouraging. One school of thought proposed that the specificity of recognition and adhesion was mediated by a kind of credit card code on the cell surface, requiring perhaps more than thousands of billions of molecules. The other school considered that adhesion was mediated by noncovalent, electrostatic, hydrophobic and Van der Waals forces. In this latter case the most stable organization would correspond to a thermodynamical equilibrium; the free energy involved would reach a minimum when cells had maximalized their contacts.

I arrived in New York City on a cold night in early January 1975, just in time for a party organized by Prof. Gerald Edelman. Quite late during that night, Prof. Edelman told me that my project on lymphocyte mitogenesis was no longer a priority in his laboratory and that I should start thinking about working on cell aggregation. In fact I did not have the opportunity to think too much about it because the next morning chicken eggs were waiting for me. You all know that French people have a good reputation for cooking, but I realized that I should not make an omelette. The only available data at that time was from Prof. Moscona’s laboratory showing that the chicken embryonic neural retina produced aggregation factors inducing larger-sized neural cell aggregates when maintained in suspension culture for several days. I spent several months trying to repeat these experiments without any success. In fact, I discovered that several irrelevant macromolecules could agglutinate these neural retina cells. We had therefore to change our strategy. First, we established a short-term quantitative aggregation assay. In this assay dissociated retina cells were allowed to recover from trypsin treatment by culture in suspension under nonreaggregating conditions. This assay allowed us to discover that retina cells could bind to other neural cells from the central nervous system, strongly suggesting that the binding mechanism was common between different neural cell types. We then developed another method based on the use of antibodies capable of inhibiting aggregation. These antibodies were obtained by immunizing rabbits with whole chicken embryonic retina cells. The next step was to find a source of surface antigen that would neutralize the inhibitory activity of the antibodies. Fractionation of these antigens led to the identification of a polypeptide chain of 140 kD which we named

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CAM (cell adhesion molecule) and later N-CAM (neural cell adhesion molecule). Newly generated antibodies to this unique molecule were in turn capable of blocking neural cell adhesion.

Many other experiments showed that this cell surface protein was directly involved in cell adhesion. Therefore by the end of 1976, we had discovered the first cell surface adhesion molecule in vertebrates. At first, these results did not receive much support; N-CAM was considered to be irrelevant or just an artifact. Perhaps I was fortunate enough to be quite naive in this field to maintain faith throughout the project which turned out to be extremely hard, considering the technical limitations at the time.

Nevertheless, after a few years, other laboratories became interested in this molecule. Finally in 1985, the cDNA encoding N-CAM was cloned and sequenced - N-CAM polypeptides were found to contain five immunoglobulin-like domains in their extracellular portion. This was certainly a surprise for Prof. Edelman who was awarded the Nobel Prize in 1972 for his decisive contribution to the establishment of the primary structure of immunoglobulins. In the last few years, N-CAM has become the prototype of an increasing number of adhesion molecules belonging to the immunoglobulin superfamily. These molecules are expressed in the nervous system, in hematopoietic cells and in cancer cells. One interesting finding is that fasciclin 2 in Drosophila is a homologue of N-CAM. Undoubtedly, immunoglobulin-like domains have appeared in evolution previous to the immunoglobulins themselves which acquired much more sophisticated features for recognition.

Calcium-dependent cell surface adhesion molecules form another major class of CAMs. L-CAM, discovered by Edelman and colleagues in chicken liver, is homologous to a molecule named uvomorulin, characterized in Prof. Jacob’s laboratory in Paris, which is identical to mouse E-cadherin, discovered by Prof. Takeichi in Kyoto. An increasing number of adhesion molecules of this family, named cadherins by Takeichi, are found in different vertebrates.

In 1983, together with Prof. Edelman and his colleagues, we were able to show that N-CAM and L-CAM are expressed together very early during development on the same blastoderm cells. Later, these primary CAMs are expressed in different tissues deriving from the three primary germ layers in a defined pattern with reiterated sequences of expression associated with morphogenetic events. These findings led us to postulate that CAMs are morphoregulators rather than merely simple markers of differentiation.

Many experiments have been designed to show that CAMs contribute
directly to morphogenesis permitting the transient or permanent association of cells into functional structures.

A third major class of cell surface adhesion molecules are the integrins. These receptors recognize different subsets of extracellular matrix adhesion molecules and these interactive pairs are called SAMs (substrate adhesion molecules). The specificity of interaction has also been defined for the 17 integrins which have already been discovered. It appears that these receptors are fairly versatile for their ability to recognize several extracellular matrix adhesive proteins, therefore one can easily guess the number of possible interactive combinations.

Together with our close colleague, Kenneth Yamada at the National Institutes of Health in Bethesda, we have been able to show that β1 integrins are essential in regulating embryonic cell motility. Fibronectin, one of the interactive pairs in the extracellular matrix contains multiple adhesive domains inducing different phenotypes. Recent results obtained by many laboratories, including ours, further emphasize the key role played by CAMs and SAMs in morphogenesis.

Indeed more adhesion and recognition molecules are going to appear. A new family, named lectin CAM, and its receptors, called addressins, may be critical in the trafficking of leukocytes. Perhaps by now we have to deal with 50-100 adhesion molecules. However, this number is very limited compared to the one proposed before to ensure the correct assembly of cells. Control mechanisms are now being uncovered; the preliminary findings suggest that they may provide an alternate mechanism to compensate for the insufficient number of recognition molecules. In addition these new modes of regulation will permit enough plasticity in time and space for histogenesis to proceed.

Although the field has made major advances over the last few years, we are still facing the same challenge: among many other issues, we have to understand how these complex regulatory mechanisms control the adhesive status of cells throughout development, repair, and in pathological states. We certainly would like to know how the adhesive signal is transduced and how cells in contact signal each other to provide positional information.

In our laboratory we are attempting to address some of these questions, focusing on the very important morphogenetic process of epithelial-mesenchymal cell interconversion and on the overwhelming problem of carcinoma invasion and metastasis. We were able to find carcinoma cells which can fully and reversibly convert into a fibroblastic morphology and

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acquire strong motility and invasive properties in the presence of collagens and/or growth factors. Our findings are suggestive of a direct connection between growth factors (which are also morphogens), adhesion, motility, and invasion and metastasis. It is hoped that in the next decade we shall be able to define to what extent adhesion molecules serve as mechanochemical links between genes and morphology. In addition we should be able to determine the role of these molecules in disease. One important issue is to try to identify master genes in morphogenesis, possibly related to the transcription factors involved in the commitment and early differentiation of muscle. Indeed CAMs and SAMs participate in a complex series of events in cooperation with other signaling molecules. It is noteworthy that proteolytic systems, which are finely regulated, are also of major importance in development and disease. To conclude it is perhaps not irrelevant to say that we shall keep our enthusiasm for fundamental research since we still have a long road ahead of us.

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