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New Perspectives in Cell Adhesion: RGD and Integrins

ERKKI RUOSLAHTI AND MICHAEL D. PIERSCHBACHER

Rapid progress has been made in the understanding of the molecular interactions that result in cell adhesion. Many adhesive proteins present in extracellular matrices and in the blood contain the tripeptide arginine-glycine-aspartic acid (RGD) as their cell recognition site. These proteins include fibronectin, vitronectin, osteopontin, collagens, thrombospondin, fibrinogen, and von Willebrand factor. The RGD sequences of each of the adhesive proteins are recognized by at least one member of a family of structurally related receptors, integrins, which are heterodimeric proteins with two membrane-spanning subunits. Some of these receptors bind to the RGD sequence of a single adhesion protein only, whereas others recognize groups of them. The conformation of the RGD sequence in the individual proteins may be critical to this recognition specificity. On the cytoplasmic side of the plasma membrane, the receptors connect the extracellular matrix to the cytoskeleton. More than ten proved or suspected RGD-containing adhesion-promoting proteins have already been identified, and the integrin family includes at least as many receptors recognizing these proteins. Together, the adhesion proteins and their receptors constitute a versatile recognition system providing cells with anchorage, traction for migration, and signals for polarity, position, differentiation, and possibly growth.

THE ATTACHMENT OF CELLS TO THEIR SURROUNDINGS IS important in determining cell shape and in maintaining proper cell function and tissue integrity. Such binding helps anchor cells and provides positional signals that direct cellular traffic and differentiation. Most cells possess multiple mechanisms for binding to the structures that surround them. For example, they can bind to extracellular matrices (*1*) or to other cells (*2*).

Extracellular matrices are made up of an insoluble meshwork of protein and carbohydrate that is laid down by cells and that fills most of the intercellular spaces. Matrices in different locations in the body consist of different combinations of collagens, proteoglycans,

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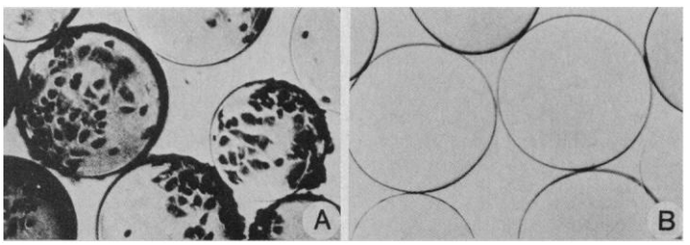

Fig. 1. Normal rat kidney cells attaching to fibronectin-coated beads. The cells attach to Sepharose particles previously coated with fibronectin (**A**). No cell attachment to control beads coated with albumin occurs (**B**). The attachment was allowed to proceed for 1 hour.

elastin, hyaluronic acid, and various glycoproteins such as fibronectin and laminin. Virtually all of the extracellular matrix glycoproteins and collagens that have been identified interact with cells, and much of the control of cellular behavior appears to originate in response to these interactions.

The most readily observable result of the interaction of cells with the extracellular matrix is cell adhesion. The adhesive properties of the extracellular matrix proteins can be easily demonstrated in vitro by plating cells onto a surface coated with extracellular matrix material or with one of the purified matrix proteins. The cells will rapidly adhere to such a surface and spread on it (Fig. 1). However, the adhesive proteins not only promote adhesion, they also stimulate cell migration (*3–5*). Moreover, when confronted with limiting concentrations of an adhesive protein applied as a gradient on a surface, cells move toward the higher concentration (*6*). These examples illustrate a principle that is important because it is likely to be valid in vivo also. The principle is that cells will migrate and localize to places favorable for their adhesion. It appears that migration is favored when a cell receives the traction needed for motility from adhesion but does not adhere strongly enough to become immobilized.

A more complex way in which extracellular matrices influence cells is to promote cell differentiation (*7*). A striking example of an effect on the expression of a differentiated cellular phenotype is the formation of neurites by neurons plated on laminin (Fig. 2). This effect has generated considerable interest in neurobiology, because it may be possible to use it to restore the function of injured nerves or even central nervous tissue (*8*). The matrix may exert its effect on cell differentiation by acting as a competence-inducing factor making cells capable of responding to hormones or other soluble factors (*9*), or the matrix itself may provide an inductive signal (*10*).

Probably the most important effect of matrices on cells is illustrated by the fact that normal cells require attachment to a substrate for survival and growth. This anchorage dependence manifests itself in the inability of normal cells to grow in semisolid media such as soft agar (*11*). Because of the discovery of a recognition sequence common to many extracellular matrix molecules and the isolation of cell surface receptors recognizing this sequence, much progress has been made recently in understanding the molecular mechanisms of the cell-extracellular matrix interactions. This article reviews some of these developments.

Tripeptide Recognition Sequence

Elucidation of the amino acid sequence of the cell-attachment domain in fibronectin and its duplication with synthetic peptides established the sequence Arg-Gly-Asp (RGD) (*12*) as the essential structure recognized by cells in fibronectin (*1, 13, 14*). When immobilized onto a surface, the RGD-containing peptides promote cell attachment in a manner similar to that of fibronectin, whereas in solution the same peptides can inhibit the attachment of cells to a surface coated with fibronectin, the peptides themselves, or other RGD-containing proteins (*14–16*). Changes in the peptides as small as the exchange of alanine for the glycine or glutamic acid for the aspartic acid, which constitute the addition of a single methyl or methylene group to the RGD tripeptide, eliminate these activities (*17*). The RGD tripeptide should occur more than 400 times among all known protein sequences. In fact, 183 RGD sequences (including 33 species duplications) were found in a computer search through the protein sequence database of the National Biomedical Research Foundation.

Most of these sequences are probably not recognized by RGD-directed cell surface receptors. However, the RGD sequence is the cell recognition site of a surprising number of extracellular matrix and platelet adhesion proteins. Vitronectin (*16*), type I collagen (*18*), fibrinogen (*19*), von Willebrand factor (*19*), and osteopontin (*20*) each contain one or more RGD sequences, and their interaction with cells can be inhibited with RGD-containing peptides. These proteins, therefore, almost certainly have the RGD sequence as their cell recognition site. Less direct evidence indicates that this site is in a number of other extracellular matrix proteins. Thus, thrombospondin and collagens other than collagen type I may also belong to this class of proteins since each of them contains one or more RGD sequences and mediates cell attachment (*17, 21*). One of the cell attachment sites of laminin may be an RGD sequence because one of the receptors that recognizes laminin is related to a fibronectin receptor (*22*).

Despite the similarity of the cell attachment sequence in the various adhesive proteins, cells can recognize them individually. This specificity is provided by a number of receptors, integrins (*23*), each of which is capable of recognizing only a single RGD-containing protein ligand, or in some cases a limited number of ligands. The RGD-containing peptides have been instrumental in the identification of these receptors.

Receptors for the RGD Sequence and the Integrin Superfamily

Isolation of RGD-directed receptors. Affinity chromatography on Sepharose that carries the appropriate, covalently bound, adhesion protein allows one to isolate RGD-directed cell surface receptors from cell extracts (*24, 25*). Specific elution of the material bound to the affinity matrix is accomplished with a peptide containing the RGD sequence. The use of fibronectin as the affinity ligand yields a fibronectin receptor that is a heterodimer of a 160-kD α subunit and a 140-kD β subunit (*24*). If vitronectin is used as the ligand, a vitronectin receptor is obtained (*25*). Yet another receptor binds to type I collagen and to an RGD-containing peptide that assumes a collagen-like triple helical structure (*18*). All these receptors have been isolated from the same cloned osteosarcoma cells. Thus, these cells possess at least three different adhesion receptors that recognize the RGD sequence in their individual ligands. The fibronectin receptor and RGD-directed receptors related to it have also been isolated from mammalian and chicken cells by using antibodies that inhibit cell attachment (*22, 26*). A complex of chicken receptors obtained in this manner recognizes fibronectin but also other adhesion proteins (*22*). Its relation to the mammalian fibronectin receptor is discussed below.

Fractionation of platelet extracts by affinity chromatography on fibrinogen-Sepharose yields a fourth RGD-directed receptor (*27, 28*). This receptor is indistinguishable from the previously characterized platelet protein gp IIb/IIIa (*29*). This protein as well as the

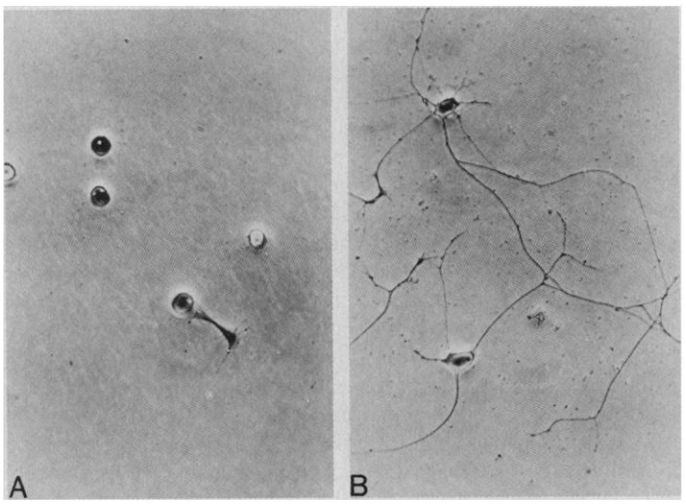

Fig. 2. Neurite-promoting activity of laminin. Chicken ciliary ganglion neurons were cultured on a plastic surface coated with polyornithine (**A**), which supports the attachment of neurons but does not promote neurite formation, or on the same polyornithine surface treated with laminin (1 μg/ml) (**B**). The laminin-treated surface supports extensive sprouting of neurites during the 24-hour culture. [Courtesy of E. Engvall and M. Manthorpe]

vitronectin receptor can also be isolated by means of Sepharose that carries an RGD-containing heptapeptide as the affinity matrix. The fibronectin receptor and the collagen receptor, however, do not have affinities for the short peptides sufficient to allow them to bind to the peptide matrix. The decrease in binding affinity of the fibronectin receptor for the GRGDSP peptide as compared to fibronectin is 100- to 1000-fold (*14, 30*), whereas the affinities of the protein and peptide ligands for the vitronectin receptor and gp IIb/IIIa differ no more than 10-fold in each case (*16, 19*).

The mammalian RGD-directed receptors are typically heterodimers of two subunits (Fig. 3) (*25, 27*). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with and without prior treatment with a reducing agent shows that the α subunits of the fibronectin receptor, vitronectin receptor, and gp IIb/IIIa consist of two disulfide-linked polypeptides. These two polypeptides arise from proteolytic cleavage of a precursor. The sizes of the α subunit polypeptides are about 20 kD for the light chain and 120 to 140 kD for the heavy chain. The β subunits, on the other hand, are single polypeptides that range in size from 90 to 140 kD. The β subunit migrates much faster in SDS-PAGE when it is not reduced, suggesting a compact structure resulting from extensive disulfide bonding. As it was realized that this heterodimeric structure was characteristic of the RGD-directed receptors, it became apparent that three other, independently identified, protein families were structurally very similar. One of these was a group of adhesive leukocyte surface proteins that comprises the molecules LFA-1, Mac-1, and p150,95 (*31*); the second one was a group of proteins called "very late antigens of activation" (*32*); and the third was the so-called "position-specific antigens" of *Drosophila* (*33*). These molecules together with the RGD receptors form the integrin superfamily.

Integrin receptor superfamily. The three leukocyte proteins mentioned above consist of a 95-kD β subunit that is identical in all three proteins and an α subunit that is distinct in each protein (*34*). Like the β subunits of the RGD-directed integrins, the leukocyte β subunit also has a compact structure caused by disulfide bonding. However, unlike the RGD-directed receptors, the α subunits of these proteins are not proteolytically processed. The leukocyte proteins also mediate adhesive interactions. The molecule LFA-1 contributes to the binding of killer T cells to target cells; Mac-1 is the macrophage receptor for complement component C3bi; and p150,95 (so-called because of the size of its polypeptides), along with the others, appears to be important for the binding of leukocytes to endothelial cells as the leukocytes exit into tissue from the circulation (*31, 35*). Whether these leukocyte receptors also recognize an RGD sequence is not known. The human C3bi contains an RGD, and the Mac-1 receptor appears to recognize the region of the molecule containing this sequence (*36*). Recent amino acid sequence data from complementary DNA (cDNA) cloning work provide definite evidence that the leukocyte receptors are related to the RGD-directed receptors and, therefore, belong to the integrin superfamily.

As with the leukocyte receptors, the other integrins can also be grouped on the basis of the identity of their β subunit. The very late antigens, or VLA proteins, are a family of proteins initially identified at the surface of stimulated T cells. These proteins are defined by a monoclonal antibody that reacts with a 130-kD β subunit shared among five proteins, each of which has a different companion α subunit (*32*). One of these proteins is the fibronectin receptor; the fibronectin receptor family, therefore, consists of at least five different proteins, each of which may itself be a receptor. The chicken adhesion receptor complex, also known as the "CSAT" complex, probably corresponds to the human fibronectin receptor family (*22, 32*). Another family of integrins includes the vitronectin receptor and gp IIb/IIIa, which appear to have very similar β subunits (*37, 38*). Table 1 lists the integrins that have been characterized in some detail.

The *Drosophila* position-specific antigens have been identified with monoclonal antibodies prepared against imaginal disk tissue from *Drosophila* larvae (*33*). These antigens are distributed in the imaginal disks following the dorsoventral boundaries of the future body structures of the fly, hence the term "position specific." They consist of a group of heterodimeric proteins that have one subunit in common and a variety of companion subunits. The companion subunits are processed into two disulfide-linked polypeptides in a manner similar to the α chains of some of the RGD-directed receptors. The NH_2-terminal amino acid sequence of one of the variable polypeptides has been determined (*33*), and it was found to

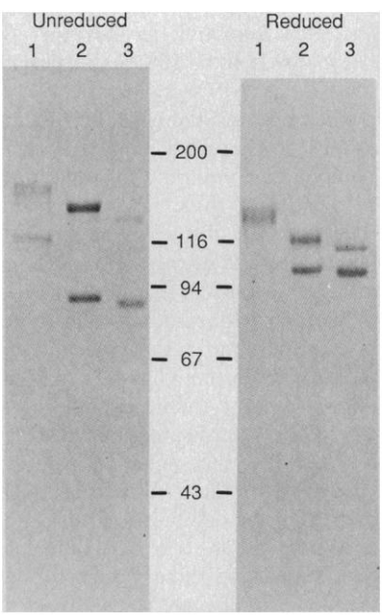

Fig. 3. Electrophoretic analysis of purified RGD-directed receptors. Three purified receptors, the fibronectin (lanes 1), the vitronectin (lanes 2) receptors isolated from human placenta, and the platelet RGD-directed receptor gp IIb/IIIa (lanes 3), were separated by SDS-PAGE when unreduced (left) or after reduction (right). Each receptor is a heterodimer, but the two subunits of the fibronectin receptor run together after reduction. The 20-kD polypeptides that separate from the larger (α) subunit under reducing conditions (Fig. 4) are not visible in the 7.5% gel used here. The double band of the fibronectin receptor α subunit is probably due to isoforms of this subunit. The positions of reduced molecular weight markers are shown in kilodaltons. [Modified from (*27*) with permission, copyright 1986 by AAAS]

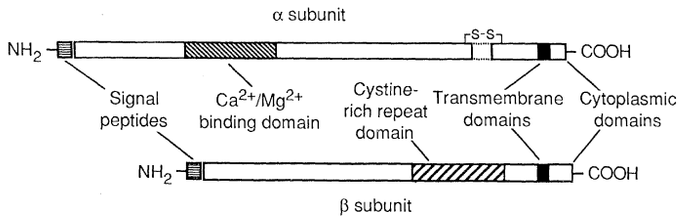

Fig. 4. General polypeptide structure of integrins. The α subunit of the integrins is translated from a single messenger RNA, and in some cases it is processed into two polypeptides that remain disulfide-bonded to one another. The α subunit as well as the β subunit contain a typical transmembrane domain that is thought to traverse the cell membrane and bring the COOH-termini of the subunits into the cytoplasmic side of the membrane. The α subunits contain a series of short sequence elements homologous to known calcium-binding sites in other proteins; the β subunit is tightly folded by numerous intrachain disulfide bonds.

be significantly homologous to the NH_2-termini of the α chains of the integrins. Thus, it seems that the position-specific antigens are related to the vertebrate integrins, although more amino acid sequence data are needed to confirm this conclusion. In a recent development, it was found that the affected gene in the *Drosophila* mutant, lethal(1)myospheroid, has strong sequence homology with the vertebrate integrin β chains (*39*). It appears from these results that it will be possible to apply the powerful techniques of *Drosophila* genetics to the study of integrin functions.

Finally, a family of proteins that mediate the reaggregation of dispersed sea urchin embryos cells may also be adhesion receptors because their polypeptide composition is somewhat similar to that of the known integrins (*40*). These molecules, however, are still incompletely characterized. Several of the vertebrate integrins have already been cloned and sequenced, and this has provided useful insight into the structure-function relations in these molecules.

Primary structure of the integrins. The amino acid sequence for the entire human fibronectin receptor has been recently determined from cDNA (*41*), and that of p150,95 has also been completed (*42*). Moreover, combination of subunit sequences (*38, 43, 44*) allows one to assemble the complete sequences of the vitronectin receptor and gp IIb/IIIa (assuming that they have the same β subunit). Finally, one polypeptide of the chicken integrin complex (*45*) has also been sequenced from cDNA. There is no sequence homology between the α and β subunits of any one of these individual integrins, but each α subunit is homologous to the other α subunits and each β subunit to the other β subunits. The extent of this homology is 40 to 50% at the amino acid level. An exception is the high degree of homology (85%) between the chicken integrin polypeptide and the β subunit of the human fibronectin receptor. Because the extent of homology in this case is far greater than the homology between the corresponding subunits of distinct integrins in the family, the chicken polypeptide probably represents the β subunit of the fibronectin receptor family.

On the basis of the amino acid sequences, each subunit of each integrin appears to contain a large extracellular domain, a membrane-spanning segment, and a short cytoplasmic domain (Fig. 4). The location within the extracellular domain of the binding site for the adhesion protein ligand is not known, but both subunits appear to contribute to the ligand binding (*46, 47*). The known receptors among the integrins require a divalent cation such as Ca^{2+} or Mg^{2+} for binding to their ligands (*29, 31, 48*) and Ca^{2+} has been shown to bind to one of the α subunits (*49*). The sequence of the extracellular domain of the α subunit of each integrin contains several sites that are homologous to Ca^{2+}-binding sites in other proteins such as calmodulin, and these sequences, therefore, are likely to represent the Ca^{2+}-binding sites.

The extracellular domains of the integrins contain one further sequence feature of interest; about one-quarter of the β subunit consists of a repeating structure with a high (20%) cysteine content. This structure is obviously responsible for the characteristic change in electrophoretic mobility displayed by the β chains upon reduction. Its function is unknown at present.

The amino acid sequences of integrins strongly suggest that both the α and β subunits span the cell membrane because each polypeptide has a segment with the characteristics of a transmembrane domain near its COOH-terminus. It is very likely that these segments are indeed embedded in the cell membrane because the isolated proteins can be readily incorporated into liposome membranes where they express their receptor activity (*24, 25, 27, 50*). Comparison of the putative transmembrane domains in the human fibronectin receptor β subunit and its chicken homolog reveals a complete conservation of the amino acid sequence, suggesting precise constraints for the structure of the transmembrane domain. This domain could transmit a signal across the cell membrane, or it might participate in the binding of the β chain with the appropriate α chain. It might also participate in the binding of lipids.

Evidence shows that gangliosides are in some way associated with the adhesion receptors. Certain sialylated gangliosides and antibodies to them can inhibit cell attachment; gangliosides codistribute at the cell surface with the receptors; and gangliosides copurify with the receptors in affinity chromatography (*51*). Perhaps gangliosides modulate the activity, or even the specificity of the receptors.

The portion of the integrin polypeptides extending from the COOH-terminal end of the transmembrane domains is probably cytoplasmic. These cytoplasmic tails of the known subunits range between 28 and 41 amino acids in length. Again, there is a complete conservation of sequence in the cytoplasmic domains of the human fibronectin receptor β subunit and its chicken homolog, suggesting a function critically dependent on that structure. The cytoplasmic tail of this subunit contains a short amino acid sequence that is homologous to a tyrosine phosphorylation site in the epidermal growth factor receptor and the insulin receptor (*45*). Phosphorylation of two of the subunits in the chicken integrin complex has been observed in virally transformed chicken fibroblasts (*52*). This integrin complex has also been shown to have an affinity for talin, a cytoskeletal protein associated with the actin filament network (*53*). Thus, the adhesion receptors may provide a link between the extracellular matrix and the cytoskeleton (*23*). Phosphorylation of

Table 1. Integrin receptor superfamily.

Protein	Ligands	Function
Fibronectin receptor*	Fibronectin	Cell attachment, phagocytosis
VLA-1 VLA-2 VLA-3 VLA-4		
Vitronectin receptor	Vitronectin	Cell attachment, phagocytosis?
gp IIb/IIIa	Fibrinogen, fibronectin, von Willebrand factor, vitronectin	Platelet aggregation
LFA-1	ICAM-1	Cell-cell adhesion
Mac-1	C3bi	Complement binding
p150,95		Cell-cell adhesion

*The integrin complex in chicken also known as the "CSAT" complex (*22, 26, 46, 53*) is likely to be equivalent to the fibronectin receptor family, but individual receptors have not yet been isolated from it.

the cytoplasmic domain may regulate the binding functions of the adhesion receptors. A regulatory mechanism involving phosphorylation could explain the apparent misregulation of receptor function in malignantly transformed cells that lack both an extracellular matrix and an organized cytoskeleton (*53, 54*). However, the β subunit of the leukocyte receptors lacks the critical tyrosine residue in the cytoplasmic tail (*42*), showing that phosphorylation at this site cannot regulate the interactions of the leukocyte receptor family.

Specificity of the adhesion receptor-ligand interactions. Affinity chromatography with various insolubilized adhesive proteins yields a different receptor from the same cell extract in each case (*27*). This indicates that although they have similar target sequences, each receptor has a mutually exclusive specificity at the protein level. Moreover, by using an assay in which one of the receptors is incorporated into liposome membranes and the binding of the liposomes to various surfaces is examined, it has been shown that the fibronectin receptor-containing liposomes bind only to a surface coated with fibronectin and not to a surface coated with vitronectin or with collagen type I (*24, 27*). The vitronectin receptor (*25*) and the collagen receptor (*18*) in liposomes are similarly specific for their own ligands. Despite this specificity, all of these interactions can be inhibited with the RGD-containing synthetic peptides, revealing common underlying mechanisms for this interaction.

The gp IIb/IIIa from platelets, on the other hand, has a different pattern of reactivity. Liposomes containing this receptor can bind to several RGD-containing proteins. These include fibrinogen, fibronectin, vitronectin, von Willebrand factor, and possibly thrombospondin (*27, 50*). Although gp IIb/IIIa appears to be exceptional in its wide specificity, other adhesion receptors may also have additional ligands. A possible example is the chicken integrin complex that can be isolated with the monoclonal antibodies CSAT and JG22 (*22, 26, 53*). These antibodies inhibit the attachment of cells to fibronectin, laminin, and type IV collagen; the receptor complex that can be isolated by affinity chromatography with these antibodies binds to at least fibronectin and laminin (*22, 26, 55*). However, as discussed above, in this case it is possible that the complex represents a mixture of several receptors.

A major question concerns the ability of the adhesion receptors to distinguish among the various ligand proteins despite the fact that many, perhaps all of them, have the same RGD cell attachment signal. One explanation for this could be that the RGD sequence serves as a shared binding site, whereas the specificity is generated by a second binding site unique to each protein ligand. Alternatively, the specificity could reside in the conformation of the RGD tripeptide, and the role of the surrounding sequences would be to force the RGD determinant into an appropriate conformation. Recent data support the latter possibility, but they also suggest that contributions to the binding come from the amino acids adjacent to the RGD sequence, especially from the residue following this sequence (*56*). That the conformation of the RGD sequence would be the main factor in determining the ligand binding would readily explain why some RGD proteins, such as γII crystallin or the *Escherichia coli* λ receptor (*14, 56*), promote cell attachment in vitro for no apparent physiological reason. It is also in agreement with the fact that the RGD sequence can take very different conformations in different proteins (Fig. 5). It may be that the RGD sequences of proteins with incidental cell attachment activity happen to be in the conformation of one of the adhesion protein sequences. An inactive RGD sequence, on the other hand, may either not be available at the surface of the molecule containing it, or, if available, its conformation may not fit any of the receptors.

The RGD sequence may not be the only binding sequence recognized by members of the receptors in the integrin superfamily. The LFA-1–mediated binding of killer lymphocytes to their target cells is not inhibited by the existing RGD-containing peptides (*42*). Perhaps, as seems to be the case with collagen type I (*18*), the LFA-1 may only recognize RGD in a very specialized presentation. Or, this receptor may have a different recognition sequence than RGD. For example, recent evidence suggests that the sequence REDV from fibronectin may also be recognized by cells (*57*). It also seems that a structure mimicking the RGD sequence can be generated by an amino acid sequence substantially different from RGD. The COOH-terminal sequence of the human fibrinogen γ chain is KQAGDV, and it has been shown that peptides containing this sequence bind to gp IIb/IIIa with a specificity similar to that of the RGD-containing peptides (*47, 58*). The corresponding sequence at the end of the γ chain of lamprey fibrinogen is RGDN (*59*), suggesting that the mammalian γ chain sequence has evolved from the RGD sequence in a primordial γ chain. Perhaps those receptors, the function of which is not affected by the existing RGD-containing peptides, recognize related sequences that have evolved from the RGD sequence.

Adhesion receptors and disease. A delicate balance probably exists between attachment and detachment of cells, determining whether a cell will remain stationary, migrate through tissues, or be a circulating cell. This balance is suggested by the fact that lymphocytes and erythroid precursor cells interact with extracellular matrix components at times during their development (*60*), although they lack this capacity when circulating. The effect of adhesion on cell migration may be an important determinant of malignancy. Most malignant cells lack their own extracellular matrix (*54*). Relieved from the constraints of an extracellular matrix of their own, the tumor cells may use their adhesion receptors to facilitate migration through tissues. The involvement of integrins in tumor cell invasiveness is suggested by the observations that RGD peptides inhibit the migration of tumor cells through tissue and restrict metastatic dissemination of tumor cells injected into the circulation (*61*).

Two diseases that stem from a genetic defect of adhesion receptors are also known. In Glanzmann's thrombasthenia, gp IIb/IIIa is missing from the patient's platelets. Such platelets fail to aggregate, and bleeding problems result (*62*). In the "leukocyte adhesion deficiency" syndrome, leukocytes lack the β subunit of the leukocyte receptor family and consequently fail to express any of the members of this receptor family (*31*). In the absence of these receptors, leukocytes cannot migrate into sites of inflammation, and the ability of an individual with this defect to fight infection is severely limited. It seems safe to predict that additional diseases resulting from abnormalities of adhesion receptors and their target ligands will be identified.

The RGD adhesion system also appears to play a role in host-parasite relations. Thus, some microorganisms and parasites such as the syphilis spirochete and trypanosome have been reported to be able to recognize fibronectin in an RGD-dependent manner (*63*). These organisms may have receptors mimicking the host receptors in specificity. The obvious advantage to the pathogenic organism is to facilitate parasitism in the host tissues.

Finally, fibronectin receptors may participate in the clearing of tissue debris through phagocytosis, a function that may be critical in situations where there is substantial tissue destruction, as in trauma cases (*64*).

Prospects

The versatility of the RGD-adhesion receptor system suggests that this system could be particularly important in providing positional signals that determine the location, polarity, and shape of cells in the body. A stylized depiction of a typical adhesion receptor

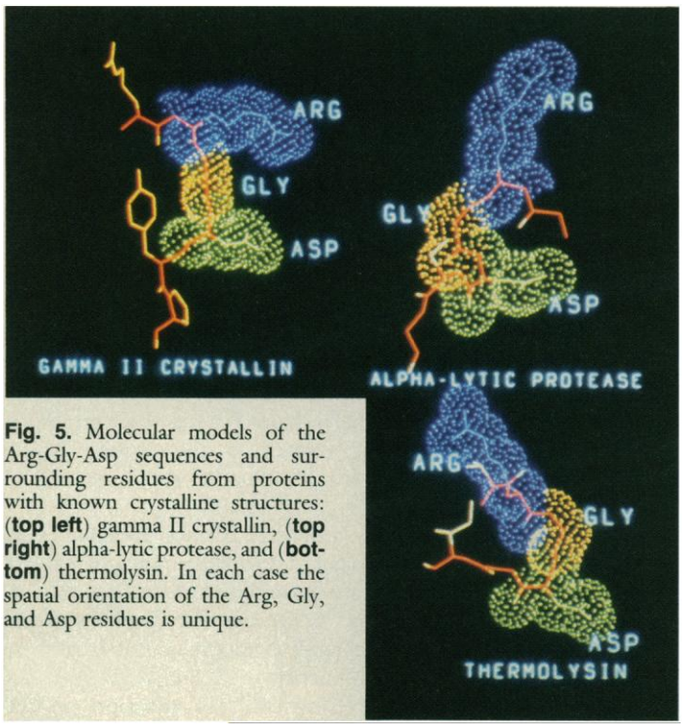

Fig. 5. Molecular models of the Arg-Gly-Asp sequences and surrounding residues from proteins with known crystalline structures: (**top left**) gamma II crystallin, (**top right**) alpha-lytic protease, and (**bottom**) thermolysin. In each case the spatial orientation of the Arg, Gly, and Asp residues is unique.

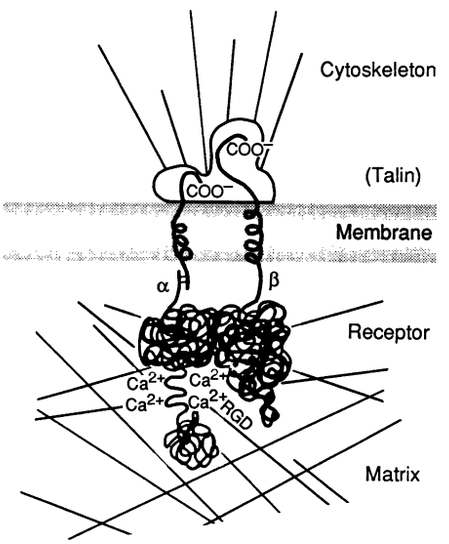

Fig. 6. A model of an adhesion receptor in the cell membrane.

connecting the extracellular matrix with the cytoskeleton is presented in Fig. 6. Whether other signals, such as those regulating cell differentiation and proliferation, are also generated through cell adhesion receptors will be an important aspect to study. Whatever the signals transmitted through the receptor connections are, one would like to know how they are transmitted through the receptors and how the signals from various receptors are integrated into information that directs cellular behavior. Finally, the expression of the adhesion receptors in development appears to be tightly regulated. Could some of the genes known to be important in the generation of the body plan such as the homeobox-containing genes be regulating adhesion receptor expression?

With regard to the ligand-binding properties of the adhesion receptors, it will be important to elucidate the crystalline structures of the adhesive proteins that serve as the ligands to derive the conformations of their RGD sequences. Such information may allow the design of peptides and nonpeptide compounds that more closely mimic the structure of this sequence within adhesive proteins and that, therefore, may have higher selectivities and affinities for the individual receptors than the current peptides. Studies with such peptides as well as gene transfer experiments should help answer the questions posed above.

Adhesion and other signals from the extracellular matrix may exert as much control over the behavior of cells as do hormones and other soluble mediators. The main difference is that the extracellular matrix is insoluble and it, therefore, exerts its effects at a short range and in a geometrically tightly controlled manner. These latter characteristics have made the extracellular matrix signals difficult to study, but, as our understanding of the biology of cell-extracellular matrix interactions develops, it is likely to change many concepts in biology and medicine.

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