Decreased Level of PDGF-stimulated Receptor Autophosphorylation by Fibroblasts in Mechanically Relaxed Collagen Matrices

Ying-Chun Lin and Frederick Grinnell

Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical School, Dallas, Texas 75235

Abstract. The goal of our studies was to characterize the interrelationship between extracellular matrix organization and fibroblast proliferation in response to growth factors. We compared fibroblasts in monolayer culture with cells in contracted collagen matrices that were mechanically stressed or relaxed. In response to platelet-derived growth factor (PDGF), DNA synthesis by fibroblasts in mechanically relaxed collagen matrices was 80–90% lower than in monolayer culture and 50% lower than in mechanically stressed matrices. Fibroblasts in monolayer and contracted collagen matrix cultures contained similar levels of PDGF receptors, but differed in their autophosphorylation response. Cells in mechanically relaxed matrices showed lowest levels of autophosphorylation, 90% less than cells in monolayer culture. Experiments comparing receptor expression and capacity for PDGF-stimulated autophosphorylation showed that cells in mechanically relaxed collagen matrices never developed normal receptor autophosphorylation. Furthermore, when mechanically stressed collagen matrices were switched to mechanically relaxed conditions, capacity for receptor autophosphorylation decreased within 1–2 h and remained low. Based on immunomicroscopic observations and studies on down-regulation of receptors by PDGF binding, it appeared that most PDGF receptors in monolayer or contracted collagen matrix cultures were localized on the cell surface and accessible to PDGF binding. In related studies, we found that EGF receptors of fibroblasts in mechanically relaxed collagen matrices also showed low levels of autophosphorylation in response to EGF treatment. Based on these results, we suggest that mechanical interactions between cells and their surrounding matrix provide regulatory signals that modulate autophosphorylation of growth factor receptors and cell proliferation.

FIBROBLASTS cultured within or on the surface of a collagen matrix reorganize collagen fibrils and contract the matrix (Bell et al., 1979; Grinnell and Lamke, 1984). After contraction, the cells show a marked decline in DNA synthesis compared to cells in monolayer culture (Sarber et al., 1981; Van Bockxmeer et al., 1984). This decline results in part from a direct effect of collagen on the fibroblasts (Elsdale and Bard, 1972; Schor, 1980; Yoshizato et al., 1985; Nishiyama et al., 1989). In addition, as will be discussed below, decreased proliferation depends on collagen matrix organization.

Collagen matrix organization varies when collagen matrices are anchored or floating during contraction. In the case of anchored matrices, tension exerted by fibroblasts is distributed asymmetrically, and mechanical stress develops during contraction. Collagen fibrils become more closely packed and organized along lines of stress, and fibroblasts spread in a bipolar morphology with prominent actin filament bundles. In the case of floating matrices, tension exerted by fibroblasts is distributed symmetrically and the matrices remain mechanically relaxed during contraction. Collagen fibril density increases without alignment, and fibroblasts spread in a stellate morphology without actin filament bundles (Nakagawa et al., 1989; Mochitate et al., 1991).

Fibroblasts in mechanically stressed collagen matrices continue to proliferate in response to growth factors (Nakagawa et al., 1989). On the other hand, cells in mechanically relaxed matrices become arrested in G_0 (Kono et al., 1990) and less responsive to addition of serum or purified growth factors (Fukamizu et al., 1990; Nishiyama et al., 1991). The mechanism underlying decreased responsiveness by fibroblasts in mechanically relaxed collagen matrices to serum stimulation probably involves changes in the PDGF signaling pathway. PDGF is the principle mitogen in serum for mesenchymal cells such as fibroblasts (Ross et al., 1990; Heldin and Westermark, 1990). Binding of PDGF to its receptors causes receptor clustering and activation of receptor tyrosine kinase, which results in autophosphorylation as well as phosphorylation of a group of cytoplasmic signaling proteins (Williams, 1989; Koch et al., 1991). Studies on receptor mutants indicate that receptor tyrosine kinase activity is required for PDGF's mitogenic activity (Mori et al., 1991). Binding of PDGF to PDGF receptors also accelerates receptor internalization and down-regulation (Heldin et al., 1982; Bowen-Pope and Ross, 1982), but this process is separate from mitogenic stimulation (Escobedo et al., 1988; Sorkin et al., 1991).

To better understand the interrelationship between extracellular matrix organization and serum stimulation of

fibroblast proliferation, we compared PDGF responsiveness by fibroblasts in monolayer culture with that of cells in contracted collagen matrix cultures that were either mechanically stressed or relaxed. Our results indicate that the decreased ability of PDGF to stimulate DNA synthesis by fibroblasts in contracted collagen matrices may result from decreased levels of PDGF receptor autophosphorylation.

Materials and Methods

Cell Culture

Maintenance of human foreskin fibroblast monolayer cultures and preparation of hydrated collagen matrices from Vitrogen "100" collagen (Celtrix Labs, Palo Alto, CA) have been described previously (Nakagawa et al., 1989). Fibroblasts were added to the neutralized collagen solutions (1.5 mg/ml) at a concentration of 5×10^5 cells/ml. Aliquots (0.2 ml) of the cell/collagen mixtures were prewarmed to 37°C for 3–4 min and then placed in 24-well culture plates (Costar Corp., Cambridge, MA). Each aliquot occupied an area outlined by a 12-mm-diameter circular score within a well. Polymerization of a collagen matrices required 60 min at 37°C, after which 1.0 ml of DME (GIBCO-BRL, Gaithersburg, MD) supplemented with 10% FBS (Intergen Co., Purchase, NY) and 50 μg/ml ascorbic acid were added to each well. The cultures were maintained in a humidified CO_2 incubator in medium containing FBS as indicated. To obtain mechanically stressed matrices, the matrices were allowed to remain anchored to the culture dishes during contraction; to obtain mechanically relaxed matrices, the matrices were dislodged from the substratum with a spatula after polymerization and allowed to float in the medium during contraction.

In some experiments, PDGF receptors were studied after stress–relaxation. That is, matrices were allowed to develop mechanical stress over 5 d and then released allowing stress to dissipate over 1 h (Mochitate et al., 1991). Since stress–relaxation requires the presence of serum (Tomasek et al., 1992), these experiments were carried out using 10% PDGF-depleted FBS, thereby allowing stress–relaxation without PDGF receptor down-regulation. PDGF-depleted FBS was prepared using CM-Sephadex (Vogel et al., 1978). Briefly, 10 ml of FBS was dialyzed against 0.1 M Tris, pH 7.4, mixed with 30 ml of CM-Sephadex (Pharmacia Fine Chemicals, Piscataway, NJ), and gently rocked overnight at 4°C. Subsequently, the mixture was poured into a Pharmacia C50 column. The liquid phase was collected and dialyzed against Dulbecco's PBS (DPBS)[1] (150 mM NaCl, 3 mM KCl, 1 mM KH_2PO_4, 6 mM Na_2HPO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.2). Protein concentration of PDGF-depleted FBS was adjusted to match FBS using a protein assay (Bio-Rad Laboratories, Cambridge, MA).

DNA Synthesis

PDGF isoform AB (human recombinant) was obtained from Upstate Biotechnology Inc. (Lake Placid, NY). DNA synthesis was measured by thymidine incorporation. Cultures were radiolabeled metabolically for 2 h with 5 μCi/ml [^{3}H]thymidine (20 Ci/mmole, New England Nuclear, Boston, MA). At the end of the incubations, cultures were rinsed twice with PBS (150 mM NaCl, 3 mM KCl, 1 mM KH_2PO_4, 6 mM Na_2HPO_4, pH 7.2) at 22°C twice and digested with 0.1 ml of 0.25% trypsin/1 mM EDTA (GIBCO-BRL) for 10 min at 37°C. Then 0.3 ml of collagenase solution (5 mg/ml type I collagenase in 130 mM NaCl, 10 mM Ca acetate, 20 mM Hepes, pH 7.2; Sigma Immunochemicals, St. Louis, MO) was added to trypsinized cultures, and the incubation continued 30–60 min until the cells dispersed completely. Aliquots of the samples were mixed with trypan blue, and cell number was measured with a hemocytometer. The remaining portions of the samples were washed once at 4°C with PBS containing 10% FBS and precipitated with 10% TCA. The precipitates were resuspended in 50 mM NaOH, precipitated again with 10% TCA, and transferred to glass fiber filters (Whatman Laboratory Products, Inc., Clifton, NJ). After washing two times with 10 ml of 5% TCA, the glass fiber filters were mixed with 10 ml Budgetsolve (R.P.I. Res. & Pres. Instr., St. Laurent, Canada), and radioactivity was measured in a scintillation counter (LS3801; Beckman Instruments, Palo Alto, CA). Each experiment was performed in triplicate, and the results were normalized by cell number.

1. *Abbreviations used in this paper:* IGF, insulin-like growth factor; PTPase, protein-tyrosine phosphatase; PVP-40, polyvinylpyrrolide 40.

Immunoblotting

Cell extracts were prepared by scraping cells or collagen matrices into 0.1–0.2 ml 0.2% NP-40 in DPBS containing proteinase inhibitors (1 μg/ml pepstatin A, 1 μg/ml leupeptin, 1 mM 4-(2-aminoethyl)-benzene-sulfonyl-fluoride HCl) and phosphatase inhibitors (2 mM sodium orthovanadate, 50 mM sodium fluoride, 1 mM ammonium molybdate, 0.2 mM microcystin-LR). Samples were homogenized (50 strokes) with a 1 ml Dounce homogenizer (B pestle; Wheaton Scientific, Millville, NJ) at 4°C, and collagen fibrils and nuclei were removed by centrifugation at 11,000 *g* (Beckman Microfuge) for 5 min.

During the 5-d culture period, cell number increased most in monolayer culture and least in mechanically relaxed collagen matrices. To compare extracts from different cultures, it was necessary to normalize the samples to constant cell numbers. Preliminary experiments using cells harvested by trypsin and collagenase (see above) showed that levels of lactate dehydrogenase (measured using the Sigma LD Diagnostic kit) were similar per cell regardless whether the cells were cultured in monolayer or collagen matrices. Therefore, aliquots of the cell extracts were analyzed for lactate dehydrogenase activity, and samples to be subjected to SDS-PAGE were adjusted to equivalent lactate dehydrogenase levels.

Samples for SDS-PAGE were mixed with reducing SDS sample buffer (62.5 mM Tris, 2% SDS, 10% glycerol, 0.01% bromophenol blue, 5% mercaptoethanol, pH 6.8), subjected to electrophoresis in 6% acrylamide mini-slab gels (Laemmli, 1970), and transferred to PVDF membranes. After blocking the membranes with 76 mM Na phosphate, 68 mM NaCl, 6% casein, 1% polyvinylpyrrolide 40 (PVP-40), 3 mM NaN_3, and 10 mM EDTA for 3 h, the membranes were incubated for 2 h with anti-phosphotyrosine (Upstate Biotechnology, Inc.) or anti-PDGF α/β-receptor antibodies (Upstate Biotechnology Inc.) in blocking solution. At the end of incubations, the blots were washed with 0.3% Tween 20, 76 mM Na phosphate, and 68 mM NaCl following by incubation for 1 h with alkaline phosphatase-conjugated goat anti-mouse or goat anti-rabbit antibodies in blocking solution. Photoactivation was initiated by addition of 50 mM sodium bicarbonate (pH 9.6), 1 mM $MgCl_2$ and 3-(4-methoxyspiro{1,2 dioxetane-3,2'-tricyclo-[3.3.1.13,7]decan}-4yi)phenylphosphate (AMPPD). Light emitted by activated AMPPD was detected with XAR x-ray film (Eastman Kodak, Rochester, NY). PDGF receptor bands were analyzed using a laser scanning densitometer (Ultrascan XL, LKB Instruments Inc., Bromma, Sweden).

Confocal Microscopy

Cultures were fixed for 10 min at 22°C with 3% paraformaldehyde in DPBS. To block non-specific staining, the samples were treated with 1% glycine and 1% BSA (Fraction V; ICN Biochemicals Inc., Irvine, CA) for 30 min. Subsequently, half the cultures were permeabilized with 0.2% NP-40 in DPBS for 10 min. Samples were incubated with anti-PGDF β-receptor antibodies (~40 μg/ml) in DPBS containing 1% BSA 20 min at 22°C, washed three times, and the incubated with ~25 μg/ml FITC-conjugated goat anti-mouse antibodies (Zymed Laboratories, South San Francisco, CA). Subsequently, the samples were mounted with glycerol/PBS (9:1) containing .001% phenylenediamine, examined, and photographed with a Bio-Rad MRC-500 confocal laser microscope. Anti-PDGF β-receptor antibodies were a generous gift from Dr. D. Bowen-Pope (University of Washington, Seattle, WA).

Results

PDGF-stimulated DNA Synthesis by Fibroblasts in Monolayer and Contracted Collagen Matrix Cultures

Fig. 1 shows DNA synthesis after PDGF stimulation of fibroblasts in monolayer cultures and collagen matrices (mechanically stressed or relaxed). Cells were cultured in DME/10% FBS for 1 d and then in DME/1% FBS for an additional 3 d. The initial day in 10% serum-containing medium was required to obtain maximal collagen matrix contraction. Mechanically stressed collagen matrices remained attached to underlying culture dishes during contraction. Mechanically relaxed collagen matrices floated in the culture medium during contraction. Culture for 3 d in

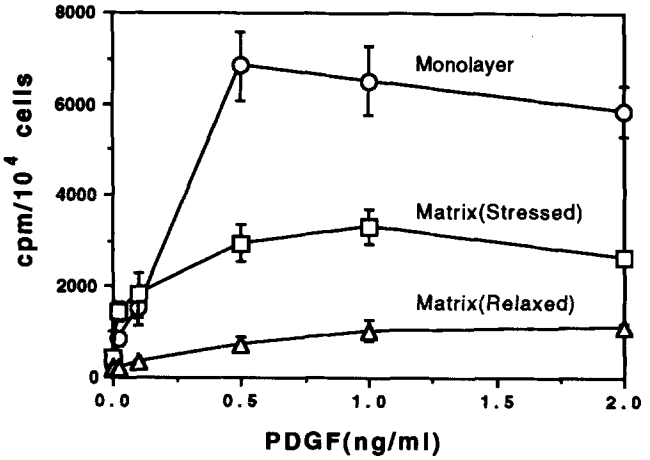

Figure 1. PDGF-stimulated DNA synthesis by fibroblasts in monolayer culture and contracted collagen matrices. Fibroblasts were cultured 1 d in DME/10% FBS followed by 3 d in DME/1% FBS in monolayer culture, in collagen matrices that were anchored to culture dishes (mechanically stressed after contraction), or in collagen matrices that were floating in medium (mechanically relaxed after contraction). At the end of the 4-d culture period, PDGF was added to the cultures at the concentrations indicated. DNA synthesis was measured 22 h later by [^{3}H]thymidine incorporation.

low (1%) serum-containing medium allowed serum down-regulated PDGF receptors to be re-expressed. Afterwards, cells were stimulated with PDGF for 22 h and then DNA synthesis was measured by [^{3}H]thymidine incorporation during 2 h.

Regardless of the culture conditions (i.e., monolayer or collagen matrix), DNA synthesis by fibroblasts reached highest levels after 0.5–1 ng/ml PDGF stimulation. The different culture conditions resulted in marked differences, however, in the magnitude of the cellular responses. Fibroblasts in mechanically relaxed collagen matrices showed 80–90% lower levels of PDGF-stimulated DNA synthesis compared to cells in monolayer culture. Fibroblasts in mechanically stressed collagen matrices showed levels of PDGF-stimulated DNA synthesis that were intermediate. Further increases in DNA synthesis were not observed even at PDGF concentrations as high as 100 ng/ml PDGF. In fact, at this level, DNA synthesis was 10–30% lower than with 1 ng/ml PDGF (data not shown).

PDGF Receptor Levels in Fibroblasts in Monolayer and Contracted Collagen Matrix Cultures

Decreased responsiveness to PDGF by fibroblasts in mechanically relaxed collagen matrices might have resulted from a decrease in the number of PDGF receptors. Fig. 2 (see also Figs. 7 and 10) compares PDGF receptor levels in cells harvested from monolayer (M), mechanically stressed (S), and mechanically relaxed (R) matrices after culture for 1 d in DME/10% FBS and 4 d in DME/1% FBS. Cells were extracted by detergent treatment and mechanical disruption, and extracts were centrifuged to remove collagen fibrils and cell nuclei. Under all three culture conditions, the major polypeptide detected by immunoblotting with polyclonal anti-PDGF α/β-receptor antibodies was ~180 kD (*arrow*), which corresponded to PDGF β receptors that predominate in fibroblasts (Kazlauskas et al., 1988; Seifert et al., 1989).

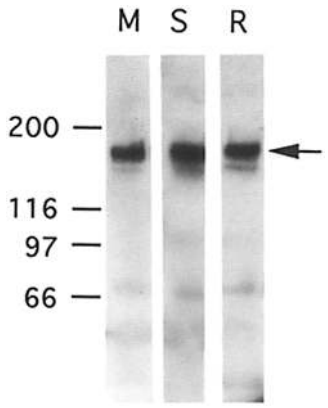

Figure 2. PDGF receptor levels in fibroblasts in monolayer and contracted collagen matrix cultures. Fibroblasts were cultured in 1 d in DME/10% FBS followed by 4 d in DME/1% FBS in monolayer culture (*M*), in collagen matrices that were anchored to culture dishes (mechanically stressed after contraction) (*S*), or in collagen matrices that were floating in medium (mechanically relaxed after contraction) (*R*). At the end of the 5-d culture period, cells extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors. Samples were subjected to SDS-PAGE, and PDGF receptors were detected by immunoblotting with anti-PDGF α/β-receptor antibodies. Samples from equivalent cell numbers were loaded onto each lane. (Arrow) 180-kD PDGF receptors.

Most of the receptors were solubilized during the extraction procedure since the pellets contained little antibody-reactive material (data not shown). Polypeptides ~160–170 kD occurred variably from experiment to experiment, and these probably corresponded to PDGF α receptors and/or PDGF β receptor precursors. In general, no significant differences in receptor levels were observed regardless of cell culture conditions.

PDGF-stimulated Receptor Autophosphorylation by Fibroblasts in Monolayer and Contracted Collagen Matrix Cultures

Since fibroblasts in collagen matrix cultures accumulated similar levels of PDGF receptors, decreased PDGF-dependent mitogenic responsiveness by fibroblasts in mechanically relaxed matrices could not be explained in terms of receptor numbers. Next, we studied the ability of PDGF to stimulate receptor autophosphorylation. Fig. 3 *A* shows dose–response studies for PDGF stimulation of tyrosine kinase activity detected by immunoblotting with anti-phosphotyrosine antibodies, and quantification of the 180-kD PDGF receptor band by scanning densitometry can be found in Fig. 3 *B*. In the absence of PDGF stimulation, little staining by the antibodies was observed. After PDGF stimulation, cells in mechanically relaxed matrices showed the lowest levels of autophosphorylation. For instance, at 10 ng/ml PDGF, there was a 90% lower level of autophosphorylation by fibroblasts in mechanically relaxed collagen matrices compared to monolayer cells. Even at 100 ng/ml PDGF, autophosphorylation by fibroblasts in mechanically relaxed collagen matrices was markedly suppressed.

In the experiment described in Fig. 3, fibroblasts were stimulated with PDGF for 10 min at 37°C. It was possible that the different levels of autophosphorylation occurred because different times were required for maximal stimulation. This was not the case, however, as shown in Fig. 4. After the addition of 50 ng/ml PDGF, peak phosphorylation occurred by 10 min (Fig. 4 *A*), and analysis by scanning densitometry (Figure 4B) showed that the pattern of activation and subse-

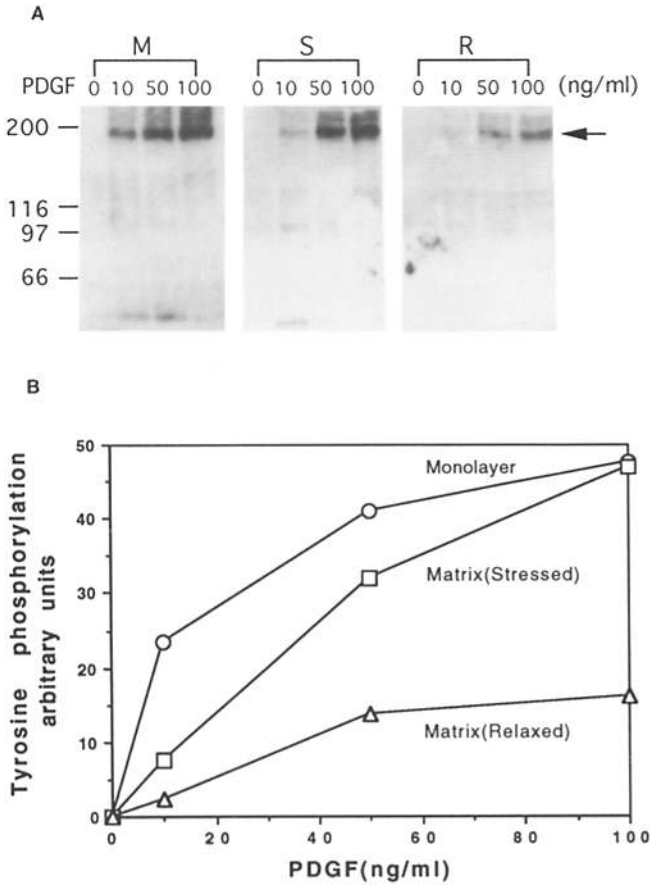

Figure 3. Dose response of PDGF-stimulated receptor autophosphorylation by fibroblasts in monolayer and contracted collagen matrix cultures. Fibroblasts were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS in monolayer culture (*M*), in collagen matrices that were anchored to culture dishes (mechanically stressed after contraction) (*S*), or in collagen matrices that were floating in medium (mechanically relaxed after contraction) (*R*). At the end of the 5-d culture period, cultures were stimulated with PDGF at the concentrations indicated for 10 min at 37°C, and cells extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors. Samples were subjected to SDS-PAGE, and phosphotyrosine containing polypeptides were detected by immunoblotting with anti-phosphotyrosine antibodies. Equivalent cell numbers were loaded onto each lane. (*A*) Immunoblotting; arrow, 180-kD PDGF receptors. (*B*) Results in *A* analyzed by scanning densitometry.

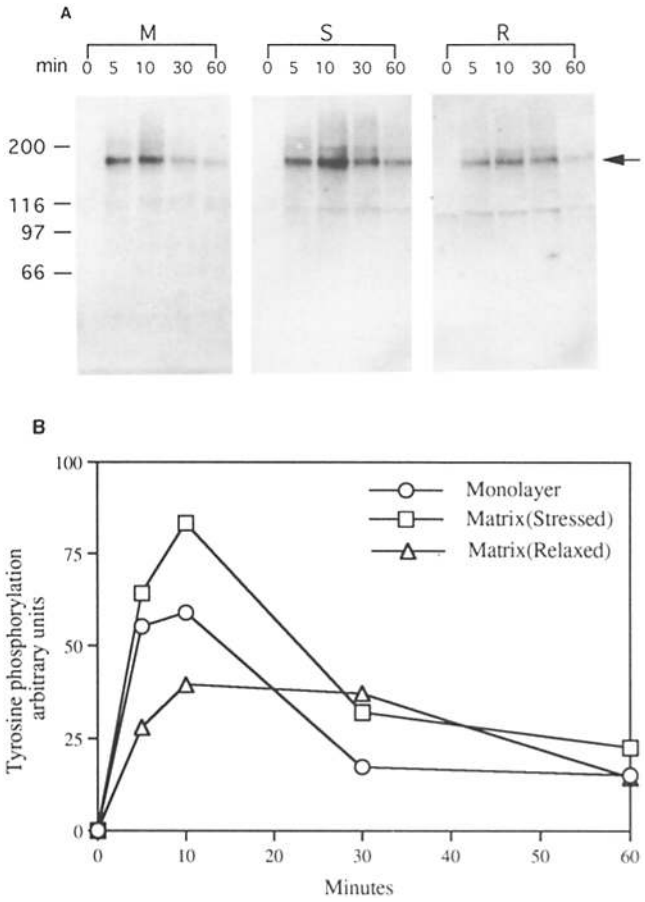

Figure 4. Time course of PDGF-stimulated receptor autophosphorylation by fibroblasts in monolayer and contracted collagen matrix cultures. Fibroblasts were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS in monolayer culture (*M*), in collagen matrices that were anchored to culture dishes (mechanically stressed after contraction) (*S*), or in collagen matrices that were floating in medium (mechanically relaxed after contraction) (*R*). At the end of the 5-d culture period, cultures were stimulated with 50 ng/ml PDGF at 37°C for the times indicated, and cells extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors. Samples were subjected to SDS-PAGE, and phosphotyrosine containing polypeptides were detected by immunoblotting with anti-phosphotyrosine antibodies. Equivalent cell numbers were loaded onto each lane. (*A*) Immunoblotting; arrow, 180-kD PDGF receptors. (*B*) Results in A analyzed by scanning densitometry.

quent decline in receptor phosphorylation levels was similar for monolayer and collagen matrix cultures.

Changes in PDGF Receptor Autophosphorylation as a Function of Receptor Synthesis and Collagen Matrix Organization

The foregoing results indicated that the PDGF receptors of fibroblasts in collagen matrices—especially mechanically relaxed matrices—had low autophosphorylation levels in response to PDGF compared to cells in monolayer culture. Since activation of PDGF tyrosine receptor kinase activity has been shown to be required for PDGF mitogenic stimulation (Mori et al., 1991), the decreased ability of fibroblasts in collagen matrices to respond to PDGF by receptor autophosphorylation may explain decreased PDGF stimulation of DNA synthesis by these cells.

In the experiments described above, fibroblasts were incubated in collagen matrices for 5 d and then receptor autophosphorylation was determined. Additional studies were carried out to compare the timing of PDGF receptor expression with the capacity for PDGF-stimulated autophosphorylation of the receptors. Fig. 5 shows that after the first day of culture, during which cells were in medium containing 10% FBS, only a low level of residual receptors were observed. Subsequently, after cells were switched to medium containing 1% FBS, PDGF receptor levels increased reaching a maximum at day 3–4 in all the cultures.

Fig. 6 shows that receptor phosphorylation occurred for fibroblasts in monolayer cultures and in mechanically stressed matrices. In mechanically relaxed matrices, however, even newly appearing PDGF receptors lacked the capacity to be

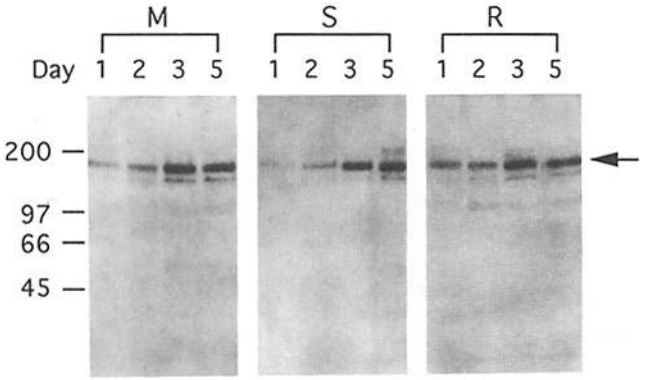

Figure 5. Accumulation of PDGF receptors during 5 d by fibroblasts in monolayer and contracted collagen matrix cultures. Fibroblasts were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS in monolayer culture (*M*), in collagen matrices that were anchored to culture dishes (mechanically stressed after contraction) (*S*), or in collagen matrices that were floating in medium (mechanically relaxed after contraction) (*R*). On the days indicated, cells extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors. Samples were subjected to SDS-PAGE, and PDGF receptors were detected by immunoblotting with polyclonal anti-PDGF α/β-receptor antibodies. Equivalent cell numbers were loaded onto each lane. Arrow, 180-kD PDGF receptors.

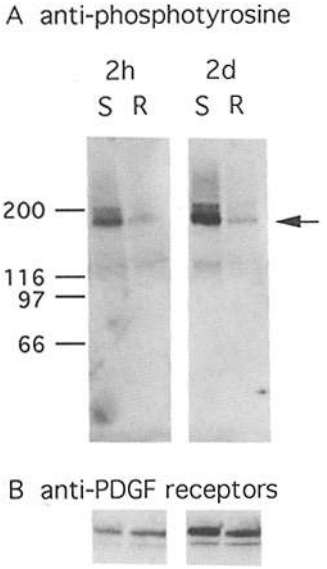

Figure 7. Decreased PDGF-stimulated receptor autophosphorylation after relaxation of mechanically stressed collagen matrices. Fibroblasts in collagen matrices anchored to culture dishes were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS (mechanically stressed). Subsequently, the cultures were switched to DME supplemented with 10% PDGF-deficient FBS and samples of the anchored matrices (*A*) were released (*R*) from the culture dishes (mechanically relaxed). At 2 h or 2 d after release, the cultures were stimulated with 50 ng/ml PDGF for 10 min at 37°C after which cells extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors, and samples were subjected to SDS-PAGE. (*A*) Immunoblotting with anti-phosphotyrosine antibodies. (*B*) Immunoblotting with anti-PDGF receptor antibodies. Arrow, 180-kD PDGF receptors.

autophosphorylated in response to PDGF. This result suggested that receptor autophosphorylation did not occur initially and then disappear after culture. Rather, loss of autophosphorylation appeared to be a function of mechanical forces on the cells. To test this possibility more directly, collagen matrices were cultured 5 d allowing stress to develop and then switched to floating conditions allowing stress-relaxation. After 1 h (data not shown) or 2 h (Fig. 7 *A*), there was a marked decrease in PDGF-stimulated receptor autophosphorylation with no change in PDGF receptor levels (Fig. 7 *B*). Similar results were observed after release for 2 d. A decrease in PDGF-stimulated receptor autophosphorylation could be detected as early as 5 min after release but the result was variable compared to release for 1–2 h. These findings show that a change in the capacity for receptor autophosphorylation occurred when the cells were switched from mechanically stressed to mechanically relaxed conditions, i.e., what has been previously called "stress-relaxation" (Mochitate et al., 1991).

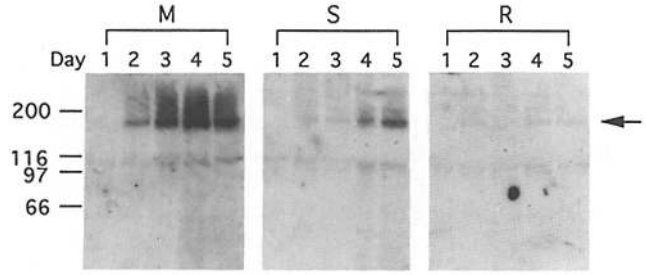

Figure 6. PDGF-stimulated receptor autophosphorylation by fibroblasts in monolayer and contracted collagen matrix cultures during the 5-d culture period. Fibroblasts were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS in monolayer culture (*M*), in collagen matrixes that were anchored to culture dishes (mechanically stressed after concentration) (*S*), or in collagen matrices that were floating in medium (mechanically relaxed after contraction) (*R*). On the days indicated, cultures were stimulated with 10 ng/ml PDGF for 10 min at 37°C, and cells extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors. Samples were subjected to SDS-PAGE, and phosphotyrosine containing polypeptides were detected by immunoblotting with anti-phosphotyrosine antibodies. Equivalent cell numbers were loaded onto each lane. Arrow, 180-kD PDGF receptors.

Localization of PDGF Receptors at the Cell Surface

Although changes in total receptor number could not account for decreased receptor autophosphorylation by fibroblasts in mechanically relaxed matrices, there could have been differences in PDGF receptor accessibility. For instance, fibroblasts in mechanically relaxed matrices may have internalized their PDGF receptors thereby making the receptor unaccessible to PDGF stimulation. Ideally, 4°C-binding studies with radiolabeled PDGF would have determined the number of accessible receptors. For fibroblasts in mechanically stressed or relaxed collagen matrices, however, saturable binding of PDGF did not occur even at PDGF concentrations as high as 500 ng/ml. Moreover, nonsaturable binding of PDGF to contracted collagen matrices occurred after the preparations were extracted with NP-40–containing buffer. On the other hand, parallel studies with cells in monolayer culture showed typical binding kinetics with half-maximal PDGF binding ~14 ng/ml and binding was lost after NP-40 treatment (data not shown).

Since direct PDGF binding studies were uninformative for the reasons described above, anti-PDGF receptor antibodies were used as an alternative approach to determining accessibility of PDGF receptors on fibroblasts in collagen matrices. Fig. 8 shows the distribution of PDGF receptors in fibroblasts in mechanically stressed or relaxed matrices observed by immunostaining and confocal microscopy. Non-permeabilized cell preparations (Fig. 8, *A* and *C*) were used to ob-

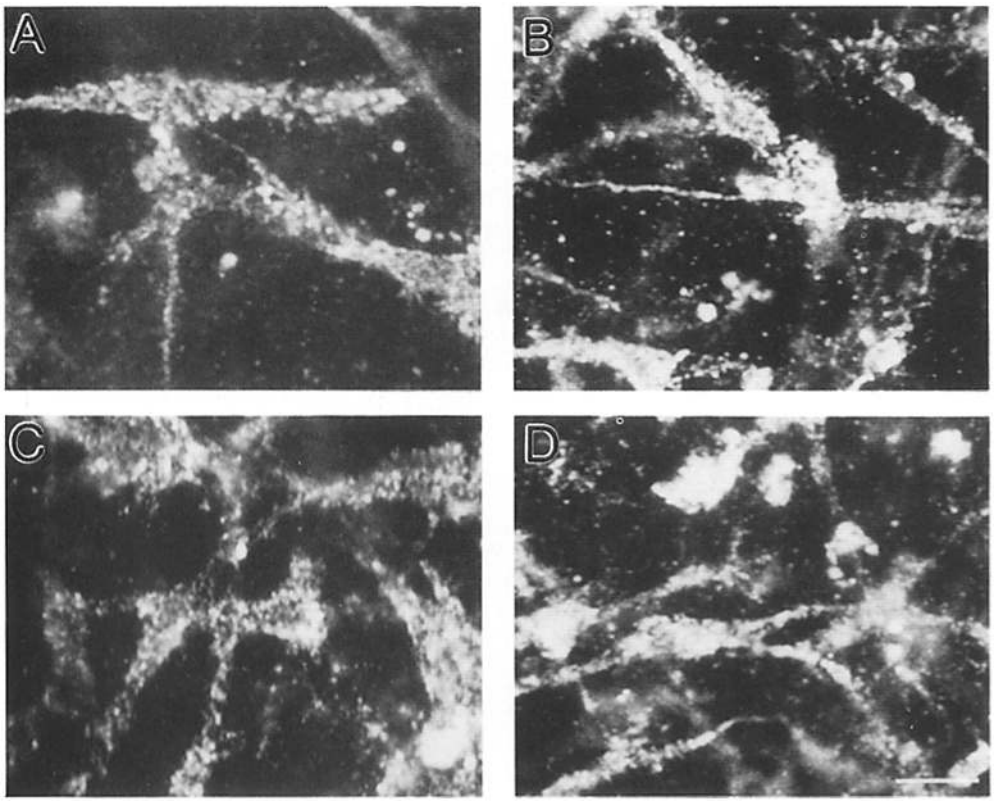

Figure 8. Distribution of PDGF receptors on fibroblasts in mechanically stressed and relaxed collagen matrices. Fibroblasts in collagen matrices anchored to culture dishes were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS (mechanically stressed). Subsequently, the cultures were switched to DMEM supplemented with 10% PDGF-deficient PBS and samples of the anchored matrices were released from the culture dishes (mechanically relaxed). Mechanically stressed or relaxed matrices were fixed (or fixed and permeabilized) and then incubated with anti-PDGF β-receptor antibodies for 20 min at 22°C followed by incubation with FITC-goat anti-mouse antibodies. The immunostained preparations were examined and photographed using a Bio-rad MRC-500 confocal laser microscope. (*A* and *B*) Fibroblasts in mechanically stressed matrices; (*C* and *D*) Fibroblasts in mechanically relaxed matrices. (*A* and *C*) Intact cells; (*B* and *D*) Permeabilized cells. Bar, 50 μm.

serve receptors on the cell surface, whereas permeabilized cell preparations (Fig. 8, *B* and *D*) were used to detect both cell surface and intracellular receptors. Control incubations using non-immune IgG in place of anti-receptor antibodies showed little background staining (data not shown). In general, most receptors appeared to be present on the cell surfaces since the staining pattern was similar with (Fig. 8, *B* and *D*) or without (Fig. 8, *A* and *C*) permeabilization. Moreover, the distribution of PDGF receptors on fibroblasts in mechanically relaxed matrices (Fig. 8, *C* and *D*) was indistinguishable from that observed on cells in mechanically stressed matrices (Fig. 8, *A* and *B*). Therefore, the cell surface distribution of PDGF receptors did not appear to change after stress–relaxation.

As an additional control, experiments were carried out to determine if antibodies bound to PDGF receptors on fibroblasts in collagen matrices could be internalized. After an initial 20-min incubation with anti-receptor antibody, the preparations were washed and incubated an additional 80 min at 37°C. Subsequently, the cells were fixed and stained with secondary antibodies. In this case, non-permeabilized cell preparations (Fig. 9, *A* and *C*) were used to localize anti-receptor antibodies remaining on the cell surface, whereas permeabilized cells (Fig. 9, *B* and *D*) were used to identify cell surface and internalized anti-receptor antibodies. There was decreased staining of anti-receptor antibodies localized on the cell surfaces (Fig. 9, *A* and *C*), compared to anti-receptor antibodies observed in permeabilized cells (Fig. 9, *B* and *D*). Therefore, the anti-receptor antibodies originally bound at the cell surface appeared to have been internalized by fibroblasts in mechanically stressed or relaxed matrices. Similar results were found with fibroblasts in monolayer culture (data not shown).

The above results showed by immunostaining procedures that PDGF receptors on fibroblasts in mechanically stressed or relaxed collagen matrices were accessible on the cell surface. Since down-regulation of PDGF receptors requires PDGF binding to the receptor but occurs independently of autophosphorylation (Escobedo et al., 1988; Sorkin et al., 1991), it was possible to compare receptor down-regulation of fibroblasts in mechanically stressed and relaxed collagen matrices as another indicator of cell surface accessibility of PDGF receptors. To make this comparison, fibroblasts in monolayer or collagen matrix cultures were treated 24 h with 100 ng/ml PDGF in the presence of 10 μg/ml cycloheximide to prevent new receptor synthesis. Subsequently, PDGF receptors were analyzed by immunoblotting. Fig. 10 shows that under all culture conditions, PDGF receptors (arrow) were markedly reduced in cells that were treated with PDGF. Therefore, most PDGF receptors of fibroblasts in mechanically relaxed collagen matrices were available for and responsive to PDGF binding.

EGF-stimulated Receptor Autophosphorylation by Fibroblasts in Monolayer and Contracted Collagen Matrix Cultures

Finally, to determine if the change in PDGF receptor auto-

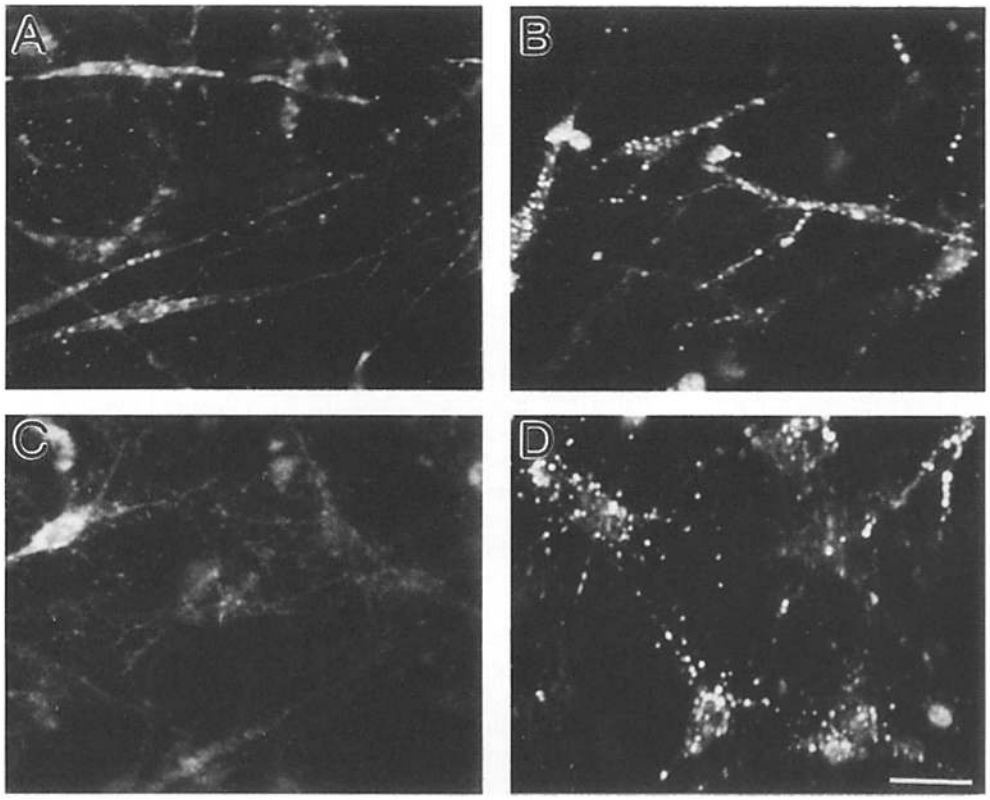

Figure 9. Internalization of anti-PDGF receptor antibodies after binding to PDGF receptors on fibroblasts in mechanically stressed and relaxed collagen matrices. Fibroblasts in collagen matrices anchored to culture dishes were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS (mechanically stressed). Subsequently, the cultures were switched to DME supplemented with 10% PDGF-deficient FBS and samples of the anchored matrices were released from the culture dishes (mechanically relaxed). Mechanically stressed or relaxed matrices were incubated 20 min at 37°C with 40 μg/ml anti-PDGF β-receptor antibodies, washed, and incubated in DPBS at 37°C for an additional 80 min. The samples were then fixed (or fixed and permeabilized) followed by incubation with FITC-goat anti-mouse. The immunostained preparations were examined and photographed using a Bio-Rad MRC-500 confocal laser microscope. (*A* and *B*) Fibroblasts in mechanically-stressed matrices; (*C* and *D*) Fibroblasts in mechanically relaxed matrices. (*A* and *C*) Intact cells; (*B* and *D*) Permeabilized cells. Bar, 50 μm.

phosphorylation was unique to PDGF receptors or if other growth factor receptors were altered as well, we analyzed EGF-stimulated autophosphorylation by fibroblasts in mechanically relaxed collagen matrix cultures. Like PDGF, EGF binding to its receptor activates the receptor's kinase activity (Ullrich and Schlessinger, 1990; Carpenter and Cohen, 1990). Fig. 11 shows autophosphorylation of 170-kD EGF receptors (arrow) detected by anti-phosphotyrosine antibodies after stimulation of cells with 0–20 ng/ml EGF for 10 min at 37°C. Fibroblasts in mechanically relaxed collagen matrices showed little autophosphorylation compared to fibroblasts in monolayer culture or mechanically stressed collagen matrices. In other experiments, we found that, as was observed for PDGF receptors, the change in capacity for EGF stimulated autophosphorylation could be detected within 1 h after stress–relaxation (data not shown). Therefore, autophosphorylation of a second growth factor receptor appeared to be unresponsive to growth factor-stimulation when the cells were switched from mechanically stressed to mechanically relaxed collagen matrices.

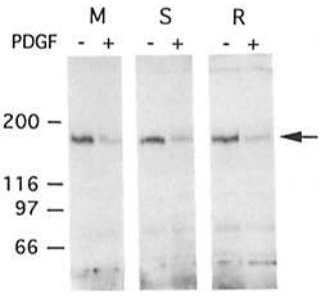

Figure 10. Down-regulation of PDGF receptors by PDGF treatment of fibroblasts in monolayer and contracted collagen matrix cultures in the presence of cycloheximide. Fibroblasts were cultured 1 d in DME/10% FBS followed by 4 d in DME/1% FBS in monolayer culture (*M*), in collagen matrices that were anchored to culture dishes (mechanically stressed after contraction) (*S*), or in collagen matrices that were floating in medium (mechanically relaxed after contraction) (*R*). During the last 24 h, the cultures were incubated with 10 μg/ml cycloheximide and without (−) or with (+) 100 ng/ml PDGF. At the end of the 5-d culture period, cell extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors. Samples were subjected to SDS-PAGE, and PDGF receptors were detected by immunoblotting with anti-PDGF α/β-receptor antibodies. Equivalent cell numbers were loaded onto each lane. Arrow, 180-kD PDGF receptors.

Discussion

The goal of our studies was to understand the interrelationship between extracellular matrix organization and fibroblast proliferation in response to growth factors. Culturing fibroblasts in mechanically relaxed (i.e., floating) collagen matrices induces a quiescent cell state (Sarber et al., 1981; Van Bockxmeer et al., 1984; Kono et al., 1990), whereas fibroblasts in mechanically stressed collagen matrices proliferate in response to serum factors (Nakagawa et al., 1989; Mochitate et al., 1991).

Since PDGF is the principle mitogen in serum for mesenchymal cells such as fibroblasts (Ross et al., 1990; Heldin and Westermark, 1990), we compared PDGF-stimulated mitogeneic response by fibroblasts in monolayer culture with that of cells in contracted collagen matrices that were mechanically stressed or relaxed. PDGF-stimulated DNA synthesis by fibroblasts in mechanically relaxed collagen ma-

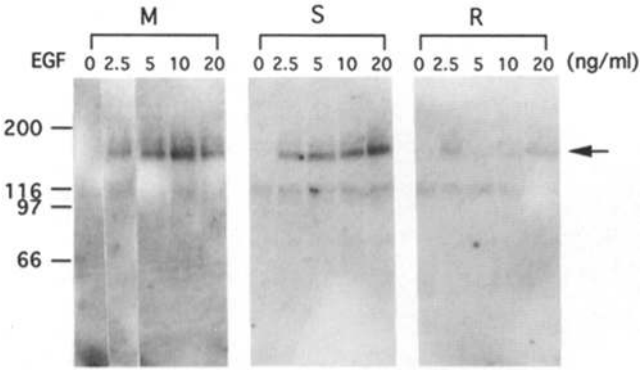

Figure 11. Dose response of EGF-stimulated receptor autophosphorylation by fibroblasts in monolayer and contracted collagen matrix cultures. Fibroblasts were cultured 1 day in DMEM/10% FBS followed by 4 days in DME/1% FBS in monolayer culture (*M*), in collagen matrices that were anchored to culture dishes (mechanically stressed after contraction) (*S*), or in collagen matrices that were floating in medium (mechanically relaxed after contraction) (*R*). At the end of the 5-d culture period, cultures were stimulated with EGF at the concentrations indicated for 10 min at 37°C, and cells extracts were prepared by homogenization in DPBS containing 0.2% NP-40 and proteinase and phosphatase inhibitors. Samples were subjected to SDS-PAGE, and phosphotyrosine containing polypeptides were detected by immunoblotting with anti-phosphotyrosine antibodies. Equivalent cell numbers were loaded onto each lane. Arrow, 170-kD EGF receptors.

trices was 80–90% lower than that of cells in monolayer culture, and 50% lower than that of cells in mechanically stressed collagen matrices. Therefore, the differential response to serum by fibroblasts in monolayer and contracted collagen matrices can be explained by differences in the ability of these cells to respond to PDGF stimulation.

The low level of responsiveness to PDGF by fibroblasts in mechanically relaxed collagen matrices could have resulted from decreased PDGF diffusion, but this seemed unlikely since PDGF-stimulated autophosphorylation peaked at 10 min for cells in collagen matrices and monolayer culture. Alternatively, PDGF may have been sequestered in the collagen matrix. Components of the extracellular matrix have been shown to bind growth factors and regulate their activity (Gospodarowicz and Cheng, 1976; Vlodavsky et al., 1987; Ruoslahti and Yamaguchi, 1991), and PDGF has been reported to interact with heparin (Fager et al., 1992) and the glycoprotein SPARC (Raines et al., 1992). If a portion of the PDGF were sequestered in the matrix, then we would have expected differences in the concentrations required for optimal stimulation and saturation of the cellular response to PDGF. Since fibroblast DNA synthesis reached maximal levels after stimulation of cells with 0.5–1 ng/ml PDGF stimulation, and further increases in DNA synthesis were not observed even at PDGF concentrations as high as 100 ng/ml PDGF, it seemed unlikely that PDGF was sequestered. Therefore, we suspected that fibroblasts in collagen matrices, especially mechanically relaxed matrices, were deficient in some aspect of their PDGF signaling pathway.

We found marked differences between fibroblasts in monolayer and contracted collagen matrix cultures in the ability of the cells' PDGF receptors to become autophosphorylated in response to PDGF. Cells in mechanically relaxed matrices showed the poorest autophosphorylation response, 90% lower than cells in monolayer culture. Even at 100 ng/ml PDGF, the extent of autophosphorylation was 60% lower for cells in mechanically relaxed matrices compared to cells in monolayer culture. When fibroblasts under mechanical stress were switched to mechanically relaxed conditions, the cells lost the capacity to become phosphorylated within 1–2 h. Loss of autophosphorylation could not be attributed to PDGF receptor numbers since immunoblotting studies showed no significant differences in PDGF receptor levels regardless of culture conditions, and no differences in receptor accessibility on the cell surface were observed by immunostaining studies and down-regulation studies.

The various assays for PDGF-stimulated autophosphorylation and mitogenesis require different time periods and different concentrations of PDGF, which makes direct comparisons impossible. Nevertheless, given the correlation between decreased autophosphorylation and mitogenesis in mechanically relaxed collagen matrices and previous studies showing that PDGF stimulated mitogenesis required PDGF receptor autophosphorylation (Williams, 1989; Mori et al., 1991; Koch et al., 1991), it seems likely that in mechanically relaxed collagen matrices fibroblasts become quiescent at least in part because their PDGF receptors no long can become autophosphorylated and initiate subsequent downstream signaling events.

The cause of decreased autophosphorylation of PDGF receptors in mechanically relaxed collagen matrices is unknown. Since EGF receptors showed a similar response to PDGF receptors, it seems likely that some sort of generic effect is involved. One possibility is activation or de-repression of a protein-tyrosine phosphatase (PTPase). PTPases have been implicated in controlling protein phosphotyrosine levels (Hunter, 1989; Fischer et al., 1991), and a membrane tyrosine phosphatase has been implicated in growth arrest caused by cell contact (Pallen and Tong, 1991). Typically, growth factor–stimulated receptor autophosphorylation activates receptor kinase function leading to downstream events. Therefore, PTPases could play a role in growth regulation by dephosphorylating growth factor receptors thereby lowering their kinase activity (Shenolikar and Nairn, 1991). Recently, this hypothesis has been tested directly by transfecting a mouse mammary tumor cell line with transmembrane PTPase CD45 (Mooney et al., 1992). In response to PDGF or insulin-like growth factor (IGF-1), transfected cells showed markedly reduced levels of receptor autophosphorylation and the mitogenic response of the cells was decreased as well.

Regardless whether increased PTPases or some other mechanism turns out to account for decreased growth factor receptor autophosphorylation by fibroblasts in collagen matrices, the effect appeared to be closely linked to mechanical stress on the cells. When mechanically stressed collagen matrices were switched to mechanically relaxed conditions, capacity for receptor autophosphorylation decreased within 1–2 h and remained low. One possibility described below is that changes in integrin receptor occupancy accompanying stress–relaxation provide the initial regulatory signals that lead to subsequent alteration in the capacity for growth factor receptor autophosphorylation.

At least two types of integrin-mediated interactions have been implicated in fibroblast interactions with collagen matrices. On one hand, cell to collagen binding required for

contraction has been shown to depend on $\alpha 2\beta 1$ integrins (Schiro et al., 1991; Klein et al., 1991). In addition, however, fibroblasts in contracted collagen matrices develop a cell surface fibronectin matrix and fibronexus junctions (Mochitate et al., 1991; Tomasek et al., 1992), which probably involve $\alpha 5\beta 1$ integrins. Allowing mechanically stressed matrices to relax results in disappearance of the junctions along with loss of cell surface fibronectin (Mochitate et al., 1991; Tomasek et al., 1992). Therefore, when fibroblasts in contracted collagen matrices are switched from mechanically stressed to mechanically relaxed conditions, their $\alpha 5\beta 1$ integrin receptors may become disengaged. Since adhesion of integrin receptors can trigger a tyrosine kinase cascade (Guan et al., 1991; Kornberg et al., 1992), differences in integrin receptor engagement by cells within mechanically stressed or relaxed collagen matrices have the potential to provide a mechanosensitive regulatory mechanism.

The importance of mechanical force for cell function pertains to a wide range of biological organisms including bacteria and plants. Recent interest in this subject is indicated by reviews on "tensegrity" (Ingber and Folkman, 1989), "mechanogenetic" (Erdos et al., 1991), and "mechanogenic" (Vandenburgh, 1992) models of cell growth regulation. Even the well-known dependence of cell growth on cell shape (Folkman and Moscona, 1978) may turn out to be an effect of cell tension (Curtis and Seehar, 1978; O'Neill et al., 1990). Our results suggest a novel mechanism by which cell proliferation can be regulated by reciprocal mechanical forces exerted between the cells and their surrounding matrix. As described below, such a mechanism may play an important role in the regulation of fibroplasia during wound repair.

In the skin, dermal fibroblasts are quiescent, biosynthetically inactive cells. Within days after cutaneous wounding, fibroblasts migrate towards the fibronectin-fibrin wound interface where granulation (i.e., wound) tissue forms. The activated fibroblasts proliferate and synthesize a new collagen-containing matrix (Peacock, 1984; Grinnell, 1984; Clark, 1985). In addition, fibroblasts in granulation tissue exert tension on the extracellular matrix, develop stress, and contract the matrix thereby bringing the wound margins moving close together (Gabbiani et al., 1972; Singer et al., 1984; Eddy et al., 1988; Welch et al., 1990). Once the wound defect is replaced, the expanded fibroblast population stops dividing and regresses and extracellular matrix remodeling begins.

Activation of fibroblasts during the early stages of repair is believed to depend on locally released growth factors such as PDGF (Ross et al., 1986). Little is known, however, about fibroblast regression at the end of wound healing. Our studies suggest that once new matrix replaces the wound and the tissue becomes mechanically relaxed, fibroblasts would no longer be able to respond PDGF and perhaps other growth factors as well. Consequently cessation of cell proliferation would occur even in the continued presence of potentially activating growth factors. Consistent with this interpretation, wounds that heal under tension show increased fibroplasia (Bunting and Eades, 1926; Burgess et al., 1990).

We are indebted to Drs. Dan Bowen-Pope and Melanie Cobb for their helpful comments and suggestions throughout the course of this research.

These studies were supported by a grant from the National Institutes of Health, GM31321.

Received for publication 16 November 1992 and in revised form 25 March 1993.

References

Bell, E., B. Ivarsson, and C. Merrill. 1979. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc. Natl. Acad. Sci. USA.* 76:1274–1278.

Bowen-Pope, D. F., and R. Ross. 1982. Platelet-derived growth factor. II. Specific binding to cultured cells. *J. Biol. Chem.* 257:5161–5171.

Bunting, C. H., and C. C. Eades. 1926. Effects of mechanical tension of the polarity of growing fibroblasts. *Exp. Med.* 44:147–149.

Burgess, L. P. A., G. V. Morin, M. Rand, J. Vossoughi, and J. O. Hollinger. 1990. Wound healing: relationship of wound closing tension to scar width in rats. *Arch. Otolaryngol. Head Neck Surg.* 116:798–802.

Carpenter, G., and S. Cohen. 1990. Epidermal growth factor. *J. Biol. Chem.* 265:7709–7712.

Clark, R. A. F. 1985. Cutaneous tissue repair: basic biological considerations. *J. Am. Acad. Dermatol.* 13:701–725.

Curtis, A. S. G., and G. M. Seehar. 1978. The control of cell division by tension or diffusion. *Nature (Lond.).* 274:52–53.

Eddy, R. J., J. A. Petro, and J. J. Tomasek. 1988. Evidence for the nonmuscle nature of the "myofibroblast" of granulation tissue and hypertrophic scar. *Am. J. Pathol.* 130:252–260.

Elsdale, T., and J. Bard. 1972. Collagen substrata for studies on cell behavior. *J. Cell Biol.* 54:626–637.

Erdos, T., G. S. Butler-Browne, and L. Rappaport. 1991. Mechanogenetic regulation of transcription. *Biochemie.* 73:1219–1231.

Escobedo, J. A., P. J. Barr, and L. T. Williams. 1988. Role of tyrosine kinase and membrane-spanning domains in signal transduction by the platelet-derived growth factor receptor. *Mol. Cell. Biol.* 8:5126–5131.

Fager, G., G. Camejo, and G. Bondjers. 1992. Heparin-like glycosaminoglycans influence growth and phenotype of human arterial smooth muscle cells in vitro. I. Evidence for reversible binding and inactivation of the platelet-derived growth factor by heparin. *In Vitro Cell Dev. Biol.* 28A:168–175.

Fischer, E. H., H. Charbonneau, and N. K. Tonks. 1991. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science (Wash. DC).* 253:401–406.

Folkman, J., and A. Moscona. 1978. Role of cell shape in growth control. *Nature (Lond.).* 273:345–349.

Fukamizu, H., and F. Grinnell. 1990. Spatial organization of extracellular matrix and fibroblast activity: effects of serum, transforming growth factor β, and fibronectin. *Exp. Cell Res.* 190:276–282.

Gabbiani, G., B. J. Hirschel, G. B. Ryan, P. R. Statkov, and G. Majno. 1972. Granulation tissue as a contractile organ: a study of structure and function. *J. Exp. Med.* 135:719–733.

Gospodarowicz, D., and J. Cheng. 1986. Heparin protects basic and acidic FGF from inactivation. *J. Cell Physiol.* 128:475–484.

Grinnell, F. 1984. Fibronectin and wound healing. *J. Cell Biochem.* 26: 107–116.

Grinnell, F., and C. R. Lamke. 1984. Reorganization of hydrated collagen lattices by human skin fibroblasts. *J. Cell Sci.* 66:51–63.

Guan, J-L., J. E. Trevithick, and R. O. Hynes. 1991. Fibronectin/integrin interaction induces tyrosine phosphorylation of a 120-kDa protein. *Cell Regul.* 2:951–964.

Heldin, C.-H., and B. Westermark. 1990. Platelet-derived growth factor: mechanism of action and possible in vivo function. *Cell Regul.* 1:555–566.

Heldin, C.-H., A. Wasteson, and B. Westermark. 1982. Interaction of platelet-derived growth factor with its fibroblast receptor. Demonstration of ligand degradation and receptor modulation. *J. Biol. Chem.* 257:4216–4221.

Hunter, T. 1989. Protein-tyrosine phosphatases: the other side of the coin. *Cell.* 58:1013–1016.

Ingber, D. E., and J. Folkman. 1989. Tension and compression as basic determinants of cell form and function. *In* Cell Shape: Determinants, Regulation, and Regulatory Role. W. D. Stein and F. Bronner, editors. Academic Press, Inc., New York. 3–31.

Kazlauskas, A., D. F. Bowen-Pope, R. Seifert, C. E. Hart, and J. A. Cooper. 1988. Different effects of homo- and heterodimers of platelet-derived growth factor A and B chains on human and mouse fibroblasts. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3727–3735.

Klein, C. E., D. Dressel, T. Steinmayer, C. Mauch, B. Eckes, T. Krieg, R. B. Bankert, and L. Weber. 1991. Integrin $\alpha 2\beta 1$ is upregulated in fibroblasts and highly aggressive melanoma cells in three-dimensional collagen lattices and mediates the reorganization of collagen I fibrils. *J. Cell Biol.* 115:1427–1436.

Koch, C. A., D. Anderson, M. F. Moran, C. Ellis, and T. Pawson. 1991. SH2 and SH3 domains: Elements that control interactions of cytoplasmic signaling proteins. *Science (Wash. DC).* 252:668–674.

Kono, T., T. Tanii, M. Furukawa, N. Mizuno, J-I. Katajima, M. Ishii, T. Hamada, and K. Yoshizato. 1990. Cell cycle analysis of human dermal fibroblasts cultured on or in hydrated type I collagen lattices. *Arch. Dermatol. Res.* 282:258–262.

Kornberg, L. J., H. S. Earp, C. E. Turner, C. Prokop, and R. L. Juliano. 1991. Signal transduction by integrins: increased protein tyrosine phosphorylation

caused by clustering of β1 integrins. *Proc. Natl. Acad. Sci. USA.* 88: 8392–9396.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Mochitate, K., P. Pawelek, and F. Grinnell. 1991. Stress relaxation of contracted collagen gels: disruption of actin filament bundles, release of cell surface fibronectin, and down regulation of DNA and protein synthesis. *Exp. Cell Res.* 193:198–207.

Mooney, R. A., G. G. Freund, B. A. Way, and K. L. Bordwell. 1992. Expression of a transmembrane phosphotyrosine phosphatase inhibits cellular response to platelet-derived growth factor and insulin-like growth factor-1. *J. Biol. Chem.* 267:23443–23446.

Mori, S., L. Classon-Welsh, and C-H. Heldin. 1991. Identification of a hydrophobic region in the carboxyl terminus of the platelet-derived growth factor β-receptor which is important for ligand-mediated endocytosis. *J. Biol. Chem.* 266:21158–21164.

Nakagawa, S., P. Pawelek, and F. Grinnell. 1989. Extracellular matrix organization modulates fibroblast growth and growth factor responsiveness. *Exp. Cell Res.* 182:572–582.

Nishiyama, T., M. Tsunenaga, Y. Nakayama, E. Adachi, and T. Hayashi. 1989. Growth rate of human fibroblasts is repressed by the culture within reconstituted collagen matrix but not by the culture on the matrix. *Matrix.* 9:193–199.

Nishiyama, T., N. Akutsu, I. Horii, Y. Nakayama, T. Ozawa, and T. Hayashi. 1991. Response to growth factors of human dermal fibroblasts in a quiescent state owing to cell-matrix contact inhibition. *Matrix.* 11:71–75.

O'Neill, C., P. Jordan, P. Riddle, and G. Ireland. 1990. Narrow linear strips of adhesive substratum are powerful inducers of both growth and total focal contact area. *J. Cell Sci.* 95:577–586.

Pallen, C. J., and P. H. Tong. 1991. Elevation of membrane tyrosine phosphatase activity in density-dependent growth-arrested fibroblasts. *Proc. Natl. Acad. Sci. USA.* 88:6996–7000.

Peacock, E. E. 1984. Wound Repair. 3rd. edition. W. B. Saunders, Co., Philadelphia.

Raines, E. W., T. F. Lane, M. L. Iruela-Arispe, and R. Ross. 1992. The extracellular glycoprotein SPARC interacts with platelet-derived growth factor (PDGF)-AB and -BB and inhibits the binding of PDGF to its receptors. *Proc. Natl. Acad. Sci. USA.* 89:1281–1285.

Roberts, C. J., T. M. Birkenmeier, J. J. McQuillan, S. K. Akiyama, S. S. Yamada, W-T. Chen, K. M. Yamada, and J. A. McDonald. 1988. Transforming growth factor β stimulates the expression of fibronectin and of both subunits of the human fibronectin receptor by cultured human lung fibroblasts. *J. Biol. Chem.* 263:4586–4592.

Ross, R., E. W. Raines, and D. F. Bowen-Pope. 1986. The biology of platelet-derived growth factor. *Cell.* 46:155–169.

Ross, R., D. F. Bowen-Pope, and E. R. Raines. 1990. Platelet-derived growth factor and its role in health and disease. *Phil. Trans. R. Soc. Lond. B.* 327:155–169.

Ruoslahti, E., and Y. Yamaguchi. 1991. Proteoglycans as modulators of growth factor activities. *Cell.* 64:867–869.

Sarber, R., B. Hull, C. Merrill, T. Soranno, and E. Bell. 1981. Regulation of proliferation of fibroblasts of low and high population doubling levels grown in collagen lattices. *Mech. Aging and Dev.* 17:107–117.

Schiro, J. A., B. M. C. Chan, W. T. Roswit, P. D. Kassner, A. P. Pentland, M. E. Hemler, A. Z. Eisen, and T. S. Kupper. 1991. Integrin α2β1 (VLA-2) mediates reorganization and contraction of collagen matrices by human cells. *Cell.* 67:403–410.

Schor, S. L. 1980. Cell proliferation and migration on collagen substrata in vitro. *J. Cell Sci.* 41:159–175.

Seifert, R. A., C. E. Hart, P. E. Phillips, J. W. Forstrom, R. Ross, M. J. Murray, and D. F. Bowen-Pope. 1989. Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. *J. Biol. Chem.* 264:8771–8778.

Shenolikar S., and A. C. Nairn. 1991. Protein phosphatases: Recent progress. Adv. Second Messenger Phosphoprotein Res. 23:1–121.

Singer, I. I., D. W. Kawka, D. M. Kazazis, and R. A. F. Clark. 1984. The in vivo codistribution of fibronectin and actin fibers in granulation tissue: immunofluorescence and electron microscopic studies of the fibronexus at the myofibroblast surface. *J. Cell Biol.* 98:2091–2106.

Sorkin, A., B. Westermark, C-H. Heldin, and L. Claesson-Welsh. 1991. Effect of receptor kinase inactivation on the rate of internalization and degradation of PDGF and the PDGF β-receptor. *J. Cell Biol.* 112:469–478.

Tomasek, J. J., C. J. Haaksma, R. J. Eddy, and M. B. Vaughan. 1992. Fibroblast contraction occurs on release of tension in attached collagen lattices: Dependency on an organized actin cytoskeleton and serum. *Anat. Rec.* 232:359–368.

Ullrich, A., and J. Schlessinger. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 61:203–212.

Van Bockxmeer, F. M,. C. E. Martin, and I. J. Constable. 1984. Effect of cyclic AMP on cellular contractility and DNA synthesis in chorioretinal fibroblasts maintained in collagen matrices. *Exp. Cell Res.* 155:413–421.

Vandenburgh, H. H. 1992. Mechanical forces and their second messengers in stimulating cell growth in vitro. *Am. J. Physiol.* 262:R350–R355.

Vlodavsky, I., J. Folkman, R. Sullivan, R. Fridman, R. Ishai-Michaeli, J. Sasse, and M. Klagsburn. 1987. Endothelial cell-derived basic fibroblast growth factor: Synthesis and deposition into subendothelial extracellular matrix. *Proc. Natl. Acad. Sci. USA.* 84:2292–2296.

Vogel, A., E. Raines, B. Kariya, M. J. Rivest, and R. Ross. 1978. Coordinate control of 3T3 cell proliferation by platelet-derived growth factor and plasma components. *Proc. Natl. Acad. Sci. USA.* 75:2810–2814.

Welch, M. P., G. F. Odland, and R. A. F. Clark. 1990. Temporal relationships of F-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction. *J. Cell Biol.* 110: 133–145.

Williams, L. T. 1989. Signal transduction by the platelet-derived growth factor receptor. *Science (Wash. DC).* 243:1564–1570.

Yoshizato, K., T. Taira, and N. Yamamoto. 1985. Growth inhibition of human fibroblasts by reconstituted collagen fibrils. *Biomed. Res.* 6:61–71.