

# Amino Acid Sequence of the Human Fibronectin Receptor

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**Abstract.** The amino acid sequence deduced from cDNA of the human placental fibronectin receptor is reported. The receptor is composed of two subunits: an  $\alpha$  subunit of 1,008 amino acids which is processed into two polypeptides disulfide bonded to one another, and a  $\beta$  subunit of 778 amino acids. Each subunit has near its COOH terminus a hydrophobic segment. This and other sequence features suggest a structure for the receptor in which the hydrophobic segments serve as transmembrane domains anchoring each subunit to the membrane and dividing each into a large ectodomain and a short cytoplasmic domain. The  $\alpha$  subunit ectodomain has five sequence elements homologous to consensus  $\text{Ca}^{2+}$ -binding sites of several calcium-binding proteins, and the  $\beta$  subunit contains a fourfold

repeat strikingly rich in cysteine. The  $\alpha$  subunit sequence is 46% homologous to the  $\alpha$  subunit of the vitronectin receptor. The  $\beta$  subunit is 44% homologous to the human platelet adhesion receptor subunit IIIa and 47% homologous to a leukocyte adhesion receptor  $\beta$  subunit. The high degree of homology (85%) of the  $\beta$  subunit with one of the polypeptides of a chicken adhesion receptor complex referred to as integrin complex strongly suggests that the latter polypeptide is the chicken homologue of the fibronectin receptor  $\beta$  subunit. These receptor subunit homologies define a superfamily of adhesion receptors. The availability of the entire protein sequence for the fibronectin receptor will facilitate studies on the functions of these receptors.

THE cell surface receptors that bind to the adhesive glycoproteins fibronectin, vitronectin, fibrinogen, and von Willebrand factor have been shown to be structurally and functionally related (Pytela et al., 1986). Furthermore, they have been shown to be members of a superfamily of receptors that include the leukocyte glycoproteins (LFA-1, Mac-1, and p150,95 (Springer et al., 1986) and another group of cell surface proteins referred to as VLA antigens (Hemler et al., 1987). Homologous forms of these receptors have been defined in various species by using antibodies that interfere with cell adhesion (Knudsen et al., 1981; Greve and Gottlieb, 1982; Horwitz et al., 1984; Brown and Juliano, 1985; Giancotti et al., 1985). A family of cell surface glycoproteins that appear to be related to the vertebrate receptors has also been found in *Drosophila* (Wilcox and Leptin, 1985) and sea urchin (Noll et al., 1985). It seems, then, that this superfamily of receptors is diversely represented in cells of vertebrate and invertebrate organisms. The receptors for fibronectin, vitronectin, fibrinogen, and von Willebrand factor all bind to Arg-Gly-Asp (RGD) sequences (Pierschbacher and Ruoslahti, 1984; Plow et al., 1985; Pytela et al., 1985a, b, 1986; Horwitz et al., 1985; Akiyama and Yamada, 1985; Hynes, 1987) within their respective ligands. It is not yet known whether RGD binding is a common functional trait among all members of the receptor superfamily.

A general structure characteristic of members of this

receptor superfamily is that they are heteromeric complexes having noncovalently associated  $\alpha$  and  $\beta$  subunits. Details of the structure of the receptors have come from recombinant DNA cloning work. The  $\beta$  subunit of the chicken fibronectin-laminin receptor complex (Tamkun et al., 1986), the  $\beta$  subunit of the human LFA-1 cell surface glycoprotein (Kishimoto et al., 1987; Law et al., 1987), and the glycoprotein IIIa of the platelet IIb/IIIa complex (Fitzgerald et al., 1987) have recently been cloned and sequenced. Comparison of primary sequences of these  $\beta$  subunits indicates that they are related, having a high overall homology, and that they share common structural features such as short cytoplasmic domains, putative transmembrane segments, and homologous cysteine-rich domains. Results from our laboratory concerning the  $\alpha$  subunits of the human fibronectin receptor and vitronectin receptor have revealed that the partial amino acid sequences deduced from cDNAs for the two subunits are homologous and have common structural elements such as potential membrane-spanning regions and short cytoplasmic domains (Argraves et al., 1986; Suzuki et al., 1986).

We have continued our efforts to determine the structure of the fibronectin receptor expecting that it will lead to an understanding of the function of this and other related receptors. Here we report the complete amino acid sequences, deduced from cDNA, of both the  $\alpha$  and  $\beta$  subunits of the human fibronectin receptor.

## Materials and Methods

### cDNA Libraries and Screening

A placental cDNA  $\lambda$ gt11 library (Millan, 1986) was immunologically screened according to the procedure of Young and Davis (1983). A rabbit antiserum prepared against purified human placental fibronectin receptor (Pytela et al., 1986) served as a source of antibodies for the screening. The antibodies were purified from this antiserum by affinity chromatography on a column of fibronectin receptor coupled to Sepharose (Pytela et al., 1987). These antibodies immunoblot both subunits of the receptor (Argraves et al., 1986) and immunoprecipitate both of the receptor subunits from cells surface labeled with  $^{125}\text{I}$  (Dedhar et al., 1987). Clones that expressed insert-encoded protein reactive with these antibodies were isolated and purified to homogeneity through successive screenings. To identify whether the selected clones corresponded to the  $\alpha$  or  $\beta$  subunit of the fibronectin receptor, their insert-encoded proteins were used to affinity select antibodies from antifibronectin receptor serum, as previously described (Argraves et al., 1986). Additional screenings were done with radiolabeled DNA fragments from the 5' portions of the previously isolated cDNA inserts as probes. DNA fragments were  $^{32}\text{P}$  labeled by random oligonucleotide priming (Feinberg and Vogelstein, 1984) and used to screen either the placental  $\lambda$ gt11 cDNA library or a primer extension cDNA library by methods described elsewhere (Maniatis et al., 1982).

The primer extension cDNA library was made based on the procedure of Krawinkel and Zobelein (1986). Placental poly (A) RNA was specifically primed with an oligonucleotide sequence which was derived from the 5' region of the fibronectin receptor  $\alpha$  subunit cDNA  $\lambda$ P7 (Argraves et al., 1986). First strand cDNA was made from 15  $\mu\text{g}$  of placental poly (A) RNA primed with 0.6  $\mu\text{g}$  of a 28-bp oligonucleotide (5' TGAATTCGAACCCTCGGGATCCAACCTCC 3'). The first strand cDNA was G-tailed and the second strand synthesis primed with the oligonucleotide TGAATTCGGATC $_{12}$ . After Eco RI digestion, the double-stranded cDNA was size fractionated on Sephacryl S-1000 (Pharmacia Fine Chemicals, Piscataway, NJ) and cDNAs greater than 500 bp were selected and ligated to Eco RI cut  $\lambda$ gt11 DNA (Promega Biotec, Madison, WI). Phage DNA was packaged using Gigapack (Vector Cloning Systems, San Diego, CA). Greater than 90% of the resulting phage were recombinants.

### DNA Sequencing

cDNA inserts from selected  $\lambda$ gt11 clones were subcloned into the phage vector M13 mp19. The cDNA inserts were sequenced by the dideoxy chain termination method (Sanger et al., 1977) by using deoxyadenosine 5'- $\alpha$ -[ $^{35}\text{S}$ ] thiotriphosphate and oligonucleotide primers that were synthesized on the basis of preceding sequences. All sequences reported here are the result of sequencing both strands of the cDNA inserts.

### RNA Hybridization Analysis

RNA was extracted from term human placenta by a guanidinium hydrochloride procedure (Adams et al., 1977) and was subjected to electrophoresis on 0.8% agarose, 2.2 M formaldehyde, 0.02 M sodium acetate gels (Lehrach et al., 1977). Blot transfer to nitrocellulose filters and hybridization with probes were done according to Thomas (1980). Filters were washed at high stringency in 0.2 $\times$  SSC (1 $\times$  SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS at 68°C.

## Results

### Isolation of cDNA Clones and Definition of Receptor Subunit mRNAs

Screening of  $\sim 5 \times 10^5$   $\lambda$ gt11 phage plaques from a placental cDNA library with affinity-purified antifibronectin receptor antibodies identified several clones producing a fusion protein recognized by the antibodies. As previously reported, a partial amino acid sequence of the fibronectin receptor  $\alpha$  subunit was deduced from the nucleotide sequences of two of these clones,  $\lambda$ P7 and  $\lambda$ P34 (Argraves et al., 1986).

The insert-encoded protein from a third clone,  $\lambda$ P32, ab-

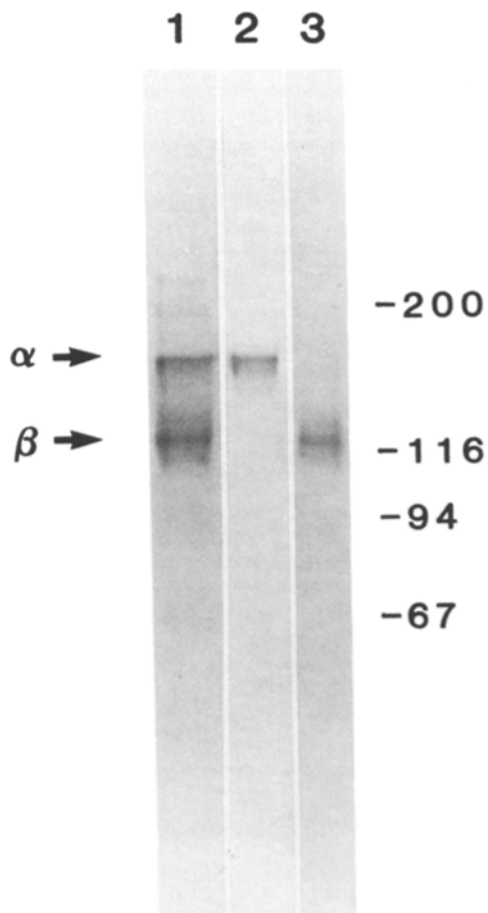
sorbed antibodies that reacted specifically with the  $\beta$  subunit of the fibronectin receptor (Fig. 1).  $\lambda$ P32 was found to contain a 2.5-kb cDNA insert that did not cross hybridize with  $\lambda$ P7 or  $\lambda$ P34. Nucleotide sequence analysis of the 2,485-bp insert of  $\lambda$ P32 showed that it had a single open reading frame of 1368 bp followed by a 3' untranslated region of 1117 bp that lacked a poly (A) tail. This sequence, presumably representing the  $\beta$  subunit, was not homologous to the  $\alpha$  subunit sequence. The cDNAs for both the  $\alpha$  and  $\beta$  subunits of the fibronectin receptor were used as probes for genomic DNA Southern hybridization analysis (Southern, 1975) and the resulting hybridization patterns indicated that both subunits are encoded by distinct, single copy genes (data not shown). RNA hybridization analysis showed that the  $\lambda$ P32  $\beta$  subunit cDNA insert hybridized to an  $\sim 4.2$ -kb placental transcript, while the  $\alpha$  subunit mRNA is  $\sim 4.9$  kb in size (Fig. 2).

To isolate cDNAs that would correspond to additional protein-encoding sequence in the mRNAs of the fibronectin receptor  $\alpha$  and  $\beta$  subunits, the placental cDNA library was rescreened with DNA fragments from the 5' portions of  $\lambda$ P7 and  $\lambda$ P32. In both cases, sets of overlapping cDNA clones were isolated and sequenced. After an additional round of screening using 5' fragments from the new clones as probes, overlapping cDNA clones encoding the complete  $\beta$  subunit were obtained. However, cDNAs encoding the amino-terminal region of the  $\alpha$  subunit were still lacking. A primer extension cDNA library was prepared from placental poly (A) RNA primed with an oligonucleotide the sequence of which was derived from the 5' portion of  $\lambda$ P7. The cDNA library that was obtained was screened with a cDNA fragment from the 5' region of a previously selected  $\alpha$  subunit cDNA that extended the farthest toward the 5' direction. As a result, several clones were isolated that completed the amino acid sequence of the  $\alpha$  subunit polypeptide.

### The $\alpha$ Subunit Sequence

The sequence of the cDNA encoding the human fibronectin receptor  $\alpha$  subunit and the deduced amino acid sequence of the  $\alpha$  subunit polypeptide is shown in Fig. 3. The cDNA sequence was derived from eight overlapping cDNA clones. The sequence deduced from these cDNAs contains the amino-terminal sequence obtained from protein sequence analysis of VLA-5 $\alpha$  (Takada et al., 1987a). VLA-5 $\alpha$  has been shown to be crossreactive with our antifibronectin receptor serum and is presumed to be the same as the fibronectin receptor  $\alpha$  subunit (Takada et al., 1987b). In our sequence the amino terminus of the mature  $\alpha$  subunit corresponds to that obtained from VLA-5 $\alpha$ . Preceding the amino terminus of the mature polypeptide is a possible signal sequence initiated by a methionine. The mature  $\alpha$  subunit is 1,008 amino acids long and has a predicted molecular mass of 110,024 daltons. The sequence contains 14 potential *N*-glycosylation sequences (Asn-Xaa-Ser/Thr). If one assumes an average molecular mass of 2,500 daltons per *N*-linked carbohydrate-side chain, the addition of 14 such moieties to the core protein would result in an estimated molecular mass of 145,024 daltons. This value differs somewhat from that estimated from the relative mobility of the  $\alpha$  subunit on SDS-polyacrylamide gels of 160,000 daltons (Pytela et al., 1986).

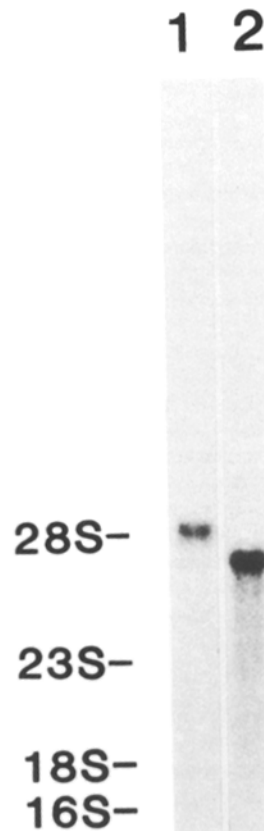
A hydrophathy analysis of the amino acid sequence deduced for the  $\alpha$  subunit revealed a hydrophobic region 29 residues



**Figure 1.** Immunoblot analysis of antibodies isolated by affinity absorption on insert-encoded proteins. Purified human placental fibronectin receptor (nonreduced) was immunologically stained with antifibronectin receptor serum (lane 1); antibodies affinity selected on cDNA-encoded protein of a fibronectin receptor  $\alpha$  subunit clone  $\lambda$ P7 (lane 2), and clone  $\lambda$ P32 (lane 3). Antibody reactive bands were visualized with horseradish peroxidase-labeled goat anti-rabbit IgG and 3,3'-diaminobenzidine tetrahydrochloride.

in length located near the carboxy terminus. This hydrophobic stretch fits criteria of a transmembrane domain (Argraves et al., 1986). It appears, therefore, that the  $\alpha$  subunit is oriented in the membrane with its amino terminus outside the cell. As a result, the bulk of the polypeptide (94%) is extracellular while on the carboxy-terminal side of the putative transmembrane domain is a 28-amino acid-long segment that is likely to constitute the cytoplasmic domain.

Further analysis of the primary sequence of the  $\alpha$  subunit revealed the presence of five sequences homologous with calcium-binding sequences of a number of calcium-binding proteins including calmodulin, troponin C, parvalbumin (Szebenyi et al., 1981), thrombospondin (Lawler and Hynes, 1986), and the myosin light chain (Reinach et al., 1986) (Fig. 4). These five potential divalent cation-binding sites of the  $\alpha$  subunit are located in the extracellular domain between amino acids 239 and 432, a region conspicuously devoid of cysteine residues. A secondary structure analysis (Garnier et al., 1978) of this region reveals that each potential binding site is likely to be surrounded by  $\beta$  structure rather than by



**Figure 2.** RNA hybridization analysis. Human placental total RNA was electrophoretically separated on 0.8% agarose-formaldehyde gels, transferred to nitrocellulose filters, and probed with a fibronectin receptor  $\alpha$  subunit cDNA ( $\lambda$ P7, 1.7 kb) (lane 1) and a fibronectin receptor  $\beta$  subunit cDNA ( $\lambda$ P32, 2.5 kb) (lane 2). The amount of RNA applied per lane was 10  $\mu$ g.

$\alpha$  helical segments as is the case with other calcium-binding proteins (Szebenyi et al., 1981). The presence of calcium-binding sites in the  $\alpha$  subunit is consistent with observations that divalent cations are required for the function and integrity of the human fibronectin-receptor as well as that of the related receptor, IIb/IIIa (Oppenheimer-Marks and Grinnell, 1984; Jennings and Phillips, 1982). The fact that bound fibronectin receptor, vitronectin receptor, and gp IIb/IIIa can all be eluted from ligand-Sepharose affinity columns using EDTA also indicates that divalent cations, e.g., calcium, are generally required for function of these adhesion receptors (Pytela et al., 1987).

A previously published comparison of partial amino acid sequences of the  $\alpha$  subunits of the human fibronectin receptor and the human vitronectin receptor showed that the two were strongly homologous (Suzuki et al., 1986). Using the now completed amino acid sequences for the  $\alpha$  subunits of the fibronectin receptor and the vitronectin receptor (Suzuki et al., 1987), a comparative analysis revealed an overall 46% amino acid identity. Conserved between both subunits are



the sizes of the ectodomains, the transmembrane, and cytoplasmic domains, as well as the relative positions of the potential divalent cation-binding sites and cysteine residues.

### The $\beta$ Subunit Sequence

The complete cDNA sequence and deduced amino acid sequence of the fibronectin receptor  $\beta$  subunit is shown in Fig. 5. The 3,614-bp cDNA sequence contains a single open reading frame of 2,394 bp encoding a polypeptide of 798 amino acids followed by a 1,113-bp untranslated 3' flanking region. The first 20 amino acids are predominantly hydrophobic, and correspond to a typical signal sequence (Watson, 1984). We have assigned the signal peptide cleavage site to a position that fits criteria described by von Heijne (1984) for eukaryotic signal-cleavage sites. The position of this signal cleavage site is analogous to those of two related  $\beta$  subunits, integrin complex band 3 (Tamkun et al., 1986) and LFA-1 (Kishimoto et al., 1987). Excluding the presumed signal sequence, the  $\beta$  subunit consists of 778 amino acids and has a predicted molecular mass of 86,248 daltons. The sequence contains 12 potential *N*-glycosylation sites. Addition of the *N*-linked carbohydrate chains (mol wt, 12) would result in an estimated molecular mass of 116,248 daltons for the mature  $\beta$  subunit. As with the  $\alpha$  chain, this value is lower than that estimated from the relative mobility of the reduced  $\beta$  subunit on SDS-polyacrylamide gels of 140,000 daltons (Pytela et al., 1985a, 1986). Located in the carboxy-terminal portion of the polypeptide is a stretch of 23 hydrophobic amino acids that are likely to constitute a transmembrane domain. All of the potential *N*-glycosylation sites are located on the amino-terminal side of this putative transmembrane domain. On the carboxy-terminal side of the hydrophobic segment are a pair of basic amino acid residues, which are typically found on the cytoplasmic side of transmembrane domains of integral membrane proteins (Sabatini et al., 1982). These features suggest that, like the  $\alpha$  subunit, the  $\beta$  subunit is oriented in the membrane with its amino terminus outside of the cell. Thus, the bulk of the  $\beta$  subunit polypeptide (93%) would be extracellular.

After the putative transmembrane domain is a 47-amino acid sequence presumed to represent the cytoplasmic domain of the  $\beta$  subunit. As in the chicken integrin band 3 subunit (Tamkun et al., 1986) and the IIb/IIIa  $\beta$  subunit (Fitzgerald et al., 1987), the cytoplasmic domain of the human fibronectin receptor  $\beta$  subunit contains a sequence having homology to a tyrosine phosphorylation site in the epidermal growth factor receptor. However, the putative tyrosine phosphorylation site is not invariant among all the adhesion receptor  $\beta$  subunits since one is not found in the LFA-1  $\beta$  subunit cytoplasmic domain (Kishimoto et al., 1987; Law et al., 1987).

The fibronectin receptor- $\beta$  subunit has a high cysteine content (7.3%), having a total of 58 cysteine residues. With the exception of two cysteines in the signal peptide, all are located in the extracellular domain. Many of the cysteines (51%) are arranged in a sequence homologously repeated four times within a 180-amino acid segment located adjacent to the transmembrane domain. When these homologous sequences are aligned, as shown in Fig. 6, it is clear that each contains nearly the same number and spacing of cysteine residues. The function of this cysteine-rich repeat is unknown.

The deduced sequence of the human fibronectin receptor  $\beta$

E	F	S	G	D	D	T	E	D	(239-247)
D	V	N	G	D	G	L	D	D	(293-301)
D	R	T	P	D	G	R	P	Q	(311-319)
D	L	D	Q	D	G	Y	N	D	(360-368)
D	L	D	G	N	G	Y	P	D	(424-432)
D	-	D	-	D	G	-	-	D	Thrombospondin consensus
D	Q	N	R	D	G	I	I	D	Myosin light chain
D	-	D	-	D	G	-	I	D	Troponin C consensus
D	-	D	G	D	G	-	I	-	Calmodulin consensus
D	-	D	-	-	G	-	I	-	Parvalbumin consensus

Figure 4. Homology comparison of divalent cation-binding sequences. Five sequences from the fibronectin receptor  $\alpha$  subunit are compared with calcium-binding consensus sequences from known calcium-binding proteins. Positions 1, 3, 5, 6, and 9 are generally conserved among all the repeats.

subunit was compared to the sequences of three other related adhesion receptor  $\beta$  subunits. The fibronectin receptor  $\beta$  subunit showed an 85% amino acid identity with the band 3 subunit of the chicken-integrin (Tamkun et al., 1986), 46% with the human LFA-1  $\beta$  subunit (Kishimoto et al., 1987), and 44% with the human gp IIIa (Fitzgerald et al., 1987). All of these  $\beta$  subunits share common structural elements including the homologous cysteine-rich repeats and similarly sized cytoplasmic and transmembrane domains. Also conserved among the  $\beta$  subunits are the number and nearly identical positions of the cysteine residues. The homology between the fibronectin receptor  $\beta$  subunit and the integrin band 3 subunit is particularly striking in the carboxy-terminal portions of the two subunits in which there is 100% identity in the amino acid sequences of the transmembrane and cytoplasmic domains.

### Discussion

We report here the complete sequence of a prototypic member of the adhesion receptor family, the human fibronectin receptor. The fibronectin receptor is defined as a heterodimeric glycoprotein isolated by affinity chromatography on fibronectin Sepharose. The purified glycoprotein can be incorporated into liposomes, and the resulting liposomes can be shown to bind specifically to fibronectin-coated substrata (Pytela et al., 1985a). As defined by the reactivities of fusion proteins synthesized from the cDNA clones with antibodies made against the purified receptor, our cDNA clones correspond to the subunits of the fibronectin receptor and provide the entire sequence of the receptor protein.

Individual subunits of other adhesion receptors have been cloned and completely or partially sequenced recently. Comparison of our sequences with these other sequences reveals several interesting relationships within the adhesion receptor superfamily. Each of the other receptor subunits, the vitronectin receptor  $\alpha$  subunit (Suzuki et al., 1986, 1987), the  $\beta$  subunit of the leukocyte receptor family (Kishimoto et al., 1987) and the platelet receptor  $\beta$  subunit, gp IIIa (Fitzgerald et al., 1987), is 40-50% homologous to the fibronectin re-

$\lambda$ 131  
 GTCCGCCAAAACCTGCGCGGATAGGGAAGAACAGCACCCCGCGCGGATGCGGTACCAAAACAGCCTAACGTCGCGCTGGGCCCGGACGCGCGCGGAAAAGATGAATTTACAACCAAT 120  
 M N L Q P I 6  
 TTTCTGGATTGGACTGATCAGTTTCAGTTGCTGTGTTGCTCAAACAGATGAAAATAGATGTTTAAAAGCAAATGCCAATCATGTGAGAAATGTATACAAGCAGGCGCAAATTTGTGG 240  
 F W I G L I S S V (C) (C) V F A Q T D E N R (C) L K A N A K S (C) G E (C) I Q A G P N (C) G  
 $\lambda$ P20  
 GTGGTGCACAAATTCACATTTTACAGGAAGGAATGGCTACTTCTGACAGATGTGATGATTTAGAAGCCTTAAAAAGAAAGGGTTGCCCTCCAGATGACATAGAAAATCCCAGAGGCTC 360  
 W (C) T N S T F L Q E G M P T S A R (C) D D L E A L K K K G (C) P P D D I E N P R G S 86  
 $\lambda$ 131  
 CAAAGATATAAAGAAAAATAAAATGTAACCAACCGTAGCAAAGGAACAGCAGAGAAGCTCAAGCCAGAGGATATTCATCAGATCCAACCCAGCAGTGGTTTTCGGATTAAGATCAGG 480  
 K D I K K N X N V T N R S K G T A E K L K P E D I H Q I Q P Q Q L V L R L R S G 126  
 GGAGCCACAGACATTTACATTAATAATCAAGAGAGCTGAAGACTATCCCATTCAGCTTACTACTTATGGACCTGTCTTATCAATGAAAGACGATTTGGAGAATGTAAAAGTCTTTGG 600  
 E P Q T F T L K F K R A E D Y P I D L Y L M D L S Y S M K D D L E N V K S L G 166  
 AACAGATCTGATGAATGAGGAGGATTTCTCGGACTTCAGAAATTTGGCTATTTGGCTCATTTGGTGAAGAAAGACTGTGATGCCCTTACATTCAGCAACACCAGCTCAGGAACCC 720  
 T D L M N E M R R I T S D F R I G F G S F V E K T V M P Y I S T P A K L R N P 206  
 TTGCACAAGTGAACAGAACTGCACCACCCCATTTAGCTACAAAATGTCTCAGTCTTACTAATAAAGGAGAAATTTAATGAACTTGTGGAAAACAGCGCATATTCGGAATTTGGA 840  
 (C) T S E Q N (C) T T P F S Y K N V L S L T N K G E V F N E L V G K Q R I S G N L D 246  
 TTCTCCAGAAGTGGTTTCGATGCCATTCAGTTCAGTTGTTGGTACTGCTGAGGAAATTTACAGGGCTGTGGTGTTCACAGATGCCGGCTTCACTTTGCTGG 960  
 S P E G G F D A I M Q V A V (C) G S L I G W R N V T R L L V F S T D A G F H F A G 286  
 AGATGGAAACCTGGTGGCATTGTTTACCAAAATGAGCAATGTCACCTGGAAAATAATGTACACAATGAGCCATTATTATGATTACTCTTATTGCTCACCTGATCCAGAACT 1080  
 D G K L G G I V L P N D G Q (C) H L E N N M Y T M S H Y Y D Y P S I A H L V Q K L 326  
 GAGTGAATAATATTACAGAAATTTTGCAGTACTGAAGAATTTTCAGCTTGTTCACAGGAGCTGAAAACCTTTGATCCCTAAGTCAGCAGTAGGAACATTATCTGCAAAATTCAGCAA 1200  
 S E N N I Q T I F A V T E E F Q P V Y K E L K N L I P K S A V G T L S A N S S N 366  
 $\lambda$ P32  
 $\lambda$ 134  
 TGTAATTCAGTTGATGATGCATACAAATCCCTTTCCSCAGAAGTCAATTTGGAAAACGGCAAATGTCAGAAGGAGTAACAATAAGTTACAAATCTTACTGCAAGACGGGGTGA 1320  
 V I Q L I I D A Y N S L S E A V T I L E N G K T G S E G V T I S Y K S Y (C) K N G V N 406  
 TGGAAACAGGGGAAAATGGAAGAAAATTTCCAAATTTCCATTTGGAGATGAGGTTCAATTTGAAATTTAGCATACTTCAAAATAAGTTCAAAAGGATTCTGACAGCTTTAAAATTTAG 1440  
 G T G E N G R K (C) S N I S I G D E V Q F E I S I T S N K (C) P K K D S D S P F K I R 446  
 GCCCTGGGCTTTACGGAGGAGTAGAGGTTATTTCTCAGTACATCTGTAATGTAATGCCAAAGCGAAGGCATCCCTGAAAGTCCCAAGTGTCAAGGAAATGGGACATTTGAGTG 1560  
 P L G F T E E V E V I L Q Y I (C) E (C) E (C) Q S E G I P E S P K (C) H E G N G T F E (C) 486  
 TGGCGGTGCAGGTGCAATGAGGCGGTGTGGTAGACATTTGTAATGAGCAGCAGATGAAATTAACAGTGAAGACATGGATGCTTACTGCGAGAAAGAAAACAGTTTCAGAAATCTCTGAG 1680  
 G A (C) R (C) N E G R V G R H (C) E (C) S T D E V N S E D M D A Y (C) R K E N S S E I I (C) S 526  
 $\lambda$ P20  
 TAACAATGGAGAGTGGCTCTCGGAGCAGTGTGTTTGTAGGAAGAGGATAAATACAAATGAAATTTATTCTGGCAAATTTCTGGAGTGTGATAATTTCAACTGTGATAGATCCAAATGGCTT 1800  
 N N G E (C) V (C) G Q (C) V (C) R K R D N T N E I Y S G K F (C) E (C) D N F N (C) D R S N G L 566  
 AATTTGGAGGAAATGGTGTGCAAGTGTGCTGTGTGAGTGCACCCCACTACTGGCAGTGCATGTGACTGTTCTTTGGATAGTACTTGTGAAGCCAGCAACGGACAGAT 1920  
 I (C) G G N G V (C) K (C) R V (C) E (C) N P N Y T G S A (C) D (C) S L D T S T (C) E A S N G Q I 606  
 CTGCAATGGCCGGGCACTCTCGAGTGTGGTGTCTGTAAGTGTACAGATCCGAAGTTCAGGGCAAACCTGTGAGATGTGTCAGACCTGCCCTGGTGTCTGTCTGAGCATAAAGAATG 2040  
 (C) N G R G I K (C) T G G V (C) T D P K F T Q G G Q (C) E M (C) Q T (C) L G V (C) A E H K E (C) 2646  
 TGTTCACTGACAGCCTTCAATAAAGGAGAAAAGAAAAGACACATGCACACAGGAATGTTCTATTTAACATTACCAAGGTAGAAGTTCGGGACAAATACCCAGCCGGTCCAACTGA 2160  
 V Q (C) R A F N K G E K K D T (C) T Q E (C) S Y F N I T K V E S R D K L P Q P V Q P D 686  
 TCCTGTGTCCTTGTAAAGGAGAGGATGTTGACGACTGTTGGTCTATTTTACGTATTTCAGTGAATGGGAACAACAGGTCATGGTTCATGTTGTGGAGAATCCAGAGTGTCCCCTGG 2280  
 P V S H (C) K E K D V D D (C) W F Y F T Y S V N G N N E V M V H V V E N P E (C) P T G 726  
 TCCAGACATTCATTCGAATTTAGCTGGTGTGGTGTGCTGGAATTTGTTTATTTGGCTTGCATTTACTGCTGATGGAAGCTTTTAATGATAATTCATGACAGAAGGGAGTTGCTAAAT 2400  
 P D I I P I V A G V V A G I V L I G L A L L L I W K L L M I I H D R R E F T A K F 766  
 TGAAGAGGAGAAAATGAATGCCAAATGGGACACGGGTGAAAATCCCTATTTATAAGAGTCCCGTAAACAACCTGGTCAATCCGAAGTATGAGGAAAATGAGTACTGCCCGTGCAAAATCCC 2520  
 E K E K M N A K W D T G E N P I Y K S A V T T V V N P K Y E G K 798  
 ACAACACTGAATGCAAGTAGCAATTTCCATAGTACAGTTAGGTTAGGCAATATTGCCATGGTTTACTCATGTGCAGGTTTGAAGAAATGTACAATGTAATAATTTTAAAA 2640  
 TGTTTATATTTTGAAGAAATGTTGTAATTCATGCCAGGACTGACAAAAGACTTGAGCAGGATGGTTATCTTGTGAGTAAAGTGCATTTGTGACCTTTTTCCTCTG 2760  
 GACTATGAAATCAAGCTTATGGATTAAGTATTTCTATAGCGATTGAAAGGGCAATAGTTAAAGTAAATGAGCATGATGAGAGTTTCTGTTAATCATGTATTAACACTGATTTTATG 2880  
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 CTGAAACAGATATGTTGAGAGTGTGCTGTTGTAATAACCTTGAATAGTTGATCTACAAGGCCATGGGAAAATTCAGAGAGTTAGGAAGGAAAACCAATAGCTTTAAAACCTGT 3120  
 GTGCCATTTTAAAGTACTTAATGTTGGTAACTTTATGCTTCACTTTTACAAATTCAGCCCTTAGATAAAGAAACCGAGCAATTTTCTGCTAAAAGTCCCTGATTTAGCACTATT 3240  
 ACATACGGCCATACTTTACAAAGTATTTGCTGAATGGGACCTTTGAGTGGAAATTTATTTATTTATTTTGTGTTAATGCTGGTGTCTTCTATCACCTTCTTAATCTTTTAA 3360  
 TGATTTGTTGCAATTTTGGGTAAGACTTTTTATGACTTTTTCTTTTGAAGTTTTCAGCGTCAATTTGCCCTTTTAAATGAACATGTGAAGTTATCTGTGGCTATGCAACAGCTC 3480  
 TCACCTACGGAGTCTTACTTTGAGTTAGTGCACATAACAGACCCTGTATGTTTACTTCTCACCATTGAGTTGCCCATCTGTTTTCACACTAGTACATTTCTGTTTAAAGTGCCTTA 3600  
 GTTTAACAGTTCA 3614  
 $\lambda$ P32

Figure 5. The cDNA sequence and deduced complete amino acid sequence of the human fibronectin receptor  $\beta$  subunit. The putative signal peptide cleavage site is indicated by an arrow pointing upward. Cysteine residues are circled. Potential N-linked glycosylation sites are designated by dots. The putative transmembrane domain is underlined. Right angle arrows denote positions of independent  $\lambda$ gt11 cDNA clones.

ceptor  $\alpha$  or  $\beta$  subunits, respectively. These receptor subunits are, therefore, likely to be the products of homologous but distinct genes derived from common ancestral  $\alpha$  and  $\beta$  subunit genes. In contrast, the band 3 subunit of the chicken-integrin RGD-directed receptor complex (Tamkun et al., 1986) is 85% homologous with our  $\beta$  subunit. This high homology strongly suggests that this polypeptide is the chicken homologue of the human fibronectin receptor  $\beta$  subunit.

The chicken receptor is a complex of three subunits, each presumably a distinct polypeptide (Horwitz et al., 1985; Buck et al., 1986). This would make the receptor different

in its subunit composition from the mammalian receptors, which are all heterodimers. Since these proteins appear to be related to the mammalian receptors, and since our results define the integrin subunit as the chicken homologue of the human fibronectin receptor  $\beta$  subunit, it is possible that one form of the adhesion receptors is a heterotrimer. However, it is known that the same  $\beta$  subunit can be shared by several heterodimeric receptors distinguished by their  $\alpha$  subunits. The human fibronectin receptor family encompasses at least five members defined immunologically as the so-called VLA proteins 1-5 (Hemler et al., 1987). An immunological com-

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466 C Q S E G I P E S P K C H E G N G T F E C G A C R C N E G R * V G * * * * * R H C E * C S T D E V N S E D M D A Y 515
516 C R K E N S * * * S E I T C S N * N G E C V C G G C V C R K R D N T N E I Y S G K F C E * C D N F N * * * * * 559
560 C D R S N G * * * L I C G * G N G V C K C R V C Z C N F N * Y T G * * * * * S A C D * C S L D T S T * * * * * 598
599 C E A S N G * * * Q I C * N G R G I T C E C G V C K C T D P K F G * * * * * Q T C E M Q Q C L G V C A E H K E C 646
1 2 3 4 5 6 7 8

```

Figure 6. Alignment of cysteine-rich repeat sequences from the fibronectin receptor  $\beta$  subunit. Identical amino acid residues are boxed. Asterisks indicate spaces inserted to optimize homology.

parison has indicated that the fibronectin receptor is VLA-5 (Takada et al., 1987b) and this result is confirmed by the identity of the amino-terminal amino acid sequence of VLA-5 $\alpha$  (Takada et al., 1987a) with the amino acid sequence deduced from our  $\alpha$  subunit cDNA clones. By analogy with the human proteins, the chicken complex could be a mixture of receptors related through a shared  $\beta$  subunit. This would agree with the finding that the chicken receptor complex has multiple specificities; it binds at least to fibronectin, laminin, and type IV collagen (Horwitz et al., 1985). However, as is most convincingly illustrated by the platelet RGD-directed receptor, it is possible that a single receptor protein can bind to more than one protein (Plow et al., 1984; Parise and Phillips, 1986; Pytela et al., 1986). It is, therefore, important that the receptors be defined as molecular entities. Our results provide such a definition for the human fibronectin receptor.

The sequences presented in this paper add new features to the known structure-function relationships of the adhesion receptors, also referred to as integrins (Hynes, 1987).<sup>1</sup> The calcium-binding sequences in the fibronectin receptor readily explain the divalent cation requirement for the RGD receptors to function. The functional impairment of the receptors in the absence of divalent cations appears to be at two levels. First, in the case of gp IIb/IIIa, the association of the subunits is dependent on the presence of calcium (Jennings and Phillips, 1982), although the other receptors may not require calcium for their subunits to remain together. Secondly, all of the receptors, including the leukocyte receptors (Springer et al., 1984) require divalent cations to function, and, in the case of the RGD-directed receptors, this is known to result in the inability of the receptor to bind its ligand in the absence of divalent cations (Pytela et al., 1987). This suggests that the calcium-binding sites of the  $\alpha$  subunit may form part of the ligand-binding site or that the occupation of these sites affects the conformation of the ligand-binding site. That the conformation of the receptor may be dependent upon calcium is suggested by the apparent sensitivity of the receptors to proteolytic digestion in the absence of diva-

lent cations (Oppenheimer-Marks and Grinnell, 1984). The location of the ligand-binding site in the RGD-directed receptors is not known, but both the  $\alpha$  and  $\beta$  subunits appear to participate in the binding (Buck et al., 1987; Santoro and Lawing, 1987). However, since  $\beta$  subunits can be shared by receptors that have different specificities (Ginsberg et al., 1987), at least the specificity of binding must be contributed by the  $\alpha$  subunit. It is interesting to note that, within the region containing the calcium-binding elements, the fibronectin receptor  $\alpha$  subunit contains an Asp-Gly-Arg (DGR) sequence. A peptide containing this sequence has been found to be a relatively nonspecific inhibitor of cell attachment to various RGD-containing proteins (Yamada and Kennedy, 1987). As additional adhesion receptors are characterized it will be possible to determine sequences serving functions common to all members of the family and those involved with unique functions such as ligand binding and signal transduction.

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<sup>1</sup>Since the fibronectin receptor binds the prototypic adhesion protein fibronectin and was the first RGD receptor to be identified, we propose that in the integrin nomenclature it would be called integrin 1.

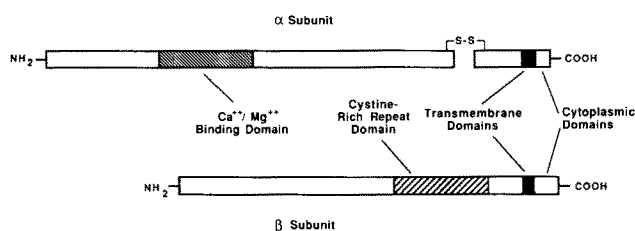


Figure 7. Structural model for the fibronectin receptor.

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