Role of Fibronectin as a Growth Factor for Fibroblasts

PETER B. BITTERMAN, STEPHEN I. RENNARD, STEVEN ADELBERG, and RONALD G. CRYSTAL
Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

ABSTRACT Fibroblast replication is regulated by exogenous signals provided by growth factors, mediators that interact with the target cell surface and signal the cell to proliferate. A useful model of growth regulation, the “dual control model,” suggests that growth factors can be grouped either as competence factors or as progression factors, and that optimal replication of fibroblasts requires the presence of both types of growth factors. Although most growth factors are soluble mediators, recent studies have demonstrated that, for some cell types, the extracellular matrix can replace the requirement for a competence factor. Since fibronectin is an important constituent of the extracellular matrix that interacts with specific domains on the fibroblast surface, we examined the ability of fibronectin to act as a competence factor to promote the growth of human diploid fibroblasts. To accomplish this, fibronectins purified from two sources, human plasma and human alveolar macrophages, were tested for their ability to (a) stimulate fibroblast replication in serum-free medium containing characterized progression factors (insulin or alveolar macrophage-derived growth factor); (b) provide a growth-promoting signal early in G1. Fibronectin stimulated fibroblast replication in a dose-dependent manner in the presence of a fixed dose of a progression factor. Conversely, fibronectin conferred on previously unresponsive fibroblasts the ability to replicate in a dose-dependent manner when cultured with increasing amounts of a progression factor. Moreover, fibronectin signaled growth-arrested fibroblasts to traverse G1 ~4 h closer to S phase. No differences were observed in the ability of plasma or macrophage fibronectins to provide a competence signal for fibroblast replication. Since fibronectin is a major component of the extracellular matrix, these observations suggest that it may provide at least one of the signals by which the matrix conveys the “competence” that permits fibroblasts to replicate in the presence of an appropriate progression signal.

Fibroblast replication requires exogenous growth factors, mediators acting with temporal specificity in the G1 phase of the cell cycle to promote DNA synthesis and cell replication (1-5). Analysis of this temporal specificity has led to the hypothesis that there are two major categories of growth factors, competence factors, and progression factors (5, 6). Competence factors, such as fibroblast growth factor or platelet-derived growth factor, act early in G1, rendering the cell responsive, for a variable number of hours, to progression factors (7). Progression factors, such as insulin, insulin-like growth factors, and alveolar macrophage-derived growth factor, act later in G1, signaling the cell to continue through the cell cycle and replicate (7, 8). Both categories of growth factors are thought to provide their signals to the target cell by interaction with specific cell surface receptors. Moreover, as a general principle, cells require a signal from both competence factors and progression factors to manifest an optimal replication rate.

Normal fibroblast replication requires, in addition to soluble factors, that the cells attach to an extracellular matrix (9). Interestingly, studies by Gospodarowicz et al. (10-12) have suggested that, in addition to providing attachment sites for cells, the extracellular matrix can, at least for some cells, act to replace the requirement for growth factors such as fibroblast growth factor or platelet-derived growth factor. Since both fibroblast growth factor and platelet-derived growth factor are competence factors, these observations suggest that the overall requirements for fibroblast growth may be met by a progression factor together with the extracellular matrix serving both as a competence factor and as a site for attachment.
In this context, we have hypothesized that fibronectin, a glycoprotein component of the extracellular matrix with specific domains that bind to the fibroblast surface and to specific regions of various components of the extracellular matrix (13-15), can act as a competence factor. This report demonstrates that fibronectin is a competence factor by two criteria: (a) the ability of fibronectin to promote fibroblast replication in a serum-free complementation test with known progression factors; and (b) the action of fibronectin early in the temporal sequence of G1.

**MATERIALS AND METHODS**

**Preparation of Growth Factors**

Plasma Fibronectin. Fibronectin was purified from human plasma by gelatin sepharose affinity chromatography as described by Engvall and Ruoslahti (16), followed by gel filtration. Briefly, 100 ml of peripheral blood were collected by venipuncture and anticoagulated with EDTA. Cells were separated from plasma by centrifugation, and the plasma was applied to a gelatin-sepharose column. Fibronectin was eluted from the column with 6 M urea followed by further purification of a 5-ml aliquot of the peak concentration (=2 mg/ml) on a Sephadex G-150 column. The fibronectin content of each fraction was quantified by an enzyme-linked immunosorbent assay as previously described (17). To reduce the probability that the fibronectin was contaminated by trace amounts of other growth factors found in plasma, some fibronectin preparations were purified by gelatin-sepharose followed by heparinagarose chromatography. The gelatin-sepharose column was eluted with 4 M urea, and the eluate was applied directly to a heparin-agarose column followed by elution with 0.5 M NaCl in 60 mM Tris-HCl, pH 7.4, 5 mM EDTA, as described by Hayashi and Yamada (18).

All fibronectin samples were adjusted to a fibronectin concentration of ~1 mg/ml with 8 M urea, and stored in aliquots in liquid nitrogen vapor. As needed, an aliquot of fibronectin was thawed, dialyzed 1:500 against Dulbecco's modified Eagle medium (DME; Biofluids, Rockville, MD) for two changes at 22°C. For each sample, fibronectin was quantified by an enzyme-linked immunosorbent assay following dialysis.

*Alveolar Macrophage Fibronectin:* To purify alveolar macrophage fibronectin, ~5 x 10^9 alveolar macrophages, obtained by bronchoalveolar lavage of patients with fibrotic lung disorders, were cultured for 24 h at 37°C in an atmosphere of 95% air-5% CO₂ in RPMI 1640 medium (Biofluids) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml) (19). Resultant supernatants were dialyzed against 50 mM NaCl, 20 mM Tris HCl, pH 7.4, 2 M urea, at 22°C, and then chromatographed on DEAE cellulose (Whatman Laboratory Products, Inc., Clifton, NJ) in the same buffer with a NaCl gradient from 50 to 300 mM. To identify the fractions containing fibronectin, an aliquot of each fraction was dialyzed (1:500) against 150 mM NaCl, 20 mM Tris HCl, pH 7.4, and fibronectin content was determined by an enzyme-linked immunosorbent assay. Fibronectin-containing fractions were stored in liquid nitrogen vapor. Prior to use in growth experiments, the DEAE-purified macrophage fibronectin was dialyzed 1:500 against DME and assayed for fibronectin content by an enzyme-linked immunosorbent assay.

*Alveolar Macrophage-derived Growth Factor (AMDGF):* AMDGF was purified from the supernatants of alveolar macrophages obtained by bronchoalveolar lavage of normal individuals as previously described (8). The macrophages were activated by surface adhesion and then cultured (24 h, 37°C) in DME supplemented with penicillin/streptomycin. The AMDGF was partially purified from the macrophage supernatants by ion exchange chromatography followed by gel filtration. Briefly, 600 ml of macrophage supernatants, containing 2.1 mg of macrophage secretory protein (determined by a colorimetric assay [Bio-Rad Laboratories, Richmond CA) using BSA [Sigma Chemical Co., St. Louis, MO) as a standard], was chromatographed on DEAE cellulose and eluted at 4°C with a NaCl gradient (150-300 mM) buffered with 20 mM Tris-HCl pH 7.0. The peak of bioactivity (~270 mM) was detected by its ability to promote the replication of human lung fibroblasts (8). This peak was lyophilized, resuspended in PBS, then chromatographed on Sephadex G-50 in PBS, pH 7.4, at 4°C. The peak of activity eluted at 18,000 daltons, and was stored in liquid nitrogen vapor at a concentration of 1.2 μg/ml. Serial dilutions of the pooled peak demonstrate the half-maximal growth response at a dilution of 10^-2 with the maximum response at a dilution of 10^-3. This represented an approximately 300- to 400-fold purification.

**Other Growth Factors:** Pituitary fibroblast growth factor (FGF) was obtained from Collaborative Research Inc. (Waltham, MA) and insulin from Sigma Chemical Co. Platelet-derived growth factor (PDGF) was partially purified as described by Vogel et al. (20).

**Evaluation of Plasma Fibronectin as a Growth Factor**

All experiments were performed on HFL-1 fibroblasts (American Type Culture Collection CCL 153), previously frozen in liquid nitrogen vapor at the tenth to twelfth population doubling. Cells were cultivated on 100-mm tissue culture dishes (Falcon Labware, Cockeyesville, MD) in DME supplemented with 10% calf serum (Colorado Serum, Denver, CO) as previously described (21), and used for growth studies between the fifteenth and twentieth population doubling.

To evaluate the effect of plasma fibronectin on fibroblast replication in a defined medium, the complementation test of Stiles et al. (7) was adapted for serum-free conditions. To accomplish this, fibroblasts were cultured on 35-mm dishes (Falcon Labware, 5 x 10^4 cells/dish) in DME plus 0.4% serum for 3 d. Cells were then rinsed three times with serum-free DME followed by cultivation for 1 d in "complementation test medium" (DME + 5 μg/ml transferrin (Sigma Chemical Co. plus 0.1% BSA [Sigma catalogue 7888]). Under these conditions, fibroblasts did not replicate without subsequent addition of growth factors. For convenience, fibroblasts cultured for 3 d in serum-free DME + 0.4% serum followed by culture for 1 d in "complementation test medium" will be referred to as nonreplicating fibroblasts.

The basic principle of the complementation test is that nonreplicating fibroblasts require both a competence factor and a progression factor for an optimal replication rate. Thus, to evaluate the role of plasma fibronectin as a growth factor, fibronectin was added to cultures of nonreplicating fibroblasts in combination with either a characterized competence factor (FGF) or a characterized progression factor (insulin or AMDGF), and fibroblast growth was assessed. To accomplish this, complementation test medium containing each growth factor individually or plasma fibronectin in combination with either FGF, insulin, or AMDGF (at the concentrations indicated in the text) was placed on nonreplicating fibroblasts, and the cultures were continued at 37°C. Cell counts were determined daily for 4 d with an electronic particle counter (Coulter Electronics, Hialeah, FL). Cell counts in complementation test medium alone served as negative controls. As a positive control, in each experiment FGF was added to nonreplicating fibroblasts with either insulin or AMDGF to verify a synergistic increase in cell number.

Since Clemmons (22) has demonstrated that confluent fetal fibroblasts can release their own competence factor(s), the nonreplicating HFL-1 cells at low density for the biosay were evaluated for production of competence factor(s) that might obscure the effect of exogenously added growth factors. To accomplish this, fibroblasts were seeded at low density in DMEM plus 0.4% serum for 3 d and rinsed as described above, then cultured in complementation test medium for 1, 2, 3, or 4 d, and the "conditioned medium" was collected each day. Culture of nonreplicating fibroblasts (37°C, 3-5 d) with conditioned medium (undiluted, 1:2, 1:4, 1:8) alone, or supplemented with insulin (10 μg/ml) or AMDGF (10 ng/ml), caused no increase in cell number above control. Thus, under the conditions used, exogenously added factors were necessary to provide competence.

One potential problem with attributing growth-promoting properties of fibronectin was the possibility that the preparation was contaminated with small amounts of PDGF. Since PDGF is heat stable (23), a fibronectin preparation (1 μg/ml) with or without supplemental PDGF (100 ng/ml), was boiled (100°C, 10 min) and then evaluated in a complementation test as described above.

To determine the effect of plasma fibronectin on the replication of fibroblasts that had been growth arrested by density inhibition rather than serum deprivation, confluent monolayers of fibroblasts were cultured with growth factors and the effect on DNA synthesis was assessed. To accomplish this, fibroblasts were seeded (5 x 10^4/well) in 0.25 mM DME plus 10% calf serum) onto 8-well glass slides (Lab-Tek, Miles Laboratories, Naperville IL) and cultured in DME plus 10% calf serum with the medium changed every other day until the cells were confluent and no mitotic cells were observed by phase contrast microscopy. The confluent cells were cultured one additional day without a medium change, rinsed three times with serum-free DME, and then cultured (30 h, 37°C) in complementation test medium containing 0.1 μCi/ml [3H]thymidine (2 Ci/mMol, Amersham Corp., Arlington Heights, IL) plus each growth factor individually, or both a competence factor (PDGF, FGF, or fibronectin at the
timepoints. These background values ranged from 200 ± 75 dpm/10^6 cells at 2 h to 900 ± 125 dpm/10^6 cells at 18 h.

Evaluation of Macrophage Fibronectin as a Growth Factor

Because of the difficulty of preparing sufficient amounts of macrophage fibronectin to perform the cell counting assay, the ability of macrophage fibronectin to act as a growth factor for fibroblasts was studied in a microassay using DNA synthesis rather than cell number as an index of fibroblast replication. The complementation test was performed in a way analogous to that described above except that the nonreplating fibroblasts were cultured in microtiter wells (10^4 fibroblasts/well, 96-well plates; Falcon Labware) in 0.2 ml of complementation test medium with or without exogenous growth factors. After a 14-h incubation at 37°C, each well was pulsed with [3H]thymidine (0.1 μCi/ml; 2 Ci/mMol, Amersham Corp.). After an additional 24-h incubation, acid-precipitable [3H]thymidine incorporation was determined by scintillation counting. [3H]Thymidine incorporation in the absence of added growth factors (1100 ± 300 dpm/10^6 fibroblasts) was subtracted from the values in the presence of growth factors to correct for the [3H]thymidine incorporation unrelated to growth factor addition.

Ability of Fibronectin to Signal Fibroblasts to Respond to Progression Factors

To evaluate the ability of fibronectin to signal fibroblasts to replicate in response to signals from progression factors, plasma fibronectin was added to fibroblasts suspended in complementation test medium at the time of seeding and then removed prior to addition of progression factors. To accomplish this, 35-mm tissue culture dishes were coated with collagen (prepared from rat skins, the gift of H. K. Kleinman, National Institute of Dental Research) as previously described, and sterilized by exposure to ultraviolet irradiation. Fibroblasts were prepared by incubation with trypsin (Gibco Laboratories, Grand Island, NY 0.25%; 1 min, 22°C) to detach them from culture dishes, washed once in DME plus soybean trypsin inhibitor to inactivate the trypsin, washed three times in complementation test medium, and resuspended in that same medium in the presence or absence of plasma fibronectin (1.0 μg/ml). Fibroblasts with or without added fibronectin were then seeded at a density of 5 x 10^5 cells/collagen-coated dish. After incubation overnight (14 h, 37°C), the fibroblasts were rinsed three times with DME to remove unbound fibronectin. Cell counts were then determined to confirm equal plating efficiency. The fibroblasts were then cultured for 3 d with either FGF, insulin, or AMDGF at the concentrations indicated. At the end of the 3-d culture, the number of cells was counted to determine the growth response.

Cell Cycle Kinetics

Previous studies have indicated that competence factors act early in the G1 phase of the cell cycle of nonreplicating BALB/C 3T3 cells (5). To determine where, in the temporal sequence of G1, fibronectin provided its growth-promoting signal to lung fibroblasts, the kinetics of fibroblast DNA synthesis after pretreatment with fibronectin was determined. Nonreplicating fibroblasts (10^4 cells/well, Falcon 96-well microtiter dishes, 0.2 ml of complementation test medium per well) were cultured for 12 h with (a) no further additions; (b) fibronectin (1.0 μg/ml); or (c) FGF (2 ng/ml). Following this 12-h incubation, calf serum was added to a final concentration of 10% (i.e., to provide the fibroblasts with a complete set of signals to replicate and to determine whether the pretreatment with fibronectin shifted the time of onset of DNA synthesis). [3H]Thymidine (0.5 μCi/ml; 2 Ci/mMol, Amersham Corp.) was added with the serum. At the time of serum and [3H]thymidine addition, and every 2 h for the subsequent 18 h, triplicate wells of fibroblasts were harvested for measurement of acid-precipitable [3H]thymidine incorporation. To establish the background for each time point in the assay, triplicate wells of fibroblasts were pretreated as described above and then cultured with [3H]thymidine but without serum. The background was then subtracted from the experimental time points. These background values ranged from 200 ± 75 dpm/10^6 cells at 2 h to 900 ± 125 dpm/10^6 cells at 18 h.

Statistical Evaluation

Each condition for the cell counting experiments was performed in duplicate, with replicates varying by <7%. Each condition in the [3H]thymidine incor-

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Dose Dependence of the Stimulation of Fibroblast Replication by Plasma Fibronectin

Nonreplicating fibroblasts cultured in complementation test medium manifested a dose-dependent increase in replication rate in response to fibronectin only when a progression factor (i.e., insulin or AMDGF) was also present (Fig. 2). The addition of fibronectin alone to the fibroblast cultures resulted in a modest increase in cell number. Furthermore, the addition of fibronectin and a fixed dose of a competence factor (FGF, 2.0 ng/ml) to the fibroblast cultures resulted in little further increase in cell number. In contrast, the addition of fibronectin along with a fixed dose of a progression factor (either insulin [10 µg/ml] or AMDGF, [10 ng/ml]) resulted in a marked increase in cell number up to ~170% above control with a fibronectin concentration of 2.0 µg/ml. This pattern of growth-promoting activity indicated that fibronectin acted as a competence factor.

In view of these findings, it was important to demonstrate that the dose-dependent competence signal provided by fibronectin was due to the fibronectin itself and not a contaminant of the fibronectin preparation. To exclude the possibility that part of the observed growth response was due to a contaminant of the fibronectin preparation, fibronectin was purified by two methods: gelatin-sepharose affinity chromatography followed by (a) gel filtration chromatography; or (b) heparin-agarose affinity chromatography. The results with both preparations were identical, suggesting that fibronectin and not a contaminant was responsible for the growth-promoting effect.

In addition, to rule out the possibility that PDGF (a heat-stable competence factor with high positive charge) might still be contaminating the preparation, the heat stability of the fibronectin-induced growth response was assessed. Boiling the fibronectin resulted in complete loss of activity in the assay described above. Supplementing the fibronectin with authentic PDGF (100 ng/ml) followed by boiling resulted in that amount of fibroblast growth observed with unboiled PDGF (data not shown). Therefore, contamination of our fibronectin with PDGF did not account for the growth-promoting effects observed.

Fibronectin not only provided a competence signal to fibroblasts that had been growth arrested at low density by serum deprivation, but also provided a dose-dependent competence signal to fibroblasts arrested in G1 by culture to saturating density (Table I). When confluent fibroblasts were cultured with the characterized competence factors, PDGF or FGF, in the presence of a progression factor (AMDGF, 10 ng/ml or insulin, 10 µg/ml) there was a dose-dependent increase in DNA synthesis. The maximum response ranged from 26 to 33% labeled nuclei representing ~50 to 75% of the DNA synthesis produced by the addition of 10% calf serum. Similarly, the addition of fibronectin to the density-arrested fibroblasts in the presence of either AMDGF or insulin resulted in a significant though smaller increase in fibroblast DNA synthesis (p < .01, compared with media alone). The maximum DNA synthesis in response to fibronectin was 16% labeled nuclei representing about one-third of the response to 10% calf serum. In contrast, DNA synthesis in response to each factor individually was far less, ranging from 1 to 7%. In addition, only for PDGF and FGF individually was there a small dose-related increase in nuclear labeling.

Fibroblast DNA Synthesis in Response to Macrophage Fibronectin

When nonreplicating fibroblasts were incubated in the presence of a progression factor there was an increase in the rate

![Figure 2](image-url)
of DNA synthesis in response to increased amounts of macrophage fibronectin (Fig. 3). As observed with plasma fibronectin, there was a macrophage fibronectin dose-dependent increase in the fibroblast growth response in the presence of either insulin (10 μg/ml) or AMDGF (10 ng/ml). The result was similar to that observed when [3H]thymidine incorporation was used as the index of fibroblast replication in response to plasma fibronectin in the presence of AMDGF or insulin (data not shown). Comparison of the dose-response relationships between macrophage fibronectin and plasma fibronectin revealed that 50% of the maximum response occurred within a twofold concentration range of fibronectin (80 ± 15 ng/ml for macrophage fibronectin and 200 ± 23 ng/ml for plasma fibronectin), indicating that there was not a major difference in growth-promoting potency of the fibronectins from these two different sources. Thus, both plasma and macrophage fibronectin provided a dose-dependent competence signal for lung fibroblasts.

Ability of Fibronectin to Signal Fibroblasts to Respond to Progression Factors

Nonreplicating fibroblasts seeded onto collagen-coated dishes in the presence of fibronectin manifested a rapid rate of replication after the addition of a progression factor (Fig. 4). Under the conditions used, fibroblasts seeded onto collagen-coated dishes in complementation test medium had similar plating efficiencies in the absence or presence of exogenous fibronectin (1.0 μg/ml). When fibroblasts were allowed to adhere in the absence of fibronectin, they did not replicate when a progression factor (insulin or AMDGF) was added. In marked contrast, when fibroblasts were allowed to adhere in the presence of fibronectin, there was a dose-related increase in replication rate after addition of insulin or AMDGF. For example, at an insulin concentration of 5 μg/ml, cell number increased 93% above control and at 50 μg/ml cell number increased 125% above control. Addition of AMDGF resulted in a similar stimulation of cell replication, with an 80% increase in cell number above control at a concentration of 0.1 ng/ml and a 140% increase at an AMDGF concentration of 10 ng/ml. Thus, fibronectin was able to provide a signal to fibroblasts that allowed them to replicate in response to a progression signal.

Further confirmation that the growth-promoting effect of fibronectin for fibroblasts was not due to a progression signal was provided by the absence of a dose-related increase in fibroblast replication in the presence of FGF, a known competence factor (Fig. 4C). Seeding fibroblasts in the presence of fibronectin did not enable FGF to substantially augment their rate of replication compared with fibroblasts seeded in the absence of fibronectin, suggesting that fibronectin was not providing a progression signal. As a positive control, fibroblasts exposed to FGF (1–2.5 ng/ml) in the presence of insulin (5 μg/ml) or AMDGF (10 ng/ml) demonstrated a significant increase in fibroblast replication, an effect that was not substantially augmented by the addition of fibronectin (1.0 μg/ml) to the cultures (data not shown).

Evaluation of the Position of Action of Fibronectin in the G1 Phase of the Cell Cycle

Evaluation of the timing of DNA synthesis under various conditions demonstrated that fibronectin acted as a competence factor by providing a growth-promoting signal early in G1 (Fig. 5). Under the conditions used in these studies, without the addition of growth factors, nonreplicating lung fibroblasts were arrested in the G1 phase of the cell cycle, about 12–14 h before the onset of S phase. Nonreplicating fibroblasts cultured for 12 h with the competence factor FGF (2.0 ng/ml) traversed about one-third of the remaining interval of G1, arresting 8–10 h from S phase. Within the framework of this temporal sequence of G1, fibronectin (1.0 μg/ml) also signaled nonreplicating fibroblasts to traverse one-third of G1, with DNA synthesis beginning 8–10 h after serum addition. In contrast, incubation of fibroblasts for 12 h with insulin or AMDGF prior to the addition of serum resulted in no traversal of G1, i.e., there was still a 12–14-h lag for DNA synthesis, a value similar to that for cells in complementation test medium alone (data not shown).
Attachment and Spreading as a function of time after addition of serum. Test medium alone (p), fibronectin (•; 1.0 µg); or FGF (O; 2.0 ng/ml). Shown is [³H]thymidine incorporation for 12 h with or without added growth factors followed by addition of reagents for fibroblast replication, and fibronectin clearly provides a signal for cellular replication.

DISCUSSION

Fibronectin, at concentrations in the nanomolar range, can, under the appropriate circumstances, stimulate nonreplicating human lung fibroblasts to replicate in a dose-dependent manner. In this context, fibronectin is able to complement the growth-promoting effects of the characterized progression factors, insulin and AMDGF, by providing a competence signal to nonreplicating fibroblasts. In providing this competence signal, fibronectin stimulates nonreplicating fibroblasts to traverse G₁ → 4 to 6 h. Thus, at least for diploid human fibroblasts, replication can be induced in serum-free conditions by a signal from the extracellular matrix component, fibronectin, plus an additional signal provided by a progression factor.

Role of Extracellular Matrix in Growth Control

There are several lines of evidence that extracellular matrix elements can provide growth promoting signal(s) to parenchymal cells. For example, in addition to stimulating the replication of human lung fibroblasts as shown in the present study, fibronectin augments the replication rate of chick heart fibroblasts (24) and human dermal fibroblasts (25). In addition, type IV collagen, a basement membrane component intimately associated with the basal surface of epithelial cells, promotes the replication of rat mammary epithelial cells (26). Furthermore, bovine corneal endothelial extracellular matrix, a highly ordered collection of several matrix elements, promotes the replication of bovine vascular endothelial cells (10). Thus, extracellular matrix components have a role in attracting and orienting parenchymal cells within an organ, and in addition there is increasing evidence that at least some of these components are able to provide a signal for cellular replication.

Mechanisms of Modulation of Cellular Replication by the Extracellular Matrix

For the process of fibroblast replication, the dual control model of Pledger and co-workers (6) suggests that most growth factors can be classified as competence factors or progression factors according to their position of action within the temporal sequence of G₁. Although this is still a working model that has not been completely validated, it emphasizes the importance of cell cycle specific signals in the control of cell replication and thus provides a useful framework in which to conceptualize growth signals. Competence factors, such as FGF, act early in G₁ and need to be present for only a few hours to render a cell responsive to a subsequent progression signal. Progression factors, such as the insulin-like growth factors and AMDGF, provide a signal later in G₁ that, under the appropriate conditions, allows the cell to proceed to DNA synthesis. In this model, both a competence signal and a progression signal are necessary for significant cell replication (7, 30).

While the precise mechanisms of action of either competence or progression factors are not known, in principle, they may act directly by initiating the sequence of events leading to replication, or indirectly by stimulating the target cell to produce their own growth factors. With regard to progression factors, several defined signals are capable of stimulating human fibroblasts to produce insulin-like growth factors (31, 32), which are active at nanomolar concentrations as progression factors. Evaluation for an analogous inducible production of competence factors by fibroblasts has not been described. However, Clemmons (22) has demonstrated that
confluent monolayers of human fibroblasts release competence factor(s) into the medium constitutively. This raises the theoretical consideration that nonreplicating fibroblasts (low density or confluent) might be signalled to divide by a growth factor that stimulates the target cell to produce its own competence factor.

Within this framework, the present study demonstrates that fibronectin acts as a competence factor by providing a signal early in G\(_1\), either directly or indirectly, permitting human lung fibroblasts to proliferate in response to progression factors. Fibronectin provides this competence signal to fibroblasts that have been arrested in G\(_1\) either by serum deprivation or by growth to saturating densities. However, while fibronectin is as potent as the well characterized competence factors FGF and PDGF with regard to stimulating the replication of fibroblasts arrested by serum deprivation, it clearly was not so potent as FGF or PDGF in stimulating DNA synthesis by density-arrested fibroblasts. This difference in potency, depending upon the method used to arrest fibroblasts in G\(_1\), distinguishes fibronectin from other competence factors such as FGF or PDGF.

While the applicability of the dual control model has not been directly assessed in cells other than fibroblasts, the concept that extracellular matrix can provide a specific signal for cellular replication is also in accord with the work of Gospodarowicz and III (10). These workers demonstrated that the requirement of bovine vascular endothelial cells for the competence factor, FGF, was eliminated by culturing these cells on an extracellular matrix provided by bovine corneal endothelial cells. Interestingly, however, in contrast to the situation with lung fibroblasts, fibronectin does not provide a good competence signal to vascular endothelial cells when compared with intact extracellular matrix. Together, these observations are consistent with the concept that matrix components provide a competence signal but that different cell types may require different matrix components.

Although the signal provided by fibronectin in stimulating lung fibroblast growth acts early in G\(_1\), the growth-promoting actions of fibronectin may occur at other times in the cell cycle for other cells. In this context, Orly and Sato (33) have demonstrated a marked increase in rat follicular cell replication in serum-free medium containing fibronectin, transferrin, and insulin; analysis of the cell cycle specificity of the growth-promoting signal provided by fibronectin demonstrated that it was required for cytokinesis during M phase. Thus, for various types of cells, fibronectin can serve an important function in the control of replication, but these functions may be cell specific.

### Various Roles for Fibronectin in Tissue Reconstruction and Fibrosis

The present study demonstrates that both plasma fibronectin and macrophage fibronectin can provide a replication signal to fibroblasts. Together with the known functions of fibronectin in promoting fibroblast chemotaxis (34-36) and attachment and spreading (27, 28), these observations suggest a fundamental role for fibronectin in the reparative processes following injury. For example, in wound healing where capillary leak is significant and macrophages play an important role (37), plasma fibronectin as well as macrophage fibronectin may contribute in concert with other growth factors to the increase in fibroblast numbers characteristic of this process. Furthermore, in human fibrotic lung disease, alveolar macrophages are releasing both fibronectin (34) and AMDGF (38), factors that, together, can recruit fibroblasts (fibronectin), attach fibroblasts (fibronectin), and signal replication of fibroblasts (fibronectin and AMDGF). Consistent with this concept, morphologic studies of the alveolar wall in these diseases have demonstrated increased numbers of fibroblasts (39). Thus fibronectin may be one of the stimuli that result in expansion of the fibroblast population in various physiological and pathological settings.

Received for publication 4 January 1983, and in revised form 18 July 1983.

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