Cell Surface Molecules That Bind Fibronectin's Matrix Assembly Domain*

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The assembly of fibronectin into disulfide cross-linked extracellular matrices requires the interaction of mesenchymal cells with two distinct sites on fibronectin, the Arg-Gly-Asp cell adhesive site and an amino-terminal site contained within the first five type I homologous repeats (Quade, B. J., and McDonald, J. A. (1988) J. Biol. Chem. 263, 19602-19609). Proteolytically derived 29-kDa fragments of fibronectin (29kDa) containing these repeats bind to monolayers of cultured fibroblasts and inhibit fibronectin matrix assembly. The cell surface molecules interacting with fibronectin's 29-kDa matrix assembly domain have resisted purification using conventional methods such as affinity chromatography. Accordingly, in order to identify molecules which bind this fragment. 29kDa was allowed to bind to fibroblast monolayers and chemically cross-linked to the cell surface with bis(sulfosuccinimidyl) suberate. Extraction of the cross-linked cell layer yielded radiolabeled complexes of 56, 150, and 280 kDa. Formation of these cross-linked complexes was specifically inhibited by the addition of excess unlabeled 29kDa but was unaffected by the presence of fibronectin fragments containing other type I repeats outside of the 29kDa matrix assembly domain. The cross-linked complexes were insoluble in non-denaturing detergents but soluble when denatured and reduced, suggesting that 29kDa may be cross-linked to components of the pericellular matrix. Immunoprecipitation of cross-linked cell extracts with a polyclonal antibody to fibronectin that does not recognize the amino terminus demonstrate that the 280-kDa band contains 29kDa cross-linked to fibronectin present on the cell surface. Formation of the 150-kDa complex was inhibited by EDTA, suggesting thativalent cations are required for its formation. Although the molecular mass and divalent cation requirement suggest that the 150-kDa complex may be related to the 29kDa matrix assembly domain of fibronectin (12-14). The affinity of amino-terminal fragment binding to cell layers is equal to or greater than that of the a5β1 integrin cell adhesive receptors for soluble fibronectin (14, 15).

Fibronectins are dimeric cell adhesion glycoproteins secreted and assembled by mesenchymal cells into extracellular matrices (1, 2). Fibronectin-rich matrices are deposited in specific locations during embryogenesis and are essential for cell attachment, migration, differentiation, and organ morphogenesis (3-6). Fibronectins are composed of multiple repeats of homologous sequences termed type I, II, and III repeats which are grouped into larger protease-resistant domains which retain their biological activity after proteolytic cleavage of the intact fibronectin molecule (7). Fibroblasts organize fibronectins into detergent-insoluble disulfide cross-linked fibrils in vitro which resemble matrix fibrils found in vivo (8).

At least two sites in fibronectin are important for fibronectin matrix assembly. The first site is the Arg-Gly-Asp cell adhesive site. Fibronectin matrix assembly is inhibited by antibodies directed against the cell adhesive domain of fibronectin or against the α5β1 integrin fibronectin receptor (9-11). This cell adhesive receptor may function by nucleating fibronectin deposition (8-10). The other site essential for matrix assembly is located within the first five type I repeats of fibronectin. Addition of amino-terminal 29kDa containing these type I repeats (9, 12), antibodies to this domain, or a recombinant staphylococcal protein that binds this domain prevents fibronectin matrix assembly by fibroblasts, perhaps by competing for molecules which recognize the amino terminus and direct fiber elongation. Amino-terminal fibronectin fragments bind only to monolayers of cultured cells which assemble fibronectin into fibrils, suggesting the existence of a second fibronectin "receptor" independent from the α5β1 integrin receptor which interacts with the amino-terminal matrix assembly domain of fibronectin (12-14). The affinity of amino-terminal fragment binding to cell layers is equal to or greater than that of the α5β1 integrin cell adhesive receptors for soluble fibronectin (14, 15).

The binding of 29kDa to fibroblastic cells is anchorage-dependent. Although 29kDa specifically binds to fibroblast monolayers, it does not bind to suspended cells or to detergent extracted cell-free matrices (12, 13, 15). Not surprisingly, affinity chromatography of cell extracts on 29kDa-Sepharose has not identified a specific cell surface binding component. Accordingly, we sought to identify components in the cell

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The abbreviations used are: 29kDa, 29-kDa fragments of fibronectin; BS2, bis(sulfosuccinimidyl)suberate; PBS, phosphate-buffered saline; 40kDa, 40-kDa collagen-binding fragment; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

B. Quade and J. A. McDonald, unpublished observations.

B. J. Quade, M. Hook, and J. A. McDonald, manuscript in preparation.

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layer which interact with the amino-terminal matrix assembly domain of fibronectin by chemically cross-linking radio-labeled 29kDa bound to cultured chicken embryo fibroblast monolayers, purifying the resulting complexes with SDS-PAGE and characterizing them with immunoprecipitation. We employed the homohifunctional cross-linker bis(sulfosuccinimidyl) suberate (BS') which covalently bonds primary amines on the ligand to molecules brought into proximity by the native binding event. Using this strategy, we identified labeled complexes migrating at 56, 150, and 280 kDa on reduced SDS-PAGE. Similar cross-linked complexes were found using matrix producing cells of different species including human (IMR-90 and WI-38) and murine (Swiss albino 3T6) fibroblasts.

The 56-kDa complex may be two cross-linked 29-kDa fragments. Immunoprecipitation reveals the 280-kDa complex to contain 29kDa cross-linked to cell surface fibronectin. Although formation of the 150-kDa complex is shown to be dependent on divalent cations, this complex was not immunoprecipitable with an anti-β1 integrin antibody. Thus, components of the 150-kDa complex may represent unique molecules involved in fibronectin matrix assembly.

### Experimental Procedures

**Materials**—The homohifunctional cross-linker BS' and IODO-HEADS were obtained from Pierce Chemical Co.; fetal bovine serum was from Cell Culture Laboratories; and carrier-free Na125I and Rainbow molecular mass standards were from Amersham Corp. All other reagents were obtained from Sigma unless otherwise specified.

**Preparation of Reagents**—Fibronectin was prepared from a fibronectin-rich by-product of human factor VIII preparation or from citrated bovine plasma (12). Fibronectin fragments containing type I repeats including the amino-terminal 29-kDa fragment, the amino-terminantype II repeats, the collagen-binding 40-kDa fragment, and the carboxy-terminal disulfide-linked dimer of 68 and 75 kDa (denoted 68/75kDa) were prepared as described previously (12). A 125I metabolically labeled recombinant amino-terminal protein containing the first five type I repeats was purified as described from conditioned medium obtained by culturing CHO-K1 cells stably transfected with a plasmid containing a 940-base pair cDNA encoding the prepro region and first five type I repeats of fibronectin (16). Cell-free deoxycholate-insoluble fibronectin matrices were prepared by sequentially extracting cell monolayers with 3% Triton X-100, 0.1 mg/ml DNase, and 2% deoxycholate (11). A polyclonal antibody reacting to epitopes other than the 29kDa matrix assembly domain was prepared by absorbing an affinity-purified polyclonal rabbit anti-human plasma fibronectin antibody (17) with 29kDa-Sepharose. This antibody was verified not to react with 29kDa by immunoprecipitation (see below) and by enzyme-linked immunosorbent assay (data not shown). A rabbit polyclonal antibody recognizing chicken αβ1 integrin fibronectin receptors was the kind gift of Dr. Kenneth Yamada (National Institute of Dental Research, National Institutes of Health).

**Cell Culture**—Diploid human fetal lung fibroblasts IMR-90 (CCL 186) and WI-38 (CCL 75) and Swiss albino 3T6 fibroblasts (CCL 96) were obtained from the American Type Culture Collection (Rockville, MD) and cultured as described previously. Chicken embryo fibroblasts (CEF-14) were prepared from day 14 embryos and cultured in MCDB 201 media supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin at 37°C in 5% CO2 in the absence of ascorbate (12).

**Binding and Cross-linking of Radiolabeled Amino-terminal 29kDa**—The 29kDa fragments and collagen-binding 40-kDa fragments (0.1-1.0 mg in 0.5 ml of PBS) were iodinated with 1 mCi of Na125I in a siliconized glass tube for 10 min at 20°C using IODO-HEADS (Pierce Chemical Co.). Free iodine was removed by centrifugation (5 min, 1,200 x g) through a 3-ml G-10 Sephadex column equilibrated with PBS. The recovered products had specific activities of approximately 6 x 106 cpm/μg and greater than 95% of the activity was precipitated by 10% cold trichloroacetic acid. 125I-Labeled 29kDa was a single band on SDS-PAGE and autoradiography. For cross-linking, CEF-14 cells were grown to confluence in 100-mm dishes, rinsed three times with Dulbecco's modified Eagle's medium containing 1 mg/ml bovine serum albumin, and incubated for 1 h at 37°C with 125I-labeled 29kDa (5-12 μg/ml), 125I-labeled 40kDa (11 μg/ml), or 35S-labeled recombinant 29kDa amino-terminal protein (2-4 μg/ml) in Dulbecco's modified Eagle's medium containing 1 mg/ml bovine serum albumin. Following incubation the cell layers were washed five times with Hank's buffered saline solution. Bound amino-terminal fragment was cross-linked to nearby free amino groups on proteins using the water-soluble membrane-impermeable homobifunctional cross-linker BS' for 15 min at 30°C (18). Unreacted BS' was removed with two washes of 20 mM Tris, 150 mM NaCl, pH 7.4. Cell layers were solubilized with solutions of SDS with or without DTT, sodium deoxycholate, CHAPS, or Triton X-100 as indicated.

**Characterization of Cross-linked Complexes**—Cross-linked complexes solubilized in 2% SDS containing 50 mM DTT were acetylated with iodoacetamide, immunoprecipitated after addition of excess Triton X-100 (4:1 ratio) to form mixed micelles, and analyzed by SDS-PAGE and autoradiography as described previously (19-21). Molecular masses of cross-linked complexes were estimated by comparing the migration of the complexes to Rainbow molecular mass standards (Amersham Corp.). In some experiments, monolayers containing bound 125I-labeled 29kDa were treated for 10 min with 5 mM EDTA or 5 μg/ml cytochalasin B prior to the cross-linking step.

**RESULTS**

**Binding and Cross-linking of Fibronectin's Matrix Assembly Domain to Fibroblast Monolayers**—As shown in Fig. 1, three radiolabeled bands of apparent molecular mass 56, 150, and 280 kDa resulted when bound 125I-labeled 29kDa was cross-linked to the cell layer by BS'. The formation of these cross-linked complexes required interaction with the cell layer, as 125I-labeled 29kDa in solution was not cross-linked with BS'. Although the smallest complex migrating at 56 kDa may result from two 29-kDa fragments bound together, the larger radiolabeled complexes do not appear to be simple multimers of 29kDa created by cross-linking.

Identical cross-linked complexes have also been obtained
using another cross-linking reagent, ethylene glycolbis(sulfosuccinimidylsulfosuccinate), further confirming the results obtained using BS² (data not shown). However, repeated attempts to use iodinateable cleavable photoaffinity cross-linkers such as sulfosuccinimidy 2-(p-azidosalicyl-amido)ethyl-1,3'-dithiopropionate (SASD), which have the ability to transfer radioactive label from the derivitized ligand to receptors which bind them, have been unsuccessful. These reagents rely on a multi-step reaction in which the ligand is first derivitized with the cross-linker, allowed to bind to the cell surface, and cross-linked using ultraviolet light. Following the initial derivatization with SASD, 29kDa loses its ability to bind to cultured fibroblasts and could not be successfully cross-linked with this reagent.

Cross-linking of Labeled 29-kDa Fragment to Fibroblast Monolayers Was Specific—To determine if the labeled complexes resulted from cross-linking of ¹²⁵I-labeled 29kDa specifically bound to the cell surface, cells were incubated with ¹²⁵I-labeled 29kDa in the presence of 100-fold excess unlabeled 29kDa and cross-linked with BS². This prevented the appearance of all labeled higher molecular weight components (Figs. 1 and 2). To determine whether type I repeats found in other regions of the fibronectin molecule would prevent binding and cross-linking of the amino-terminal matrix assembly domain, fragments containing the other seven type I repeats, including the 40-kDa collagen-binding fragment and the carboxyl-terminal heparin-binding 65/75kDa dimer, were added during the binding of 29kDa to cell monolayers (12). Neither fragment affected formation of cross-linked complexes of ¹²⁵I-labeled 29-kDa fragment (Fig. 2).

To confirm that the cross-linked complexes represented specific binding of 29kDa to components of the cell monolayers rather than the concentration by cells of trace amounts of labeled polypeptides in the proteolytically derived 29kDa preparation, ³⁵S-labeled recombinant amino terminus was expressed in CHO-K1 cells and purified. The radiolabeled recombinant amino-terminal protein gave an identical pattern of cross-linked complexes when bound to fibroblasts and treated with BS² (Fig. 3).

As further demonstration of the specificity, cross-linking studies were performed with another fragment containing type I repeats that is not involved in matrix assembly by itself. The 40-kDa collagen-binding fragment contains four type I, two type II repeats, and has no effect on matrix assembly at concentrations as high as 25 µM (12). The 40-kDa fragment was labeled to a specific activity comparable with that of the labeled 29kDa. Binding of the labeled 40kDa to the chicken embryo fibroblast monolayer was approximately 3% that of 29kDa, and the bound 40kDa was not cross-linked into distinct complexes (Fig. 4). All cell lines tested to date that assemble fibronectin matrices bind the 29kDa matrix assembly domain, whereas other cell lines that are incapable of assembling a matrix do not (12). This suggests a common underlying mechanism of
to those obtained with chick embryo fibroblasts, with major plexes with 29kDa. Supporting this concept, 125I-labeled cells from differing species should form similar-sized compation of similar molecules in this process, matrix-forming Triton X-100. Immunoprecipitated extracts were evaluated on 5-15% the 125I-labeled 29kDa probe alone.

Figure 5. The 280-kDa complex contains 29kDa bound and cross-linked to cell surface fibronectin. 125I-labeled 29kDa was incubated with WI-38 fibroblasts for 1 h, cross-linked to the cell surface with 50 μM BS3, and extracted with 2% SDS containing 50 mM DTT. Cross-linked cell extracts were acetomethyalted with iodoacetamide and mixed micelles formed by the addition of 4-fold excess Triton X-100. Immunoprecipitated extracts were evaluated on 5-15% gradient SDS-PAGE and autoradiography performed. Lane A, total cross-linked extract demonstrating complexes migrating at 56 and 280 kDa. At this concentration of cross-linker the 150-kDa band was not prominent. Lane B, immunoprecipitation with a non-immune control rabbit IgG. Lane C, immunoprecipitation of 280-kDa complex with rabbit polyclonal antifibronectin antibody which was affinity purified to recognize only epitopes outside the 29kDa amino-terminal domain. Lane D, 125I-labeled 29kDa probe alone in solution. Lane E, the preabsorbed antifibronectin antibody does not immunoprecipitate the 125I-labeled 29kDa probe alone.

Characerization of the Cross-linked Complexes—The cross-linked complex at 280 kDa migrates as a closely spaced doublet (Fig. 2). Cellular fibronectin migrates as such a doublet on SDS-PAGE with a molecular mass of 250 kDa, suggesting that 29kDa may be cross-linked to fibronectin on the surface of the cells. To test this possibility, cross-linked complexes were immunoprecipitated with a polyclonal antibody to fibronectin that does not recognize the 29kDa matrix receptor. The 150-kDa complex was not immunoprecipitable using a polyclonal rabbit anti-chicken α5β1 integrin fibronectin receptor. The 150-kDa complex was not immunoprecipitable using this antibody (Fig. 8). To ensure that β1 integrins on cross-linked cells were recognized by the antibody, we surface-labeled cells with 125I, added cold 29kDa, cross-linked with BS3, and immunoprecipitated the detergent-solubilized cells as described. This antibody did immunoprecipitate β1 integrin from cell extracts, verifying its ability to recognize cell surface β1 integrins after cross-linking.

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Since the 280-kDa complex contains 29kDa cross-linked to fibronectin present on the surface of cells, we questioned whether cell-free fibronectin matrices alone would bind 29kDa. Accordingly, 125I-labeled 29kDa was bound to isolated cell-free deoxycholate-insoluble fibronectin matrices. The 125I-labeled 29kDa bound to isolated fibronectin matrices was only 1.7% that bound specifically to intact cell monolayers, strongly suggesting that additional cell surface components are required for 29kDa to interact with fibronectin.

The cross-linked complexes were sparingly soluble in non-denaturing detergents such as CHAPS and Triton X-100 but were soluble in denaturing agents, including SDS and sodium deoxycholate, and solubility was enhanced by the presence of DTT (Fig. 6). The requirement for denaturation to solubilize the cross-linked complexes is compatible with 29kDa being cross-linked to fibronectin in the pericellular matrix but also suggests that 29kDa might be cross-linked to components of the cytoskeleton. To test whether cytoskeletal components are involved in 29kDa binding, 125I-labeled 29kDa was bound to cell monolayers, the monolayers treated with cytochalasin B, rinsed, the eluted 29kDa quantified, and the bound fraction cross-linked with BS3. Cytochalasin B (5 μg/ml) treatment of cell-associated 125I-labeled 29kDa neither resulted in any significant loss of bound 29kDa nor altered the pattern of radiolabeled complexes obtained after cross-linking (Fig. 7).

To determine the potential role of divalent cations in the binding events resulting in cross-linked complexes, cell monolayers were allowed to bind 29kDa and then were treated with 5 mM EDTA in PBS prior to cross-linking. EDTA treatment resulted in loss of the 150-kDa complex without change in the 56- or 280-kDa bands (Fig. 7). Thus, formation of the 150-kDa complex on the cell surface is dependent on divalent cations. In light of the divalent cation dependence of the 150-kDa complex, its predicted molecular mass, and the role of β1 integrins in amino-terminal binding to cells (11), we hypothesized that this band might contain β1 integrin receptors. Accordingly, immunoprecipitated cross-linked cell extracts using a polyclonal rabbit anti-chicken α5β1 integrin fibronectin receptor. The 150-kDa complex was not immunoprecipitable using this antibody (Fig. 8). To ensure that β1 integrins on cross-linked cells were recognized by the antibody, we surface-labeled cells with 125I, added cold 29kDa, cross-linked with BS3, and immunoprecipitated the detergent-solubilized cells as described. This antibody did immunoprecipitate β1 integrin from cell extracts, verifying its ability to recognize cell surface β1 integrins after cross-linking.

Figure 6. The cross-linked complexes are insoluble in non-denaturing detergents but soluble in denaturing detergents. Confluent monolayers of CEF-14 were incubated with 125I-labeled 29kDa and cross-linked. One 100-μm plate was extracted with 2% SDS and 50 mM DTT. Another plate was extracted by scraping the monolayer with an equal volume of 1% Triton X-100 (TX-100) in PBS. The Triton X-100 extract was pelleted (10 min, 12,000 × g). To the Triton X-100-insoluble fraction, concentrated electrophoresis sample buffer containing SDS and DTT was added. To the Triton X-100-insoluble fraction, 1× SDS-PAGE sample buffer was added to a final volume one-half that of the Triton X-100-soluble fraction. SDS-PAGE was performed with a 5-15% denaturing polyacrylamide gel after loading equal volumes of each sample. Note the enrichment of the cross-linked complexes in the Triton X-100-insoluble fraction. Similar to Triton X-100 and SDS, the cross-linked 29kDa and the higher molecular mass complexes were insoluble in 20 mM CHAPS in PBS, but soluble in 2% sodium deoxycholate in 50 mM Tris, pH 8.8 (data not shown).
terminal binding activity to monolayer cultured cells was components involved. Because cell extracts and suspended cells lose 29kDa binding activity (12, 13, 15), a chemical cross-linking technique causing minimal perturbation of amino-terminal binding activity to monolayer cultured cells was employed. When $^{125}$I-labeled 29kDa was bound to confluent chicken embryo fibroblasts and cross-linked, specific labeled complexes of 56, 150, and 280 kDa were formed.

The formation of these cross-linked complexes was specific as assessed by: 1) fragments other than 29kDa containing type I repeats had no effect on binding and cross-linking of 29kDa on the cell surface. 2) Similar-sized cross-linked complexes were formed when fibroblasts from two other species were employed. 3) A fragment not involved in matrix assembly but containing type I repeats (the 40-kDa collagen-binding fragment) did not yield specific complexes when cross-linked to fibroblast monolayers. 4) A recombinant amino-terminal protein containing the first five type I repeats resulted in an identical pattern of complexes when cross-linked to matrix forming cells. It is therefore highly unlikely that the cell monolayers are simply concentrating minor proteolytic fragments contaminating the radiolabeled 29kDa preparations, since identical complexes were obtained when cross-linking was performed using this recombinant 29-kDa protein.

Fibronectin itself may act to direct the further assembly of fibronectin matrices. This is suggested by the demonstration that the 280-kDa cross-linked complex is composed of 29kDa bound to cell surface fibronectin. Clearly, fibronectin-fibronectin interactions are essential to the formation of the pericellular matrix. The location of this fibronectin-fibronectin interactive site is presently unknown, although a potential homophilic interactive site may exist in the first type III repeat of fibronectin, as antibodies directed against this region inhibit the binding of fibronectin to fibroblasts and the subsequent incorporation of this exogenous fibronectin into the pericellular matrix.

The formation of the 150-kDa cross-linked complex is dependent on divalent cations. It is therefore possible that this complex may contain an integrin receptor responsible for matrix assembly. Although this complex could not be immunoprecipitated with a polyclonal anti-$\beta_1$ integrin antibody, it remains possible that it may be composed of an alternate integrin receptor of the $\beta_1$ or even a new $\beta$ class. Integrins are a family of divalent cation-dependent cell surface molecules involved in cell-matrix and cell-cell interactions. Two fibronectin receptors, the $\alpha\beta_1$ and $\alpha\beta_1$, are members of the very late activation antigens group of integrins. These receptors recognize the Arg-Gly-Asp sequence and adjacent amino-terminal sequences (24–26). The $\alpha\beta_1$ receptor is required for efficient deposition of fibronectin matrices (9–11). Antibodies directed against $\beta_1$ integrins recently have been shown to partially inhibit fibronectin matrix assembly (11). Fogarty and co-workers (11) have also shown that antibodies directed against $\beta_1$ integrins inhibit the binding of amino-terminal fragments of fibronectin to fibroblasts, even though these fragments do not appear to bind directly to $\beta_1$ integrin (11). The matrix assembly domain contained in the amino-terminal region of fibronectin does not contain an Arg-Gly-Asp-like sequence, does not promote mesenchymal cell adhesion, and has not been shown to bind integrin receptors (13).

A key assumption implicit in using a chemical cross-linking strategy is that the labeled 29kDa and its receptor must have two free amino groups within the 12-Å span which can be bridged by the reactive groups of BS3 (21). This method is usually effective in detecting protein receptors, and trypsin treatment reduces amino-terminal fragment binding to cells, indicating that proteins are involved (12). However, cross-linking analysis will not identify small molecular mass or non-proteinaceous molecules, like gangliosides also implicated in the matrix assembly phenotype (27, 28). Gangliosides are

\[ \text{DISCUSSION} \]

The assembly of fibronectin into an insoluble disulfide cross-linked matrix depends on the interaction between an amino-terminal matrix assembly domain of fibronectin and the surface of matrix forming cells (12, 13). In contrast to the entropy-driven assembly of collagen fibrils, fibronectin matrix assembly is a cell-mediated process (22). To better understand this mechanism, we sought to identify the cell surface components involved. Because cell extracts and suspended cells lose 29kDa binding activity (12, 13, 15), a chemical cross-linking technique causing minimal perturbation of amino-terminal binding activity to monolayer cultured cells was employed. When $^{125}$I-labeled 29kDa was bound to confluent chicken embryo fibroblasts and cross-linked, specific labeled complexes of 56, 150, and 280 kDa were formed.

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known to also alter integrin function (29,30) and may affect fibronectin matrix assembly through other more complex mechanisms.

How these various amino-terminal binding components function together to direct fibronectin matrix assembly remains a puzzle. Clearly, the αβ₁ cell adhesive receptor as well as a yet to be determined site on fibronectin present on the cell surface are important for fibronectin matrix assembly. Fibronectin fibers assembled in the absence of transglutaminase are stabilized by disulfide bonds (13,31). The two cryptic free sulfhydryl in fibronectin are not essential for the disulfide-mediated stabilization, implying that disulfide bonds are catalytically rearranged during fiber assembly, possibly by the amino-terminal binding protein(s) (23). It is therefore highly likely that another receptor molecule which binds the amino terminus and affects disulfide rearrangement is critical for fibronectin matrix assembly. Understanding this novel post-translational processing will require further characterization of these matrix assembly domain binding proteins. We hope that identification of 29-kDa binding proteins by cross-linking will be a useful first step in understanding this process.

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