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VIEWPOINT

The Saga of Adhesion Molecules

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The field of cell adhesion has reached a stage of formidable complexity. Cell adhesion seems to contribute to all morphogenetic events and is also linked to many other key processes including the control of cell division, cell differentiation, and apoptosis. Much effort is devoted to understanding the role of cell adhesion in normal organisms and in many different pathological states. Although not covered in this issue, cell adhesion also operates in bacteria and in unicellular eukaryotes and plays an essential role during the interaction between pathogen and host. Cell adhesion can be mediated by numerous surface glycoproteins and glycoconjugates that can be grouped into families of structurally related molecules.

Among the best studied families are the cell adhesion molecules (CAM) involved in cell-cell adhesion that belong to the immunoglobulin superfamily (CAM-Ig) and to the cadherin superfamily. Many studies have contributed to the identification and functions of a large number of the members of the CAM-Ig class. For example, an increasing number of these CAM-Ig glycoproteins have been characterized in developing nervous systems. Functional studies in vitro and in vivo have stressed their importance in the migration of neuroblasts, axon guidance, fasciculation, and synapse formation. In this volume, Baldwin et al. discuss this important issue. Several studies support the notion that the neural cell adhesion molecules N-CAM are involved in the initial contact between a growth cone and its target cell and in the mechanisms that promote neurite outgrowth. These authors have made the intriguing observation that either the homophilic binding of these molecules or the activation of FGF receptors by FGF-2 lead independently to a very similar transduction pathway involving the formation of the second messenger arachidonic acid and voltage-dependent calcium channels through the activation of PLCγ. The data support the notion that initial homophilic binding of a CAM in *trans* will activate FGF-receptor through an interaction in *cis* involving a CAM homology domain located on FGF-receptor.

Work is in progress in different laboratories to ablate systematically the different members of the CAM-Ig superfamily. Results obtained with N-CAM clearly demonstrate their importance in the development of the nervous system although the defects produced by each ablation are more limited than had been expected on the basis of the widespread expression of these molecules during early embryogenesis. A phenomenon often encountered as a result of knocking out a gene, tentatively explained by compensatory mechanisms provided by other closely related members of the same family.

Major breakthroughs have been made recently in describing the structure and function of cadherins. Perhaps most important has been the establishment of the three dimensional structure of the N-cadherin and E-cadherin-amino terminal repeat [Overduin et al., 1995; Shapiro et al., 1995]. Surprisingly, the folding of the polypeptide chain closely resembles the Ig domain with seven anti-parallel β strands. A model for cell adhesion has been proposed in which dimers of cadherins interacting in *cis* through one face of their five Ig domain are able to form a ribbon when establishing homophilic contact with dimers in *trans*. In this issue, Aberle et al. present a comprehensive proposal to describe how the cytoplasmic domain of cadherin is connected to the cytoskeleton through the catenins. Much work has been done recently to localize the sites of interaction both between β-catenin or plakoglobin and the cytoplasmic domain of cadherin and between β-catenin and α-catenin. A major emphasis is put upon β catenin as a key

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regulator of both cell adhesion and the Wint signal transduction pathway. When β-catenin is ablated, the mesoderm does not form. This result is consistent with the possibility that β-catenin is involved in signaling prior to gastrulation. Mareel et al. address important aspects of the regulation of adhesion mediated by cadherins that can be modulated during the transition from in situ carcinoma to the invasive phase.

The recent observation that the inactivation of E-cadherin leads to embryonic death before the implantation stage stress the importance of this cadherin in the formation of the first epithelia. The prevailing view is that E-cadherin can also be considered as a tumor suppressor and that components of this adhesive system might be manipulated to restore adhesion in invasive carcinoma. Indeed, there is hope of restoring functional adhesion in malignant tumors because the genes for E-cadherin and catenin are rarely the target of mutations; rather their transcription may be arrested. There are even cases where E-cadherin is expressed on the cell surface but is blocked by steric hindrance because of the presence of large proteoglycans. Several agents such as tamoxifen seem to be helpful in restoring the function of E-cadherin.

In his essay, Suzuki analyzes the large evolutionary diversity of the cadherins. He proposes that all cadherins are derived from an ancestral precursor of the protocadherins, an extensive, newly discovered family of surface glycoproteins closely resembling classical cadherins but differing in some parts of the extracellular domain and in their highly divergent cytoplasmic domains. He emphasized that type II classical cadherins and desmosomal cadherins have very weak binding activity when they are expressed in cells. This observation leads to the question of what other functions cadherins may have. As already stated in the two other reviews on cadherins, the possibility that β-catenin may be a critical element in signal transduction mediated by wingless/Wnt-1 is discussed.

Another major adhesion system is involved in cell-extracellular matrix interactions. Over the last years, many adhesive glycoproteins of the extracellular matrix (ECM) have been identified and sequenced and their biological activity studied in vitro and in vivo. The best characterized family of these receptors is that of the integrins. These non-covalent heterodimers comprise more than 20 members, most of which recognizing a small subset of ECM components. Some integrins, however, are able to bind to surface receptors of the CAM-Ig superfamily thereby mediating cell-cell adhesion.

Recent work has largely focused on the mechanism of signal transduction associated with the integrins which are often found in inactivated states such as those of the platelet integrin α IIb β 3 and the leukocyte integrins sharing the β 2 chain. In addition, the co-stimulation of other surface receptors including growth factor receptors considerably enhances the mitogenic or differentiation promoting activities of the integrins. In their review, Lafrenie and Yamada describe these signal transductions in a variety of cell types. These events involve the immediate early genes IL-1b, c-fos, and myc. The coincident up-regulation of cyclin A, which allows normal cells in suspension to enter the S phase, clearly emphasizes the importance of adhesion for cell growth. Many different cell types either differentiate or maintain their differentiated phenotype when grown on ECM components, and ECM response elements are now being characterized in the promoters of genes specifically associated with programs of differentiation. The early events of signal transduction occur during the assembly of structural and enzymatic components at specific cellular sites in contact with the substratum. These events can be studied in model systems where the integrin receptors are occupied either or with or without subsequent clustering. Directly or indirectly, the stimulation of integrins can lead to the activation of most of the already known pathways for cellular stimulation such as those involving tyrosine kinases and adaptor proteins belonging to the ras-raf pathway and other pathways particularly those involving small G proteins such as *rho* and *rac*. Given the complexity of the system it is not surprising that the interplay between the structural proteins responsible for the connection between the cytoskeleton and the enzymatic machinery in the control of cell behavior and gene transcription is far from being understood.

Perhaps the most rapid advances in our understanding of how integrins can be activated has come from numerous studies made with hemopoietic cells. In their review, Stewart and Hogg describe important structural features of integrins focusing on the I domain which is found in integrin *a* chains expressed by leukocytes. A molecular model of this domain has now been completed, and this structure may lead to an

understanding of how the divalent cation binding site can interact with its counter-receptor. Much work has been devoted to examining the role of divalent cations, ligands, and antibodies as potential inducers of a population of high affinity integrin receptors. Integrins can also be activated from the cytoplasmic side. Sequences in the cytoplasmic domain of the α and β subunits that maintain a low affinity by default have now been characterized. The notion that the avidity of these domains may be increased by local clustering of integrin receptors rather than by increasing the affinity of individual receptors is receiving experimental support and now has to be examined under physiological conditions in vivo.

Many ECM adhesion molecules are large modular glycoproteins constructed from distinct functional domains. They have the capacity to assemble into very complex supramolecular structures that are still poorly understood. Major efforts have been made to analyze these large extracellular glycoproteins. One class of these molecules is that of the laminin isoforms. Engvall and Wewer provide an updated summary of the structure and function of the different laminin domains. A mutation in domain VI of laminin α 2 chain results in muscular dystrophy. Major progress has been made in identifying binding sites on the laminins for different ECM proteins and surface receptors. The sequences involved in the binding of integrins and dystroglycan, however, have not yet been identified. Studies on mice that are lacking one of the different laminin genes are underway. The first results are encouraging since they have produced phenotypes that resemble already known genetic diseases. These results demonstrate that although the different laminin isoforms share structural similarities, one isoform may not be able to compensate for another one.

Tenascin, a large, complex extracellular glycoprotein with multiple functional domains is the first adhesion molecule found to contain anti-adhesive sites. The adhesive and anti-adhesive domains have now been mapped to distinct regions of the molecule. In her review, Crossin describes the numerous receptors that have been identified for tenascin. Integrin receptors can recognize distinct domains in this molecule and through these receptors it induces different responses according to the cell type. The gene products associated with the response to a specific domain of tenascin are now being identified. Tenascin is also remarkable in its pattern of expression during development and in diseases. Recent studies have permitted the identification of binding sites for homeoproteins on the tenascin gene promoter.

Classical studies in cell adhesion and morphogenesis using marine sponges were initiated early in this century, but it is only recently that the glycosaminoglycan complex involved in the adhesion of sponge cells has been characterized at the molecular level. The high binding strength of the long carbohydrate chains on these glycosaminoglycans is thought to result from the fact that they form multimers and that each protomer contains multiple repeats of short oligosaccharide motifs. The proper spacing of the interactive sites favors homophilic adhesion, and a strong synergistic effect is obtained when all these multiple weak binding sites are simultaneously engaged. As reviewed by Spillmann and Burger, carbohydrate-carbohydrate interactions may play a major role in development and in adult tissues of many different species. Appropriate physical methods are being developed to measure these interactions.

Much attention has been paid in the past to hyaluronic acid, a large heteropolysaccharide that has been shown to contribute to the maintainance of large extracellular spaces. More recently, studies have focused on the identification of its surface receptors and the mechanisms of signal transduction mediated by those receptors. In the report by Entwistle et al., three distinct receptors are described. One of these, CD44, is widely expressed in tissues, but specific isoforms have a more restricted distribution. Some of these isoforms may be important in promoting tumor metastasis. It is unclear, however, whether these isoforms bind in different ways to hyaluronic acid. The second receptor discussed, the RHAMM receptor, is often upregulated in normal cell migrations and in transformed cells. Intracellular RHAMM receptors have now been identified suggesting novel functions in signalling mechanisms involving hyaluronic acid. This glycosaminoglycan can also be internalized at different sites in the cytoplasm and possibly also in the nucleus. The third, I-CAM-1, which recognizes the β 2 integrins, can also bind to hyaluronic acid.

A high degree of complexity in adhesion receptors is attained with syndecans. As described in the review of Couchman and Woods, each of the four different syndecans recognize multiple ECM

component but can also bind and store growth factors and protease inhibitors and may be involved in viral entry. The binding sites are carried by the glycosaminoglycans and not by the core protein of syndecans; these oligosaccharides present a great variability, and this variability offers a novel mechanism for the regulation of the specificity and the strength of adhesion. As with other adhesion molecules, syndecans may act at the cell surface as oligomers, a state also favoring a more stable connection to the cytoskeleton.

The importance of protein-carbohydrate interactions in cell adhesion has been particularly well demonstrated in interactions between leukocytes and blood vessels. In his review, Vestweber describes three well-known selectins and their ligands, which include glycolipids, glycoproteins, and sialomucins. Interestingly, the carbohydrate ligands, which often contain the sialyl Lewis X motif, can be carried by a protein core the expression of which is not restricted only to cells interacting with cells expressing selectin. Therefore, specific posttranslational modifications of the selectin counter receptors must occur in these cells. One newly described specific receptor for E-selectin, the ESL-1 receptor, is closely related to a novel type of cysteine-rich fibroblast growth factor receptor. One of the critical questions is whether or not this strict specificity is ensured by a second binding site carried by the protein in addition to a common carbohydrate binding site for both E and P selectins.

These reviews provide an overview of recent developments in the field of cell adhesion. As stated by Edelman [1993], cell adhesion has reached a golden age. Many families have now been defined and are being studied extensively. Most of these families have been conserved throughout evolution. For instance, the immunoglobulin-like fold is often found in adhesive domains. There are many common features that apply to both cell-cell and cell-matrix interactions. The mechano-chemical transducing machinery associated with distinct adhesion systems share some common principles whether these molecules are connected to the actin microfilaments or to the keratin-intermediate filaments. Much effort will be devoted in the next few years in this area to define the hierarchy of interaction on the cytoplasmic side, the crosstalk between the different adhesive systems, and the signalling mechanisms that control the adhesive behavior of cells both at the epigenetic and genetic levels. How a cell expressing multiple adhesion systems can integrate the signals from the many different adhesive contacts and respond in a unique manner to its environment remains a mystery. It is certainly a very challenging question that will not be answered easily even though we have now access to sophisticated methods in biophysics such as atomic force microscopy, laser tweezers, new X-ray crystallographic techniques, and NMR spectroscopy as well as genetic approaches such as conditional knock-out of genes and numerous other techniques of molecular and cell biology. Perhaps one of the most difficult problems to solve is that of understanding the many combinatorial events that are operating in concert at the cell surface and within the cell to control adhesion.

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