

**Abstract.** Previous studies have suggested that the assembly of fibronectin into the extracellular matrix of cultured fibroblasts is mediated by specific matrix assembly receptors that recognize a binding site in the amino terminus of the fibronectin molecule (McKeown-Longo, P. J., and D. E Mosher, 1985, J. Cell Biol., 100:364–374). In the presence of dexamethasone, human fibrosarcoma cells (HT-1080) acquired the ability to specifically bind exogenous plasma fibronectin and incorporate it into a detergent-insoluble extracellular matrix. Dexamethasone-induced fibronectin binding to HT-1080 cells was time dependent, dose dependent, and inhibited by cycloheximide. Saturation binding curves indicated that dexamethasone induced the appearance of 7.7 × 10^4 matrix assembly receptors per cell. The induced receptors exhibited a dissociation constant (K_d) for soluble fibronectin of 5.0 × 10^{-8} M. In parallel experiments, normal fibroblasts exhibited 4.1 × 10^5 receptors (K_d = 5.3 × 10^{-8} M) per cell. In the presence of cycloheximide, the induced fibronectin-binding activity on HT-1080 cells returned to uninduced levels within 12 h. In contrast, fibronectin-binding activity on normal fibroblasts was stable in the presence of cycloheximide for up to 54 h. The first-order rate constant (K_t = 2.07 × 10^{-4} min^{-1}) for the transfer of receptor-bound fibronectin to extracellular matrix was four- to fivefold less than that for normal fibroblasts (K_t = 1.32 × 10^{-3} min^{-1}). Lactoperoxidase-catalyzed iodination of HT-1080 monolayers indicated that a 48,000-mol-wt cell surface protein was enhanced with dexamethasone. The results from these experiments suggest that dexamethasone induces functional matrix assembly receptors on the surface of HT-1080 cells; however, the rate of incorporation of fibronectin into the matrix is much slower than that of normal fibroblasts.

**Fibronectin** is a prominent plasma protein that is primarily synthesized by the liver (45). In the plasma, fibronectin is found in a soluble form as a 440-kD disulfide-bonded dimer. An insoluble form of fibronectin is found in the extracellular matrix of loose connective tissues, basement membranes, and granulation tissue. Fibronectin may have both structural and adhesive functions in the matrix. Fibronectin has been shown to bind a number of matrix molecules including collagen, heparin/heparan, hyaluronic acid, and fibrin and may therefore have a role in the deposition and/or organization of these molecules in the matrix. When coated onto plastic or glass, fibronectin promotes the attachment and spreading of cells. This activity may provide fibronectin with the ability to mediate cell–cell and cell–tissue interactions. In addition, fibronectin can effect a number of cellular parameters including morphology, differentiation, migration, and proliferation (reviewed in references 24, 25, and 58).

In cultured fibroblasts, the extracellular matrix completely surrounds the cells, is insoluble in low ionic strength detergents and is stabilized by disulfide bonds (8). Components of the fibroblast matrix that have been identified include fibronectin, collagens (types I, III, and VI), thrombospondin (8, 18, 19, 27, 48), and proteoglycans (17). Fibronectin in the matrix is customarily found in the form of 5–10-nm fibrils (9, 12, 13, 18, 26, 51) composed of fibronectin derived from both the cells and the serum in which the cells are grown (16). Very little is known about precise mechanisms of extracellular matrix assembly. Fibronectin is the only matrix protein for which a specific, cell-mediated, assembly mechanism has been described (37–39). According to this model, soluble fibronectin is bound to cell surface receptors (matrix assembly receptors), which mediate the transfer of fibronectin into the detergent-insoluble extracellular matrix. The matrix assembly receptor recognizes a cell binding site in the amino terminus of the fibronectin molecule (39) and is distinct from the 140-kD receptor complex (cell attachment receptor) that functions in the attachment of cells to fibronectin matrices (1, 20, 47).

In vitro, many transformed cell lines do not contain an extracellular fibronectin matrix. This loss has been correlated with decreased fibronectin synthesis (11, 22, 42) and increased fibronectin degradation (42). In some systems, the addition of exogenous cellular fibronectin has been shown to cause transformed cells to assume a more normal phenotype.
which had been electrophoresed in the presence and absence of reducing agent.

**Determination of the Transfer Rate Constant \( (K_t) \) for Matrix Assembly**

This constant is based on the endocytic rate constant of Wiley and Cunningham (57). The transfer rate constant for matrix assembly defines the probability of receptor-bound fibronectin (pool I) being transferred into the matrix (pool II) over a 1-min period, based on the following equation:

\[
\frac{[\text{FN}]_n}{[\text{FN}]_I} = K_t, \quad \text{where} \quad [\text{FN}]_I \quad \text{and} \quad [\text{FN}]_n \quad \text{represent the concentration of fibronectin in pool I and pool II, respectively, at time } t. \]

This equation can be solved by a straight line with a slope \( K_t \).

**Indirect Immunofluorescence of Fibronectin in Cell Layers**

Cell layers in 35-mm plastic culture dishes were rinsed three times with 1.0 ml serum-free MEM and fixed for 10 min with 1.0 ml of 3.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After fixation, monolayers were rinsed three times with 1.0 ml of PBS. Rabbit antiserum against human plasma fibronectin was diluted 1:500 in PBS and incubated on monolayers for 1 h at room temperature. Rabbit antifibronectin was a gift from Dr. Deane Mosher. After staining with primary antibody, cell layers were washed for 30 min with three changes of PBS. Secondary antibody, fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Cooper Biomedical, Inc., Cockeysville, PA) was diluted 1:100 in PBS and incubated with monolayers for 1 h at room temperature. After a second antibody staining, monolayers were washed for 30 min with three changes of PBS and viewed under an Olympus IMT-2 inverted microscope (Olympus Corporation of America, New Hyde Park, NY) equipped with reflected light fluorescence. Controls using normal rabbit serum instead of primary antibody showed no fluorescence.

**Binding of \(^{125}\text{I}\)-Fibronectin to Cultured Cells**

All binding experiments were done using either F-12 (fibroblasts) or MEM (HT-1080) supplemented with either 5.0% FBS or 0.2% BSA. In some experiments medium was supplemented with dexamethasone, 1 x 10^{-7} M, or cycloheximide, 20 gg/ml. Specific protocols are provided in the figure legends. Binding medium contained between 350,000-800,000 cpm of \(^{125}\text{I}\)-fibronectin per ml. After incubation with labeled medium at 37°C, cultures were rinsed four times in cold (4°C) PBS, and cell layers were either sequentially extracted in 1% deoxycholate followed by 4% SDS or directly solubilized in 1.0 N sodium hydroxide. The sequential extraction procedure was used to distinguish cell surface-associated radioactivity (deoxycholate soluble, pool I) from radioactivity incorporated into the extracellular matrix (deoxycholate insoluble, pool II) as previously described (37). Deoxycholate-soluble and -insoluble material were separated by centrifugation at 35,000 g for 30 min.

Integrity of radioactivity extracted from cell layers was judged by SDS gel electrophoresis and autoradiography. Only intact fibronectin was associated with the monolayers. Degradation of \(^{125}\text{I}\)-fibronectin was assessed by SDS gel electrophoresis of medium containing labeled protein and by quantification of the percentage of labeled protein soluble in 10% trichloroacetic acid. Minimal degradation of labeled protein, 5% over a 24-h period, was observed in the medium conditioned by both cell types.

To determine specific binding of iodinated preparations, cell layers were incubated for 30 min at 37°C with \(^{125}\text{I}\)-fibronectin in the absence (total binding) or presence (nonspecific binding) of excess unlabeled fibronectin (350 gg/ml). The range of the specific binding of \(^{125}\text{I}\)-fibronectin was 60-70%.

**Quantification of Fibronectin Synthesis**

The amount of fibronectin secreted by the cell monolayers was measured in conditioned medium using an ELISA. 96-well microtiter plates were incubated overnight at room temperature with 200 ll of purified human plasma fibronectin at 4 gg/ml in Tris-buffered saline (TBS) containing 0.1% BSA. Samples to be tested, and fibronectin standard solutions, were diluted in PBS containing 5 mM EDTA and 0.5% Triton X-100. Samples (125 ll) were incubated with 125 ll of rabbit antifibronectin antibody solution (competition mixtures). Antifibronectin antibody was diluted 1:10,000 in 1. Abbreviation used in this paper: TBS, Tris-buffered saline.
TBS containing 10% normal goat serum. These mixtures were incubated overnight at 4°C. After overnight incubation, fibronectin-coated plates were blocked with 1.0% BSA in TBS for 30 min at room temperature, and rinsed twice for 5 min with TBS containing 0.05% Tween-20. Competition mixtures (200 μl) were transferred to coated wells and incubated 2 h at room temperature. Wells were then washed twice with TBS containing 0.05% Tween-20, and 200 μl of alkaline phosphatase-conjugated goat anti-rabbit IgG diluted 1:1,000 in TBS containing 0.1% BSA was added. Incubation with secondary antibody was for 2 h at room temperature. Wells were then washed twice with TBS containing 0.05% Tween-20. Alkaline phosphatase substrate was diluted to 1 mg/ml in TBS, pH 9.0, and 200 μl was added to each well. Color development was measured by Absorbance at 405 nm on an E1-307 microtiter plate reader (Bio-Tek Instruments, Inc., Burlington, VT). Materials for ELISA were purchased from Sigma Chemical Co.

Figure 1. Fluorescent staining of the fibronectin matrix of dexamethasone-treated HT-1080 cells. Monolayers of (a) HT-1080 cells, (b) HT-1080 cells pretreated for 72 h with 10^{-7} M dexamethasone, and (c) fibroblasts were stained for extracellular fibronectin by indirect immunofluorescence. Cell layers were fixed with 3.0% paraformaldehyde and stained with rabbit antiserum against human plasma fibronectin (1:500 dilution), followed by a second antibody staining using FITC-conjugated goat anti-rabbit IgG (1:100 dilution). (c-e) Phase contrasts of the same fields shown in a-c, respectively. Bars, 20 μm.

Lactoperoxidase-catalyzed Iodination of Cell Layers

HT-1080 cell monolayers in 35-mm plastic tissue culture dishes were rinsed four times with 2 ml of MEM at 37°C. Labeling was done in 600 μl of PBS containing 5.5 mM glucose, 120 μCi Na ^{125}I, 0.12 U glucosoxidase (Sigma Chemical Co.), and 12 μg of lactoperoxidase (Sigma Chemical Co.). Iodination was carried out for 10 min at room temperature. Iodinated monolayers were rinsed four times with 2 ml of MEM and dissolved into 200 μl of gel sample buffer (0.025 M Tris-HCl, pH 6.8, containing 2% SDS, and 10% glycerol).

Polyacrylamide Gel Electrophoresis

Samples were analyzed by SDS gel electrophoresis on linear (5–16%) polyacrylamide gels using a discontinuous buffer system (32). Iodinated proteins were visualized by autoradiography using XAR-5 film (Eastman Kodak Co., Rochester, NY). Materials for electrophoresis were purchased from Bio-Rad Laboratories.

Results

Effect of Dexamethasone on Fibronectin Matrix Assembly

Previous studies have shown that a human fibrosarcoma cell line, HT-1080, synthesizes small amounts of fibronectin and

McKeown-Longo and Etzler Dexamethasone-induced Matrix Assembly
does not assemble an extracellular fibronectin matrix. In the presence of $1 \times 10^{-7}$ M dexamethasone, these cells increased their rate of fibronectin biosynthesis, organized a fibronectin matrix, and exhibited changes in cellular adhesiveness and morphology (43). The results shown in Fig. 1 are in agreement with this previous report. In the absence of dexamethasone, HT-1080 cells grew as a monolayer of substrate-attached, spread cells. Within the monolayer, a clump of rounded, refractile cells was occasionally present (Fig. 1 d). These cell layers did not stain for fibronectin by indirect immunofluorescence (Fig. 1 a). In the presence of $1 \times 10^{-7}$ M dexamethasone, the HT-1080 cells flattened, appeared larger, and were less phase dense (Fig. 1 e). The HT-1080 cells exhibited an epitheloid morphology rather than the elongated, spindle shape typical of fibroblasts (Fig. 1 f). After 3 d in dexamethasone, HT-1080 cells accumulated a fibronectin-containing matrix that could be stained by indirect immunofluorescence (Fig. 1 b). The fibronectin was in the form of short fine fibrils and in diffuse amorphous patterns. Fibronectin appeared to be localized to regions of cell-cell contact. In contrast, normal fibroblasts exhibited a more extensive fibronectin matrix (Fig. 1 c) consisting of longer, more well-defined fibrils surrounding the cells.

The process of dexamethasone-induced fibronectin matrix assembly was also followed using $^{125}$I-fibronectin. We have previously shown that matrix assembly in cultured cell monolayers can be monitored by the incorporation of $^{125}$I-fibronectin into a detergent-insoluble binding pool within the cell layer (37–39). Based on data presented in these earlier studies, we have proposed that $^{125}$I-fibronectin does not deposit directly into the extracellular matrix, but must first bind to a detergent-soluble pool (pool I) within the monolayer. Pool I binding is believed to represent specific cell surface receptors (matrix assembly receptors). As a consequence of this binding, fibronectin can then be transferred to a detergent-insoluble pool (pool II) which represents fibronectin in the form of extracellular fibrils.

When HT-1080 cell layers were incubated with $^{125}$I-fibronectin for up to 24 h, no specific binding of $^{125}$I-fibronectin to the cell layers was observed (Fig. 2 a). After a 48-h pretreatment with dexamethasone, HT-1080 cell layers specifically bound $^{125}$I-fibronectin in both pool I and pool II (Fig. 2 b). Although dexamethasone restored the ability of HT-1080 cells to acquire a fibronectin matrix, accumulation of fibronectin in the cell layer represented only 30% of the levels incorporated by normal fibroblasts (Fig. 2 c). The kinetics of $^{125}$I-fibronectin binding in pool I were similar in dexamethasone-treated HT-1080 and normal fibroblasts, with an apparent steady state being reached between 3 and 6 h; however, absolute amounts of fibronectin bound were different. At steady state, normal fibroblast monolayers bound 2.5-fold more radioactivity in pool I than dexamethasone-treated HT-1080 cells. The incorporation of $^{125}$I-fibronectin into pool II was linear in both dexamethasone-treated HT-1080 cells and fibroblasts. However, fibronectin accumulated in pool II at a much slower rate in the HT-1080 monolayers over the 24-h period, resulting in fivefold less $^{125}$I-fibronectin incorporated into the matrix of HT-1080 monolayers than fibroblast monolayers.

Because two distinct cell surface receptors for fibronectin have been proposed (37, 39), a cell attachment receptor and a matrix assembly receptor, it was of interest to determine whether the dexamethasone-induced fibronectin binding by these cells was a function of induced matrix assembly receptor activity. Matrix assembly receptors specifically bind a 70-kD amino-terminal fragment of fibronectin, which binds to the receptor but does not become incorporated into the extracellular matrix (39). Because this fragment does not contain the cell attachment domain, it can be used to distinguish fibronectin binding to matrix assembly receptors from fibronectin binding to cell attachment receptors. The results shown in Fig. 2 represent radioactivity that could be competed for with excess unlabeled 70-kD fragment. Similar results were obtained using intact fibronectin as competitor (data not shown). A previously described (39) 150-kD fragment of fibronectin containing the cell attachment, but not the matrix assembly site, was unable to compete for the binding of $^{125}$I-fibronectin to the cell layers (data not shown). Therefore, the dexamethasone-induced incorporation of fibronectin into the detergent-insoluble extracellular matrix of HT-1080 cells represented matrix assembly via matrix assembly receptor activity.

The effect of the protein synthesis inhibitor, cycloheximide, on this process was tested to see whether the dexamethasone-induced binding of fibronectin to HT-1080 cells required the synthesis of new proteins. In the presence of 20

Figure 2. Time course of specific $^{125}$I-fibronectin binding to HT-1080 and fibroblast monolayers. Confluent cultures of (a) HT-1080 cells, (b) HT-1080 cells pretreated for 48 h with $10^{-7}$ M dexamethasone (DEX), and (c) fibroblasts (FIB) were incubated with 2.0 ml of MEM containing 5% FBS and $^{125}$I-fibronectin ($5 \times 10^{4}$ cpm/ml). Duplicate plates received binding medium containing the unlabeled 70-kD fragment of fibronectin (25 μg/ml). Dexamethasone-treated cells continued to receive dexamethasone during the 24-h binding period. At the indicated time points, labeled medium was removed and monolayers were processed as described in Materials and Methods to determine specific binding of $^{125}$I-fibronectin in pool I (open circles) and pool II (solid circles). Points are the average from duplicate plates and represent the radioactivity that could be competed for by the 70-kD fragment.
µg/ml cycloheximide, incorporation of 125I-fibronectin into dexamethasone-treated HT-1080 cells was inhibited by >70% (Fig. 3a). This suggested that the synthesis of some protein is important in the binding of fibronectin to these monolayers. The 30% binding, which was not inhibited, probably reflected nonspecific binding by the monolayers (see Fig. 4). In contrast, normal fibroblasts showed a slight increase in 125I-fibronectin binding in the presence of cycloheximide. This difference was apparent within 3 h, and became more pronounced after 5 h (Fig. 3b). Because these cells were pretreated for 48 h with cycloheximide, any proteins required for the assembly of fibronectin into the matrix must be relatively long-lived on normal fibroblasts. In addition, the synthesis and accumulation of some protein may decrease the binding of 125I-fibronectin to monolayers. Such proteins may include endogenous cellular fibronectin that may compete for the binding of putative matrix assembly receptors on the surface of these cells, or molecules that bind to fibronectin and decrease the affinity of fibronectin for the receptor.

**Kinetics of Induction and Deinduction of Fibronectin Binding**

The kinetics of induction of 125I-fibronectin binding to HT-1080 cells was examined by pretreating monolayers for various times with dexamethasone. Increased 125I-fibronectin binding was seen after 7 h of incubation with dexamethasone and a maximum level of binding was achieved after 14 h of incubation (Fig. 4). At this time, specific binding was enhanced 10-fold over control levels. Longer incubations (24-72 h) resulted in binding levels that were decreased 20-25% from maximum. A duplicate set of plates, which had received cycloheximide, showed no dexamethasone-dependent increase in binding (Fig. 4). In fact, binding was slightly decreased from control levels. Cells were treated with dexamethasone concentrations ranging from 10^{-5} to 10^{-3} M and maximal fibronectin binding occurred at 1 × 10^{-7} M (data not shown).

The deinduction of fibronectin-binding activity was exam-
of incubation in dexamethasone-free medium, the medium was replaced with 1.0 ml of MEM containing 5% FBS, and usI-fibronectin (6 × 10^6 cpm/ml). Those plates that had received cycloheximide continued to receive it during the 2-h labeling period. After the 2-h incubation with usI-fibronectin, the medium was removed and the monolayers were rinsed and scraped into 1.0 ml of complete medium containing no cycloheximide (solid circles) or 20 μg/ml (empty circles) cycloheximide. During the last 2 h of incubation in dexamethasone-free medium, the medium was replaced with 1.0 ml of MEM containing 5% FBS, and ^125I-fibronectin (6 × 10^6 cpm/ml). Those plates that had received cycloheximide continued to receive it during the 2-h labeling period. After the 2-h incubation with ^125I-fibronectin, the medium was removed and the monolayers were rinsed and scraped into 1.0 ml of 1.0 N sodium hydroxide for determination of bound radioactivity. Points represent the mean of triplicate plates and the standard error of the mean is represented by the bars. The dashed line represents ^125I-fibronectin binding to uninduced HT-1080 monolayers.

Kinetics of Induction of Fibronectin Biosynthesis

Because a previous report indicated that the primary effect of dexamethasone treatment on HT-1080 cells was an increase in the rate of fibronectin synthesis (43), we were interested in the relationship between the kinetics of induction of fibronectin synthesis and fibronectin-binding activity in these cells. As shown in Fig. 6, the time course of dexamethasone-induced increase in fibronectin synthesis correlated fairly well with the time course of fibronectin-binding activity (Fig. 4). The increased rate of fibronectin synthesis was detectable within 9 h after dexamethasone treatment, and reached a maximum between 13 and 20 h. At this time, the rate of fibronectin biosynthesis (1.31 μg/ml per 3 h) was almost 20 times greater than the uninduced rate (0.07 μg/ml per 3 h), and almost four times the rate of fibronectin synthesis in normal fibroblasts (0.38 μg/ml per 3 h) (data not shown). The increase in fibronectin biosynthetic rate is in agreement with a previous report (43). After 34 h in dexamethasone, the rate of fibronectin biosynthesis had dropped to about half of the maximum rate and remained at about this level for the next 18 h.

Saturation Binding of Fibronectin to Dexamethasone-induced HT-1080 Cells

The effect of increasing concentrations of fibronectin on binding to both dexamethasone-induced HT-1080 and normal fibroblasts was tested. Saturable binding was demonstrated in both systems, with half-maximal binding being achieved with about 10 μg/ml fibronectin (Fig. 7). HT-1080 cells that were not treated with dexamethasone showed no concentration dependence of fibronectin binding (data not shown). These data were replotted for Scatchard analysis (inset) and binding constants and receptor number per cell were determined. The dexamethasone-treated HT-1080 cells contained 7.7 × 10^4 receptor sites per cell. The induced apparent receptor had a dissociation constant (K_d) for fibronectin of 5.03 × 10^{-4} M. Normal fibroblasts contained 4.1 × 10^5 receptor sites per cell. The K_d for the normal fibroblast receptor for fibronectin was 5.34 × 10^{-4} M. These data were consistent with a single class of receptor. Therefore, dexamethasone-induced HT-1080 cells contain fivefold fewer receptors than normal fibroblasts; however, the induced receptors have the same affinity for fibronectin as the recep-
Figure 7. Saturation curve and Scatchard analysis of fibronectin binding to (a) dexamethasone-treated HT-1080 monolayers and (b) fibroblast monolayers. HT-1080 cells were treated with $10^{-7}$ M dexamethasone 18 h before incubation with $^{125}$I-fibronectin. Monolayers in 35-mm culture dishes were incubated for 3 h in MEM containing 0.2% BSA, $^{125}$I-fibronectin (7 x $10^5$ cpm/ml), and increasing concentrations of unlabeled fibronectin. After 3 h, monolayers were washed and scraped into 1.5 ml of 1.0% deoxycholate for determination of pool I binding. Data were analyzed by the method of Scatchard (insets) and fitted with a straight line ($r = 0.91$ [a], $r = 0.95$ [b]) to determine dissociation constants ($K_d$) and receptor number (sites).

Figure 8. Transfer rate constant for fibronectin matrix assembly. Confluent monolayers, in 35-mm culture dishes, of (a) HT-1080 cells and (b) fibroblasts were incubated with $10^{-3}$ M dexamethasone 18 h before incubation with $^{35}$S-fibronectin. Monolayers in 35-mm culture dishes were incubated for 3 h in MEM containing $10^{-7}$ M dexamethasone (HT-1080) or F-12 (FIB) containing 0.2% BSA, $^{35}$S-fibronectin ($7 \times 10^5$ cpm/ml), and increasing concentrations of unlabeled fibronectin. After 3 h, monolayers were washed and scraped into 1.5 ml of 1.0% deoxycholate for determination of pool I binding. Data were analyzed by the method of Scatchard (insets) and plotted as a function of time (inset).

Kinetics of Fibronectin Transfer into the Matrix

Previous studies (37, 39) have shown that the incorporation of exogenous plasma fibronectin into the extracellular matrix proceeds in a stepwise fashion where fibronectin is first bound to cell surface receptors (pool I) and subsequently transferred to a detergent-insoluble extracellular matrix (pool II). This implies that the rate of fibronectin accumulation in pool II depends both on the concentration of fibronectin bound in pool I and the rate at which pool I-bound fibronectin is transferred into pool II. As outlined in Materials and Methods, the rate of transfer of receptor-bound fibronectin into the matrix can be described by a transfer rate constant, $K_t$, which measures the probability of receptor-bound fibronectin being transferred into the matrix over a 1-min period. $K_t$ determinations for both fibroblasts and dexamethasone-treated HT-1080 cells were determined over a 6-h period (Fig. 8). As shown in the inset of Fig. 8, the $K_t$ for dexamethasone-induced HT-1080 cells was $2.07 \times 10^{-4}$ min$^{-1}$. The $K_t$ for normal fibroblasts was $1.32 \times 10^{-3}$ min$^{-1}$. These results indicated that once fibronectin is bound to the receptor, the HT-1080 cells are five- to sixfold less efficient than normal cells in transferring receptor-bound fibronectin into the matrix.

Dexamethasone-induced Cell Surface Proteins

The effect of dexamethasone on cell surface proteins was analyzed by lactoperoxidase-catalyzed iodination. In several experiments, only one cell surface protein was consistently enhanced after treatment with dexamethasone. This protein had an apparent molecular mass of 48,000 (Fig. 9), and was enhanced fivefold over uninduced levels as judged by densitometric scanning of the autoradiograph. A protein of the same molecular mass on the surface of normal fibroblasts was also iodinated (data not shown). The relative mobility of the 48-kD protein was unchanged in the presence of reducing agent, indicating that this protein was not involved in disulfide-bonded complexes, nor did it contain extensive intramolecular disulfide bonding (data not shown).

Discussion

Previous studies have shown that glucocorticoids can induce the formation of a fibronectin-containing extracellular matrix in some transformed cell lines (6, 13, 14, 43). In some cases, this was accompanied by increased cell flattening and adhesion (6, 43). Similar results have been reported in cultured rat hepatocytes (36), where dexamethasone also increases the rate of insulin receptor synthesis (50). Both an increased rate of fibronectin biosynthesis and changes in cell surface molecules have been postulated as possible mechanisms involved in mediating the phenotypic changes observed with glucocorticoid treatment. Glucocorticoid-induced increases in levels of fibronectin biosynthesis have been demonstrated in the human fibrosarcoma cell line, HT-1080 (43), and in a rat hepatoma cell line (7) as well as normal fibroblasts (34, 43). In the HT-1080 cells the increased rate...
and inhibited by cycloheximide; suggesting that fibronectin synthesis and have provided evidence for the induction of specific fibronectin-binding molecule(s), matrix assembly receptors. These receptors recognize a binding site in the amino terminus of the fibronectin molecule, termed the matrix assembly site. In the present study, we have confirmed the dexamethasone-induced increase in fibronectin synthesis and have provided evidence for the induction of specific fibronectin-binding molecule(s), matrix assembly receptors on the surface of these cells.

The induction of fibronectin-binding activity by dexamethasone in HT-1080 cells was dose dependent, time dependent, and inhibited by cycloheximide; suggesting that fibronectin binding required de novo protein synthesis. Specific binding of fibronectin to induced receptors was completely inhibited by a 70-kD fibronectin fragment containing the amino-terminal one-third of the molecule. This fragment binds to matrix assembly receptors and not to cell attachment receptors (39). Therefore, the induced receptors appeared to be receptors for fibronectin matrix assembly and not receptors for cell attachment. HT-1080 cells apparently contained functional cell attachment receptors, because they attached and spread normally on fibronectin-coated substrata (37). The induced receptor activity on HT-1080 cells exhibited equivalent binding affinity for fibronectin as the receptor on normal fibroblasts, \( K_D = 5.0 \times 10^{-8} \) M. However, HT-1080 cells contained fivefold fewer (77,000) receptor sites as compared with fibroblasts (410,000). The decreased number of receptor sites may be a consequence of the dexamethasone-induced receptor having a very short half-life on the cell surface. Fibronectin-binding activity on normal fibroblasts was stable for 54 h in the presence of cycloheximide, suggesting that the half-life of the receptor was on the order of several days. In contrast, the dexamethasone-induced fibronectin-binding activity had an apparent half-life of only 3.5 h in the presence of cycloheximide, indicating that either the receptor or some protein required for its activity had a very short half-life. The reason for this short half-life is not known. Transformed cells are known to be more proteolytically active than their non-transformed counterparts (3). It is possible, therefore, that receptor activity may be sensitive to proteolysis. However, the receptor activity of normal fibroblasts and induced HT-1080 cells was insensitive to mild trypsinization (unpublished observations). Transformed cells, including some clones of HT-1080 cells, are known to synthesize increased amounts of plasminogen activator (30), and dexamethasone has been shown to induce an inhibitor of plasminogen activator in hepatoma cells (10). Therefore, dexamethasone could have a stabilizing effect on a plasmin-sensitive receptor. This seems unlikely because \( ^{125}I \)-fibronectin, a plasmin-sensitive protein (28), was stable in the medium of HT-1080 cell cultures for at least 24 h (unpublished observations). Alternatively, the unstable association of the induced receptor with the cell surface could result from a defect in either the receptor itself or another molecule (membrane or cytoskeletal) required for receptor activity.

The kinetics of binding of fibronectin to the dexamethasone-induced receptor (pool I binding) were similar to those obtained using normal fibroblasts. However, once fibronectin was bound to the receptors, its rate of transfer (\( K_I \)) into the extracellular matrix was fivefold lower than that of normal fibroblasts. Therefore, when compared with normal fibroblasts, the slower rate of accumulation of fibronectin into the matrix of dexamethasone-treated HT-1080 cells (Fig. 2) resulted from decreased fibronectin binding in pool I (fewer receptors) and slower transfer of receptor-bound fibronectin into pool II (lower \( K_I \)). This difference was also reflected in the immunofluorescent staining patterns (Fig. 1), which showed that a relatively small proportion of the fibronectin in the matrix of dexamethasone-induced HT-1080 cells was in the form of fibrils, as compared with normal. The reason for the lower transfer rate constant in HT-1080 cells is not known. In fact, very little is known about the processes in normal fibroblasts that render fibronectin detergent insoluble. Once bound to the receptors, fibronectin molecules can form disulfide-bonded aggregate structures through intramolecular disulfide exchange within their amino termini (38). This process may be important in fibronectin-forming fibrils and in becoming detergent insoluble. In addition, a number of forces may regulate the formation and organization of fibronectin fibrils, including cell surface gangliosides (31, 52, 53, 59), the cytoskeleton (35), and other matrix molecules (29, 49). Molecules representing all these categories have been shown to be altered during transformation (4). Alternatively, the decreased rate of fibronectin transfer into the extracellular matrix of HT-1080 cells may reflect differences in the cell cycle between normal fibroblasts, which are contact inhibited, and HT-1080 cells, which are actively divid-

**Figure 9.** Autoradiograph of iodinated cell surface molecules from HT-1080 monolayers. HT-1080 cell monolayers were either (a) untreated or (b) pretreated for 24 h with 10\(^{-7}\) M dexamethasone before lactoperoxidase-catalyzed iodination. Cell layers were dissolved in gel sample buffer (2.0% \( \beta \)-mercaptoethanol, boiled, and electrophoresed into linear (5–16%) polyacrylamide gels. After electrophoresis gels were fixed and dried and radioactivity was visualized by autoradiography. Slashes indicate molecular mass standards. Arrow indicates the 48-kD dexamethasone-sensitive protein.
ing. It has been shown that actively growing cells contain less fibronectin matrix than cells that are growth arrested (23). Using lactoperoxidase-catalyzed iodination of cell monolayers we have identified only one cell surface protein that is enhanced after treatment with dexamethasone. This protein has a molecular mass of 48,000. The dexamethasone-induced 48-kD protein on the surface of the HT-1080 cells may be related to a 47/48-kD cell surface protein described by others. Such a protein has been identified on the surface of baby hamster kidney (BHK) cells, and has been implicated in mediating the spreading of these cells on fibronectin (5, 21). In one study (5), this protein was preferentially cross-linked to fibronectin during attachment and spreading of BHK cells to fibronectin-coated glass coverslips. Antibodies prepared against a fraction enriched for this protein were subsequently shown to block spreading, but not initial attachment of cells to fibronectin (21). In a second study, antibodies prepared against BHK cell wheat germ agglutinin receptors caused rounding and detachment of cells from fibronectin substrata. This study suggested that a trypsin-resistant 48-kD cell surface protein was important in the adhesion of cells to fibronectin (44). A recent report has identified a similar class of cell surface antigen on murine and human fibroblasts: immunoperoxidase and immunoferritin localization. J. Histochem. Cytochem. 28:1319-1333.

The function of the dexamethasone-sensitive 48-kD protein on the surface of HT-1080 cells is not known. It is induced on the cell surface within 14 h and is lost from the surface during a 10-h chase, where it is recovered, apparently intact, from the conditioned medium (unpublished observations). The kinetics of 48-kD induction and loss are similar to those shown for fibronectin binding, suggesting that this molecule may be involved in the binding of soluble fibronectin to cell surfaces. Alternatively, the 48-kD protein may play a role in the dexamethasone-induced spreading of these cells and may accumulate in the cell layer secondary to the deposition of a fibronectin matrix. A third possibility is that the assembly of the extracellular matrix is coupled to the process of cell spreading and that the 48-kD protein is involved in both processes. A recent report using variants of a ganglioside-deficient cell line have correlated the expression of complex gangliosides with the ability of these variant cell types to organize fibronectin into extracellular fibrils (52). These authors have postulated that the matrix assembly receptor may be a complex ganglioside. Consistent with this hypothesis, Thompson et al. (54) have identified a ganglioside-binding site in the amino terminus of fibronectin (54). Because these studies were performed using a fibronectin-binding subset of oligosaccharides purified from brain gangliosides, the physiological significance of this binding remains to be determined. However, it does appear that the ability of cells to organize a fibronectin matrix may require the proper set of interactions among several cellular structures including the cell surface and the cytoskeleton.

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References

1. Akiyama, S. K., S. S. Yamada, and K. M. Yamada. 1986. Characterization of a 140 Kd avian cell surface antigen as a fibronectin-binding molecule. J. Cell Biol. 102:442-448.

2. Alti, J. V., V. Manurer, R. Lanza, and R. O. Hynes. 1977. Restoration of normal morphology, adhesion and cytoskeleton in transformed cells by addition of a transformation-sensitive surface protein. Cell. 11:115-126.

3. Alitalo, K., J. Keski-Oja, K. Hedman, and A. Vaheri. 1982. Loss of different pericellular matrix components of rat cells transformed with a T-class is mutant of rous sarcoma virus. Virology. 119:347-357.

4. Alitalo, K., and A. Vaheri. 1982. Pericellular matrix in malignant transformation. Adv. Cancer Res. 37:111-158.

5. Apolin, J. D., R. C. Hughes, C. L. Jaffe, and N. Sharon. 1981. Reversible cross-linking of cellular components of adherent fibroblasts to fibronectin and lectin-coated substrata. Exp. Cell Res. 134:488-494.

6. Armelin, M. C. S., and H. A. Armelin. 1983. Glucocorticoid hormone modulation of both cell surface and cytoskeleton related to growth control of rat glioma cells. J. Cell Biol. 97:459-465.

7. Baumann, H., and D. Eldredge. 1982. Dexamethasone increases the synthesis and secretion of a partially active fibronectin in rat hepatoma cells. J. Cell Biol. 95:29-40.

8. Carter, W. G., and S. Hakomori. 1981. A new cell surface determinant-dissoluble glycoprotein matrix of human and hamster fibroblasts. J. Biol. Chem. 256:6960-6962.

9. Chen, L. B., A. Murray, B. Segal, and M. L. Walsh. 1978. Studies on intercellular LETS glycoprotein matrices. Cell. 14:377-391.

10. Cwikel, B., P. A. Barouski-Miller, P. L. Coleman, and T. D. Geletrides. 1984. Dexamethasone induction of an inhibitor of plasminogen activator in HT-29 cells. Virology. 259:6847-6851.

11. Fagan, J. B., M. E. Sobel, K. M. Yamada, B. Decrombrugghe, and I. Pastan. 1981. Effects of transformation on fibronectin gene expression using cloned fibronectin cDNA. J. Biol. Chem. 256:520-525.

12. Furcht, L. T., D. Smith, G. Wendelschafer-Crabb, D. F. Mosher, and J. M. Foidart. 1980. Fibronectin present in native collagen fibrils of human fibroblasts: immunoperoxidase and immunoferritin localization. J. Histochem. Cytochem. 28:1319-1333.

13. Furcht, L. T., D. F. Mosher, G. Wendelschafer-Crabb, and J. F. Foidart. 1979. Reversal by glucocorticoid hormones of the loss of a fibronectin and procollagen matrix around transformed human cells. Cancer Res. 39:2077-2083.

14. Furcht, L. T., D. F. Mosher, G. Wendelschafer-Crabb, P. A. Woodbridge, and J. M. Foidart. 1979. Dexamethasone-induced accumulation of a fibronectin and collagen extracellular matrix in transformed human cells. Nature (Lond.). 277:393-395.

15. Gobel, E. G., and E. Ruoslahti. 1981. Concomitant loss of cell surface fibronectin and laminin from transformed rat kidney cells. J. Cell Biol. 88:352-357.

16. Hayman, E. G., and E. Ruoslahti. 1979. Distribution of fetal bovine serum fibronectin and endogenous rat cell fibronectin extracellular matrix. J. Cell Biol. 93:255-259.

17. Hedman, K., S. Johansson, T. Vartio, L. Kjellin, A. Vaheri, and M. Hook. 1982. Structure of the pericellular matrix: association of heparan and chondroitin sulfates with fibrinogen-procollagen fibers. Cell. 28:663-671.

18. Hedman, K., M. Krontinen, K. Alitalo, A. Vaheri, S. Johansson, and M. Hedman. 1979. Isolation of the pericellular matrix of human fibroblast cultures. J. Cell Biol. 81:83-91.

19. Helder-Rosler, R. A., and W. G. Carter. 1984. Pepsin-generated Type VI collagen is a degradation product of GP140. J. Biol. Chem. 259:6858-6864.

20. Horwitz, A. K., R. Greggs, C. Decker, and C. Buck. 1985. The cell substrate attachment antigen has properties of a receptor for laminin and fibronectin. J. Cell Biol. 101:2134-2144.

21. Hughes, R. C., T. D. Butters, and J. D. Apolin. 1981. Eur. J. Cell Biol. 26:198-207.

22. Hynes, R. O., I. V. Ali, A. T. Destrue, V. Maunter, M. E. Perkins, D. R. Singer, D. D. Wuyten, and K. K. Smith. 1978. A large glycoprotein lost from the surfaces of transformed cells. Ann. N.Y. Acad. Sci. 312:317-323.

23. Hynes, R. O., and J. M. Bye. 1974. Density and cell cycle dependence of cell surface proteins in hamster fibroblasts. Cell. 3:113-120.

24. Hynes, R. O. 1983. Fibronectin and its relation to cellular structure and behavior. In Cell Biology of Extracellular Matrix. E. D. Hay, editor. Plenum Press, New York. 295-326.

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

26. Irish, P. S., and D. L. Hasty. 1983. Immunocytochemical localization of fibronectin in human fibroblast cultures using a cell surface replica technique. J. Histochem. Cytochem. 31:69-77.

27. Jaffe, E. A., J. T. Rochlin, L. L. K. Leung, M. J. Doyle, P. J. McKeown-Longo, and D. F. Mosher. 1983. Cultured human fibroblasts synthesize and secrete thrombospondin and incorporate it into extracellular matrix. Proc. Natl. Acad. Sci. USA. 80:998-1002.

28. Ilark, F., and H. Hormann. 1977. Cold-insoluble globulin II. Plasminolysis of cold-insoluble globulin. Hoppe-Seylers Z. Physiol. Chem. 358:133-136.

29. Johansson, S., and M. Hook. 1984. Substrate adhesion of rat hepatocytes: on the mechanism of attachment to fibronectin. J. Cell Biol. 98:810-
polymers.

exogenously added cellular fibronectin. adhesion sites in monensin-exposed cultured human fibroblasts in response to containing structures on the surface of lamelloplasm of flbroblasts; hypothesis of receptor-mediated assembly of fibronectin structures.

tocytes in vitro.

cell-substratum adhesion receptors against proteolysis.

Infect. Dis.

bly of the head of bacteriophage. Nature (Lond.).

817.

30. Jones, P. A., W. E. Laug, and W. F. Benedict. 1975. Fibrinolytic activity in a human fibrosarcoma cell line and evidence for the induction of plasminogen activator secretion during tumor formation. Cell. 6:245-252.31. Kleinman, H. K., G. R. Martin, and P. H. Fishman. 1979. Ganglioside inhibition of fibronectin-mediated cell adhesion to collagen. Proc. Natl. Acad. Sci. USA. 76:3367-3371.

32. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature (Lond.). 277:680-685.

33. Lehto, V. P., and I. Virtanen. 1985. Formation of stress fibers and focal adhesion sites in monensin-exposed cultured human fibroblasts in response to exogenously added cellular fibronectin. Exp. Cell Res. 158:563-569.

34. Lien, Y., M. J. Wong, M. S. Golbas, and R. Stein. 1984. Hydrocortisone stimulates fibronectin synthesis in cultured fibroblasts. 1984. J. Cell. Physiol. 120:103-107.

35. Lyubimova, A. V., and J. M. Vasiliev. 1982. Distribution of fibronectin-containing structures on the surface of lamelloplasm of fibroblasts; hypothesis of receptor-mediated assembly of fibronectin structures. Cell Biol. Int. Rep. 6:105-112.

36. Marceau, N., R. Goyette, J. P. Valet, and J. Deschenes. 1980. The effect of dexamethasone on formation of a fibronectin extracellular matrix by rat hepatocytes in vitro. Exp. Cell. Res. 125:497-502.

37. McKeown-Longo, P. J., and D. F. Mosher. 1983. Binding of plasma fibronectin to cell layers of human skin fibroblasts. J. Cell Biol. 97:466-472.

38. McKeown-Longo, P. J., and D. F. Mosher. 1984. Mechanism of formation of disulfide-bonded multimers of plasma fibronectin in cell layers of cultured human fibroblasts. J. Biol. Chem. 259:12210-12215.

39. McKeown-Longo, P. J., and D. F. Mosher. 1985. Interaction of the 70,000-mol-wt amino-terminal fragment of fibronectin with the matrix-assembley receptor of fibroblasts. J. Cell Biol. 100:364-374.

40. McKeown-Longo, P. J. 1987. Fibronectin-cell surface interactions. Rev. Infect. Dis. In press.

41. Mosher, D. F., and P. J. McKeown-Longo. 1985. Assembly of fibronectin-containing extracellular matrix: a glimpse of the machinery. Bio polymers. 24:199-210.

42. Olden, K., and K. M. Yamada. 1977. Mechanism of the decrease in the major cell surface protein of chick embryo fibroblasts after transformation. Cell. 11:957-969.

43. Oliver, N., R. F. Newby, L. T. Furcht, and S. Bourgeois. 1983. Regulation of fibronectin biosynthesis by glucocorticoids in human fibrosarcoma cells and normal fibroblasts. Cell. 33:287-296.

44. Oppenheimer-Marks, N., and F. Grinnell. 1984. Calcium ions protect cell-substratum adhesion receptors against proteolysis. Exp. Cell Res. 152:467-475.

45. Owens, M. R., and C. D. Cinimino. 1982. Synthesis of fibronectin by the isolated perfused rat liver. Blood. 59:1305-1309.

46. Perkins, M. E., T. H. Ji, and R. O. Hynes. 1979. Cross-linking of fibronectin to sulfated proteoglycans at the cell surface. Cell. 16:941-955.

47. Pytel, R., M. D. Pierschbacher, and E. Rousslah. 1985. Identification and isolation of a 140 kD cell surface glycoprotein with properties expected of a fibronectin receptor. Cell. 40:191-198.

48. Raugi, G. J., S. M. Mumby, D. Abbott-Brown, and P. Bornstein. 1982. Thrombospondin: synthesis and secretion by cells in culture. J. Cell Biol. 95:351-354.

49. Rennard, S. I., M. L. Wind, A. T. Hewitt, and H. K. Kleinman. 1981. Effect of collagen and cell shape of the binding of fibronectin to cells. Arch. Biochem. Biophys. 206:205-212.

50. Salhanick, A. I., N. M. Krupp, and J. M. Amatruda. 1983. Dexamethasone stimulates insulin receptor synthesis in cultured rat hepatocytes. J. Biol. Chem. 258:14130-14135.

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

52. Spiegel, S., K. M. Yamada, B. E. Hon, J. Moss, and P. H. Fishman. 1986. Fibrillar organization of fibronectin is expressed coordinately with cell surface gangliosides in a variant murine fibroblast. J. Cell Biol. 102:1898-1906.

53. Spiegel, S., J. Schlessinger, and P. H. Fishman. 1984. Incorporation of fluorescent gangliosides into human fibroblasts: mobility, fate and interaction with fibronectin. J. Cell Biol. 99:699-705.

54. Thompson, L. K., P. M. Horowitz, K. L. Bentley, D. D. Thomas, J. F. Alderete, and R. J. Klebe. 1986. Localization of the ganglioside-binding site of fibronectin. J. Biol. Chem. 261:5209-5214.

55. Urasihara, H., and K. M. Yamada. 1986. Evidence for involvement of more than one class of glycoprotein in cell interactions with fibronectin. J. Cell. Physiol. 126:323-332.

56. Wagner, D. D., R. Ivatt, A. T. Destree, and R. O. Hynes. 1981. Similarities and differences between the fibronectins of normal and transformed hamster cells. J. Biol. Chem. 256:11708-11715.

57. Wiley, H. S., and D. D. Cunningham. 1982. The endocytic rate constant, A cellular parameter for quantitating receptor-mediated endocytosis. J. Biol. Chem. 257:4222-4229.

58. Yamada, K. M. 1983. Cell surface interactions with extracellular materials. Annu. Rev. Biochem. 52:761-799.

59. Yamada, K. M., D. R. Critchley, P. H. Fishman, and J. Moss. 1983. Exogenous gangliosides enhance the interaction of fibronectin with ganglioside-deficient cells. Exp. Cell. Res. 143:295-302.

60. Yamada, K., S. S. Yamada, and J. Pastan. 1976. Cell surface protein partially restores morphology, adhesiveness and contact inhibition of movement to transformed fibroblasts. Proc. Natl. Acad. Sci. USA. 73:1217-1221.