

## FIBRONECTIN-INTEGRIN INTERACTIONS

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### 1. ABSTRACT

Fibronectin is recognized by at least ten cell surface receptors of the integrin family. Most cell types in the body can adhere to fibronectin via these receptors, and thereby fibronectin becomes involved in many different biological processes. Three areas related to fibronectin and its receptors which have developed rapidly during the last few years are summarized in this review: the mechanisms of interactions between fibronectin and integrins, fibronectin polymerization, and *in vivo* functions of the proteins as studied by gene targeting in mice.

### 2. INTRODUCTION

The interaction of cells with adhesion proteins in the extracellular matrix (ECM) provides signals which affect the morphology, motility, gene expression and survival of adherent cells (1-3). Many cell adhesion proteins have been identified and extensively characterized. In general, they are large, multifunctional mosaic proteins; some of them can be grouped into protein families, but otherwise they are structurally very diverse. Since each cell adhesion protein has specific effects on cells, their temporal and spatial deposition in ECMs is important for various normal and pathological processes such as formation, maintenance and remodeling of tissues, lymphocyte recirculation, thrombus formation, tumor formation and metastasis.

In contrast to the ligands, most cellular receptors for adhesion proteins of the ECM belong to recirculation, thrombus formation, tumor formation and metastasis.

One protein family, the integrins (1). Additional adhesion receptors for the ECM clearly exist, *e.g.* dystroglycan (4, 5) and syndecans (6, 7), but the integrins have a dominating role in the anchoring of cells to the ECM. At present, 22 different integrins and several additional splice variants have been identified, a specific subset of them being expressed by each cell. Thus, the appropriate capacity for receiving information from ECMs of varying composition is available for the cells.

The topic of this review is the interaction of one cell adhesion protein, fibronectin, with its various integrin receptors. The focus is on the mechanisms of ligand binding, and the importance of the interactions for fibronectin fibril formation. In addition, the *in vivo* effects in mouse of disruption of the genes for fibronectin and integrins subunits are discussed. For information about the intracellular responses following fibronectin-integrin interactions, other recent reviews are recommended (8-10).

### 3. DISCUSSION

#### 3.1. Fibronectin

Fibronectin is a prototype cell adhesion protein, widely distributed in the tissues of all vertebrates and a potential ligand for most cell types (11). It is present as a polymeric fibrillar network in the ECM and as soluble protomers in body fluids. The protomer consists of two subunits linked in an antiparallel manner by a pair of disulfide bridges at

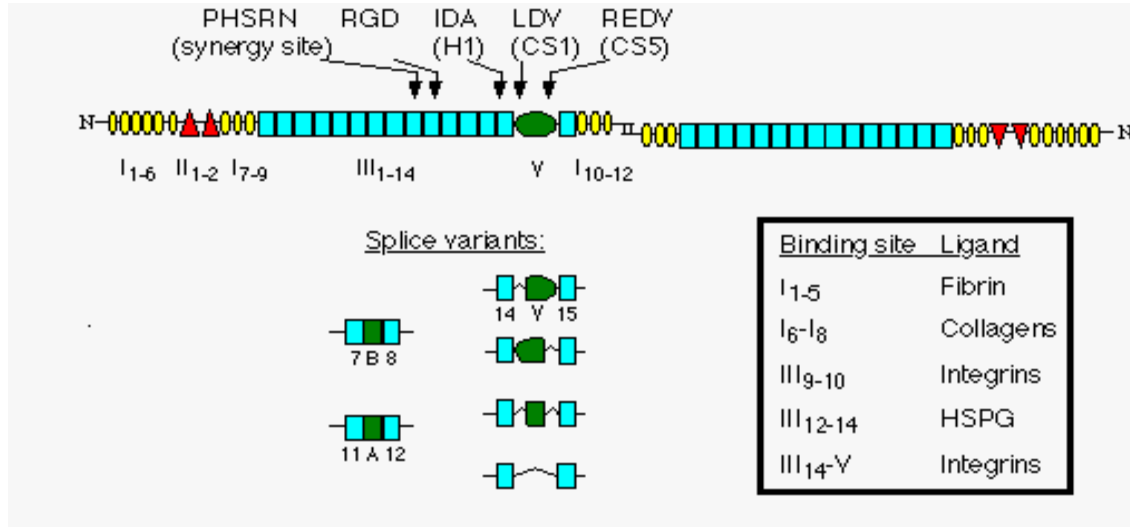
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**Figure 1.** Schematic model of a plasma fibronectin protomer. The protein is a dimer of two subunits which are identical except for the inclusion of the V-segment in one of the chains. The subunits are covalently connected close to the C-termini by two disulfide bridges. Three types of homologous structural units called I, II, and III make up most of the protein (represented by small ovals, triangles, and squares, respectively). Integrin binding sites are indicated in one of the subunits by arrows. The fibronectin found in blood plasma is a major form of the protein, but other splice variants are present at other locations and at specific developmental stages. The alternatively spliced exons (III<sub>A</sub>, III<sub>B</sub>, and V) are marked in green and may be present in one or both of the subunits. The known variants of the splicing reactions are illustrated.

the C-termini. Fibronectin is a good example of a mosaic protein since essentially the entire primary sequence, except for the V segment, is comprised of three types of repeating amino acid motifs (Fig. 1). Several variants of the protein are formed by alternative splicing of the pre-mRNA at three positions: the type III repeat units A and B can independently be included or excluded, while the V segment is subject for a more complex splicing pattern (Fig. 1).

Several of the repeating units function together as distinct domains with specific biological activities (12). The domains are separated by short hinge regions which allow the molecule to adopt different overall shapes. The native soluble protomer is believed to have a globular configuration (13, 14), while an elongated form would be adopted during polymerization to fibrils (15, 16). However, it should be noted that the procedures for isolation of fibronectin require unphysiological conditions (4 M urea is commonly used), hence the native state of the protein is really not known. Conformational changes in the fibronectin molecule have been demonstrated to be key events for some of its functions (17-20), but it is not known if the regulation of function is related to the gross shape alterations or to more subtle structural rearrangements.

Two regions in each fibronectin subunit possess cell binding activity: III<sub>9-10</sub> and III<sub>14-V</sub>. The amino acid sequence RGD, a widely occurring cell

adhesive motif originally discovered in fibronectin (21), is located in III<sub>10</sub>. The RGD motif in fibronectin and other cell adhesion proteins is the most important recognition site for about half of all known integrins. The affinity for short peptides containing the RGD sequence varies significantly among these integrins (22, 23), but for all receptors the affinity for larger protein fragments or the intact proteins is orders of magnitude higher than for short RGD-containing peptides (24, 25). This indicates that areas in the vicinity of the RGD site contribute to the contact surface between ligands and integrins. In fibronectin, one such site of major importance for the interaction with some of the fibronectin binding integrins has been located to III<sub>9</sub> (26). This site, which acts in synergy with the RGD site, has the minimal amino acid sequence PHSRN (27) (Table 1, Figs. 1 and 2).

The III<sub>14-V</sub> region is recognized by two integrins (Table 1), and for both of these receptors three binding sites have been identified within this region (Fig. 1). The so called CS1 site has approximately 20-fold higher affinity for the integrins than the other two sites, namely CS5 and H1 (32). However, CS1 as well as CS5 can be spliced out and are therefore available only in some fibronectin molecules, while H1 is present in all forms of the protein. The related sequence motifs LDV and IDA are the key cell binding structures in CS1 and H1, respectively (33, 34). The active site in CS5 has been identified as REDV in human fibronectin, corresponding to RGDV in the rat and

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**Table 1.** Fibronectin-binding integrins.

Receptor	Main binding site in FN <sup>2</sup>	Additional binding sites in FN	Other protein ligands
$\alpha 3\beta 1$	RGD		LN 5
$\alpha 4\beta 1$	LDV in CS1	H1, CS5, (RGD)	VCAM-1
$\alpha 5\beta 1$	RGD	PHSRN	
$\alpha 8\beta 1$	RGD		VN, TN
$\alpha V\beta 1$	RGD		
$\alpha V\beta 3$	RGD		VN, OP, vWF, FG, TSP
$\alpha IIb\beta 3^1$	RGD	PHSRN	FG, vWF, VN
$\alpha V\beta 6$	RGD		TN
$\alpha 4\beta 7$	LDV in CS1		MAdCAM-1, VCAM-1
$\alpha ?\beta 8$	CS1		

<sup>1</sup>Unactivated  $\alpha IIb\beta 3$  in resting platelets does not bind soluble ligands except short RGD peptides, but it can mediate adhesion to surfaces of immobilized fibrinogen or RGD peptide (28). The activated receptor can bind all indicated ligands in soluble as well as immobilized form (29, 30).

<sup>2</sup>Abbreviations: FN, fibronectin; FG, fibrinogen; LN, laminin; MAdCAM, mucosal addressin cell adhesion molecule; OP, osteopontin; TN, tenascin; TSP, thrombospondin; VCAM, vascular cell adhesion molecule; VN, vitronectin; vWF, von Willebrand factor.

bovine proteins (35). Thus, the binding sequence in CS5 shows similarity to both the RGD and the LDV motifs. It is worth noting that integrin binding sites in fibronectin as well as in other proteins usually contain an aspartic acid as one of the critical amino acids.

### 3.2. Integrins

Most integrins function in cell-matrix contacts, some mediate cell-cell interactions, and a few participate in both types of contacts. (1) All integrins are composed of two noncovalently associated subunits, denoted  $\alpha$  and  $\beta$ , which both span the plasma membrane. The combination of  $\alpha$  and  $\beta$  subunits determine the specificity for extracellular ligands as well as intracellular signaling events.

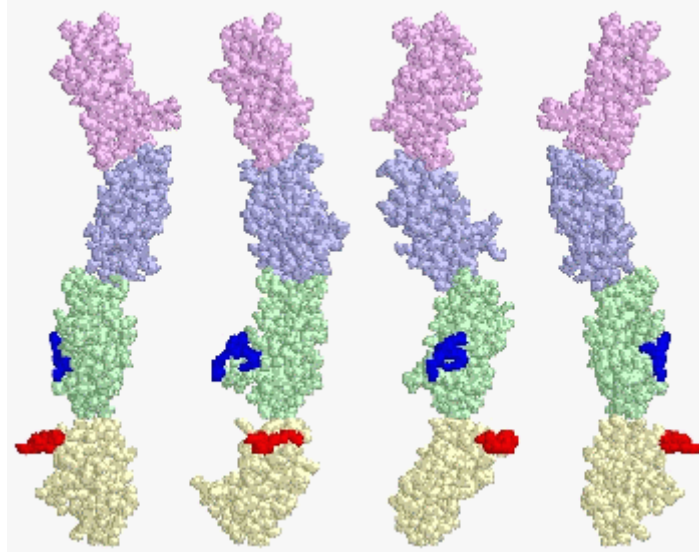
A striking feature of many integrins is the ability to bind multiple ligands. For example, integrin  $\alpha V\beta 3$  serves as a receptor for vitronectin, fibronectin, fibrinogen, von Willenbrand factor, thrombospondin, and osteopontin (36). Since each of these proteins contain an RGD sequence as the dominating recognition motif for integrin  $\alpha V\beta 3$ , the ligands can be envisaged to bind to one common binding site in the receptor. In the case of integrin  $\alpha 1\beta 1$ , laminins and collagens are recognized as ligands, although the two groups of proteins have no known structural similarities. In spite of this and several other examples, there are no indications that there would be separate binding sites for different ligands on an integrin (although the possibility remains open).

It is also commonly found that a particular cell adhesion protein can bind to more than one type of integrin. Fibronectin is an extreme case, for which

binding to ten different integrins have been described (Table 1). This should not be interpreted as redundancy of receptors which could compensate for each other if necessary; rather, it is clear that different intracellular signals are generated by the ligand depending on which integrin it interacts with. The point is most convincingly illustrated by the strikingly different mouse phenotypes obtained after disruption of the various integrin genes (see below) (37, 38).

### 3.3. Molecular mechanisms of ligand-integrin interactions

A central concept for the function of integrins is their ability to shift between active and inactive ligand binding states, by alteration of the conformation of the extracellular domain (39). Transitions between the two states are dynamically regulated by the cell through energy dependent events involving the cytoplasmic tails of the integrin (40). A third conformational state is acquired after ligand-occupation, as demonstrated by exposure of epitopes called ligand-induced binding sites (LIBS) for antibodies. Experimentally, the three conformations can be induced or stabilized by the binding of different monoclonal antibodies to the  $\alpha$  or  $\beta$  subunits at epitopes distinct from the ligand binding site (39). In adherent cells the integrins are mainly in the active state, but possibly become inactivated during specific situations, *e.g.* cell migration and mitosis. In circulating cells the integrins are present on the cell surface in an inactive conformation until the cells are exposed to factors which trigger intracellular reactions leading to activation of the integrins (41, 42).



**Figure 2.** Structure of the fibronectin fragment III<sub>7-10</sub> as determined by X-ray crystallography of the recombinant protein fragment. The fragment is presented in four different angles with a stepwise rotation of 60 degrees. The four repeats are shown in different colors: pale purple, blue, green, and yellow for III<sub>7</sub>-III<sub>10</sub>, respectively. The synergy site (PHSRN) in III<sub>9</sub> repeat is marked in blue color, and the GRGDS loop in III<sub>10</sub> is marked in red. In fibronectin, as well as in many other proteins, several type III units link to each other as an extended array. The interfaces between the units in fragment III<sub>7-10</sub> are highly variable, suggesting that they may be important for the structure and interactions of the protein. The interdomain surface between III<sub>9</sub> and III<sub>10</sub> is smaller than between other units in FN<sub>7-10</sub>. Therefore, they are likely to interact less strongly, and this junction may represent a flexible hinge region. Furthermore, the link between III<sub>9</sub> and III<sub>10</sub> has an unusually small rotation, resulting in the exposure of the RGD loop in III<sub>10</sub> and the synergy site in III<sub>9</sub> on the same side of the molecule. The two sites are separated by 30-40Å, a distance which one integrin molecule could span. The RGD loop is well exposed extending ~10Å away from the body of the molecule. These structure data were obtained from Leahy *et al.*, 1996 (31).

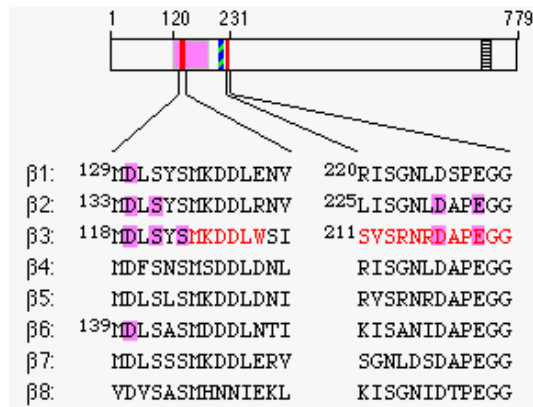
Intimately related to the conformational rearrangements in integrins is their requirements for extracellular divalent cations for ligand binding ability. There is evidence for at least three functionally important coordination sites for divalent cations in the extracellular integrin domains, each one exhibiting different ion preference (43). Mg<sup>2+</sup> is most likely the dominating ion which supports ligand binding to integrins under normal physiological conditions. Mn<sup>2+</sup> stimulates ligand binding to integrins even more strongly, but it is questionable whether this is of physiological relevance. The Mn<sup>2+</sup> effect is due to the fact that the ion can support ligand binding, possibly by the same mechanism as Mg<sup>2+</sup>. Additionally, Mn<sup>2+</sup> is capable of inducing/stabilizing a ligand binding conformation of otherwise inactive receptors (43, 44). Thus, Mn<sup>2+</sup> can activate integrins independently of the intracellular events which normally regulate the extracellular conformation of the receptor (*i.e.* in the presence of Mg<sup>2+</sup>). In contrast, Ca<sup>2+</sup> at millimolar concentrations has an inhibitory effect on the ligand binding ability of most integrins, by inducing the inactive conformation. However, the role of Ca<sup>2+</sup> is complex since low concentrations (μM) appear to stimulate ligand binding. This indicates that Ca<sup>2+</sup> can bind to at least two different sites in integrins (43-45). The number of binding

sites for Mn<sup>2+</sup> has been directly investigated in one study; by equilibrium gel filtration three Mn<sup>2+</sup> ions were found to bind to each αIIbβ3 molecule (46). It is unknown whether Mg<sup>2+</sup> binds to more than one site in integrins, and it is also unclear if any of the metal ion (Me<sup>2+</sup>) sites can accept alternative divalent ions.

Several good candidates for ion coordination sites have been identified in fibronectin binding integrins; different α subunits contain three or four homologous sequences (DxDxDGxxD) which are similar to the Ca<sup>2+</sup>-binding EF-hand motif (47), and a second type of cation binding motif, DxSxS, is located in the β subunits (Fig. 3) (48, 49).

Direct binding of divalent cations has been demonstrated for the latter site; a 14 amino acid peptide from the β3 subunit (β3<sub>118-131</sub>; shown in Fig. 3), which is strongly conserved among all β subunits, was found to bind Mn<sup>2+</sup>, Mg<sup>2+</sup>, or Ca<sup>2+</sup> with 1:1 stoichiometry (46). It is not known if this site has an equally broad ion specificity in the intact receptor. Interestingly, β3<sub>118-131</sub> could also bind RGD peptides (46). It had previously been suggested that one of the integrin-bound ions may be directly involved in bridging to an aspartic acid residue of the ligand (*e.g.* RGD or LDV sites) (50). However, the RGD

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**Figure 3.** Schematic illustration of the integrin subunit  $\beta 1$ . The numbers refer to amino acids starting from the N-terminus of the mature protein. The horizontal black stripes represent the transmembrane part. Both inhibiting and activating monoclonal antibodies bind to the small segment of the protein marked with alternating blue and green lines. The dotted pink and red boxes indicate regions corresponding to those which have been implicated in ligand binding in  $\alpha \text{IIb}\beta 3$ ; RGD peptides have been crosslinked mainly to the pink region, while direct binding of ligands have been demonstrated for the isolated red regions. The red regions are highly conserved among all known  $\beta$  subunits, as shown by the aligned amino acid sequences. Mutation of anyone of the oxygenated amino acids shaded in pink to alanine has been shown to result in inability of the receptor to bind ligand, presumably because coordination of a divalent cation by these amino acids is required for the active protein conformation.

peptides bound also to a mutant form (D119A) of  $\beta 3_{118-131}$  which lacks the ion binding ability. Furthermore, the ion bound to the normal  $\beta 3_{118-131}$  was displaced upon addition of RGD peptides. Ligand binding to an intact receptor had a similar effect, causing two of the three  $\text{Mn}^{2+}$  ions bound to  $\alpha \text{IIb}\beta 3$  to be released (46). Thus, the interactions between integrins and RGD-containing ligands appear to be independent of a direct involvement of divalent ions, but ions and ligands mutually influence their interactions with the receptor through allosteric regulation of the receptor conformation.

### 3.4. Ligand specificity of fibronectin binding integrins

Integrins  $\alpha 5\beta 1$ ,  $\alpha \text{IIb}\beta 3$ , and  $\alpha 4\beta 1$  have been studied in much greater detail than the other fibronectin-binding integrins. These receptors are therefore discussed first, followed by the others in the same order as they appear in Table 1.

**Integrin  $\alpha 5\beta 1$ :** This integrin is a prototype fibronectin receptor in the sense that it was the first to be identified (51), and, in contrast to most other fibronectin binding integrins, it is specialized for

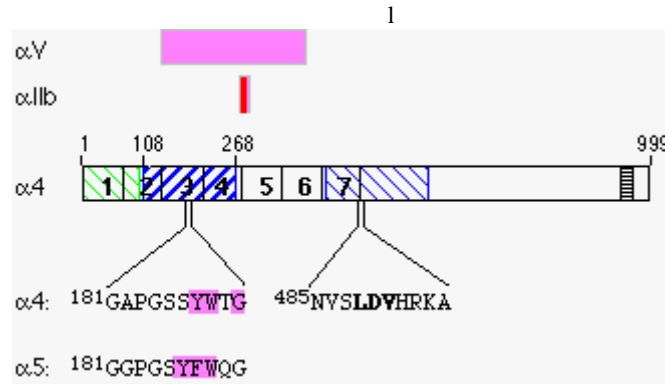
binding this ligand.  $\alpha 5\beta 1$  is expressed by many cell types and is probably the major fibronectin receptor in several of these.

The RGD loop in  $\text{III}_{10}$  is the critical recognition site for  $\alpha 5\beta 1$  (21), but the synergy site PHSRN in  $\text{III}_9$  is required for high affinity binding (27). Screening of peptide libraries for binding to isolated  $\alpha 5\beta 1$  by the phage display technique enriched peptides containing the sequence RGDGW/F (52). Notably, the replacement of the R for a K abolished the binding to  $\alpha 5\beta 1$ , while  $\alpha \text{IIb}\beta 3$  bound almost equally well to both variants (53).

A curious finding from the phage display screening was the strong and specific binding of the peptide RRETAWA to  $\alpha 5\beta 1$  (52). The peptide did not bind to  $\alpha \text{V}\beta 3$  and only weakly to  $\alpha \text{V}\beta 1$ . There is no sequence homology between this peptide and fibronectin (or any other known ECM protein), but still it competes with RGD for binding to the receptor. However, the interaction may be limited to the human integrin since the peptide was not recognized by mouse or hamster  $\alpha 5\beta 1$  (52, 54).

Several different approaches have been taken in order to localize ligand binding sites in  $\alpha 5\beta 1$ . One such approach was the use of phage display screening of peptide libraries for binding to the  $\text{FNIII-10}$  fragment. The peptide sequence WDDG/LWL (55), which is similar to a conserved region in RGD-binding  $\beta$  subunits (135-140 in  $\beta 1$ ; 124-129 in  $\beta 3$ ), was found to be enriched in this screening. Furthermore, this sequence is located within the RGD/ $\text{Me}^{2+}$  binding 14 amino acid peptide of  $\beta 3$  (see above, Fig. 3) which was identified by an independent method (55) (see further under  $\alpha \text{IIb}\beta 3$ ). Cyclic WDDGWL peptide was found to bind directly to RGD peptides; this interaction was independent of cations (55), confirming the result with the D119A mutant of  $\beta 3_{118-131}$ . Both activating and inhibiting monoclonal antibodies have been mapped to a small region of  $\beta 1$  (amino acids 207-218) close to the above suggested ligand binding site (56). A sharp and flexible turn is predicted to form within this region by the sequence TNKG (amino acids 206-209). The likely contribution of the  $\alpha$  subunit to the ligand recognition has been investigated by systematic mutagenesis within an  $\alpha 5$  segment corresponding to a region in  $\alpha 4$  in which epitopes for several inhibiting antibodies against  $\alpha 4\beta 1$  are located (Fig. 4). Ala-scanning of the third N-terminal repeat of  $\alpha 5$  showed that mutations Y186A, F187A, and W188A within a predicted  $\beta$ -turn inhibit ligand binding (57). The strongest effect was obtained by the mutation of F187 (corresponding to Y187 in  $\alpha 4$ , see below). It is yet not known if these mutations indicate the location of an important contact surface with fibronectin, or if they prevent ligand binding indirectly due to conformational alterations of the receptor.

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**Figure 4.** Schematic illustration of the integrin subunit  $\alpha 4$ . The numbers above the bar refer to amino acids starting from the N-terminus of the mature protein. The squares numbered 1-7 represent repeating sequences of ~70 amino acids which are present in the N-terminus of integrin  $\alpha$  subunits. The last three, or in some subunits four, of these repeats each contain a putative divalent cation binding motif. The horizontal black stripes represent the transmembrane part. The epitopes for antibodies which inhibit ligand binding have been mapped to the region marked with bold blue lines. Within this region, the amino acids shaded in pink have been demonstrated to be important for the ligand binding ability of the receptor. Analogous results were obtained for the corresponding residues in  $\alpha 5$ . The location of the cross-linking regions in  $\alpha V$  for RGD peptides and in  $\alpha IIb$  for fibrinogen  $\gamma$ -chain 12 amino acid peptide, respectively, are indicated by the pink boxes above  $\alpha 4$ . The isolated red region of  $\alpha IIb$  has been shown to bind directly to fibrinogen via the  $\gamma$ -chain peptide. Antibodies which induce homotypic aggregation of lymphocytes bind to the N-terminal part (thin green lines), while epitopes for antibodies which can block this activity are located in the region marked with thin blue lines. An LDV sequence which may be the binding site for a homotypic receptor interaction is located within this region.

**Integrin  $\alpha IIb\beta 3$ :** Apart from platelets, in which  $\alpha IIb\beta 3$  is the major cell surface protein (29, 58), this integrin has only been detected on megakaryocytes (59). Its most important function is to bind fibrinogen during thrombus formation. However, the recognition of fibronectin and several other RGD-containing proteins is probably also physiologically relevant for haemostasis (30).

Both the RGD site and the synergy site DRVPHSRNSIT contribute to the binding of fibronectin to  $\alpha IIb\beta 3$  (60, 61), resembling the binding to  $\alpha 5\beta 1$ . However, the direct binding of the isolated synergy region can only be demonstrated for  $\alpha IIb\beta 3$ , even though the interaction is ~30-fold weaker compared to RGD peptides (60). Each one of the peptides can efficiently inhibit the binding of fibronectin (or fibrinogen) to isolated receptor or to platelets. Interestingly, the two fibronectin peptides cross-inhibit each other in binding to  $\alpha IIb\beta 3$  (60), but still they appear to have separate binding sites (see below). A possible explanation for these findings would be that the integrin acts as a closing trap after triggering at either of the two ligand-binding sites. According to this model the unoccupied site would become closed as a result of the conformational rearrangements induced by binding of one of the peptides, while the corresponding event may result in cooperative binding at both sites when the intact fibronectin molecule is available.

Since short RGD peptides bind with higher affinity to  $\alpha IIb\beta 3$  and  $\alpha V\beta 3$  than to other integrins, it has been possible to use the peptides in cross-linking experiments to localize binding sites within these receptors. The RGD-containing peptides were found to cross-link mainly to amino acids 109-172 in  $\beta 3$  of  $\alpha IIb\beta 3$  (62), and to 61-203 in  $\beta 3$  of  $\alpha V\beta 3$  (63). Subsequently, the synthetic peptide  $\beta 3_{118-131}$  was identified as an RGD-binding site (46). This region also contains the DXSXS motif which is involved in coordination of one  $Me^{2+}$  (46). Based on molecular modeling and mutagenesis by Ala-scanning, two additional amino acids, D217 and E220, were suggested to contribute to the ion binding site. Mutation of either one of these five oxygenated amino acids results in inactivation of  $\alpha IIb\beta 3$  (49). The importance of the region containing D217 and E220 was further demonstrated when overlapping synthetic peptides spanning the N-terminal 288 amino acids were tested for the ability to inhibit binding of fibronectin and fibrinogen to  $\alpha IIb\beta 3$  (64).

The fact that  $\alpha V\beta 3$  does not recognize the synergy site of fibronectin suggests that the  $\alpha$  unit in  $\alpha IIb\beta 3$  contains an important contact surface for the binding of that region. There is no direct data available to support this assumption, but the information obtained for the interaction of fibrinogen with  $\alpha IIb\beta 3$  may be relevant for this issue. Fibrinogen binds to the receptor via RGD site(s) as well as by a 12 amino acid peptide from the C-terminus of the  $\gamma$  chain (a non-RGD site). The  $\gamma$  chain



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peptide was found to crosslink mainly to amino acids 294-314 in repeat 5 of  $\alpha$ Ib (65). Further, the peptide  $\alpha$ Ib<sub>296-306</sub> binds directly to fibrinogen and inhibits binding of fibrinogen to  $\alpha$ Ib $\beta$ 3 (66). It remains to be tested if  $\alpha$ Ib<sub>296-306</sub> is the binding site also for the fibronectin synergy peptide. The peptide represents the second metal ion binding site in  $\alpha$ Ib, corresponding to the first in  $\alpha$ 4 (amino acids 278-297). It has been speculated that the second cation which is displaced from  $\alpha$ Ib $\beta$ 3 upon ligand binding would be released from  $\alpha$ Ib<sub>296-306</sub> (see above) (46).

**Integrin  $\alpha$ 4 $\beta$ 1:** The function of  $\alpha$ 4 $\beta$ 1 has been studied mainly in white blood cells, but it is expressed also by several types of adherent cells (67-69). It can mediate cell-cell contacts as well as cell-matrix contacts through the two known ligands, VCAM-1 and fibronectin, respectively (34). Both these interactions are important for the process of leucocyte extravasation, and thereby for a number of immunological and inflammatory events. Activating signals are required to induce strong binding of  $\alpha$ 4 $\beta$ 1 to VCAM-1 or fibronectin, but even in an unactivated state  $\alpha$ 4 $\beta$ 1 is able to mediate a weak binding to VCAM-1, by which lymphocytes can roll along activated endothelial cells (70, 71).

$\alpha$ 4 $\beta$ 1 interacts primarily with the III<sub>14</sub>-V region in fibronectin. Among the three known binding sites within this region, CS1 is predominant in the fibronectin splice variants where it is present (32). CS1 and CS5 cross-inhibit each other for binding to  $\alpha$ 4 $\beta$ 1 (35), but the affinity of recombinant fibronectin fragments for the receptor is higher when both of the sites are included (32), suggesting that they are not competing for the same binding site. The results thus resemble those obtained for the interactions of the RGD and synergy sites with  $\alpha$ 5 $\beta$ 1 and  $\alpha$ Ib $\beta$ 3, although the combined effect of CS1 and CS5 is less dramatic and therefore said to be additive rather than synergistic. H1 was reported not to inhibit the binding of CS1 to  $\alpha$ 4 $\beta$ 1 (72), but higher concentrations of H1 have to be tested, as well as the reverse inhibition experiment, before a firm conclusion can be made. Obviously, determination of the structure of the III<sub>14</sub>-V region at atomic resolution would greatly increase our understanding of how the multiple cell-binding sites may cooperate.

$\alpha$ 4 $\beta$ 1 can be induced to recognize also the RGD region of fibronectin (73). The interaction is detectable only in the presence of integrin activating antibodies, hence its physiological relevance remains to be determined. Nevertheless, under these conditions the binding of large RGD-containing fibronectin fragments to the integrin can be inhibited by short peptides with the efficiency decreasing in the order CS1>GRGDSPC>H1 (73).

Monoclonal antibodies against  $\alpha$ 4 which inhibit binding to CS-1, as well as VCAM-1, map to amino acids 108-268 (the so called B epitopes) (74, 75). Ala-scanning mutagenesis within this region revealed that Y187A, W188A, and G190A inhibit binding of CS-1 and VCAM-1 (57). These amino acids are part of a predicted  $\beta$ -turn between two  $\beta$ -sheets in the third N-terminal repeat of  $\alpha$ 4 (76). This repeat lacks cation binding motifs. Mutations in the corresponding turn in  $\alpha$ 5 also inhibits binding to fibronectin (see above) (57).

Another activity of  $\alpha$ 4 $\beta$ 1 which is inducible by certain monoclonal antibodies against  $\alpha$ 4 is aggregation of lymphoid cells (77-79). These monoclonal antibodies have all been mapped to the N-terminal 100 amino acids (A epitopes) (74, 75). Antibodies capable of blocking the induced aggregation bind to amino acids 422-606 of  $\alpha$ 4 (C epitopes) (74, 75). Since there are no known ligand for  $\alpha$ 4 $\beta$ 1 on lymphocytes, the possibility of homotypic binding of this receptor to itself has been suggested. In support of this concept,  $\alpha$ 4 $\beta$ 1 on lymphocytes was shown to bind to an LDV-containing region of the isolated  $\alpha$ 4 chain (LDV<sub>488-490</sub> located within the C epitope region) (80). Although the data are convincing, they raise several questions: In which physiological situations would homotypic  $\alpha$ 4 $\beta$ 1 interactions occur? Could the interactions reflect lateral interactions between integrins in the same cell (allowing high avidity binding to an unknown ligand) rather than binding between integrin molecules on different cells? Is homotypic binding of  $\alpha$ 4 $\beta$ 1 restricted to certain cells (*i.e.* lymphocytes), since expression of  $\alpha$ 4 $\beta$ 1 in K562 (erythroleukemia) or CHO cells did not induce aggregation of the cells (81). Is the ability of homotypic interaction unique for  $\alpha$ 4 $\beta$ 1 or shared by other integrin(s)?

**Integrin  $\alpha$ 3 $\beta$ 1:** The normal distribution of this integrin *in vivo* is limited mainly to epithelial cells in the skin, endothelial cells of the digestive tract, and kidney mesangial cells (82-85). In contrast, it is expressed by most cells in culture and by many tumors *in vivo* (86, 87). Laminin 5 (kalinin) appears to be an important ligand for the receptor (88, 89), while a weak binding to fibronectin and several other ligands has been observed only under certain conditions (82, 90-92). Further,  $\alpha$ 3 $\beta$ 1 is unable to mediate initial cell adhesion to any of these proteins except for laminin 5, indicating that it does not function as a fibronectin receptor in intact cells (93, 94). The issue is confused by the fact that  $\alpha$ 3 $\beta$ 1 localizes to focal contacts in cells seeded on fibronectin; however, this localization was shown to occur independently of which protein the cells adhered to (85).  $\alpha$ 3 $\beta$ 1 has therefore been suggested to serve as a secondary receptor with post adhesion functions (*e.g.* signaling for growth?) (85). It is unknown whether low affinity interactions with

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fibronectin or other matrix molecules would be of any importance for such functions.

**Integrin  $\alpha 8 \beta 1$ :** This receptor is expressed mainly in epithelial cells, smooth muscle cells, myofibroblasts, and embryonic neural cells (95, 96). It has been shown to bind to RGD sites in fibronectin and vitronectin (97). In addition, tenascin-C has been reported to bind to  $\alpha 8 \beta 1$  via both RGD-dependent and RGD-independent interactions (97).  $\alpha 8 \beta 1$  is able to mediate cell adhesion and neurite outgrowth on these ligands (98, 99). Several other RGD-containing proteins, collagens, and CAMs were not recognized by  $\alpha 8 \beta 1$  (97).

**Integrin  $\alpha V \beta 1$ :**  $\alpha V \beta 1$  is present on the surface of some cell lines, and appears to recognize fibronectin as its major ligand (100, 101). Although the subunits  $\alpha V$ ,  $\beta 1$ , and  $\beta 3$  are expressed simultaneously by many cells, the combination  $\alpha V \beta 1$  is seldom detected. This raises the question of how the formation of multiple integrins is regulated inside a cell. Is it just the relative amounts of the different subunits that determine which heterodimeric receptors are formed (simple competition on equal terms), or do the  $\alpha$  and  $\beta$  subunits have some preferred partners?

**Integrin  $\alpha V \beta 3$ :** This integrin is abundantly expressed by many cultured cells, but its distribution *in vivo* is restricted mainly to activated endothelial cells, osteoclasts, and tumor cells (36). It has been demonstrated to be important for angiogenesis and is therefore a potential target for inhibition of tumor growth (102-104).  $\alpha V \beta 3$  was the first vitronectin receptor to be identified (105), but later it was found to bind also fibronectin and several other cell adhesion proteins (28, 106, 107). In all cases the RGD motif in the ligands is of critical importance for binding of  $\alpha V \beta 3$ . As discussed above,  $\alpha V \beta 3$  does not require the synergy site of fibronectin for stable interaction (60, 108).

**Integrin  $\alpha V \beta 6$ :**  $\alpha V \beta 6$  is expressed by epithelial cells during development and wound healing, and in many epithelial tumors (109). In this context it is interesting that the C-terminal 11 amino acids of the cytoplasmic part, which is unique to  $\beta 6$ , contains a proliferation promoting activity (110, 111). The cytoplasmic tail of  $\beta 6$  also contains three regions required for localization of  $\alpha V \beta 6$  to focal contacts (the membrane proximal region and two NPXY motifs) (110); the corresponding regions in  $\beta 1$  and  $\beta 3$  share the same function.

Fibronectin appears to be the main ligand for  $\alpha V \beta 6$  (112), but a weaker interaction with tenascin-C has also been described (113).  $\alpha V \beta 6$  binds fibronectin via the RGD site, and like  $\alpha V \beta 3$ , it does not require the synergy site in fibronectin (114).

**Integrin  $\alpha 4 \beta 7$ :** This integrin is expressed on subsets of lymphocytes (115). Similar to  $\alpha 4 \beta 1$ , it binds to the III<sub>14</sub>-V region in fibronectin, VCAM, and the LDV site in the  $\alpha 4$  subunit (116). In addition, it recognizes MAdCAM-1, an interaction which is important for homing of specific lymphocyte populations to mucosal sites (117).

**Integrin  $\alpha ? \beta 8$ :** The  $\beta 8$  subunit is expressed mainly in brain, spinal cord, kidney, and embryonic muscle (118). The sequence of  $\beta 8$  is rather different from other integrin subunits; for example, the cytoplasmic domain has no similarity to the other  $\beta$  subunits (118). The  $\alpha$  subunit(s) associated with  $\beta 8$  unit has not yet been identified for the fibronectin receptor.  $\alpha V \beta 8$  can form in  $\beta 8$  transfected 293 cells, but it does not bind fibronectin (118, 119). Antibodies specific for the  $\beta 8$  subunit co-immunoprecipitated one or more  $\alpha$  components from sensory neurons (not  $\alpha V$ ) having unreduced Mr ~110-120kD in SDS-PAGE. The antibody could inhibit neurite outgrowth from these cells seeded on either fibronectin, laminin 1, or collagen IV (120). Since this is an unusual combination of ligands for integrins, it appears likely that  $\beta 8$  can combine with several different  $\alpha V$ .

Besides mediating cell adhesion, all integrins can participate in one or more of the

outgrowth, organization of the cytoskeleton and focal contacts, and signaling which affects gene expression,

new extracellular matrix is an activity so far only described for fibronectin binding integrins. In contrast *e.g.* collagens,

spontaneously *in vitro*

Instead, the reaction occurs on the cell surface and involves interaction of fibronectin with integrins and

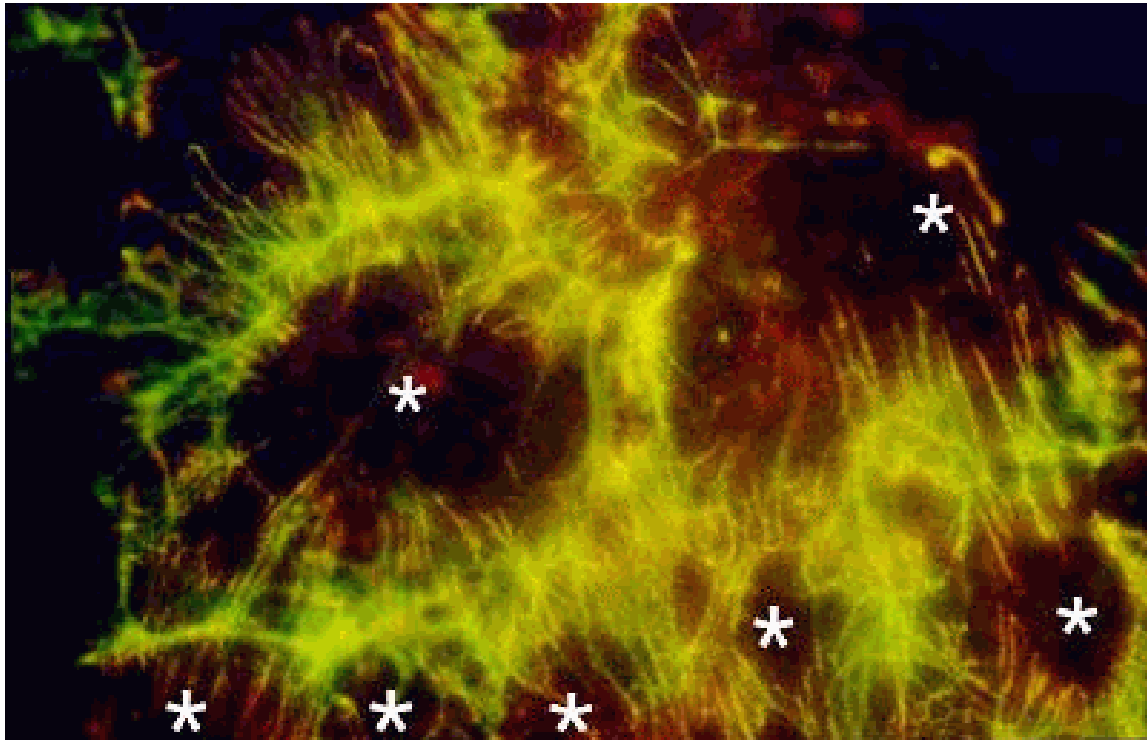
major ECM components such as collagens, fibulins, and proteoglycans (121, 122) may be dependent on interactions with fibronectin for their organized

reaction of soluble fibronectin protomers to insoluble networks is central for matrix formation. The general

fibronectin polymerization (123, 124) further highlights the importance of the process.

$\alpha 5 \beta 1$  was the first integrin identified to be involved in fibronectin network formation. It localizes to the sites where fibronectin fibrils are in contact with the cell , and antibodies to  $\alpha 5$  or  $\beta 1$  can inhibit the polymerization in fibroblast cultures (126). Furthermore, overexpression of  $\alpha 5$  in CHO cells increases the deposition of fibronectin in the matrix (124). However, mouse embryos deficient in the  $\alpha 5$





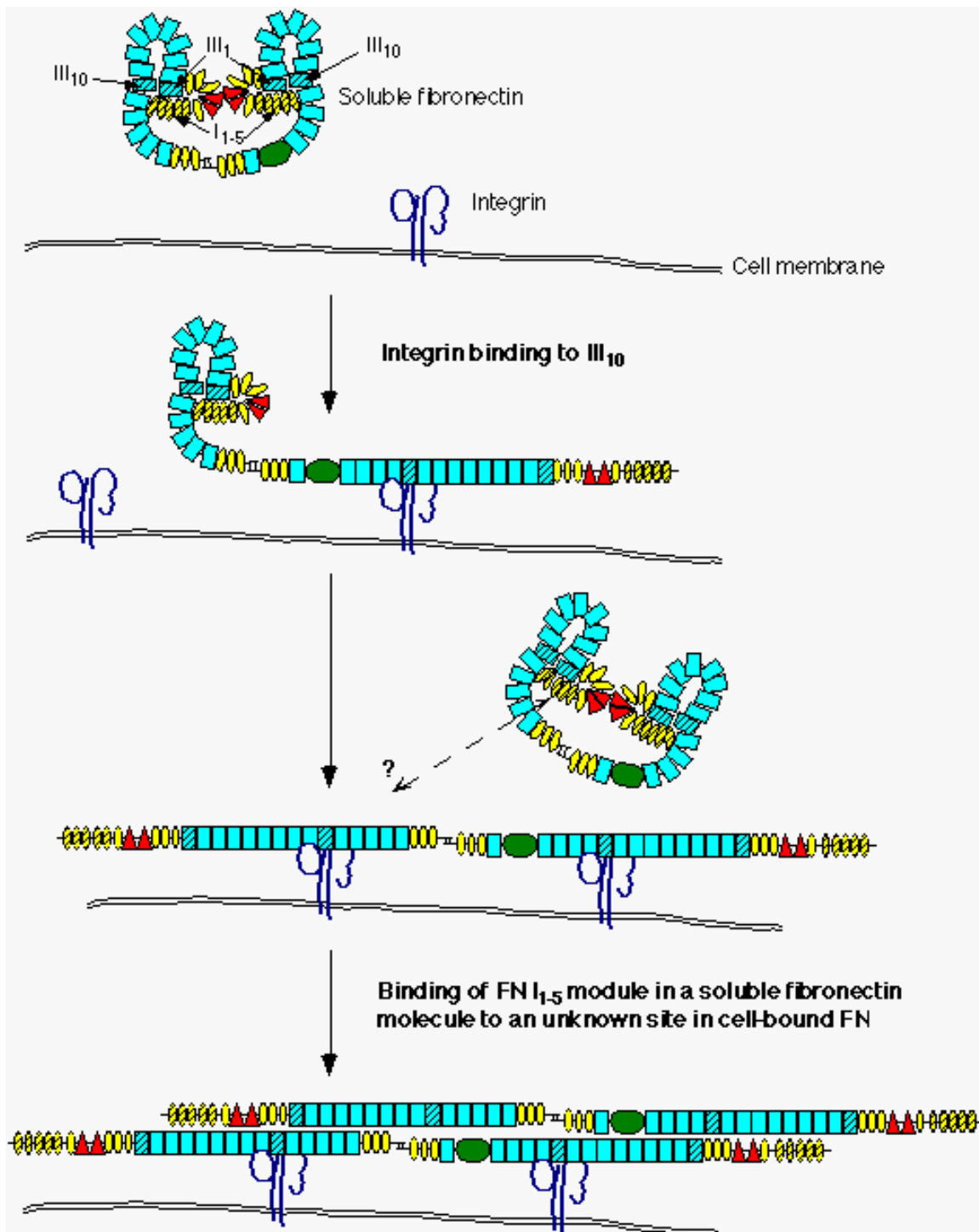
**Figure 5.** Double immunofluorescent staining for fibronectin (green) and integrin subunit  $\alpha 5$  (red) in a culture of GD25- $\beta 1A$  cells (107), grown on coverslips for 5 days before fixing and staining. Cells are marked with asterisks (\*). Note that most fibrils either seem to be stretched between two cells or are positioned at cell-cell borders, and that the ends of the fibrils colocalize with  $\alpha 5$ . The staining was performed as described (107).

gene still contained extracellular fibronectin networks (127), indicating that also other integrins could promote the polymerization process. At present,  $\alpha V\beta 3$  (107) and  $\alpha IIb\beta 3$  (128) have been shown to have this potential, although  $\alpha V\beta 3$  was markedly less efficient than  $\alpha 5\beta 1$ , and  $\alpha IIb\beta 3$  probably never serves this function *in vivo*. Expression of  $\alpha V\beta 1$  (101) or  $\alpha 4\beta 1$  (129) in CHO cells did not promote fibronectin polymerization, illustrating that mere binding of fibronectin to the cell surface by any integrin is not sufficient. A connection of the  $\beta$  subunit to actin filaments is known to be required for fibronectin fibril formation on the cell surface (128, 130). Other factors which may influence whether an integrin has the fibril promoting activity include the recognition site preference in the fibronectin molecule (RGD, LDV, etc.) and the receptor-ligand binding affinity.

The polymerization process can be separated into two phases, initiation (nucleation) and extension, both of which are poorly understood. It has been reported in one study that the fibrils grow at only one end, indicating that they are polarized (131). Alternatively, the direction of fibril growth may reflect the migration of the cell. It is not known if new protomers are added to the fibril at the cell

surface, or somewhere else along the fibril. The issue is further confused by the fact that the fibrils often appear to be anchored at both ends to cell surfaces, either on one cell or on two different cells (Fig. 5). The inability of monomeric fibronectin chains to become incorporated into the fibrils (132) may be relevant in this context.

Several regions in fibronectin are involved in one or the other phases of polymerization, through binding to cell surface components or to a neighbouring fibronectin molecule (Fig. 6). Binding of the RGD region to integrins may be the initial event which triggers the subsequent reactions (126), possibly by induction of an altered fibronectin conformation. The inability of recombinant fibronectin lacking the RGD motif to initiate fibril formation supports this idea (133). However, it is unclear whether fibronectin in its soluble folded conformation can bind to  $\alpha 5\beta 1$ . On hepatocytes  $\alpha 5\beta 1$  was found to recognize the protein only after treatments which are known to unfold fibronectin (*i.e.* interaction with a specific collagen fragment or heparin, limited proteolytic cleavage) (17). Furthermore, fibronectin fragments rather than the intact protein are commonly chosen for affinity isolation of  $\alpha 5\beta 1$  (51, 108, 134). It can be speculated that folding at the suggested hinge between III<sub>9</sub> and



**Figure 6.** Hypothetical model of the initial events in fibronectin polymerization. A soluble fibronectin molecule is bound to the surface of a cell via III<sub>10</sub> to fibronectin-binding integrins. The interaction induces a conformational change in the fibronectin molecule which becomes elongated. The cell bound fibronectin exposes binding sites (unidentified) for I<sub>1.5</sub> in another soluble fibronectin molecule. This interaction will “open up” the second fibronectin molecule, and so on. Pulling force from the actin filament system may be required for the postulated conformational changes. Domains in fibronectin which may be involved in interactions with other fibronectin domains are marked with black stripes.

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III<sub>10</sub> in soluble fibronectin (31) would prevent  $\alpha 5\beta 1$  from reaching both the synergy site and the RGD loop. Thus, modulation of the fibronectin structure by some other interaction may precede its binding to  $\alpha 5\beta 1$ .

The isolated N-terminal I<sub>1-5</sub> domain has been shown to bind cell layers at sites of fibronectin fibril formation, and to inhibit further incorporation of intact fibronectin molecules (135, 136). Deletion or disruption of this functional unit in recombinant fibronectin prevents incorporation of the protein into fibrils (132, 137). The N-terminal domain has been suggested to bind to a non-integrin "matrix assembly receptor" (135), but the nature of the receptor still remains unknown (138). Instead, accumulating evidence indicates that I<sub>1-5</sub> binds either to conformationally altered fibronectin (139, 140) or to a site on  $\alpha 5\beta 1$  which becomes exposed after binding RGD ligands (141). In either of the latter two models, I<sub>1-5</sub> would be involved in fibril extension rather than initiation. The postulated conformational changes could result directly from the interactions or from a pulling force of the actin filament system. The importance of the cytoskeleton has been demonstrated by use of cytochalasin B, and fibronectin fibrils running between two cells appear to be under tension as indicated by immunofluorescent staining (Fig. 5).

A cryptic site in III<sub>1</sub> can bind to I<sub>1-5</sub> (139, 140) and a similar interaction was described between unfolded III<sub>10</sub> and III<sub>1</sub> (142). At present it is not known which of these potential interactions mediate inter- and/or intramolecular bridging between fibronectin domains. The difficulties in studying these events is a major reason why the exact mechanism of the polymerization process still is unsolved.

### 3.6. *In vivo* functions of fibronectin and its integrins receptors

During embryogenesis, fibronectin appears before or at the onset of gastrulation in all vertebrates examined, and it is abundant at times and sites of cell migration: during gastrulation, neural crest cell migration and the migration of primordial germ cells (143). Alternative mRNA splicing is used during development as a mechanism to create different forms of fibronectin within the extracellular matrix by inclusion or exclusion of the III<sub>A</sub>, III<sub>B</sub>, and V segments (144-146). The expression of integrins has also been demonstrated to be developmentally regulated, where some of the fibronectin receptor subunits are continuously expressed, while others are not (147). Injection of antibodies to fibronectin or RGD-containing peptides inhibits gastrulation of several species, indicating that the interactions between fibronectin and integrins are important during that particular stage of development (148-151). To investigate the role of fibronectin and each of its receptors, the genes of both fibronectin and several

integrin subunits have been knocked out by homologous recombination (37, 38, 152). Up to now, disruption of the genes for five  $\alpha$ -subunits ( $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 8$  and  $\alpha V$ ) (127, 153, 154) and three  $\beta$  subunits ( $\beta 1$ ,  $\beta 6$  and  $\beta 7$ ) (155-159) of fibronectin binding integrins has been reported. Since several of the knock-outs are lethal at embryonic stages, the generation of chimeric mice have been necessary in order to investigate the effects on later stages of development and in adult animals. To generate chimeric animals, embryonic stem (ES) cells in which both alleles of the gene of interest have been disrupted are injected into a normal blastocyst. The resulting mouse will have a mixture of cells which are normal and which are homozygous for the disrupted gene. This enables the investigation of the contribution of the null-cells in different organs and in different processes. Further information about the function of the disrupted gene product can be obtained by *in vitro* studies of the ES cells. For example, the differentiation process of the mutated ES cells into specific cell types can conveniently be followed and manipulated in cell cultures.

**Fibronectin:** Mouse embryos lacking fibronectin (fibronectin-null) die at embryonic day 8.5, and they have defects in the development of mesoderm, neural tube and blood vessels (152, 160). Although fibronectin is expressed in normal preimplantation blastocysts, the fibronectin-null blastocysts hatch and implant into the uterine wall. They initiate gastrulation and form mesoderm in the complete absence of embryo derived fibronectin. Nevertheless, the presence of fibronectin at these early stages is believed to be important, since the oocyte alone is probably contributing with enough maternal fibronectin for these processes to occur. From embryonic day 8.0 and onwards the mutant embryos develop deformities and deteriorate during day 10-11 of gestation. Fibronectin-null embryos show shortened anterior-posterior axes, fail to develop certain mesodermally derived structures like notochord and somites, and develop abnormal heart and blood vessels, all probably a result of a deficit in the mesodermal layer. The lack of notochord and somites has later been shown to be a result of fibronectin being critical for the organization or maintenance of the notochord precursor cells and for the condensation of precursor cells into somites (160). Neural folds are formed in the absence of fibronectin, while the neural tube becomes kinked. Primitive red blood cells do develop in the fibronectin-null embryos, while blood vessels do not, strongly suggesting a role for fibronectin in vasculogenesis but not in hematopoiesis.

Since there are several receptors for fibronectin, the total effect of the fibronectin-null mutation is likely to be made up of separate effects due to lack of binding between fibronectin and its individual receptors. Therefore, studies on knock-outs

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of each of the fibronectin receptor subunits make it easier to determine where the different receptors are active, what functions they have, and what their roles are during embryonic development.

**$\beta 1$ :** The  $\beta 1$  subunit can dimerize with at least 10  $\alpha$  subunits, of which five have been shown to bind fibronectin ( $\alpha 3\beta 1$ ,  $\alpha 4\beta 1$ ,  $\alpha 5\beta 1$ ,  $\alpha 8\beta 1$ , and  $\alpha V\beta 1$ ). The homozygous  $\beta 1$ -null embryos develop normally to the blastocyst stage, implant, but die at embryonic day 5.5 (155, 156, 158). The  $\beta 1$ -null trophoblast cells are able to invade the uterine stroma and survive longer than the inner cell mass, suggesting that the inner cell mass requires  $\beta 1$  integrins for survival while the trophoblasts do not. Analysis of the chimeric embryos demonstrated the presence of  $\beta 1$  integrin-deficient cells in all germ layers, indicating that  $\beta 1$ -null cells can differentiate and migrate in the environment provided by most normal tissue. For example, chimeric mice show patches of myocytes that are  $\beta 1$ -null in cardiac muscle, and myotubes show some  $\beta 1$ -null contribution as a result of fusion between wild-type and mutant myoblasts into mixed myotubes. However, lack of the  $\beta 1$  subunit markedly retards the formation of cardiac and skeletal muscles (161, 162) when the differentiation from ES cells was studied *in vitro*. In conflict with previous reports (68, 163), studies using *in vitro* and *in vivo* differentiated myoblasts have proven that the  $\beta 1$  integrin is not necessary for sarcomer or myotube formation (162), events which represent late stages of the differentiation process. Migration of neuronal cells as well as neural crest cells can occur in the absence of  $\beta 1$  integrins. In contrast, the chimeric mice lack  $\beta 1$ -null cells in blood and in haematopoietic organs such as spleen, thymus and bone marrow as a consequence of the inability of  $\beta 1$ -null cells to invade the fetal liver (164). The  $\beta 1$ -null embryos die before the fibronectin-null embryos. The reason for this can be that  $\beta 1$  containing integrins that bind ligands other than fibronectin are important at earlier stages, before the  $\beta 1$ /fibronectin interactions take place. Alternatively, it can be due to the different life-times of the  $\beta 1$  and the fibronectin maternal mRNAs provided by the oocyte.

**$\alpha 5$ :** The  $\alpha 5$  subunit is found only in combination with  $\beta 1$ . The knockout of the  $\alpha 5$  subunit resulted in nearly the same defects as the fibronectin-null mutation did (127), suggesting that the majority of the defects seen in the fibronectin-null embryos probably are due to the lack of  $\alpha 5\beta 1$ /fibronectin binding. The  $\alpha 5$  mutant embryos start to show defects by embryonic day 8.5 and die around day 10-11. The  $\alpha 5$ -null embryos have defects in the posterior trunk and yolk sac mesodermal structures, suggesting a role for  $\alpha 5\beta 1$  integrin in mesoderm formation, movement or function. The morphogenesis in  $\alpha 5$ -null embryos seems to extend further compared to the fibronectin-

null embryos, including formation of notochord, somites and heart, considerable development of the brain, optic and otic anlagen and formation of branchial arches. The anterior part of the  $\alpha 5$ -null embryo develops relatively normally up to about the tenth somite. However, the posterior somites are absent, the paraxial mesoderm are defective, and the neural tube becomes kinked as it does in the fibronectin-null embryos. The  $\alpha 5\beta 1$  defective embryo develops a heart but shows defects in vascular development, although again the defects due to the absence of  $\alpha 5\beta 1$  are less severe and more limited to the posterior part than those due to the absence of fibronectin. It seems that  $\alpha 5\beta 1$  is required for proper formation and maintenance of blood vessels, while other fibronectin receptors are involved in initial steps of vasculogenesis (127).

**$\alpha 4$ :** The  $\alpha 4$  integrins ( $\alpha 4\beta 1$  and  $\alpha 4\beta 7$ ) are both capable of binding fibronectin as well as VCAM-1. The  $\alpha 4$ -null embryos die at embryonic day 11 due to the lack of a functional placenta (154). The allantois fail to fuse with the chorion during placentation and the development of the epicardium and the coronary vessels are impaired leading to cardiac haemorrhage. However, the  $\alpha 4$  subunit seems not to be essential for the formation of either cardiac or skeletal muscle (154, 165). Studies of the VCAM-1 knockout suggest that these  $\alpha 4$ -null phenotypes are a result of abolished  $\alpha 4$ /VCAM-1 interactions rather than  $\alpha 4$ /fibronectin interactions (166, 167). Chimeric mice were also used to study blood cell development (168). During fetal life, T-cell development is  $\alpha 4$  independent, but after birth further production of T-cells becomes  $\alpha 4$  dependent. Precursors for both T- and B-cells require  $\alpha 4$  integrins for normal development within the bone marrow, while monocytes and natural killer cells can develop normally without  $\alpha 4$  integrins.

**$\beta 7$ :** The  $\beta 7$  subunit can dimerize with  $\alpha 4$  and the integrin  $\alpha 4\beta 7$  is expressed on a subset of lymphocytes together with  $\alpha 4\beta 1$ . It has been shown that lack of  $\beta 7$  leads to an impaired formation of the gut-associated lymphoid tissues (159). This is probably a result of inability of the lymphocytes to adhere to the endothelium and subsequently to populate the gut-associated lymphoid tissues. Whether this is a result of disrupted interactions of  $\alpha 4\beta 7$  with VCAM-1, MAdCAM, or fibronectin is not known.

**$\alpha 3$ :** The  $\alpha 3$ -subunit can dimerize with the  $\beta 1$  subunit, and  $\alpha 3\beta 1$  is a receptor for laminin 5 and possibly for fibronectin. The homozygous  $\alpha 3$  mutant mice die during the first day after birth (153). The  $\alpha 3$  mutants have several kidney and lung abnormalities, consistent with the epithelial expression in these organs of normal mice. The tubules in the medullary region of the kidney are dilated, and the bronchial

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airways of the mutant lung are wider than normal. The mutant kidney cells have a greatly decreased number of foot processes along the glomerular basement membrane, and the basement membrane is wider and more disorganized when compared with the normal kidney. Since a recent report indicates that  $\alpha 3\beta 1$  does not really bind fibronectin but that it rather has a secondary function (85) that may be important for fibronectin assembly (169), further investigation is needed to determine if the defects in the  $\alpha 3$ -knockout is a consequence of lack of binding to its major ligand laminin 5, rather than a fibronectin related effect.

**$\alpha 8$ :** The  $\alpha 8\beta 1$  integrin binds fibronectin and is expressed in adult smooth muscle and epithelia, and in mesenchymal and neural cells during development. The  $\alpha 8$  gene has been knocked out, but the original results have not been published yet. Apparently, lack of  $\alpha 8$  results in defect development of the kidney, indicating a role for  $\alpha 8\beta 1$  in kidney morphogenesis (37, 38).

**$\alpha V$ :** The  $\alpha V$  subunit can dimerize with five  $\beta$  subunits, three of which are able to bind fibronectin ( $\alpha V\beta 1$ ,  $\alpha V\beta 3$ , and  $\alpha V\beta 6$ ). The  $\alpha V$  subunit has been knocked out, but the original data has not yet been published. Apparently, the mouse die on the first day after birth, probably as a cause of vascular haemorrhage (37, 38). The  $\alpha V$  containing integrins have a wide range of ligands in addition to fibronectin, and this complicates the interpretation of the results with regard to fibronectin and its functions.

**$\beta 3$ :** The  $\beta 3$  subunit has, to our knowledge, not been knocked out. However, the lack of, or mutations in,  $\beta 3$  lead to bleeding disorders in man (Glanzmann's disease) due to the impaired ability of the platelets to aggregate at injury sites. Surprisingly few other abnormalities are observed in these patients.

**$\alpha IIb$ :** The  $\alpha IIb$  subunit has not been reported knocked out (however, see  $\beta 3$ ).

**$\beta 6$ :** The integrin  $\alpha V\beta 6$  is the only integrin expressed exclusively in epithelial cells and has been found to bind fibronectin and tenascin-C (112, 113). The expression of  $\alpha V\beta 6$  is rapidly induced following epithelial injury while only barely detectable on normal adult epithelia. During embryogenesis,  $\alpha V\beta 6$  is expressed in lung, skin, and kidney. The  $\beta 6$ -null mice have juvenile baldness associated with degenerated hair follicles and infiltration of macrophages into the skin, and accumulation of activated and cytokine producing B- and T-cells around conducting airways in the lungs (157). The lungs show enhanced sensitivity to acetylcholine, a hallmark feature of asthma, suggesting that  $\alpha V\beta 6$

participates in the modulation of epithelial inflammation. On the other hand, the  $\beta 6$ -null mice were capable of healing cutaneous wounds, which had been suggested to be  $\alpha V\beta 6$  dependent. Further studies are needed to determine whether it is fibronectin or tenascin-C that is involved in these conditions, but the lack of the corresponding abnormalities after disruption of the tenascin-C gene strongly indicate that the interaction between  $\alpha V\beta 6$  and tenascin-C is of minor importance (170).

Taken together, these knock-out experiments of both fibronectin and its integrin receptors clearly demonstrate that these proteins are of vital importance for the organism. They also show that the different integrins have distinct functions and distribution, and that they in most cases do not compensate for each other. Nevertheless,  $\alpha V$  integrins have been found to be able to compensate for  $\alpha 5\beta 1$  integrin function with regard to adhesion to fibronectin and fibronectin matrix assembly *in vitro* (107, 165). The other important aspect of these knock-out investigations is that they complement the earlier antibody or peptide inhibition studies, by providing new and often contradictory conclusions of the *in vivo* functions of fibronectin and integrins. They also show that a protein can be present while not necessarily have a critical function at that particular location at that particular time. Further studies using tissue specific knock-outs, knock-ins, and disruption of one splice variant at the time, will hopefully give even more detailed and interpretable information about the *in vivo* roles of fibronectin and its integrin receptors.

### 3.7. Perspectives

Integrin  $\alpha 5\beta 1$  was isolated in 1985 as the first fibronectin receptor (51) and its cDNA sequence was reported two years later (171). Since then an impressive amount of information on the structure, interactions, and functions of fibronectin and its receptors has accumulated. As convincingly demonstrated by gene knock-out technology, these proteins are of fundamental importance in various physiological situations. Therefore, they will continue to be subject for intense research, and the progress rate will probably be faster than we can foresee. Some of the more immediate goals to reach include information on the three dimensional structures of whole integrins and fibronectin, and understanding of how conformational changes in these proteins are regulated. One area where such information would be of practical use is in development of reagents which could modulate receptor function during treatment of various adhesion-related disorders. These reagents could potentially be designed to act directly on the ligand binding sites, to affect conformation, or to modulate interactions with integrin associated proteins inside or outside of the cells.

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#### **5. REFERENCES**

1. R. O. Hynes: Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69, 11-25 (1992)
2. E. Ruoslahti & J. C. Reed: Anchorage dependence, integrins, and apoptosis. *Cell* 77, 477-478 (1994)
3. P. Huhtala, M. J. Humphries, J. B. McCarthy, P. M. Tremble, Z. Werb & C. H. Damsky: Cooperative signaling by  $\alpha 5 \beta 1$  and  $\alpha 4 \beta 1$  integrins regulates metalloproteinase gene expression in fibroblasts adhering to fibronectin. *J Cell Biol* 129, 867-879 (1995)
4. K. P. Campbell: Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage. *Cell* 80, 675-679 (1995)
5. A. M. Belkin & N. R. Smalheise: Localization of crinin (dystroglycan) at sites of cell-matrix and cell-cell contact: recruitment to focal adhesions is dependent upon extracellular ligands. *Cell Adhes Commun* 4, 281-296 (1996)
6. M. Bernfield, R. Kokenyesi, M. Kato, M. T. Hinkes, J. Spring, R. L. Gallo & E. J. Lise: Biology of syndecans: A family of transmembrane heparan sulfate proteoglycans. *Ann Rev Cell Biol* 8, 365-393 (1992)
7. A. Woods & J. R. Couchman: Syndecan 4 heparan sulfate proteoglycan is a selectively enriched and widespread focal adhesion component. *Mol Biol Cell* 5, 183-192 (1994)
8. E. A. Clark & J. S. Brugge: Integrins and signal transduction pathways: the road taken. *Science* 268, 233-239 (1995)
9. M. A. Schwartz, M. D. Schaller & M. H. Ginsberg: Integrins: emerging paradigms of signal transduction. *Ann Rev Cell Dev Biol* 11, 549-599 (1995)
10. S. Dedhar & G. E. Hannigan: Integrin cytoplasmic interactions and bi-directional transmembrane signaling. *Curr Opin Cell Biol* 8, 657-669 (1996)
11. R. O. Hynes: *Fibronectins*. New York: Springer Verlag. (1990)
12. S. Johansson: Non-collagenous matrix proteins. In: W. E. Comper, ed. *Extracellular matrix*, 1 Ed, vol 2. Amsterdam: Harwood academic publishers, 68-94 (1996)
13. H. P. Erickson & N. A. Carrell: Fibronectin in extended and compact conformations. Electron microscopy and sedimentation analysis. *J Biol Chem* 258, 14539-14544 (1983)
14. M. Rocco, E. Infusini, M. G. Daga, L. Gogioso & C. Cuniberti: Models of fibronectin. *EMBO J* 6, 2343-2349 (1987)
15. E. C. Williams, P. A. Janmey, J. D. Ferry & D. F. Mosher: Conformational states of fibronectin. Effects of pH, ionic strength, and collagen binding. *J Biol Chem* 257, 14973-14978 (1982)
16. N. M. Tooney, M. W. Mosesson, D. L. Amrani, J. F. Hainfeld & J. S. Wall: Solution and surface effects on plasma fibronectin structure. *J Cell Biol* 97, 1686-1692 (1983)
17. S. Johansson: Demonstration of high affinity fibronectin receptors on rat hepatocytes in suspension. *J Biol Chem* 260, 1557-1561 (1985)
18. A. Morla & E. Ruoslahti: A fibronectin self-assembly site involved in fibronectin matrix assembly: reconstruction in a synthetic peptide. *J Cell Biol* 118, 421-429 (1992)
19. A. Morla, Z. Zhang & E. Ruoslahti: Superfibronectin is a functionally distinct form of fibronectin. *Nature* 367, 193-196 (1994)
20. T. P. Ugarova, C. Zamarron, Y. Veklich, R. D. Bowditch, M. H. Ginsberg, J. W. Weisel & E. F. Plow: Conformational transitions in the cell binding domain of fibronectin. *Biochemistry* 34, 4457-4466 (1995)
21. M. D. Pierschbacher & E. Ruoslahti: Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* 309, 30-33 (1984)
22. J. W. Smith, Z. M. Ruggeri, T. J. Kunicki & D. A. Cheresh: Interaction of integrins  $\alpha V \beta 3$  and glycoprotein IIb-IIIa with fibrinogen. Differential peptide recognition accounts for distinct binding sites. *J Biol Chem* 265, 12267-12271 (1990)
23. E. Koivunen, D. A. Gay & E. Ruoslahti: Selection of peptides binding to the  $\alpha 5 \beta 1$  integrin from phage display library. *J Biol Chem* 268, 20205-20210 (1993)
24. S. Akiyama & K. M. Yamada: Synthetic peptides competitively inhibit both direct binding to fibroblasts and functional biological assays for the

## Fibronectin - Integrin interaction

- purified cell-binding domain of fibronectin. *J Biol Chem* 260, 10402-10405 (1985)
25. S. Johansson, E. Forsberg & B. Lundgren: Comparison of fibronectin receptors from rat hepatocytes and fibroblasts. *J Biol Chem* 262, 7819-7824 (1987)
26. S. Aota, T. Nagai & K. M. Yamada: Characterization of regions of fibronectin besides the arginine-glycine-aspartic acid sequence required for adhesive function of the cell-binding domain using site-directed mutagenesis. *J Biol Chem* 266, 15938-15943 (1991)
27. S. Aota, M. Nomizu & K. M. Yamada: The short amino acid sequence Pro-His-Ser-Arg-Asn in human fibronectin enhances cell-adhesive function. *J Biol Chem* 269, 24756-24761 (1994)
28. N. Kieffer, L. A. Fitzgerald, D. Wolf, D. A. Cheresh & D. R. Phillips: Adhesive properties of the  $\beta 3$  integrins: comparison of GP IIb-IIIa and the vitronectin receptor individually expressed in human melanoma cells. *J Cell Biol* 113, 451-461 (1991)
29. S. J. Shattil: Function and regulation of the  $\beta 3$  integrins in hemostasis and vascular biology. *Thromb Haemost* 74, 149-155 (1995)
30. B. Savage, E. Saldivar & Z. M. Ruggeri: Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell* 84, 289-297 (1996)
31. D. J. Leahy, I. Aukhil & H. P. Erickson: 2.0 Å crystal structure of a four-domain segment of human fibronectin encompassing the RGD loop and synergy region. *Cell* 84, 155-164 (1996)
32. A. P. Mould, J. A. Askari, S. E. Craig, A. N. Garratt, J. Clements & M. J. Humphries: Integrin  $\alpha 4 \beta 1$ -mediated melanoma cell adhesion and migration on vascular cell adhesion molecule-1 (VCAM-1) and the alternatively spliced IIICS region of fibronectin. *J Biol Chem* 269, 27224-27230 (1994)
33. A. Komoriya, L. J. Green, M. Mervic, S. S. Yamada, K. M. Yamada & M. J. Humphries: The minimal essential sequence for a major cell type-specific adhesion site (CS1) within the alternatively spliced type III connecting segment domain of fibronectin is leucine-aspartic acid-valine. *J Biol Chem* 266, 15075-15079 (1991)
34. M. J. Humphries, J. Sheridan, A. P. Mould & P. Newham: Mechanisms of VCAM-1 and fibronectin binding to integrin  $\alpha 4 \beta 1$ : implications for integrin function and rational drug design. *Ciba Found Symp* 189, 177-191 (1995)
35. A. P. Mould, A. Komoriya, K. M. Yamada & M. J. Humphries: The CS5 peptide is a second site in the IIICS region of fibronectin recognized by the integrin  $\alpha 4 \beta 1$ . Inhibition of  $\alpha 4 \beta 1$  function by RGD peptide homologues. *J Biol Chem* 266, 3579-3585 (1991)
36. J. A. Varner, P. C. Brooks & D. A. Cheresh: REVIEW: the integrin  $\alpha V \beta 3$ : angiogenesis and apoptosis. *Cell Adhes Commun* 3, 367-374 (1995)
37. R. Fässler, E. Georges-Labouesse & E. Hirsch: Genetic analyses of integrin function in mice. *Curr Opin Cell Biol* 8, 641-646 (1996)
38. R. O. Hynes: Targeted Mutations in Cell Adhesion Genes: What Have We Learned from Them? *Dev Biol* 180, 402-412 (1996)
39. M. J. Humphries: Integrin activation: the link between ligand binding and signal transduction. *Curr Opin Cell Biol* 8, 632-640 (1996)
40. T. E. O'Toole, Y. Katagiri, R. J. Faull, K. Peter, R. Tamura, V. Quaranta, J. C. Loftus, S. J. Shattil & M. H. Ginsberg: Integrin cytoplasmic domains mediate inside-out signal transduction. *J Cell Biol* 124, 1047-1059 (1994)
41. T. A. Springer: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76, 301-314 (1994)
42. C. Weber, R. Alon, B. Moser & T. A. Springer: Sequential regulation of  $\alpha 4 \beta 1$  and  $\alpha 5 \beta 1$  integrin avidity by CC chemokines in monocytes: implications for transendothelial chemotaxis. *J Cell Biol* 134, 1063-1073 (1996)
43. A. P. Mould, S. K. Akiyama & M. J. Humphries: Regulation of integrin  $\alpha 5 \beta 1$ -fibronectin interactions by divalent cations. Evidence for distinct classes of binding sites for  $Mn^{2+}$ ,  $Mg^{2+}$ , and  $Ca^{2+}$ . *J Biol Chem* 270, 26270-26277 (1995)
44. G. Bazzoni, D. T. Shih, C. A. Buck & M. E. Hemler: Monoclonal antibody 9EG7 defines a novel  $\beta 1$  integrin epitope induced by soluble ligand and manganese, but inhibited by calcium. *J Biol Chem* 270, 25570-25577 (1995)
45. D. D. Hu, C. F. Barbas & J. W. Smith: An allosteric  $Ca^{2+}$  binding site on the  $\beta 3$ -integrins that regulates the dissociation rate for RGD ligands. *J Biol Chem* 271, 21745-21751 (1996)
46. S. E. D'Souza, T. A. Haas, R. S. Piotrowicz, V. Byers-Ward, D. E. McGrath, H. R. Soule, C. Cierniewski, E. F. Plow & J. W. Smith: Ligand and cation binding are dual functions of a discrete segment of the integrin  $\beta 3$  subunit: cation



## Fibronectin - Integrin interaction

displacement is involved in ligand binding. *Cell* 79, 659-667 (1994)

47. D. Gulino, C. Boudignon, L. Y. Zhang, E. Concord, M. J. Rabiet & G. Marguerie: Ca(2+)-binding properties of the platelet glycoprotein IIb ligand- interacting domain. *J Biol Chem* 267, 1001-1007 (1992)

48. J. O. Lee, P. Rieu, M. A. Arnaout & R. Liddington: Crystal structure of the A domain from the  $\alpha$  subunit of integrin CR3 (CD11b/CD18). *Cell* 80, 631-638 (1995)

49. E. C. Tozer, R. C. Liddington, M. J. Sutcliffe, A. H. Smeeton & J. C. Loftus: Ligand binding to integrin  $\alpha$ IIb $\beta$ 3 is dependent on a MIDAS-like domain in the  $\beta$ 3 subunit. *J Biol Chem* 271, 21978-21984 (1996)

50. M. J. Humphries: The molecular basis and specificity of integrin-ligand interactions. *J Cell Sci* 97, 585-592 (1990)

51. R. Pytela, M. D. Piersbacher & E. Ruoslahti: Identification and isolation of a 140 kD cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell* 40, 191-198 (1985)

52. E. Koivunen, B. Wang & E. Ruoslahti: Isolation of a highly specific ligand for the  $\alpha$ 5 $\beta$ 1 integrin from a phage display library. *J Cell Biol* 124, 373-380 (1994)

53. R. M. Scarborough, M. A. Naughton, W. Teng, J. W. Rose, D. R. Phillips, L. Nannizzi, A. Arfsten, A. M. Campbell & I. F. Charo: Design of potent and specific integrin antagonists. Peptide antagonists with high specificity for glycoprotein IIb-IIIa. *J Biol Chem* 268, 1066-1073 (1993)

54. K. T. O'Neil, R. H. Hoess, S. A. Jackson, N. S. Ramachandran, S. A. Mousa & W. F. DeGrado: Identification of novel peptide antagonists for GPIIb/IIIa from a conformationally constrained phage peptide library. *Proteins* 14, 509-515 (1992)

55. R. Pasqualini, E. Koivunen & E. Ruoslahti: A peptide isolated from phage display libraries is a structural and functional mimic of an RGD-binding site on integrins. *J Cell Biol* 130, 1189-1196 (1995)

56. Y. Takada & W. Puzon: Identification of a regulatory region of integrin  $\beta$ 1 subunit using activating and inhibiting antibodies. *J Biol Chem* 268, 17597-17601 (1993)

57. A. Irie, T. Kamata, W. Puzon-McLaughlin & Y. Takada: Critical amino acid residues for ligand binding are clustered in a predicted  $\beta$ -turn of the third N-terminal repeat in the integrin  $\alpha$ 4 and  $\alpha$ 5 subunits. *EMBO J* 14, 5550-5556 (1995)

58. R. J. Faull, X. Du & M. H. Ginsberg: Receptors on platelets. *Methods Enzymol.* 245, 183-194 (1994)

59. M. Poncz, R. Eisman, R. Heidenreich, S. M. Silver, G. Vilaire, S. Surrey, E. Schwartz & J. S. Bennett: 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 (1987)

60. R. D. Bowditch, M. Hariharan, E. F. Tominna, J. W. Smith, K. M. Yamada, E. D. Getzoff & M. H. Ginsberg: Identification of a novel integrin binding site in fibronectin. Differential utilization by  $\beta$ 3 integrins. *J Biol Chem* 269, 10856-10863 (1994)

61. H. Mohri, J. Tanabe, K. Katoh & T. Okubo: Synthetic peptides demonstrate RGD-independent platelet glycoprotein IIb/IIIa recognition site in cell binding domain of human fibronectin. *Peptides* 16, 263-268 (1995)

62. S. E. D'Souza, M. H. Ginsberg, T. A. Burke, S. C.-T. Lam & E. F. Plow: Localization of an Arg-Gly-Asp recognition site within an integrin adhesion receptor. *Science* 242, 91-93 (1988)

63. J. W. Smith & D. A. Cheresh: The Arg-Gly-Asp binding domain of the vitronectin receptor. Photoaffinity cross-linking implicates amino acid residues 61-203 of the  $\beta$  subunit. *J Biol Chem* 263, 18726-18731 (1988)

64. I. F. Charo, L. Nannizzi, D. R. Phillips, M. A. Hsu & R. M. Scarborough: Inhibition of fibrinogen binding to GP IIb-IIIa by a GP IIIa peptide. *J Biol Chem* 266, 1415-1421 (1991)

65. S. E. D'Souza, M. H. Ginsberg, T. A. Burke & E. F. Plow: The ligand binding site of the platelet integrin receptor GPIIb-IIIa is proximal to the second calcium binding domain of its  $\alpha$  subunit. *J Biol Chem* 265, 3440-3446 (1990)

66. S. E. D'Souza, M. H. Ginsberg, G. R. Matsueda & E. F. Plow: A discrete sequence in a platelet integrin is involved in ligand recognition. *Nature* 350, 66-68 (1991)

67. S. M. Albelda, S. A. Mette, D. E. Elder, R. Stewart, L. Damjanovich, M. Herlyn & C. A. Buck: Integrin distribution in malignant melanoma: association of the  $\beta$ 3 subunit with tumor progression. *Cancer Res* 50, 6757-6764 (1990)

68. G. D. Rosen, J. R. Sanes, R. LaChance, J. M. Cunningham, J. Roman & D. C. Dean: Roles for the integrin VLA-4 and its counter receptor VCAM-1 in myogenesis. *Cell* 69, 1107-1119 (1992)

## Fibronectin - Integrin interaction

69. M. A. Stepp, L. A. Urry & R. O. Hynes: Expression of  $\alpha 4$  integrin mRNA and protein and fibronectin in the early chicken embryo. *Cell Adhes Commun* 2, 359-375 (1994)
70. R. Alon, P. D. Kassner, M. W. Carr, E. B. Finger, M. E. Hemler & T. A. Springer: The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J Cell Biol* 128, 1243-1253 (1995)
71. C. Berlin, R. F. Bargatze, J. J. Campbell, U. H. von Andrian, M. C. Szabo, S. R. Hasslen, R. D. Nelson, E. L. Berg, S. L. Erlandsen & E. C. Butcher:  $\alpha 4$  integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell* 80, 413-422 (1995)
72. H. Fujita, H. Mohri, H. Kanamori, A. Iwamatsu & T. Okubo: Binding site in human plasma fibronectin to HL-60 cells localizes in the C-terminal heparin-binding region independently of RGD and CS1. *Exp Cell Res* 217, 484-489 (1995)
73. A. P. Sanchez, J. C. Dominguez & P. A. Garcia: Activation of the  $\alpha 4 \beta 1$  integrin through the  $\beta 1$  subunit induces recognition of the RGDS sequence in fibronectin. *J Cell Biol* 126, 271-279 (1994)
74. T. Kamata, W. Puzon & Y. Takada: Identification of putative ligand-binding sites of the integrin  $\alpha 4 \beta 1$  (VLA-4, CD49d/CD29). *Biochem J* 305, 945-951 (1995)
75. S. G. Schiffer, M. E. Hemler, R. R. Lobb, R. Tizard & L. Osborn: Molecular mapping of functional antibody binding sites of  $\alpha 4$  integrin. *J Biol Chem* 270, 14270-14273 (1995)
76. D. S. Tuckwell, M. J. Humphries & A. Brass: A secondary structure model of the integrin  $\alpha$  subunit N-terminal domain based on analysis of multiple alignments. *Cell Adhes Commun* 2, 385-402 (1994)
77. J. L. Bednarczyk & B. W. McIntyre: A monoclonal antibody to VLA-4  $\alpha$ -chain (CDw49d) induces homotypic lymphocyte aggregation. *J Immunol* 144, 777-784 (1990)
78. M. R. Campanero, R. Pulido, M. A. Ursa, M. Rodriguez-Moya, M. O. de Landazuri & F. Sanchez-Madrid: An alternative leukocyte homotypic adhesion mechanism, LFA-1/ICAM-1- independent, triggered through the human VLA-4 integrin. *J Cell Biol* 110, 2157-2165 (1990)
79. R. Pulido, M. J. Elices, M. R. Campanero, L. Osborn, S. Schiffer, A. Garcia-Pardo, R. Lobb, M. E. Hemler & F. Sanchez-Madrid: Functional evidence for three distinct and independently inhibitable adhesion activities mediated by the human integrin VLA-4. Correlation with distinct  $\alpha 4$  epitopes. *J Biol Chem* 266, 10241-10245 (1991)
80. P. Altevogt, M. Hubbe, M. Ruppert, J. Lohr, P. von Hoegen, M. Sammar, D. P. Andrew, L. McEvoy, M. J. Humphries & E. C. Butcher: The  $\alpha 4$  integrin chain is a ligand for  $\alpha 4 \beta 7$  and  $\alpha 4 \beta 1$ . *J. Exp. Med.* 182, 345-355 (1995)
81. J. B. Weitzman, A. Chen & M. E. Hemler: Investigation of the role of  $\beta 1$  integrins in cell-cell adhesion. *J Cell Sci* 108, 3635-3644 (1995)
82. E. A. Wayner, W. G. Carter, R. S. Piotrowicz & T. J. Kunicki: The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J Cell Biol* 107, 1881-1891 (1988)
83. J. Peltonen, H. Larjava, S. Jaakkola, H. Gralnick, S. K. Akiyama, S. S. Yamada, K. M. Yamada & J. Uitto: Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. Variable expression in basal and squamous cell carcinomas. *J Clin Invest* 84, 1916-1923 (1989)
84. N. Patey, L. Halbwachs-Mecarelli, D. Droz, P. Lesavre & L. H. Noel: Distribution of integrin subunits in normal human kidney. *Cell Adhes Commun* 2, 159-167 (1994)
85. C. M. DiPersio, S. Shah & R. O. Hynes:  $\alpha 3 \beta 1$  integrin localizes to focal contacts in response to diverse extracellular matrix proteins. *J Cell Sci* 108, 2321-2336 (1995)
86. W. J. Rettig, V. V. Murty, M. J. Mattes, R. S. Chaganti & L. J. Old: Extracellular matrix-modulated expression of human cell surface glycoproteins A42 and J143. Intrinsic and extrinsic signals determine antigenic phenotype. *J Exp Med* 164, 1581-1599 (1986)
87. M. E. Hemler, C. Huang & L. Schwarz: The VLA protein family. Characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight  $\beta$  subunit. *J Biol Chem* 262, 3300-3309 (1987)
88. W. G. Carter, M. C. Ryan & P. J. Gahr: Epiligrin, a new cell adhesion ligand for integrin  $\alpha 3 \beta 1$  in epithelial basement membranes. *Cell* 65, 599-610 (1991)
89. P. Rouselle, G. P. Lunstrum, D. R. Keene & R. E. Burgeson: Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J Cell Biol* 114, 567-576 (1991)

90. E. A. Wayner & W. G. Carter: Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique  $\alpha$  and common  $\beta$  subunits. *J Cell Biol* 105, 1873-1884 (1987)
91. M. J. Elices, L. A. Urry & M. E. Hemler: Receptor functions for the integrin VLA-3: fibronectin, collagen, and laminin binding are differentially influenced by Arg-Gly-Asp peptide and by divalent cations. *J Cell Biol* 112, 169-181 (1991)
92. L. C. Plantefaber & R. O. Hynes: Changes in integrin receptors on oncogenically transformed cells. *Cell* 56, 281-290 (1989)
93. J. B. Weitzman, R. Pasqualini, Y. Takada & M. E. Hemler: The function and distinctive regulation of the integrin VLA-3 in cell adhesion, spreading, and homotypic cell aggregation. *J Biol Chem* 268, 8651-8657 (1993)
94. G. O. Delwel, A. A. de Melker, F. Hogervorst, L. H. Jaspars, D. L. Fles, I. Kuikman, A. Lindblom, M. Paulsson, R. Timpl & A. Sonnenberg: Distinct and overlapping ligand specificities of the  $\alpha 3\beta 1$  and  $\alpha 6\beta 1$  integrins: recognition of laminin isoforms. *Mol Biol Cell* 5, 203-215 (1994)
95. B. Bossy, E. Bossy Wetzel & L. F. Reichardt: Characterization of the integrin  $\alpha 8$  subunit: a new integrin  $\beta 1$ -associated subunit, which is prominently expressed on axons and on cells in contact with basal laminae in chick embryos. *EMBO J* 10, 2375-2385 (1991)
96. L. M. Schnapp, J. M. Breuss, D. M. Ramos, D. Sheppard & R. Pytela: Sequence and tissue distribution of the human integrin  $\alpha 8$  subunit: a  $\beta 1$ -associated a subunit expressed in smooth muscle cells. *J Cell Sci* 108, 537-544 (1995)
97. L. M. Schnapp, N. Hatch, D. M. Ramos, I. V. Klimanskaya, D. Sheppard & R. Pytela: The human integrin  $\alpha 8\beta 1$  functions as a receptor for tenascin, fibronectin, and vitronectin. *J Biol Chem* 270, 23196-23202 (1995)
98. U. Muller, B. Bossy, K. Venstrom & L. F. Reichardt: Integrin  $\alpha 8\beta 1$  promotes attachment, cell spreading, and neurite outgrowth on fibronectin. *Mol Biol Cell* 6, 433-448 (1995)
99. B. Varnum-Finney, K. Venstrom, U. Muller, R. Kypta, C. Backus, M. Chiquet & L. F. Reichardt: The integrin receptor  $\alpha 8\beta 1$  mediates interactions of embryonic chick motor and sensory neurons with tenascin-C. *Neuron* 14, 1213-1222 (1995)
100. B. E. Vogel, G. Tarone, F. G. Giancotti, J. Gailit & E. Ruoslahti: A novel fibronectin receptor with an unexpected subunit composition ( $\alpha V\beta 1$ ). *J Biol Chem* 265, 5934-5937 (1990)
101. Z. Zhang, A. O. Morla, K. Vuori, J. S. Bauer, R. L. Juliano & E. Ruoslahti: The  $\alpha V\beta 1$  integrin functions as a fibronectin receptor but does not support fibronectin matrix assembly and cell migration on fibronectin. *J Cell Biol* 122, 235-242 (1993)
102. M. Friedlander, P. C. Brooks, R. W. Shaffer, C. M. Kincaid, J. A. Varner & D. A. Cheresh: Definition of two angiogenic pathways by distinct  $\alpha v$  integrins. *Science* 270, 1500-1502 (1995)
103. P. C. Brooks, S. Strömblad, L. C. Sanders, T. L. von Schalscha, R. T. Aimes, W. G. Stetler-Stevenson, J. P. Quigley & D. A. Cheresh: Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin  $\alpha V\beta 3$ . *Cell* 85, 683-693 (1996)
104. J. A. Varner & D. A. Cheresh: Integrins and cancer. *Curr Opin Cell Biol* 8, 724-730 (1996)
105. R. Pytela, M. D. Pierschbacher & E. Ruoslahti: 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 USA* 82, 5766-5770 (1985)
106. I. F. Charo, L. Nannizzi, J. W. Smith & D. A. Cheresh: The vitronectin receptor  $\alpha V\beta 3$  binds fibronectin and acts in concert with  $\alpha 5\beta 1$  in promoting cellular attachment and spreading on fibronectin. *J Cell Biol* 111, 2795-2800 (1990)
107. K. Wennerberg, L. Lohikangas, D. Gullberg, M. Pfaff, S. Johansson & R. Fässler:  $\beta 1$  integrin-dependent and -independent polymerization of fibronectin. *J Cell Biol* 132, 227-238 (1996)
108. S. K. Akiyama, S. Aota & K. M. Yamada: Function and receptor specificity of a minimal 20 kilodalton cell adhesive fragment of fibronectin. *Cell Adhes Commun* (1995)
109. J. M. Breuss, J. Gallo, H. M. DeLisser, I. V. Klimanskaya, H. G. Folkesson, J. F. Pittet, S. L. Nishimura, K. Aldape, D. V. Landers, W. Carpenter & *et al.*: Expression of the  $\beta 6$  integrin subunit in development, neoplasia and tissue repair suggests a role in epithelial remodeling. *J Cell Sci* 108, 2241-2251 (1995)
110. M. Agrez, A. Chen, R. I. Cone, R. Pytela & D. Sheppard: The  $\alpha V\beta 6$  integrin promotes proliferation of colon carcinoma cells through a unique region of

## Fibronectin - Integrin interaction

- the  $\beta 6$  cytoplasmic domain. *J Cell Biol* 127, 547-556 (1994)
111. R. B. Dixit, A. Chen, J. Chen & D. Sheppard: Identification of a sequence within the integrin  $\beta 6$  subunit cytoplasmic domain that is required to support the specific effect of  $\alpha V\beta 6$  on proliferation in three-dimensional culture. *J Biol Chem* 271, 25976-25980 (1996)
  112. M. Busk, R. Pytela & D. Sheppard: Characterization of the integrin  $\alpha V\beta 6$  as a fibronectin-binding protein. *J Biol Chem* 267, 5790-5796 (1992)
  113. A. L. Prieto, G. M. Edelman & K. L. Crossin: Multiple integrins mediate cell attachment to cytotactin/tenascin. *Proc Natl Acad Sci USA* 90, 10154-10158 (1993)
  114. J. Chen, T. Maeda, K. Sekiguchi & D. Sheppard: Distinct structural requirements for interaction of the integrins  $\alpha 5\beta 1$ ,  $\alpha V\beta 5$ , and  $\alpha V\beta 6$  with the central cell binding domain in fibronectin. *Cell Adhes Commun* 4, 237-250 (1996)
  115. A. A. Postigo, P. Sanchez-Mateos, A. I. Lazarovits, F. Sanchez-Madrid & M. O. de Landazuri:  $\alpha 4\beta 7$  integrin mediates B cell binding to fibronectin and vascular cell adhesion molecule-1. Expression and function of  $\alpha 4$  integrins on human B lymphocytes. *J Immunol* 151, 2471-2483 (1993)
  116. C. Ruegg, A. A. Postigo, E. E. Sikorski, E. C. Butcher, R. Pytela & D. J. Erle: Role of integrin  $\alpha 4\beta 7/\alpha 4\beta P$  in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J Cell Biol* 117, 179-189 (1992)
  117. C. Berlin, E. L. Berg, M. J. Briskin, D. P. Andrew, P. J. Kilshaw, B. Holzmann, I. L. Weissman, A. Hamann & E. C. Butcher:  $\alpha 4\beta 7$  integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* 74, 185-185 (1993)
  118. M. Moyle, M. A. Napier & J. W. McLean: Cloning and expression of a divergent integrin subunit  $\beta 8$ . *J Biol Chem* 266, 19650-19658 (1991)
  119. S. L. Nishimura, D. Sheppard & R. Pytela: Integrin  $\alpha V\beta 8$ . Interaction with vitronectin and functional divergence of the  $\beta 8$  cytoplasmic domain. *J Biol Chem* 269, 28708-28715 (1994)
  120. K. Venstrom & L. Reichardt:  $\beta 8$  integrins mediate interactions of chick sensory neurons with laminin 1, collagen IV, and fibronectin. *Mol Biol Cell* 6, 419-431 (1995)
  121. K. Hedman, T. Vartio, S. Johansson, L. Kjellen, M. Hook, A. Linker, E. M. Salonen & A. Vaheri: Integrity of the pericellular fibronectin matrix of fibroblasts is independent of sulfated glycosaminoglycans. *EMBO J* 3, 581-584 (1984)
  122. J. Roman & J. A. McDonald: Fibulin's organization into the extracellular matrix of fetal lung fibroblasts is dependent on fibronectin matrix assembly. *Am J Respir Cell Mol Biol* 8, 538-545 (1993)
  123. A. Vaheri, J. Keski-Oja & T. Vartio: Fibronectin and malignant transformation. In: D. F. Mosher, ed. *Fibronectin*. New York: Academic Press, 255-271 (1989)
  124. F. G. Giancotti & E. Ruoslahti: Elevated levels of the  $\alpha 5\beta 1$  fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell* 60, 849-859 (1990)
  125. J. Roman, R. M. LaChance, T. J. Broekelmann, C. J. Kennedy, E. A. Wayner, W. G. Carter & J. A. McDonald: The fibronectin receptor is organized by extracellular matrix fibronectin: implications for oncogenic transformation and for cell recognition of fibronectin matrices. *J Cell Biol* 108, 2529-2543 (1989)
  126. F. J. Fogerty, S. K. Akiyama, K. M. Yamada & D. F. Mosher: Inhibition of binding of fibronectin to matrix assembly sites by anti-integrin ( $\alpha 5\beta 1$ ) antibodies. *J Cell Biol* 111, 699-708 (1990)
  127. J. T. Yang, H. Rayburn & R. O. Hynes: Embryonic mesodermal defects in  $\alpha 5$  integrin-deficient mice. *Development* 119, 1093-1105 (1993)
  128. C. Wu, V. M. Keivens, T. E. O'Toole, J. A. McDonald & M. H. Ginsberg: Integrin activation and cytoskeletal interaction are essential for the assembly of a fibronectin matrix. *Cell* 83, 715-724 (1995)
  129. C. Wu, A. J. Fields, B. A. Kapteijn & J. A. McDonald: The role of  $\alpha 4\beta 1$  integrin in cell motility and fibronectin matrix assembly. *J Cell Sci* 108, 821-829 (1995)
  130. V. Mautner & R. O. Hynes: Surface distribution of LETS protein in relation to the cytoskeleton of normal and transformed cells. *J Cell Biol* 75, 743-768 (1977)
  131. R. Winklbauer & C. Stoltz: Fibronectin fibril growth in the extracellular matrix of the *Xenopus* embryo. *J Cell Sci* 108, 1575-1586 (1995)
  132. J. E. Schwarzbauer: Identification of the fibronectin sequences required for assembly of a fibrillar matrix. *J Cell Biol* 113, 1463-1473 (1991)

133. J. L. Sechler, Y. Takada & J. E. Schwarzbauer: Altered rate of fibronectin matrix assembly by deletion of the first type III repeats. *J Cell Biol* 134, 573-583 (1996)
134. S. Johansson, S. Gustafson & H. Persson: Identification of a fibronectin receptor specific for rat liver endothelial cells. *Exp Cell Res* 172, 425-431 (1987)
135. P. J. McKeown-Longo & D. F. Mosher: Interaction of the 70,000-mol-wt amino-terminal fragment of fibronectin with the matrix-assembly receptor of fibroblasts. *J Cell Biol* 100, 364-374 (1985)
136. B. J. Quade & J. A. McDonald: Fibronectin's amino-terminal matrix assembly site is located within the 29-kDa amino-terminal domain containing five type I repeats. *J Biol Chem* 263, 19602-19609 (1988)
137. J. Sottile, J. Schwarzbauer, J. Selegue & D. F. Mosher: Five type I modules of fibronectin form a functional unit that binds to fibroblasts and *Staphylococcus aureus*. *J Biol Chem* 266, 12840-12843 (1991)
138. Q. Zhang & D. F. Mosher: Cross-linking of the NH<sub>2</sub>-terminal region of fibronectin to molecules of large apparent molecular mass. Characterization of fibronectin assembly sites induced by the treatment of fibroblasts with lysophosphatidic acid. *J Biol Chem* 271, 33284-33292 (1996)
139. K. M. Aguirre, R. J. McCormick & J. E. Schwarzbauer: Fibronectin self-association is mediated by complementary sites within the amino-terminal one-third of the molecule. *J Biol Chem* 269, 27863-27868 (1994)
140. D. C. Hocking, J. Sottile & P. J. McKeown-Longo: Fibronectin's III-1 module contains a conformation-dependent binding site for the amino-terminal region of fibronectin. *J Biol Chem* 269, 19183-19187 (1994)
141. B. J. Dzamba, H. Bultmann, S. K. Akiyama & D. M. Peters: Substrate-specific binding of the amino terminus of fibronectin to an integrin complex in focal adhesions. *J Biol Chem* 269, 19646-19652 (1994)
142. D. C. Hocking, R. K. Smith & P. J. McKeown-Longo: A novel role for the integrin-binding III-10 module in fibronectin matrix assembly. *J Cell Biol* 133, 431-444 (1996)
143. J. C. Adams & F. M. Watt: Regulation of development and differentiation by the extracellular matrix. *Development* 117, 1183-1198 (1993)
144. C. ffrench-Constant & R. O. Hynes: Alternative splicing of fibronectin is temporally and spatially regulated in the chicken embryo. *Development* 106, 375-388 (1989)
145. C. ffrench-Constant: Alternative splicing of fibronectin-many different proteins but few different functions. *Exp Cell Res* 221, 261-271 (1995)
146. J. H. Peters & R. O. Hynes: Fibronectin isoform distribution in the mouse. I. The alternatively spliced EIII<sub>B</sub>, EIII<sub>A</sub>, and V segments show widespread codistribution in the developing mouse embryo. *Cell Adhes Commun* 4, 103-125 (1996)
147. A. E. Sutherland, P. G. Calarco & C. H. Damsky: Developmental regulation of integrin expression at the time of implantation in the mouse embryo. *Development* 119, 1175-1186 (1993)
148. J. C. Boucaut, T. Darribere, H. Boulekbache & J. P. Thiery: Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos. *Nature* 307, 364-367 (1984)
149. J. C. Boucaut, T. Darribere, T. J. Poole, H. Aoyama, K. M. Yamada & J. P. Thiery: Biologically active synthetic peptides as probes of embryonic development: a competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cell migration in avian embryos. *J Cell Biol* 99, 1822-1830 (1984)
150. T. Darribere, K. M. Yamada, K. E. Johnson & J. C. Boucaut: The 140-kDa fibronectin receptor complex is required for mesodermal cell adhesion during gastrulation in the amphibian *Pleurodeles waltlii*. *Dev Biol* 126, 182-194 (1988)
151. F. M. Bronner, C. D. Stern & S. Fraser: Analysis of neural crest cell lineage and migration. *J Craniofac Genet Dev Biol* 11, 214-222 (1991)
152. E. L. George, L. E. Georges, K. R. Patel, H. Rayburn & R. O. Hynes: Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development* 119, 1079-1091 (1993)
153. J. A. Kreidberg, M. J. Donovan, S. L. Goldstein, H. Rennke, K. Shepherd, R. C. Jones & R. Jaenisch:  $\alpha 3 \beta 1$  integrin has a crucial role in kidney and lung organogenesis. *Development* 122, 3537-3547 (1996)
154. J. T. Yang, H. Rayburn & R. O. Hynes: Cell adhesion events mediated by  $\alpha 4$  integrins are essential in placental and cardiac development. *Development* 121, 549-560 (1995)

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155. R. Fässler, M. Pfaff, J. Murphy, A. A. Noegel, S. Johansson, R. Timpl & R. Albrecht: Lack of  $\beta 1$  integrin gene in embryonic stem cells affects morphology, adhesion, and migration but not integration into the inner cell mass of blastocysts. *J Cell Biol* 128, 979-988 (1995)
156. R. Fässler & M. Meyer: Consequences of lack of  $\beta 1$  integrin gene expression in mice. *Genes Dev* 9, 1896-1908 (1995)
157. X. Z. Huang, J. F. Wu, D. Cass, D. J. Erle, D. Corry, S. G. Young, R. V. Farese, Jr. & D. Sheppard: Inactivation of the integrin  $\beta 6$  subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. *J Cell Biol* 133, 921-928 (1996)
158. L. E. Stephens, A. E. Sutherland, I. V. Klimanskaya, A. Andrieux, J. Meneses, R. A. Pedersen & C. H. Damsky: Deletion of  $\beta 1$  integrins in mice results in inner cell mass failure and peri-implantation lethality. *Genes Dev* 9, 1883-1895 (1995)
159. N. Wagner, J. Lohler, E. J. Kunkel, K. Ley, E. Leung, G. Krissansen, K. Rajewsky & W. Muller: Critical role for  $\beta 7$  integrins in formation of the gut-associated lymphoid tissue. *Nature* 382, 366-370 (1996)
160. E. N. Georges-Labouesse, E. L. George, H. Rayburn & R. O. Hynes: Mesodermal development in mouse embryos mutant for fibronectin. *Dev Dyn* 207, 145-156 (1996)
161. R. Fässler, J. Rohwedel, V. Maltsev, W. Bloch, S. Lentini, K. Guan, D. Gullberg, J. Hescheler, K. Addicks & A. M. Wobus: Differentiation and integrity of cardiac muscle cells are impaired in the absence of  $\beta 1$  integrin. *J Cell Sci* 109, 2989-2999 (1996)
162. E. Hirsch, L. Lohikangas, D. Gullberg, S. Johansson & R. Fässler: *Unpublished results* (1997)
163. T. Volk, L. I. Fessler & J. H. Fessler: A role for integrin in the formation of sarcomeric cytoarchitecture. *Cell* 63, 525-536 (1990)
164. E. Hirsch, A. Iglesias, A. J. Potocnik, U. Hartmann & R. Fässler: Impaired migration but not differentiation of haematopoietic stem cells in the absence of  $\beta 1$  integrins. *Nature* 380, 171-175 (1996)
165. J. T. Yang & R. O. Hynes: Fibronectin receptor functions in embryonic cells deficient in  $\alpha 5 \beta 1$  integrin can be replaced by  $\alpha V$  integrins. *Mol Biol Cell* 7, 1737-1748 (1996)
166. G. C. Gurtner, V. Davis, H. Li, M. J. McCoy, A. Sharpe & M. I. Cybulsky: Targeted disruption of the murine VCAM1 gene: essential role of VCAM-1 in chorioallantoic fusion and placentation. *Genes Dev* 9, 1-14 (1995)
167. L. Kwee, H. S. Baldwin, H. M. Shen, C. L. Stewart, C. Buck, C. A. Buck & M. A. Labow: Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (VCAM-1) deficient mice. *Development* 121, 489-503 (1995)
168. A. G. Arroyo, J. T. Yang, H. Rayburn & R. O. Hynes: Differential requirements for  $\alpha 4$  integrins during fetal and adult hematopoiesis. *Cell* 85, 997-1008 (1996)
169. C. Wu, A. E. Chung & J. A. McDonald: A novel role for  $\alpha 3 \beta 1$  integrins in extracellular matrix assembly. *J Cell Sci* 108, 2511-2523 (1995)
170. Y. Saga, T. Yagi, Y. Ikawa, T. Sakakura & S. Aizawa: Mice develop normally without tenascin. *Genes Dev* 6, 1821-1831 (1992)
171. W. S. Argraves, S. Suzuki, H. Arai, K. Thompson, M. D. Pierschbacher & E. Ruoslahti: Amino acid sequence of the human fibronectin receptor. *J Cell Biol* 105, 1183-1190 (1987)