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Landmark Essay

The emergence of integrins: a personal and historical perspective

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I never intended to work on cell adhesion. My involvement in this fascinating field for the past 30 years originated in a desire to find molecular differences between the surfaces of normal and tumor cells. In the early 1970s, viral transformation of cultured cells had been shown to yield tumorigenic cells that differed in their growth properties, in their dependence on serum factors and on attachment to substrates and in their morphology in culture. These transformed cells grew more than their normal progenitors and we did not know why. In 1971, I went to Michael Stoker's laboratory at the Imperial Cancer Research Fund (ICRF) in London as a postdoctoral fellow. He was an expert on transformation by polyoma virus and I planned to investigate early biochemical alterations induced by the virus that might explain the altered cell behavior. It soon became clear that the so-called "abortive transformation" by polyoma virus was not amenable to biochemical analyses so I looked around for something else to do. Many people in Stoker's lab were working on purifying growth factors from serum but that did not appeal to me. On the other hand, these serum factors promoted cell growth, presumably by binding to "receptors" on cell surfaces. There were also indications from studies with carbohydrate-binding lectins that the surfaces of transformed cells differed from those of normal cells in some way (Burger, 1973) although the molecular basis for these differences was completely unclear. In fact, we knew rather little indeed about the molecular structure of the cell surface of any but a few simple cells such as erythrocytes. The fluid-mosaic model proposing proteins floating in a lipid bilayer had just been formulated (Singer and Nicolson, 1972).

So, I decided to try and find out what proteins were on the surfaces of normal and transformed cells and whether they differed. My underlying hypothesis was that some alteration in the surface might underlie the difference in growth control. I tried various surface labeling reagents including synthesizing "Bretscher's reagent" from ^{35}S-methionine (Bretscher, 1971), with the side product of liters of radioactive ether and cooling fluid from the paper electrophoresis tanks—that did not seem to be a practical approach, especially since the reagent only worked effectively above pH 8 and that made the cells leaky. Then I tried the ^{125}I-iodide/lactoperoxide/glucose oxidase system developed in work on erythrocytes (Phillips and Morrison, 1971). We had no fume hood so I took seaweed pills to preblock my thyroid each day and labeled dishes of cells on the open bench. The initial results were encouraging—I saw multiple labeled bands on the, then newly invented, SDS slab gels but initially no differences between normal and virally transformed cells. One day early in 1973, I was looking over my results and realized that all the normal cell lanes had a lot of ^{125}I label stuck at the top of the 10% polyacrylamide gels, whereas the transformed cells had much less or none. The next day I ran some lower percentage cells and, "eureka," there was a major high molecular weight band (~250 kDa) in the normal cell lanes but it was greatly reduced in polyoma-transformed cells and absent in cells transformed by hamster sarcoma virus (now known to harbor H-ras). Control experiments showed that this protein was removed from cells by low doses of trypsin, an additional criterion for cell surface location. This was fibronectin, although at the time I called it LETS protein (large, external transformation-sensitive). Its loss on transformation or proteolysis seemed to correlate with changes in the lectin agglutinability of cells and in their proliferation, known to be stimulated by transformation or by proteolysis (Hynes, 1973). This was all very exciting and, for a while, I

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iodinated any cell I could get my hands on until I eventually achieved a minor ^{125}I contamination of my thyroid and ICRF built a fume hood so that I could continue. The loss of LETS protein seemed to be a consistent correlate of oncogenic transformation and we initially thought that we had discovered a surface growth regulatory molecule. It soon became clear that it did not behave like a detergent-soluble integral membrane protein (Graham et al., 1975). Antibodies, first in the hands of Antti Vaheri and Erkki Ruoslahti (Wartiovaara et al., 1974), and later in my own lab at MIT (Hynes et al., 1976; Mautner and Hynes, 1977), showed a fibrillar pattern of staining. When we purified the protein and added it back to cells that lacked it, that had little effect on their growth rate but it had a major effect on their adhesion and morphology (Ali et al., 1977) and on cell migration (Ali and Hynes, 1978). Others also reported effects on cell adhesion and motility (Willingham et al., 1977).

So, rather than identifying an integral membrane protein involved in growth control, it seemed clear that we had in our hands an extracellular matrix molecule involved in adhesion. Years later it became clear that fibronectin and integrins do, after all, play very important roles in controlling cell proliferation and survival but, in the mid-1970s, our attention switched to cell adhesion, cytoskeletal morphology and cell migration, all of which were affected by LETS protein/fibronectin. How did that lead to integrins?

1. The road to integrins

The concept that there must exist transmembrane connections between extracellular matrix proteins and the actin-based cytoskeleton developed during the 1970s. By 1976 significant circumstantial evidence had accumulated that suggested some relationship between extracellular fibronectin-containing fibrils and intracellular actin filaments and we suggested that an integral membrane protein or proteins might link the two (Hynes, 1976). Further evidence soon appeared demonstrating clearly that these two sets of fibrils, one inside the cell and one outside, are physically connected (Hynes and Destree, 1978; Heggeness et al., 1978; Singer, 1979) and we showed that assembly of fibronectin fibrils could induce rearrangement of actin filaments (Ali et al., 1977), whereas disassembly of actin led to loss of surface fibronectin (Ali and Hynes, 1977). The evidence was sufficiently suggestive that review articles included diagrams depicting unknown transmembrane linker proteins (Hynes, 1981a,b; Hynes and Yamada, 1982). However, the identities of such integral membrane linkers remained obscure and it was not until the mid-1980s that they were clearly identified. The postulated “fibronectin receptor” became a Holy Grail for the field for a number of years.

Several candidate molecules were considered, based on a variety of experimental approaches. These included generation of polyclonal antisera that blocked cell adhesion to matrix proteins, attempts to crosslink fibronectin to putative receptors and numerous efforts to isolate binding proteins by affinity chromatography. In retrospect, some of these candidate molecules did eventually turn out to be, or at least to include, genuine receptors, but the results, while tantalizing, were not convincing. I will discuss later why this proved to be such a difficult search.

The eventual identification of the proteins we were all seeking relied on several independent lines of investigation. One was the ongoing dissection of the structure of fibronectin, a large glycoprotein with many independent binding sites. Proteolytic fragmentation studies in many labs defined separable domains of fibronectin responsible for binding other extracellular proteins such as fibrin and gelatin and, importantly, others for mediating cell adhesion (Hynes and Yamada, 1982). This progressive dissection of fibronectin eventually yielded a small fragment of fibronectin (108 amino acids out of over 2500) that would promote cell adhesion, albeit inefficiently (Pierschbacher et al., 1982). Synthetic peptides based on the sequence of this cell-binding fragment eventually showed that a tetrapeptide sequence, RGDS, was the minimal cell-binding sequence (Pierschbacher and Ruoslahti, 1984). As we shall see, this provided one key to the puzzle.

A second important key, which was crucial for our own subsequent work, came from a couple of monoclonal antibodies that had been raised against chicken myoblasts, in attempts to block myoblast fusion (Greve and Gottlieb, 1982; Neff et al., 1982). Fusion-blocking antibodies were not found but two antibodies, JG22 and CSAT, which interfered with adhesion of myoblasts to matrix-coated surfaces, were obtained. Each antibody precipitated a complex of several proteins, which was initially confusing. It just so happened that during early 1984 I gave seminars at Howard University and at the Wistar Institute and during those visits I met first with Wen-Tien Chen, who was working on JG22, and then with Caroline Damsky from Clayton Buck’s lab, who was working on CSAT, isolated in the neighboring lab of Rick Horwitz at the University of Pennsylvania (Neff et al., 1982; Knudsen et al., 1985). Both Wen-Tien and Caroline showed me beautiful immunofluorescence pictures demonstrating that the antigens recognized by these two monoclonals lined up both with fibronectin and with actin and some other cytoskeletal proteins (Chen et al., 1985; Damsky et al., 1985). For me these then-unpublished results were a revelation. Having spent many hours at the microscope exploring the codistribution of fibronectin and actin, and several years trying to find the molecules we believed must link them, I was convinced that the JG22 and CSAT antigens must be the ones we wanted. Furthermore, we were in a position to exploit the antibodies because we had become adept at cDNA cloning using antibodies and the λgt11 system. John Tamkun in our laboratory had made some excellent λgt11 libraries and Jean Schwarzbauer had recently used that

approach to clone fibronectin (Schwarzbauer et al., 1983). So I promptly established a collaboration with the Philadelphia group and Tamkun set about making a chicken embryo fibroblast λgt11 library and screening for antibody-reactive clones. Cloning and sequencing were not so easy in those days and isolating and validating cDNA clones took us a while (Tamkun et al., 1986). In the meantime, the other approaches moved forward. The Ruoslahti lab exploited their discovery of the RGD binding site in fibronectin and used the peptide to make affinity columns and as a hapten to elute from columns of fibronectin. They isolated several binding proteins that appeared to be receptors for fibronectin and vitronectin (Pytela et al., 1985a,b).

Meanwhile, we had been following a different lead. Several years earlier we had shown that fibronectin bound to platelets and promoted their spreading (Hynes et al., 1978). Mark Ginsberg and Ed Plow at Scripps had been studying platelet receptors for fibrinogen and they and others had accumulated evidence that a complex of two platelet surface proteins, known as GPIIb/IIIa, originally defined by David Phillips (Phillips and Agin, 1977) served as an inducible fibrinogen receptor on platelets (Bennett et al., 1982; Marguerie et al., 1984). Ginsberg and Plow also provided suggestive evidence that GPIIb/IIIa might be a fibronectin receptor as well (Ginsberg et al., 1983). We had been using monoclonal antibodies and chemical crosslinkers in our attempts to define fibronectin receptors on both fibroblasts and platelets. The platelet system turned out to be more tractable and we were able to crosslink the cell-binding fragment of fibronectin to an inducible receptor on platelets and identify it as GPIIb/IIIa (Gardner and Hynes, 1985). We also used affinity chromatography and RGD peptide elution to confirm that identification, as did the Ruoslahti lab (Pytela et al., 1986).

By late 1985, we had cloned and sequenced the first complete subunit of the CSAT complex and were struggling to prove it was the right thing, which we eventually did. The sequence revealed a transmembrane protein with several novel and interesting structural features (Tamkun et al., 1986) so we decided the time had come to give it a name. Believing firmly that it is best to name proteins by structure, which is unlikely to change on further investigation, rather than by inferred function, which often does, we suggested the name "integrin" for the integral membrane protein complex linking the extracellular matrix to the cytoskeleton. That much seemed already clear. We also hoped that the name could denote a role in the integrity of both structures and an integrating function for those receptors. As it turned out, those hopes were realized in coming years and the name has been universally adopted.

2. The integrin family emerges

We were fascinated by the possibility that the CSAT complex of avian fibroblasts, now called integrin, might be homologous with the GPIIb/IIIa complex of human platelets. They were similar in size and behavior on gels, although there was the nagging problem that the CSAT complex appeared to have at least three components, whereas GPIIb/IIIa was clearly a heterodimer, but their common function as receptors for extracellular matrix proteins (we viewed fibrinogen as a facultative matrix protein) was very suggestive. However, the story was about to become more intriguing yet. While writing the discussion for our integrin cloning paper, we came across a paper (Cosgrove et al., 1986) that suggested some sort of relationship between GPIIb/IIIa and a set of cell surface proteins on lymphoid and myeloid cells, called LFA-1/Mac-1. It turned out later that the basis for the suggestion was completely incorrect but it was nonetheless a valuable stimulus to us. Interestingly, LFA-1 and Mac-1 appeared to be heterodimers like GPIIb/IIIa. I knew quite a bit about GPIIb/IIIa but I had never heard of LFA-1 or Mac-1 so I looked them up on Medline and found only a few papers, mostly from someone called Springer. I had never heard of him either but it turned out he was just across the river at Harvard Medical School. So I called him up and suggested that we should meet, since it seemed we might be working on related proteins. We went over to visit Tim Springer and his fellows at the Dana Farber Cancer Institute. Our sequencing paper was about to come out and we had spent ages poring over our sequence; we knew it intimately. It turned out that Springer's lab was in the middle of sequencing the shared β subunit of LFA-1 and Mac-1 and they pulled out their reams of computer printout. It took maybe 10 to 15 s to realize that they had a homologue of our chicken integrin subunit—the novel, characteristic pattern of cysteine-rich repeats was unmistakable (Kishimoto et al., 1987; Law et al., 1987). It was another of those special moments in science—I realized that we had found a family of cell adhesion receptors in a diverse array of cell types. Over the next few months this became ever clearer as cDNA sequences were obtained for GPIIIa (Fitzgerald et al., 1987a), GPIIb (Poncz et al., 1987; Fitzgerald et al., 1987b) and subunits of the human fibronectin and vitronectin receptors (Argraves et al., 1987; Suzuki et al., 1987). It became clear that there were at least three distinct β subunits and a variety of α subunits. Another scientist at Dana Farber, Martin Hemler, had another set of heterodimeric lymphocyte proteins that he called VLA proteins (Hemler et al., 1985, 1987). They were distinct from Springer's heterodimers but each set had a common β subunit and a variety of α subunits. We now know that the VLA proteins are human β1 integrins, the mammalian equivalents of the chicken CSAT proteins, although that took a couple of years to resolve completely.

As it happened, I was organizing a Gordon conference on fibronectin in California for February 1987 so I invited everyone I could identify who was working on one of these receptors. These included cancer and developmental biologists, hematologists, immunologists and even one *Droso-*

phila person, Michael Wilcox, who had some interesting proteins called PS antigens that looked like fly integrins (Wilcox et al., 1984; Wilcox and Leptin, 1985). At a meeting earlier that year he had shown me a few amino acids of N-terminal sequence of one of his PS antigen subunits and it looked to me like the fragmentary N-terminal sequences of several vertebrate α subunits. It became clear at the Gordon conference that there was indeed a family of receptors present in mammals, birds and insects. It was one of the most exciting meetings that I can recall attending—people from widely divergent fields met each other for the first time. Each field was approaching these receptors from rather different viewpoints. The people studying extracellular matrix in development and cancer were intrigued by the transmembrane linkage I have discussed earlier, by cell–matrix adhesion and cell migration. The platelet people were more interested in cell–cell aggregation and in the interesting phenomenon of activation of GPIIb/IIIa, whereas the immunologists were interested in leukocyte cell–cell interactions and adhesion and Wilcox was interested in the PS antigens because of their intriguing patterns of expression in imaginal disks. This convergence was incredibly stimulating—all of a sudden each discipline had a whole new set of ideas to apply in their system. I tried to capture this integration of the field in a brief review in Cell (Hynes, 1987) that has been cited over 3000 times in the intervening years.

3. Why were integrins so hard to identify?

In retrospect, it is puzzling that it took us so long to identify integrins. The idea of transmembrane links between extracellular matrix and actin microfilaments was well established by the mid-1970s but it took nearly a decade before they were definitively identified. I think it is instructive to ask why that was so—the answers are, in part, technical and, in part, conceptual. As I reviewed above, monoclonal antibodies were key reagents in the discovery of integrins and cDNA cloning (still challenging for large molecules in the mid-1980s) provided the clear evidence for a homologous family of receptors. Both approaches were subsequently very important in expanding the integrin family.

If one looks back to the years before those technological breakthroughs, one can see why we failed to identify the "receptors" we were seeking. The obvious approaches were all attempted. Polyclonal antisera that blocked cell adhesion had been used to identify some adhesion receptors such as proteins mediating slime mold aggregation (Muller and Gerisch, 1978) and N-CAM (Thiery et al., 1977). When this approach was applied to cell–matrix adhesion, antisera were obtained which blocked cell–substratum adhesion (Damsky et al., 1981; Knudsen et al., 1981; Tarone et al., 1982). The trouble was that they blocked adhesion to multiple matrix proteins, raising the specter of non-specific effects. This concern was exacerbated by the fact that they recognized multiple protein species. We were expecting specific receptors and were not anticipating multiple subunits.

When we and others attempted to raise monoclonal antibodies that blocked fibroblast adhesion to matrix, we purposefully screened for antibodies that specifically blocked adhesion to fibronectin and not to laminin and discarded hybridomas that blocked both. We must have thrown away many good blocking antibodies to the β1 subunit common to several fibronectin and laminin receptors. It also proved challenging to find antibodies that effectively blocked adhesion of fibroblasts to fibronectin although, later on, once we knew the nature of integrins, many such antibodies were isolated. In retrospect, we now know that fibroblasts typically express both α5β1 and αvβ3, both active fibronectin receptors, so no one antibody can block all adhesion to fibronectin—we were to some extent trapped by the implicit assumption that there was such a thing as *the fibronectin receptor*. It is striking that the two monoclonals that were key, JG22 and CSAT, were both raised against chicken myoblasts in screens for anti-fusion monoclonals, not in screens for blockers of cell–substration adhesion. Myoblasts have fewer integrins than fibroblasts and are not so strongly adherent, so it is easier to find single monoclonals that block their adhesion. Both these monoclonals are against the chicken β1 subunit and both block adhesion to fibronectin and to laminin (Horwitz et al., 1985) and both immunoprecipitate multiple integrin subunits (β1 plus several α subunits). That was initially puzzling—again because of unstated presumptions about specificity and singularity of cell–matrix receptors. As I mentioned above, the clincher for me was their striking codistribution with both fibronectin and the actin-based cytoskeleton,whose striking colinearity had started us on the quest in the first place.

Another obvious approach to identify receptors was crosslinking and we invested a lot of effort in that, including designing new crosslinkers (Schwartz et al., 1982). The trouble was that our ligand, fibronectin, was so large and bound to many other things in addition to cell surface receptors. So we identified interactions with proteoglycans (Perkins et al., 1979) and with thrombospondin (Lahav et al., 1982) but it was not until we used the small cell-binding fragment of fibronectin that we finally detected crosslinking to GPIIb/IIIa (Gardner and Hynes, 1985). The identification of the small cell-binding fragment and its active sequence, RGDS (Pierschbacher et al., 1982; Pierschbacher and Ruoslahti, 1984) was also key in facilitating another obvious approach, affinity chromatography, as I described above.

Earlier attempts in our lab, and I am certain in others, to use fibronectin affinity columns to isolate "receptors" failed dismally—too many proteins stuck and were eluted from such columns and it was impossible to distinguish specific from non-specific binding. It is now clear that this was, in part, due to the relatively low affinity of integrins

for their ligands (K_d=10^{-6} to 10^{-7}). All of that changed with the discovery of RGD and its use as a hapten to elute integrins specifically from affinity columns of RGD, fibronectin and other proteins (Pytela et al., 1985a,b). So, by 1985, all the approaches were finally working—monoclonal antibodies, affinity columns and crosslinking; setting the stage for cDNA cloning and the realization that there were multiple homologous receptors and that they were heterodimers. The latter had long been clear for GPIIb/IIIa and, in the early 1980s, studies of LFA-1 and Mac-1, both originally identified by monoclonals (Springer, 1985), as were the VLA antigens (Hemler et al., 1985, 1987), introduced the idea of sets of α subunits with a common β subunit (β1 for VLA and β2 for LFA-1/Mac-1). In addition, there were human genetic diseases affecting GPIIb/IIIa (Glanzmann thrombasthenia) and β2 integrins (leukocyte adhesion deficiency) and both clearly implicated these receptors in cell–cell adhesion of platelets and leukocytes, respectively.

So by early 1987 the existence of a family of αβ heterodimeric cell adhesion receptors involved in both cell–matrix adhesion and cell–cell adhesion was clear (Hynes, 1987) and one could begin to see the emergence of an "integrin field."

4. Subsequent developments

Additional monoclonals and cDNA cloning revealed many more integrins. Highly cross-reactive polyclonal antisera to cytoplasmic domains confirmed that integrins were conserved between vertebrates and invertebrates (Marcantonio and Hynes, 1988). Subsequent genomic sequences have demonstrated that all metazoa, and only metazoa, express integrins—one can argue that integrins are a necessary component of metazoan multicellularity, providing the attachment to basement membranes essential to forming a multilayered organism and it is intriguing that a laminin-specific and an RGD-specific integrin appear to have evolved well before the Cambrian explosion (Hynes and Zhao, 2000; Hynes, 2002a).

Subsequent evolutionary divergence has generated 8 β subunits and 18 α subunits in vertebrates. Although this could, in principle, yield a gross of integrins, there is only limited promiscuity and only 24 αβ heterodimers have been identified to date. These 24 receptors mediate adhesion to a wide array of matrix proteins as well as to cell surface counterreceptors and other extracellular ligands (Plow et al., 2000; van der Flier and Sonnenberg, 2001). Contrary to earlier assumptions, most integrins are not specific for single ligands—in general, each integrin binds several ligands and many of those ligands are recognized by multiple integrins. Implicit in the original identification of integrins is their role as transmembrane linkers (integrators) between the extracellular matrix and the cytoskeleton. That connection has been explored extensively leading to a detailed, elaborate and still-expanding array of associated proteins (Zamir and Geiger, 2001; van der Flier and Sonnenberg, 2001).

Another vitally important property of integrins that was not fully appreciated at the outset is their role as signaling receptors. Indeed, initially some questioned whether they should really be called "receptors" since it was not known that they signaled like "true receptors" such as the G-protein-coupled or tyrosine kinase receptors. This always seemed to me to be a semantic argument and, in any case, it is now moot. It became clear over the 5 years following the definition of the integrin receptor family that they transmit signals both into and out of cells (Hynes, 1992). That review has been cited over 6000 times as many researchers in the field explored integrin-mediated signaling showing that they trigger signal transduction pathways at least as complex and important as receptors for soluble ligands. It is now abundantly clear that receptors for soluble mediators (hormones, growth factors, cytokines, etc.) and cell adhesion receptors, especially integrins but also others, cooperate in transducing information about the extracellular milieu into cells and controlling cell proliferation, survival and behavior in an intimately integrated fashion (see reviews cited in Hynes, 2002a).

One important aspect of integrin function that was apparent early, at least for platelets and leukocytes, is that integrins can be in inactive and active states. It is obvious for these blood cells that they need to switch rapidly from non-adherent to adherent and it is clear that their main adhesion receptors are integrins that, while expressed on the surfaces of resting, non-adherent cells need to be activated to mediate cell adhesion. Early evidence for conformational changes underlying this activation has recently culminated in the determination of the three-dimensional structure of αvβ3 (Xiong et al., 2001, 2002) and a series of studies based on that insight have clearly shown the occurrence of major conformational changes in several integrins associated with their activation (reviewed in Hynes, 2002a; Shimaoka et al., 2002; Takagi and Springer, 2002; Liddington and Ginsberg, 2002). These ancient metazoan adhesion receptors were evidently already complex molecular machines well before the divergence of invertebrates and vertebrates. Their fundamental importance is shown by the early lethality of null mutants in the shared β subunits of nematodes and flies or of the β1 subunit of mammals. Ablation of other mammalian integrin subunits that are less broadly expressed yields more subtle and selective defects. I have already mentioned the platelet and leukocyte adhesion defects caused by mutations in β3 or β2, respectively. This selectivity of mutations in blood cell integrins has been exploited, using blocking antibodies, or peptides or peptidomimetics selective for particular integrins of platelets, leukocytes or lymphocytes to develop effective drugs targeting thrombosis, inflammation and autoimmune diseases and more are on the way. Alterations in expression of integrins on cancer cells (Plantefaber and Hynes, 1989 and

many subsequent reports; see reviews in Ruoslahti, 1999; Felding-Habermann, 2003; Jin and Varner, 2004) and on angiogenic blood vessels (see reviews in Hynes et al., 2002; Hynes, 2002b; Eliceiri and Cheresh, 2001) offer the promise of further integrin-based therapeutic interventions in coming years.

5. Conclusion

It has been an exciting and endlessly stimulating journey from the initial discovery of the loss of fibronectin on transformation, the physical relationship between extracellular matrix and cytoskeleton, the discovery of the integrin family and the deciphering of their signal transduction mechanisms to seeing the application of anti-integrin reagents in the clinic. Thirty years ago, extracellular matrix was largely viewed from a structural perspective. The discovery of fibronectin and its effects on cell behavior and of other matrix glycoproteins and then of integrins as the links (both physical and informational) between the matrix and the interior of the cell brought the matrix into the center of the mainstream of cell biology. Integrins can indeed be viewed as integrators of spatial cues provided by the matrix with those provided by soluble cues.

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References

Ali, I.U., Hynes, R.O., 1977. Effects of cytochalasin B and colchicine on attachment of a major surface protein of fibroblasts. Biochim. Biophys. Acta 471, 16–24.

Ali, I.U., Hynes, R.O., 1978. Effects of LETS glycoprotein on cell motility. Cell 14, 439–446.

Ali, I.U., Mautner, V.M., Lanza, R.P., Hynes, R.O., 1977. Restoration of normal morphology, adhesion and cytoskeleton in transformed cells by addition of a transformation-sensitive surface protein. Cell 11, 115–126.

Argraves, W.S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M.D., Ruoslahti, E., 1987. Amino acid sequence of the human fibronectin receptor. J. Cell Biol. 105, 1183–1190.

Bennett, J.S., Vilaire, G., Cines, D.B., 1982. Identification of the fibrinogen receptor on human platelets by photoaffinity labeling. J. Biol. Chem. 257, 8049–8054.

Bretscher, M.S., 1971. Human erythrocyte membranes: specific labelling of surface proteins. J. Mol. Biol. 58, 775–781.

Burger, M.M., 1973. Surface changes in transformed cells detected by lectins. Fed. Proc. 32, 91–101.

Chen, W.T., Hasegawa, T., Hasegawa, C., Weinstock, C., Yamada, K.M., 1985. Development of cell surface linkage complexes in cultivated fibroblasts. J. Cell Biol. 100, 1103–1114.

Cosgrove, L.J., Sandrin, M.S., Rajasekariah, P., McKenzie, I.F.C., 1986. A genomic clone encoding the chain of the OKM1, LFA-1, and platelet glycoprotein IIb–IIIa molecules. Proc. Natl. Acad. Sci. U. S. A. 83, 752–756.

Damsky, C., Knudsen, K., Dorio, R., Buck, C., 1981. Manipulation of cell–cell and cell–substratum interactions in mouse mammary tumor epithelial cells using broad spectrum antisera. J. Cell Biol. 89, 173–184.

Damsky, C.M., Knudsen, K.A., Bradley, D., Buck, C.A., Horwitz, A.F., 1985. Distribution of the CSAT cell–matrix antigen on myogenic and fibroblastic cells in culture. J. Cell Biol. 100, 1528–1539.

Eliceiri, B.P., Cheresh, D.A., 2001. Adhesion events in angiogenesis. Curr. Opin. Cell Biol. 13, 563–568.

Felding-Habermann, B., 2003. Integrin adhesion receptors in tumor metastasis. Clin. Exp. Metastasis 20, 203–213.

Fitzgerald, L.A., Steiner, B., Rall, S.C., Lo, S.-S., Phillips, D.R., 1987a. Protein sequence of endothelial glycoprotein IIIa derived from a cDNA clone: identity with platelet glycoprotein IIIa and similarity to integrin. J. Biol. Chem. 262, 3936–3939.

Fitzgerald, L.A., Poncz, M., Steiner, B., Rall, S.C., Bennett, J.S., Phillips, D.R., 1987b. Comparison of cDNA-derived protein sequence of the human fibronectin and vitronectin receptor alpha subunits and platelet glycoprotein IIb. Biochemistry 26, 8158–8165.

Gardner, J.M., Hynes, R.O., 1985. Interaction of fibronectin with its receptor on platelets. Cell 42, 439–448.

Ginsberg, M., Forsyth, J., Lightsey, A., Chediak, J., Plow, E., 1983. Reduced surface expression and binding of fibronectin by thrombin-stimulated thrombasthenic platelets. J. Clin. Invest. 71, 619–624.

Graham, J.M., Hynes, R.O., Davidson, E.A., Bainton, D.F., 1975. The location of proteins labelled by the 125I-lactoperoxidase system in the NIL8 hamster fibroblast. Cell 4, 353–365.

Greve, J.M., Gottlieb, D.I., 1982. Monoclonal antibodies which alter the morphology of cultured chick myogenic cells. J. Cell Biochem. 18, 221–230.

Heggeness, M.H., Ash, J.F., Singer, S.J., 1978. Transmembrane linkage of fibronectin to intracellular actin-containing filaments in cultured human fibroblasts. Ann. N.Y. Acad. Sci. 312, 414–417.

Hemler, M.E., Jacobson, J.G., Strominger, J.L., 1985. Biochemical characterization of VLA-1 and VLA-2. J. Biol. Chem. 260, 15246–15252.

Hemler, M.E., Huang, C., Schwarz, L., 1987. The VLA protein family: characterization of five distinct cell surface heterodimers each with a common 130,000 Mr subunit. J. Biol. Chem. 262, 3300–3309.

Horwitz, A., Duggan, K., Greggs, R., Decker, C., Buck, C., 1985. The cell substrate attachment (CSAT) antigen has properties of a receptor for laminin and fibronectin. J. Cell Biol. 101, 2134–2144.

Hynes, R.O., 1973. Alteration of cell-surface proteins by viral transformation and by proteolysis. Proc. Natl. Acad. Sci. U. S. A. 70, 3170–3174.

Hynes, R.O., 1976. Cell surface proteins and malignant transformation. Biochim. Biophys. Acta 458, 73–107.

Hynes, R.O., 1981a. Fibronectin and its relation to cellular structure and behavior. In: Hay, E.D. (Ed.), The Cell Biology of the Extracellular Matrix. Plenum Press, pp. 295–333.

Hynes, R.O., 1981b. Relationships between fibronectin and the cytoskeleton. In: Poste, G., Nicolson, G.L. (Eds.), Cytoskeletal Elements and

Plasma Membrane Organization, Cell Surface Reviews, vol. 7. Elsevier-Biomedical Press, pp. 97–139.

Hynes, R.O., 1987. Integrins: a family of cell surface receptors. Cell 4, 549–554.

Hynes, R.O., 1992. Integrins: versatility, modulation and signalling in cell adhesion. Cell 69, 11–25.

Hynes, R.O., 2002a. Integrins: bi-directional, allosteric, signaling machines. Cell 110, 673–687.

Hynes, R.O., 2002b. A reevaluation of integrins as regulators of angiogenesis. Nat. Med. 8, 918–921.

Hynes, R.O., Destree, A.T., 1978. Relationships between fibronectin (LETS protein) and actin. Cell 15, 875–886.

Hynes, R.O., Yamada, K.M., 1982. Fibronectins: multifunctional modular glycoproteins. J. Cell Biol. 95, 369–377.

Hynes, R.O., Zhao, Q., 2000. The evolution of cell adhesion. J. Cell Biol. 150, F89–F95.

Hynes, R.O., Destree, A.T., Mautner, V.M., 1976. Spatial organization at the cell surface. In: Marchesi, V.T. (Ed.), Membranes and Neoplasia: New Approaches and Strategies. Alan R. Liss, New York, pp. 189–201.

Hynes, R.O., Ali, I.U., Destree, A.T., Mautner, V.M., Perkins, M.E., Senger, D.R., Wagner, D.D., Smith, K., 1978. A large glycoprotein lost from the surfaces of transformed cells. Ann. N.Y. Acad. Sci. 312, 317–342.

Hynes, R.O., Lively, J.C., McCarty, J.H., Taverna, D., Francis, S.E., Hodivala-Dilke, K., Xiao, Q., 2002. Diverse roles of integrins and their ligands in angiogenesis. Cold Spring Harbor Symp. Quant. Biol. 67, 143–153.

Jin, H., Varner, J., 2004. Integrins: roles in cancer development and as treatment targets. Br. J. Cancer 90, 561–565.

Kishimoto, T.K., O'Connor, K., Lee, A., Roberts, T.M., Springer, T.A., 1987. Cloning the subunit of the leukocyte adhesion proteins: homology to an extracellular matrix receptor defines a novel supergene family. Cell 48, 681–690.

Knudsen, K.A., Rao, P.E., Damsky, C.H., Buck, C.A., 1981. Membrane glycoproteins involved in cell–substratum adhesion. Proc. Natl. Acad. Sci. U. S. A. 78, 6071–6075.

Knudsen, K.A., Horwitz, A.F., Buck, C.A., 1985. A monoclonal antibody identifies a glycoprotein complex involved in cell–substratum adhesion. Exp. Cell Res. 157, 218–228.

Lahav, J., Schwartz, M.A., Hynes, R.O., 1982. Analysis of platelet adhesion using a radioactive chemical crosslinking reagent: interaction of thrombospondin with fibronectin and collagen. Cell 31, 253–262.

Law, S.K.A., Gagnon, J., Hildreth, J.E.K., Wells, C.E., Willis, A.C., Wong, A.J., 1987. The primary structure of the β-subunit of the cell surface adhesion glycoproteins LFA-1, CR3 and p150,95 and its relationship to the fibronectin receptor. EMBO J. 6, 915–919.

Liddington, R.C., Ginsberg, M.H., 2002. Integrin activation takes shape. J. Cell Biol. 158, 833–839.

Marcantonio, E.E., Hynes, R.O., 1988. Antibodies to the conserved cytoplasmic domain of the integrin β1 subunit react with proteins in vertebrates, invertebrates and fungi. J. Cell Biol. 106, 1765–1772.

Marguerie, G.A., Thomas-Maison, N., Ginsberg, M.H., Plow, E.F., 1984. The platelet–fibrinogen interaction. Evidence for proximity of the A chain of fibrinogen to platelet membrane glycoproteins IIb/IIIa. Eur. J. Biochem. 139, 5–11.

Mautner, V.M., Hynes, R.O., 1977. Surface distribution of LETS protein in relation to the cytoskeleton of normal and transformed fibroblasts. J. Cell Biol. 75, 743–768.

Muller, K., Gerisch, G., 1978. A specific glycoprotein as the target site of adhesion blocking Fab in aggregating Dictyostelium cells. Nature 274, 445–449.

Neff, N.T., Lowrey, C., Decker, C., Tovar, A., Damsky, C., Buck, C., Horwitz, A.F., 1982. A monoclonal antibody detaches embryonic skeletal muscle from extracellular matrices. J. Cell Biol. 95, 654–666.

Perkins, M.E., Ji, T.H., Hynes, R.O., 1979. Crosslinking of fibronectin to proteoglycans at the cell surface. Cell 16, 941–952.

Phillips, D.R., Agin, P.P., 1977. Platelet plasma membrane glycoproteins. Evidence for the presence of nonequivalent disulfide bonds using non-reduced–reduced two-dimensional gel electrophoresis. J. Biol. Chem. 252, 2121–2126.

Phillips, D.R., Morrison, M., 1971. Exposed proteins on the intact human erythrocyte. Biochemistry 10, 1766–1771.

Pierschbacher, M.D., Ruoslahti, E., 1984. The cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 309, 30–33.

Pierschbacher, M.D., Ruoslahti, E., Sundelin, J., Lind, P., Peterson, P.A., 1982. The cell attachment domain of fibronectin. Determination of the primary structure. J. Biol. Chem. 7, 9593–9597.

Plantefaber, L.C., Hynes, R.O., 1989. Changes in integrin receptors on oncogenically transformed cells. Cell 56, 281–290.

Plow, E.F., Haas, T.A., Zhang, L., Loftus, J., Smith, J.W., 2000. Ligand binding to integrins. J. Biol. Chem. 275, 21785–21788.

Poncz, M., Eisman, R., Heidenreich, R., Silver, S.M., Vilaire, G., Surrey, S., Schwartz, E., Bennett, J.S., 1987. Structure of the platelet membrane glycoprotein IIb: homology to the alpha subunits of the vitronectin and fibronectin membrane receptors. J. Biol. Chem. 262, 8476–8482.

Pytela, R., Piershbacher, M.D., Ruosahti, E., 1985a. Idetification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. Cell 40, 191–198.

Pytela, R., Pierschbacher, M.D., Ruoslahti, E., 1985b. A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine–glycine–aspartic acid adhesion sequence derived from fibronectin. Proc. Natl. Acad. Sci. U. S. A. 82, 5766–5770.

Pytela, R., Pierschbacher, M.D., Ginsberg, M.H., Plow, E.F., Ruoslahti, E., 1986. Platelet membrane glycoprotein IIb/IIIa: member of a family of arg–gly–asp-specific adhesion receptors. Science 231, 1559–1562.

Ruoslahti, E., 1999. Fibronectin and its integrin receptors in cancer. Adv. Cancer Res. 76, 1–20.

Schwartz, M.A., Das, O.P., Hynes, R.O., 1982. A new radioactive crosslinking reagent for studying the interactions of proteins. J. Biol. Chem. 257, 2343–2349.

Schwarzbauer, J.E., Tamkun, J.W., Lemischka, I.R., Hynes, R.O., 1983. Three different fibronectin mRNAs arise by alternative splicing within the coding region. Cell 35, 421–431.

Shimaoka, M., Takagi, J., Springer, T.A., 2002. Conformational regulation of integrin structure and function. Annu. Rev. Biophys. Biomol. Struct. 31, 485–516.

Singer, I.I., 1979. The fibronexus: a transmembrane association of fibronectin-containing fibers and bundles of 5 nm microfilaments in hamster and human fibroblasts. Cell 16, 675–685.

Singer, S.J., Nicolson, G.L., 1972. The fluid mosaic model of the structure of cell membranes. Science 175, 720–731.

Springer, T.A., 1985. The LFA-1, Mac-1 glycoprotein family and its deficiency in an inherited disease. Fed. Proc. 44, 2660–2663.

Suzuki, S., Argraves, W.S., Arai, H., Languino, L.R., Pierschbacher, M.D., Ruoslahti, E., 1987. Amino acid sequence of the vitronectin receptor a subunit and comparative expression of adhesion receptor mRNAs. J. Biol. Chem. 262, 14080–14085.

Takagi, J., Springer, T.A., 2002. Integrin activation and structural rearrangement. Immunol. Rev. 186, 141–163.

Tamkun, J.W., DeSimone, D.W., Fonda, D., Patel, R.S., Buck, C., Horwitz, A.F., Hynes, R.O., 1986. Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. Cell 42, 271–282.

Tarone, G., Galetto, G., Prat, M., Comoglio, P., 1982. Cell surface molecules and fibronectin-mediated cell adhesion: Effect of proteolytic digestion of membrane proteins. J. Cell Biol. 94, 179–186.

Thiery, J.P., Brackenbury, R., Rutishauser, U., Edelman, G.M., 1977. Adhesion among neural cells of the chick embryo: II. Purification and characterization of a cell adhesion molecule from neural retina. J. Biol. Chem. 252, 6841–6845.

van der Flier, A., Sonnenberg, A., 2001. Function and interactions of integrins. Cell Tissue Res. 305, 285–298.

Wartiovaara, J., Linder, E., Ruoslahti, E., Vaheri, A., 1974. Distribution of fibroblast surface antigen: association with fibrillar structures of normal cells and loss upon viral transformation. J. Exp. Med. 140, 1522–1533.

Wilcox, M., Leptin, M., 1985. Tissue-specific modulation of a set of related cell surface antigens in Drosophila. Nature 316, 351–354.

Wilcox, M., Brown, N., Piovant, M., Smith, R.J., White, R.A.H., 1984. The Drosophila position-specific antigens are a family of cell surface glycoprotein complexes. EMBO 3, 2307–2313.

Willingham, M.C., Yamada, K.M., Yamada, S.S., Pouyssegur, J., Pastan, I., 1977. Microfilament bundles and cell shape are related to adhesiveness to substratum and are dissociable from growth control in cultured fibroblasts. Cell 10, 375–380.

Xiong, J.P., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D.L., Joachimiak, A., Goodman, S., Arnaout, M.A., 2001. Crystal structure of the extracellular segment of integrin αvβ3. Science 294, 339–345.

Xiong, J.P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S., Arnaout, M.A., 2002. Crystal structure of the extracellular segment of integrin αvβ3 in complex with an Arg–Gly–Asp ligand. Science 296, 151–155.

Zamir, E., Geiger, B., 2001. Components of cell–matrix adhesions. J. Cell Sci. 114, 3577–3579.