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The origins of the molecular era of adhesion research

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Abstract

Recognition of the importance of cell adhesion grew steadily during the twentieth century as it promised answers to fundamental questions in diverse fields that included cell biology, developmental biology, tumorigenesis, immunology and neurobiology. However, the route towards a better understanding of its molecular basis was long and difficult, with many false starts. Major progress was made in the late 1970s to late 1980s with the identification of the major families of adhesion molecules, including integrins and cadherins. This in turn set the stage for the explosive growth in adhesion research over the past 25 years.

25 years ago, a seminal review article¹ and a highly influential Gordon Research Conference heralded the birth of the integrin family of adhesion receptors. Around this time, other major families of adhesion molecules were also emerging. The discoveries of these adhesion molecules were the culmination of many decades of effort and, while highly anticipated, the new molecular era in adhesion research that followed greatly exceeded most expectations. It brought unexpected new insights not only into basic cell biology but also into a multitude of fundamental developmental, homeostatic and pathological phenomena. Exciting discoveries continue to this day with no end in sight. We are now beginning to understand how adhesions hold tissues together and generate signals that regulate cell proliferation, survival, migration and gene expression. Through these fundamental cellular processes, adhesions mediate tissue formation and regeneration and target immune responses through a highly orchestrated interplay among integrins, selectins and the immunoglobulin superfamily of cell adhesion molecules (IgSF-CAMs). They also mediate homeostatic mechanisms such as bone turnover and platelet aggregation. Altered adhesion through receptors such as integrins and cadherins drive tumour formation, invasion and metastasis among other pathological processes. Finally, adhesion figures prominently in efforts to form new tissues either through regeneration or stem cell transplantation, and the role of adhesion in disease may provide routes to the design of diagnostic, prognostic and therapeutic strategies.

This Timeline article outlines some key developments that motivated and moved adhesion research from initial observations to the molecular level. It is written from a personal perspective, which I hope reflects that of many others who entered this field early and led it into the molecular era. I begin with studies that demonstrated the importance of adhesion for holding tissues and organisms together and implicated adhesion in tissue morphogenesis. Next, I discuss the tissue culture studies that demonstrated the importance of adhesion in cell growth and sociology and that revealed altered adhesion in cancer cells. Finally, I review the

development of approaches used to identify and purify the first cell–cell and cell–substrate adhesion molecules.

Setting the stage

The importance of adhesion emerged around the turn of the twentieth century from multiple sources. Early tissue culture studies, initiated by Harrison in 1907, showed convincingly that most tissues were not a syncytium but rather were comprised of individual cells². Thus, the presence of tissues required cell adhesion. In 1922, Lewis, an embryologist and early practitioner of tissue culture, captured this well:

“We have long known, for example, of the adhesive quality of white blood-cells for glass. The importance of this adhesive quality in the various body cells, however, has been largely ignored. Yet most of our tissues and organs are made up of cells that are merely stuck together. Were these various types of cells to lose their stickiness for one another and for the supporting white fibres, reticuli, etc., our bodies would at once disintegrate and flow off onto the ground in a mixed stream of ectodermal, muscle, mesenchyme, endothelial, liver, pancreatic and many other cell types.”³

Studies of tissue dissociation and reassociation in invertebrates also pointed to the importance of adhesion. In 1900, Herbst showed that sea urchin blastomeres fall apart when calcium is removed and then reassociate in normal seawater to form embryo-like structures⁴. Soon after, Wilson observed that sponges could similarly be dissociated into single cells and then reassociate to form a small differentiated sponge⁵ (FIG. 1). Furthermore, when dissociated cells from genera of different coloured sponges were mixed, they initially formed mixed genera aggregates but then moved apart, or sorted, to form genus-specific aggregates. These experiments also revealed cell–cell and cell–substratum (matrix) adhesion and the importance of cell migration^{5–7}.

Holtfreter moved adhesion research ahead enormously by showing that embryonic tissues could form through adhesion-based self-organization^{8,9}. He first observed that dissociated embryonic amphibian tissues can reassociate to recapitulate their initial morphology. He also found that gastrula layers can be dissociated into single cells by high pH and then reassociate and organize to recapitulate the early embryonic layers when the pH is reduced. Thus, the notion emerged that tissues can self-organize by sorting out individual cells through differential adhesion with other cells. Moscona generalized this to other organisms by showing that tissues can be dissociated using trypsin, and this led to many dissociation and reaggregation studies using different vertebrate species and tissues^{10,11}. From these findings, the hypothesis arose and soon dominated models of morphogenesis that tissues are formed through the migration and sorting of cells with different adhesive properties.

Tyler, Weiss and Sperry began to place this concept on a molecular footing. Tyler and Weiss proposed that the adhesion between cells arises from an antibody–antigen-like interaction between cell surface molecules^{12,13}. Consistent with this, Spiegel showed that antibodies against the surfaces of both sponges and amphibian embryos inhibited the reaggregation of dissociated cells in a cell type- and species-dependent manner, respectively^{14,15}. On the basis of studies in the 1940s of neural regeneration, Sperry proposed the chemoaffinity (also known as chemospecificity) hypothesis^{16,17}. He suggested that neural connections arise in part through the recognition of “identification tags” and that each axon becomes selectively attached to specific neurons depending on “specific chemical affinities”¹⁷.

Interest in adhesion also came from studies of disease. Analyses of squamous cell carcinomas showed decreased cell–cell adhesion forces and implicated adhesion in invasion

and metastasis¹⁸. Studies in the 1950s and 1960s using fibroblast cell lines and their viral transformants as a model for tumorigenic transformation also observed changes at the cell surface and in cell adhesion during tumorigenesis¹⁹. Whereas normal cells required anchorage to a substratum for growth, some viral transformants did not²⁰. Furthermore, transformed fibroblasts often did not show the density-dependent inhibition of growth seen in untransformed fibroblasts²¹. They also showed an abnormal morphology, migrated faster and did not exhibit ‘contact inhibition’ of movement (that is, the local inhibition of movement observed when normal cells contact each other and stops them from ‘walking’ over each other); this loss of contact inhibition was implicated in tumour invasion^{22,23}. The idea that the cell surface changed during transformation was also supported by changes in both the agglutination response to plant lectins and the carbohydrates that were present on the cell surface^{24,25}.

All of this fuelled much speculation about the molecular basis of adhesion and the need to identify the implicated adhesion molecules. At the same time, this early work provided novel quantitative techniques (such as Moscona’s dissociation–reaggregation system and the quantification of cell attachment to dishes) and powerful strategies for the identification of these putative adhesion molecules. The early focus was on agglutinins and cell surface carbohydrates, either acting directly via a carbohydrate ‘code’ or through a lectin-like binding protein or glycosyltransferases²⁶. But, despite a diversity of approaches and strategies to identify molecules that stimulate or inhibit adhesion, progress was slow.

Cell–cell adhesion molecules

CsA and NCAM

The Tyler–Weiss hypothesis and Spiegel’s observations led Gerisch to raise antisera against adhesion-competent (aggregating) *Dictyostelium discoideum* cells, which acquire this phenotype during their transition from a growth phase to a multicellular slug. Using the monovalent antibodies (Fab fragments) that were not adsorbed by aggregation-incompetent (growth phase) cells, an antigen, contact site A (csA) protein, was purified and shown to mediate adhesion^{27,28}.

Edelman and co-workers adapted and refined this strategy (FIG. 2) to isolate a neural cell adhesion molecule (NCAM)²⁹. Fab’ fragments from antibodies raised against embryonic retinal cells inhibited the reaggregation of the embryonic tissues. Proteins in culture supernatants were fractionated biochemically and assayed for their ability to block the adhesion-inhibiting activity of the antibodies. The active protein fraction was then used to prepare a new antiserum, and Fab fragments from this were used to immunopurify the 140 kDa NCAM cell surface adhesion molecule. The transient, localized expression of NCAM during central nervous system (CNS) development suggested that it has a major role in this process³⁰. NCAM is now viewed as one of many adhesion molecules that mediate nervous system development.

Cadherins

The earliest studies of cell separation and reaggregation pointed to a calcium-dependent cell adhesion mechanism. Using a lung cell line from Chinese hamsters, Takeichi showed that cell–cell interactions were mediated by calcium-dependent and calcium-independent systems. Both mechanisms were inhibited by trypsin. However, if calcium was present, trypsin no longer inhibited the calcium-dependent system, and calcium also inhibited the release of certain cell surface components^{31,32}. This observation led to an experimental strategy, based on the approach used by Gerisch and Edelman, and allowed the identification of a 134 kDa cell surface component and a 34 kDa proteolytic fragment that were responsible for cell–cell adhesion in F9 teratocarcinoma cells³³. The calcium-dependent

adhesion properties of this molecule led to the name 'cadherin'. Introduction of its cDNA into L cells induced calcium-mediated adhesion³⁴.

Edelman had previously identified a 124 kDa protein that mediated adhesion of chicken hepatocytes³⁵. Others, using related cell adhesion systems, such as mouse embryo compaction^{36,37} and a mammary carcinoma³⁸, also identified 34 kDa, 80 kDa and 130 kDa molecules that were implicated in cell–cell adhesion. We now know that these molecules are all related and form the cadherin protein family³⁹. Each of these cadherins was characterized as having different tissue distribution and adhesion specificities. These proteins include: N-cadherin, which is expressed in the brain and mesenchyme; E-cadherin, which is expressed in epithelia; P-cadherin, which is expressed in the placenta; and VE-cadherin, which is expressed in the endothelium. Cadherin-mediated adhesions, like those mediated by NCAM, are homophilic interactions.

The discovery of the protocadherins expanded the cadherins into a very large superfamily of adhesion molecules^{40,41}. Within this family, the classic cadherins regulate cytoskeletal associations via catenins, which were discovered in co-immunoprecipitation studies with E-cadherin⁴². They link to actin filaments via α -catenin–vinculin⁴³ and regulate WNT signalling through β -catenin⁴⁴. Classic cadherins mediate cell rearrangements that result in the formation of tissues, for example tubule and ductal morphogenesis in epithelia and gastrulation during development⁴⁵, and they regulate barrier function in epithelia and endothelia⁴⁵. It is now clear that some cadherins are sensitive to local forces such as shear stress in endothelial cells⁴⁶. N-cadherin has a tissue-specific role in synapse formation and brain morphogenesis, and there is also a plethora of protocadherins, which may contribute to synaptic specificity in the brain⁴⁷. Finally, cadherins are strongly implicated in tumorigenesis, partly through effects on epithelial-to-mesenchymal transition⁴⁸.

Subsequently, other cell–cell adhesion families emerged, including: lectin cell adhesion molecules (LEC-CAMs; for example selectins), which mediate lymphocyte homing and leukocyte–endothelial interactions^{49,50}; and IgSF-CAMs, which mediate various cell–cell interactions⁵¹. IgSF-CAMs include intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein (VCAM), which are also involved in leukocyte–endothelial interactions, as well as NCAM.

Cell–matrix adhesion molecules

Meanwhile, a search was also ongoing for cell–substrate adhesion molecules, but the path to their discovery was different. As cell culture became more widely used in the 1960s and early 1970s, particularly to study cancer and cell differentiation, the importance of cell–substrate adhesion became increasingly evident. Whereas untransformed cells would grow only when adhered to a substratum, this limiting property was lost in many cancer cells. Furthermore, specific matrix components, and even their physical nature, were implicated in the *in vitro* differentiation of muscle cells⁵², hepatocytes⁵³ and mammary epithelial cells^{54,55}. Cell culture also opened the door to new quantitative microscopy methods to study the morphologies and migration of individual cells, the subcellular localization of specific molecules that they expressed and the changes in these properties that were observed in transformed cells. These methods greatly facilitated the search for the molecular mediators of cell–matrix adhesion.

Identification of focal adhesions

Two major discoveries moved this quest ahead dramatically. First, interference reflection microscopy revealed that adhesions are discrete focal regions of close contact with the tissue culture substratum and thereby provided an easy localization-based assay for cell–substrate

adhesion molecules^{56,57}. The combined use of interference reflection and electron microscopy showed that these adhesions were sites where actin filament bundles terminate^{57,58}. Second, the molecular era was accelerated by the discovery that molecules such as actin could be removed from SDS–PAGE gels and used to raise antibodies for immunolocalization⁵⁹. Using this method, antibodies were generated against chicken gizzard smooth muscle α -actinin and shown to distribute along actin filament bundles and in the (adhesion) plaques observed at the ends of the bundles⁶⁰. Vinculin⁶¹ and talin⁶² were then identified as new molecules that localize specifically in adhesion plaques (FIG. 3). These studies founded the molecular era of cell–matrix adhesion research, although all of the molecules identified with this approach resided on the cytoplasmic face of the membrane.

Fibronectin is a matrix adhesion molecule

Independently, a search for cell surface molecules that changed during cell transformation revealed the secreted matrix component fibronectin as a large external transformation-sensitive protein (LETS)⁶³. When fibronectin was added back to cultures of virally transformed cells, these cells reverted to a normal phenotype: cell–substrate adhesion increased; the cells assumed a flatter morphology; and contact inhibition of movement was restored⁶⁴. Fibronectin resided in cell–substrate adhesions and also co-aligned with actin filament bundles⁶⁵. This showed that cells were adhering to a specific matrix protein rather than to the tissue culture substratum. It also pointed to the presence of transmembrane molecules that link actin filaments and fibronectin and mediate cell adhesion to fibronectin.

Integrins

The identification of cell–matrix adhesion receptors was achieved using both biochemical and immunological techniques. Affinity chromatography using matrix components such as fibronectin dominated the biochemical approach, whereas antibodies that inhibit adhesion formed the basis for the immunological approach. In retrospect, however, the low affinity of the receptor for its ligand, the large number of binding domains on extracellular matrix (ECM) components such as fibronectin, the presence of multiple related receptors and the heterodimeric nature of the receptors impeded progress. As a result, mixtures of proteins, often in the 140 kDa range⁶⁶, were purified rather than the single protein seen in purifications of cell–cell adhesion components.

It was the development of monoclonal antibodies that moved the field forward. With them it was possible to produce, *in vitro* individual cell lines secreting an antibody that recognized a single epitope⁶⁷. A monoclonal antibody that inhibits substratum adhesion could then be used for ‘one step’ affinity purification and immuno fluorescence (FIG. 2b). Two monoclonal antibodies were isolated that inhibited adhesion of embryonic skeletal muscle. They bound a complex in the 140 kDa range that localized along actin filament bundles and in adhesions and also dissociated embryo explants into single cells^{68–71}. The complex also bound to fibronectin and talin, which is an actin-associated cytoplasmic protein that binds vinculin⁷². cDNA cloning of one subunit (the β -subunit) revealed a transmembrane domain and thus fit the role of the transmembrane linker proposed previously^{72,73}. This subunit was named integrin to reflect its integral membrane protein nature and its role in linking the cytoskeleton and fibronectin. The name also presaged the integrative nature of integrins in cell signalling⁷³. The biochemical problem of assaying putative membrane receptors for binding to large multidomain matrix proteins was aided by the identification of a peptide sequence, Arg-Gly-Asp-Ser (RGDS), in fibronectin that mediates cell adhesion⁷⁴. This major discovery also led to an affinity approach for identifying a 140 kDa receptor complex for fibronectin⁷⁵ (FIG. 2c).

Membrane protein complexes with similar molecular weights were also emerging in other contexts. For example, a monoclonal antibody raised against T cells revealed a complex that inhibited T cell function by blocking cell–cell adhesion between the killer T cell and its target cell⁷⁶. The antibody precipitated a small set of heterodimeric lymphocyte receptors (leukocyte function-associated antigen 1 (LFA1), macrophage 1 antigen (MAC1), P150,95) that share a common subunit and are involved in adhesion-related processes⁷⁷, and a mutation in the common subunit leads to leukocyte adhesion deficiency^{78,79}. Another group of monoclonal antibodies (VLA-1-5) was raised that recognized antigens appearing very late after T cell activation, and the heterodimeric receptors that they recognized also shared a common subunit⁸⁰. In addition, a pair of cell surface proteins present on platelets were shown to be greatly reduced in patients with Glanzmann thrombasthenia^{81,82}, a platelet aggregation defect, and these proteins were identified as receptors for several RGD-containing molecules including fibrinogen and fibronectin^{83,84}. Finally, *Drosophila melanogaster* position-specific antigens, which showed varying expression throughout development, were also characterized as a heterodimeric complex of glycoproteins that shared a common subunit⁸⁵.

All these findings came together in 1986 to 1987. Several common properties, including molecular weight similarities, sequence homology by cDNA and partial peptide analyses and antibody cross-reactivity, revealed that these receptors were related heterodimeric complexes^{1,86}. Families were defined by common β -subunits, and the different specificities within a family were dictated by the α -subunit. In 1987, Hynes organized a key Gordon Research Conference (FIG. 4) and wrote a pivotal review that brought this family together and named it the integrins¹, the name assigned previously to the $\beta 1$ subunit that defined matrix adhesion receptors⁷³. The identification of this large family brought together people from various fields of research including immunology, cancer, vascular biology, cell biology and development. It created the integrin field as we now know it (BOX 1). The result was an enormous synergy; each integrin had its own story, and when conjoined with the others, research leapt ahead. At the same time, other adhesion families including cadherins, selectins (LEC-CAMs) and IgSF-CAMs were also emerging.

The large range of ECM ligands and cell surface receptors implied that integrins comprised a major adhesion system not only for cell–matrix adhesion but also for cell–cell adhesion. Moreover, knockout and mutant mouse studies began to reveal integrin functions that spanned from development to death in many cell types and organisms⁸⁷. It soon became clear that integrins not only hold cells together but also initiate and organize signalling networks that regulate proliferation, gene expression, migration and cell survival. In this respect, integrin adhesions function like growth factor receptors with which they synergize⁸⁸. The emergence of the specific signals that are generated from integrin signalling, the complexes and post-translational events that regulate integrins and the mechanisms by which integrins on resting platelets and leukocytes become activated were characterized in great detail and reviewed in a previous perspective article⁸⁹. The conformational changes in integrins that drive their activation and their regulation are now understood in exquisite detail⁹⁰.

These signalling networks established a molecular framework for understanding some of the phenomena, such as anchorage-dependent growth, that originally motivated the search for cell–matrix receptors. For some cellular processes such as migration and gene expression, the role of adhesion was unexpectedly complex. In migrating cells, for example, adhesions not only form the traction points by which cells move, but they also generate the transient, local signals that regulate compartmentalized processes that drive migration such as protrusion, contraction, front–back polarity and even adhesion⁹¹. The forces on adhesions, through a poorly understood signalling loop, in turn, regulate these signals. They also

regulate gene expression and explain the early observations that epithelial cells, for example, develop better on compliant substrates^{92,93}. The many downstream effects that integrin signalling can have and the complex nature of its regulation remain a challenge for understanding how adhesions affect different cellular processes.

A bright outlook

Breathtaking progress has been made over the past 25 years; however, much still remains to be learned, and some of the questions that originally motivated adhesion research have not been answered. For example, synaptic specificity and the mechanism of tissue morphogenesis and regeneration are complex and not understood. Although the role of adhesion in diseases such as cancer, thrombosis, cardiovascular disease and inflammation is being addressed, only a handful of therapeutics has emerged so far, and the potential of adhesion research for diagnostics, prognostics and therapeutics has not been fully exploited. Finally, the dominant role proposed for cell surface carbohydrates in adhesive specificity still remains unclear²⁶.

The cell biology of adhesion also faces major challenges. The mechanism by which physical forces and local pliability regulate adhesion and the signals they produce is not understood. Nonetheless, pliability is a crucial component of the cellular microenvironment and determines cell function, and this is a particularly pressing issue in tumour biology⁹⁴. The structure of the supramolecular assemblies that comprise adhesions and how these structures vary among different adhesions and in response to the local environment are also not known. Recent advances in super-resolution microscopy and multiscale structural analyses that combine nuclear magnetic resonance (NMR), X-ray crystallography, tomography and light microscopy are only just beginning to unravel these mysteries⁹⁵. Although optical methods, reagents and analytic tools are being developed that can localize, measure and correlate cellular activities, measure intra- and extracellular forces (biosensors) and perturb these activities using photoactivating and photoinhibiting reagents, the repertoire needs to be expanded and extended to cells *in vivo*. Finally, all that we have learned about adhesion will need to be translated to complex activities and diseases at the organismal level.

When I became interested in adhesion as a graduate student in the late 1960s, adhesion research had great promise for a young biophysicist looking for a career trajectory. However, I did not anticipate the extent to which it would exceed my already enormous expectations. I also did not foresee the embracing and open nature of the adhesion community. Watching this field mature from description to molecule to mechanism has been exhilarating, and all of us who have contributed to the field have had a wonderful ride.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The scope, brevity and nature of the perspective format preclude presenting all of the important contributions and citations; the author apologizes to his colleagues for these omissions. He thanks D. DeSimone, M. Schwartz, R. Hynes and B. Gumbiner for their suggestions. A.R.H. was supported by the US National Institutes of Health (NIH).

References

1. Hynes RO. Integrins: a family of cell surface receptors. *Cell*. 1987; 48:549–554. [PubMed: 3028640]

2. Harrison RG. Observations on the living developing nerve fiber. *Proceedings of the society for experimental biology and medicine*. 1907; 4:140–143.
3. Lewis WH. The adhesive quality of cells. *Anat. Record*. 1922; 23:387–392.
4. Herbst K. Über das Auseinandergehen von Furchungs- und Gewebezellen in Ca-freiem Medium. *Arch. Entwicklungsmech*. 1900; 9:424–463.
5. Wilson HV. On some phenomena of coalescence and regeneration in sponges. *J. Exp. Zool*. 1907; 5:245–258.
6. Galtsoff PS. The amoeboid movement of dissociated sponge cells. *Biol. Bull*. 1923; 57:153–161.
7. Galtsoff PS. Regeneration after dissociation (an experimental study on sponges). *J. Exp. Zool*. 1925; 42:183–221.
8. Holtfreter J. Gewebeaffinität, ein Mittel der embryonalen Formbildung. *Arch. Exp. Zellforsch. Gewebezucht*. 1939; 23:169–209.
9. Townes PL, Holtfreter J. Directed movements and selective adhesion of embryonic amphibian cells. *J. Exp. Zool*. 1955; 128:53–120.
10. Moscona A, Moscona H. The dissociation and aggregation of cells from organ rudiments of the early chick embryo. *J. Anat*. 1952; 86:287–301. [PubMed: 12980879]
11. Moscona A. Rotation-mediated histogenetic aggregation of dissociated cells. A quantifiable approach to cell interactions in vitro. *Exp. Cell Res*. 1961; 22:455–475. [PubMed: 13773012]
12. Tyler A. The auto-antibody concept of cell structure, growth and differentiation. *Growth*. 1947; 10:7–19.
13. Weiss P. The problem of specificity in growth and development. *Yale J. Biol. Med*. 1947; 19:235–278. [PubMed: 20284280]
14. Spiegel M. The role of specific surface antigens in cell adhesion. Part I. The reaggregation of sponge cells. *Biol. Bull*. 1954; 107:130–148.
15. Spiegel M. The role of specific surface antigens in cell adhesion. Part II. Studies on embryonic amphibian cells. *Biol. Bull*. 1954; 107:149–155.
16. Sperry RW. Optic nerve regeneration with return of vision in anurans. *J. Neurophysiol*. 1944; 7:57–69.
17. Sperry RW. Chemoaffinity in the orderly growth of nerve fiber patterns and connections. *Proc. Natl Acad. Sci. USA*. 1963; 50:703–710. [PubMed: 14077501]
18. Coman DR. Decreased mutual adhesiveness, a property of cells from squamous cell carcinomas. *Cancer Res*. 1944; 4:625–629.
19. Stoker MG. The Leeuwenhoek lecture, 1971. Tumour viruses and the sociology of fibroblasts. *Proc. R. Soc. Lond. B Biol. Sci*. 1972; 181:1–17. [PubMed: 4402333]
20. Stoker M, O'Neill C, Berryman S, Waxman V. Anchorage and growth regulation in normal and virus-transformed cells. *Int. J. Cancer*. 1968; 3:683–693. [PubMed: 5749478]
21. Stoker MG, Rubin H. Density dependent inhibition of cell growth in culture. *Nature*. 1967; 215:171–172. [PubMed: 6049107]
22. Abercrombie M. Contact inhibition and malignancy. *Nature*. 1979; 281:259–262. [PubMed: 551275]
23. Abercrombie M, Heaysman JE. Observations on the social behaviour of cells in tissue culture. I. Speed of movement of chick heart fibroblasts in relation to their mutual contacts. *Exp. Cell Res*. 1953; 5:111–131. [PubMed: 13083622]
24. Warren L, Fuhrer JP, Buck CA. Surface glycoproteins of cells before and after transformation by oncogenic viruses. *Fed. Proc*. 1973; 32:80–85. [PubMed: 4346326]
25. Burger MM. Surface changes in transformed cells detected by lectins. *Fed. Proc*. 1973; 32:91–101. [PubMed: 4346328]
26. Roseman S. Reflections on glycobiology. *J. Biol. Chem*. 2001; 276:41527–41542. [PubMed: 11553646]
27. Beug H, Katz FE, Stein A, Gerisch G. Quantitation of membrane sites in aggregating *Dictyostelium* cells by use of tritiated univalent antibody. *Proc. Natl Acad. Sci. USA*. 1973; 70:3150–3154. [PubMed: 4522296]

28. Muller K, Gerisch G. A specific glycoprotein as the target site of adhesion blocking Fab in aggregating *Dictyostelium* cells. *Nature*. 1978; 274:445–449. [PubMed: 566857]
29. Thiery JP, Brackenbury R, Rutishauser U, Edelman GM. Adhesion among neural cells of the chick embryo. II. Purification and characterization of a cell adhesion molecule from neural retina. *J. Biol. Chem.* 1977; 252:6841–6845. [PubMed: 893446]
30. Rutishauser U, Thiery JP, Brackenbury R, Edelman GM. Adhesion among neural cells of the chick embryo. III. Relationship of the surface molecule CAM to cell adhesion and the development of histotypic patterns. *J. Cell Biol.* 1978; 79:371–381. [PubMed: 569155]
31. Takeichi M. Functional correlation between cell adhesive properties and some cell surface proteins. *J. Cell Biol.* 1977; 75:464–474. [PubMed: 264120]
32. Takeichi M, Atsumi T, Yoshida C, Uno K, Okada TS. Selective adhesion of embryonal carcinoma cells and differentiated cells by Ca^{2+} -dependent sites. *Dev. Biol.* 1981; 87:340–350. [PubMed: 6793433]
33. Yoshida C, Takeichi M. Teratocarcinoma cell adhesion: identification of a cell-surface protein involved in calcium-dependent cell aggregation. *Cell*. 1982; 28:217–224. [PubMed: 7060128]
34. Nagafuchi A, Shirayoshi Y, Okazaki K, Yasuda K, Takeichi M. Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. *Nature*. 1987; 329:341–343. [PubMed: 3498123]
35. Bertolotti R, Rutishauser U, Edelman GM. A cell surface molecule involved in aggregation of embryonic liver cells. *Proc. Natl Acad. Sci. USA*. 1980; 77:4831–4835. [PubMed: 6933535]
36. Kemler R, Babinet C, Eisen H, Jacob F. Surface antigen in early differentiation. *Proc. Natl Acad. Sci. USA*. 1977; 74:4449–4452. [PubMed: 270688]
37. Hyafil F, Morello D, Babinet C, Jacob F. A cell surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. *Cell*. 1980; 21:927–934. [PubMed: 6159985]
38. Damsky CH, Richa J, Solter D, Knudsen K, Buck CA. Identification and purification of a cell surface glycoprotein mediating intercellular adhesion in embryonic and adult tissue. *Cell*. 1983; 34:455–466. [PubMed: 6352050]
39. Takeichi M. Cadherins: a molecular family important in selective cell–cell adhesion. *Annu. Rev. Biochem.* 1990; 59:237–252. [PubMed: 2197976]
40. Suzuki ST. Protocadherins and diversity of the cadherin superfamily. *J. Cell Sci.* 1996; 109:2609–2611. [PubMed: 8937978]
41. Yagi T, Takeichi M. Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. *Genes Dev.* 2000; 14:1169–1180. [PubMed: 10817752]
42. Ozawa M, Baribault H, Kemler R. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.* 1989; 8:1711–1717. [PubMed: 2788574]
43. Yonemura S. Cadherin–actin interactions at adherens junctions. *Curr. Opin. Cell Biol.* 2011; 23:515–522. [PubMed: 21807490]
44. Nelson WJ, Nusse R. Convergence of Wnt, β -catenin, and cadherin pathways. *Science*. 2004; 303:1483–1487. [PubMed: 15001769]
45. Gumbiner BM. Regulation of cadherin-mediated adhesion in morphogenesis. *Nature Rev. Mol. Cell Biol.* 2005; 6:622–634. [PubMed: 16025097]
46. Leckband DE, le Duc Q, Wang N, de Rooij J. Mechanotransduction at cadherin-mediated adhesions. *Curr. Opin. Cell Biol.* 2011; 23:523–530. [PubMed: 21890337]
47. Takeichi M. The cadherin superfamily in neuronal connections and interactions. *Nature Rev. Neurosci.* 2007; 8:11–20. [PubMed: 17133224]
48. Yang J, Weinberg RA. Epithelial–mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev. Cell*. 2008; 14:818–829. [PubMed: 18539112]
49. Lasky LA. Lectin cell adhesion molecules (LEC-CAMs): a new family of cell adhesion proteins involved with inflammation. *J. Cell Biochem.* 1991; 45:139–146. [PubMed: 1711527]
50. Rosen SD, Bertozzi CR. The selectins and their ligands. *Curr. Opin. Cell Biol.* 1994; 6:663–673. [PubMed: 7530461]

51. Wai Wong C, Dye DE, Coombe DR. The role of immunoglobulin superfamily cell adhesion molecules in cancer metastasis. *Int. J. Cell Biol.* 2012; 2012:340296. [PubMed: 22272201]
52. Hauschka SD, Konigsberg IR. The influence of collagen on the development of muscle clones. *Proc. Natl Acad. Sci. USA.* 1966; 55:119–126. [PubMed: 5220860]
53. Michalopoulos G, Pitot HC. Primary culture of parenchymal liver cells on collagen membranes. Morphological and biochemical observations. *Exp. Cell Res.* 1975; 94:70–78. [PubMed: 243]
54. Emerman JT, Enami J, Pitelka DR, Nandi S. Hormonal effects on intracellular and secreted casein in cultures of mouse mammary epithelial cells on floating collagen membranes. *Proc. Natl Acad. Sci. USA.* 1977; 74:4466–4470. [PubMed: 270692]
55. Lee EY, Parry G, Bissell MJ. Modulation of secreted proteins of mouse mammary epithelial cells by the collagenous substrata. *J. Cell Biol.* 1984; 98:146–155. [PubMed: 6707082]
56. Curtis AS. The mechanism of adhesion of cells to glass. A study by interference reflection microscopy. *J. Cell Biol.* 1964; 20:199–215. [PubMed: 14126869]
57. Izzard CS, Lochner LR. Cell-to-substrate contacts in living fibroblasts: an interference reflexion study with an evaluation of the technique. *J. Cell Sci.* 1976; 21:129–159. [PubMed: 932106]
58. Heath JP, Dunn GA. Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated interference-reflexion and high-voltage electron-microscope study. *J. Cell Sci.* 1978; 29:197–212. [PubMed: 564353]
59. Lazarides E, Weber K. Actin antibody: the specific visualization of actin filaments in non-muscle cells. *Proc. Natl Acad. Sci. USA.* 1974; 71:2268–2272. [PubMed: 4210210]
60. Lazarides E, Burridge K. α -actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. *Cell.* 1975; 6:289–298. [PubMed: 802682]
61. Geiger B. A 130K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells. *Cell.* 1979; 18:193–205. [PubMed: 574428]
62. Burridge K, Connell L. Talin: a cytoskeletal component concentrated in adhesion plaques and other sites of actin–membrane interaction. *Cell. Motil.* 1983; 3:405–417. [PubMed: 6319001]
63. Hynes RO. Alteration of cell-surface proteins by viral transformation and by proteolysis. *Proc. Natl Acad. Sci. USA.* 1973; 70:3170–3174. [PubMed: 4361679]
64. Yamada KM, Yamada SS, Pastan I. Cell surface protein partially restores morphology, adhesiveness, and contact inhibition of movement to transformed fibroblasts. *Proc. Natl Acad. Sci. USA.* 1976; 73:1217–1221. [PubMed: 177979]
65. Hynes RO, Destree AT. Relationships between fibronectin (LETS protein) and actin. *Cell.* 1978; 15:875–886. [PubMed: 365353]
66. Wylie DE, Damsky CH, Buck CA. Studies on the function of cell surface glycoproteins. I. Use of antisera to surface membranes in the identification of membrane components relevant to cell–substrate adhesion. *J. Cell Biol.* 1979; 80:385–402. [PubMed: 457748]
67. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature.* 1975; 256:495–497. [PubMed: 1172191]
68. Neff NT, et al. A monoclonal antibody detaches embryonic skeletal muscle from extracellular matrices. *J. Cell Biol.* 1982; 95:654–666. [PubMed: 6183279]
69. Greve JM, Gottlieb DI. Monoclonal antibodies which alter the morphology of cultured chick myogenic cells. *J. Cell Biochem.* 1982; 18:221–229. [PubMed: 7068780]
70. Damsky CH, Knudsen KA, Bradley D, Buck CA, Horwitz AF. Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. *J. Cell Biol.* 1985; 100:1528–1539. [PubMed: 3921554]
71. Chen WT, Greve JM, Gottlieb DI, Singer SJ. Immunocytochemical localization of 140 kD cell adhesion molecules in cultured chicken fibroblasts, and in chicken smooth muscle and intestinal epithelial tissues. *J. Histochem. Cytochem.* 1985; 33:576–586. [PubMed: 3889142]
72. Horwitz A, Duggan K, Buck C, Beckerle MC, Burridge K. Interaction of plasma membrane fibronectin receptor with talin — a transmembrane linkage. *Nature.* 1986; 320:531–533. [PubMed: 2938015]
73. Tamkun JW, et al. Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. *Cell.* 1986; 46:271–282. [PubMed: 3487386]

74. Pierschbacher MD, Ruoslahti E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature*. 1984; 309:30–33. [PubMed: 6325925]
75. Pytela R, Pierschbacher MD, Ruoslahti E. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell*. 1985; 40:191–198. [PubMed: 3155652]
76. Springer TA, et al. LFA-1 and Lyt-2,3, molecules associated with T lymphocyte-mediated killing; and Mac-1, an LFA-1 homologue associated with complement receptor function. *Immunol. Rev.* 1982; 68:171–195. [PubMed: 6184305]
77. Sanchez-Madrid F, Nagy JA, Robbins E, Simon P, Springer TA. A human leukocyte differentiation antigen family with distinct α -subunits and a common β -subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. Exp. Med.* 1983; 158:1785–1803. [PubMed: 6196430]
78. Springer TA, Thompson WS, Miller LJ, Schmalstieg FC, Anderson DC. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J. Exp. Med.* 1984; 160:1901–1918. [PubMed: 6096477]
79. Dana N, Todd RF 3rd, Pitt J, Springer TA, Arnaout MA. Deficiency of a surface membrane glycoprotein (Mo1) in man. *J. Clin. Invest.* 1984; 73:153–159. [PubMed: 6361068]
80. Hemler ME, Huang C, Schwarz L. The VLA protein family. Characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight β -subunit. *J. Biol. Chem.* 1987; 262:3300–3309. [PubMed: 3546305]
81. Nurden AT, Caen JP. An abnormal platelet glycoprotein pattern in three cases of Glanzmann's thrombasthenia. *Br. J. Haematol.* 1974; 28:253–260. [PubMed: 4473996]
82. Phillips DR, Agin PP. Platelet membrane defects in Glanzmann's thrombasthenia. Evidence for decreased amounts of two major glycoproteins. *J. Clin. Invest.* 1977; 60:535–545. [PubMed: 70433]
83. Pytela R, Pierschbacher MD, Ginsberg MH, Plow EF, Ruoslahti E. Platelet membrane glycoprotein IIb/IIIa: member of a family of Arg-Gly-Asp-specific adhesion receptors. *Science*. 1986; 231:1559–1562. [PubMed: 2420006]
84. Ginsberg MH, Forsyth J, Lightsey A, Chediak J, Plow EF. Reduced surface expression and binding of fibronectin by thrombin-stimulated thrombasthenic platelets. *J. Clin. Invest.* 1983; 71:619–624. [PubMed: 6826726]
85. Leptin M, Aebersold R, Wilcox M. *Drosophila* position-specific antigens resemble the vertebrate fibronectin-receptor family. *EMBO J.* 1987; 6:1037–1043. [PubMed: 2954814]
86. Plow EF, et al. Immunologic relationship between platelet membrane glycoprotein GPIIb/IIIa and cell surface molecules expressed by a variety of cells. *Proc. Natl Acad. Sci. USA.* 1986; 83:6002–6006. [PubMed: 2426709]
87. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell*. 1992; 69:11–25. [PubMed: 1555235]
88. Schwartz MA, Ginsberg MH. Networks and crosstalk: integrin signalling spreads. *Nature Cell Biol.* 2002; 4:E65–E68. [PubMed: 11944032]
89. Miranti CK, Brugge JS. Sensing the environment: a historical perspective on integrin signal transduction. *Nature Cell Biol.* 2002; 4:E83–E90. [PubMed: 11944041]
90. Kim C, Ye F, Ginsberg MH. Regulation of integrin activation. *Annu. Rev. Cell Dev. Biol.* 2011; 27:321–345. [PubMed: 21663444]
91. Ridley AJ, et al. Cell migration: integrating signals from front to back. *Science*. 2003; 302:1704–1709. [PubMed: 14657486]
92. Bershadsky AD, Balaban NQ, Geiger B. Adhesion-dependent cell mechanosensitivity. *Annu. Rev. Cell Dev. Biol.* 2003; 19:677–695. [PubMed: 14570586]
93. Hoffman BD, Grashoff C, Schwartz MA. Dynamic molecular processes mediate cellular mechanotransduction. *Nature*. 2011; 475:316–323. [PubMed: 21776077]
94. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. *J. Cell Biol.* 2012; 196:395–406. [PubMed: 22351925]
95. Hanein D, Horwitz AR. The structure of cell–matrix adhesions: the new frontier. *Curr. Opin. Cell Biol.* 2012; 24:134–140. [PubMed: 22196929]

96. Wilson HV. Development of sponges from dissociated tissue cells. Bull. US Bureau Fisheries. 1910; 30:1–30.

Box 1**Recollections from the adhesion community****Richard Hynes**

“The convergence of multiple fields — cancer, development, haemostasis, immunology — and phyla (vertebrates, insects) where people had found receptors mediating different functions was very exciting as it became clear that there was a family of related genes/proteins. The different fields had different questions and different insights — cell migration, cell–matrix adhesion, cell–cell adhesion, activatable receptors, patterning in embryos. This meant that each field acquired new ways of thinking about the functions of their receptors and could feed off the insights of the other fields. That meeting nucleated a field with common goals and the culture of sharing of ideas and reagents was enormously fruitful in subsequent years.” (regarding the 1987 Gordon Research Conference).

Mark Ginsberg

“As a Physician-Scientist, the integrin field provided me with validation of the critical role of basic science in new therapies; it is a classic ‘bedside to bench and back again’ story. Basic studies of Glanzmann Thrombasthenia and Leukocyte Adhesion Deficiency, recessive diseases due to mutations affecting $\beta 3$ or $\beta 2$ integrins, respectively, were instrumental in the discovery of the integrin family, elucidation of mechanisms of ligand binding and activation of integrins, and in assignment of integrin functions in humans. This basic research provided the essential foundation and impetus for the development of integrin-targeted therapeutics for diseases such as heart attack, stroke, and multiple sclerosis.”

Masatoshi Takeichi

“I recall that integrin and cadherin were cloned in similar years (1986 for integrin and 1987 for E-cadherin). Thus, the studies of cell–substrate and cell–cell adhesion advanced in parallel — both complementing and synergizing with each other. Thanks to this lucky history, we could quickly establish a clear, definite, and more complete picture of cell adhesion.”

Kenneth M. Yamada

“Once integrins were identified, our hopes that complicated biology could be explained by specific protein–protein interactions came true, both for receptor-type binding at the cell surface and aggregates of cytoskeletal and signalling proteins inside cells. What was surprising, however, was the broad importance of integrins to so many different kinds of signalling.”

Figure 1. Early observations that adhesion mediates tissue association in invertebrates
a | Sponges can be triggered to dissociate by removing calcium, and then their reaggregation into minisponges is observed. Freshly dissociated cells that were pressed out through bolting cloth are shown. **b** | The morphogenesis of aggregates in open sea water. Images are reproduced from REF. 96.

Figure 2. Experimental strategies that allowed the identification of adhesion molecules

a | The isolation of neural cell adhesion molecule (NCAM) using antibody purification. Embryonic retinal cells were dissociated using trypsin and then allowed to reaggregate. Fab' fragments from antibodies raised against retinal cells bound to the cells and prevented their reaggregation. Proteins released into tissue culture supernatants were fractionated and assayed for fractions that relieved this antibody inhibition. Immunization against the active protein fraction then allowed the isolation of a highly specific blocking antibody that was used to purify the 140 kDa antigen as NCAM²⁹. **b |** The isolation of integrins using monoclonal antibodies. After immunizing mice with embryonic muscle cells, hybridomas were prepared and cloned. The supernatants from single cell lines were then assayed for those that inhibit the adhesion of embryonic skeletal muscle cells to the substratum. The active monoclonal antibodies were then used for immunolocalization and immunoprecipitation, which led to the isolation of $\beta 1$ integrins^{68,69}. **c |** Isolation of a fibronectin receptor. The identification of a specific adhesion-binding motif, RGDS, from fibronectin provided the means for an affinity purification strategy. A large cell adhesion-promoting chymotryptic fragment from fibronectin was conjugated onto an affinity column support, which would retain binding proteins upon the addition of a cell extract. Addition of Arg-Gly-Asp-Ser (RGDS) but not Arg-Gly-Glu-Ser (RGES) peptides eluted a specific membrane protein that, when isolated and reconstituted in liposomes, mediated binding to fibronectin^{74,75}. ECM, extracellular matrix.

Figure 3. Localization of the adhesion component vinculin using fluorescence microscopy
 Comparison of immunofluorescence images of vinculin with interference reflection microscope (IRM) images and actin immunofluorescence in chicken gizzard cells. **a** | The dark areas in the interference reflection image (white boxes) are areas of close association with the substratum (adhesion plaques). The correspondence between the vinculin image and the insets (white boxes) shows that vinculin resides in adhesions. The arrows point to the same areas in the IRM and fluorescence images. **b** | The localization of actin is shown, using an actin-specific antibody. **c** | The localization of vinculin is shown, using a vinculin-specific antibody. The arrows show that vinculin resides at actin filament termini primarily near cell edges. Images are taken, with permission, from REF. 61 © (1979) Cell Press.

Figure 4. Participants in the 1987 Fibronectin Gordon Research Conference

This meeting, held in Santa Barbara, California, in February 1987, brought together researchers working on immunology, cancer, vascular biology, cell biology and development, and precipitated the formation of a new research community. For the participant list, see Supplementary information S1 (figure). The image is reproduced, with permission, from Gordon Research Conferences © 1987.