Gelatinase A Activation Is Regulated by the Organization of the Polymerized Actin Cytoskeleton*

(Received for publication, October 10, 1996, and in revised form, December 19, 1996)

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Gelatinase A (GL-A) is a matrix metalloproteinase (MMP) involved in both connective tissue remodeling and tumor invasion. GL-A activation is mediated by a membrane-type MMP (MT-MMP) that cleaves the GL-A propeptide. In this study, we examined the role of the actin cytoskeleton in regulating GL-A activation and MT-MMP-1 expression. Human palmar fascia fibroblasts and human fetal lung fibroblasts were cultured on a planar substratum or within different types of collagen lattices. Fibroblasts that formed stress fibers, either on a planar substratum or within an attached collagen lattice, showed reduced GL-A activation compared with fibroblasts lacking stress fibers, within either floating or stress-released collagen lattices. To determine whether changes in the organization of the actin cytoskeleton could promote GL-A activation, fibroblasts with stress fibers were treated with cytochalasin D. Within 24 h after treatment, GL-A activation was dramatically increased. Associated with this GL-A activation was an increase in MT-MMP-1 mRNA as determined by Northern blot analysis. Treatment with nocodazole, which induced microtubule depolymerization and cell shape changes without affecting stress fibers, did not promote GL-A activation. These results suggest that the extracellular matrix and the actin cytoskeleton transduce signals that modulate GL-A activation and regulate tissue remodeling.

MMPs are among the key extracellular enzymes involved in turnover of the ECM during normal and pathological processes. Changes in MMP expression by cells in vitro and in vivo occur in response to different environmental cues, but MMPs must become activated in order for them to exert their biochemical functions. The latency of MMPs, which are secreted as proenzymes, is conferred by the presence of propeptides which contain a free cysteine residue that is thought to associate with the active-site zinc molecule. Activation of MMPs occurs when this association is disrupted by cleavage of the propeptide or by disruption of the cysteine-zinc bond. Recently, a sub-family of MMPs, the MT-MMPs, has been identified as having a potential role in activating GL-A (MMP-2, 72-kDa gelatinase), a MMP previously shown to be refractive to most proteolytic activation mechanisms. MT-MMPs are unique in that they possess a transmembrane domain that anchors them to the cell surface. In addition, MT-MMPs have a putative activation site cleavable by furin-like intracellular enzymes and may therefore appear on the cell surface already activated. Thus, GL-A, which is constitutively expressed by many cell types, may be regulated through its interaction with the MT-MMPs, which themselves may be regulated transcriptionally. Expression of MT-MMPs would lead to the subsequent activation of GL-A.

GL-A activation may be regulated, in part, by the physical properties of the ECM to which cells adhere. Previous studies have demonstrated that fibroblasts cultured on a planar substratum activate little, if any, of the secreted GL-A (5). Cellular activation of GL-A by cultured fibroblasts is promoted by agents which alter the organization of the actin cytoskeleton, including phorbol esters, concanavalin A, and cyto D (5–8), suggesting that the actin cytoskeleton plays a role in regulating GL-A activation. In addition, the binding of type I collagen fibrils to the surfaces of fibroblasts, possibly through the α2β1 integrin, can promote GL-A activation (5, 9). However, the three-dimensional organization of the collagen lattice may be critical to this process, as only fibroblasts cultured within floating collagen lattices and not on a thin coating of collagen can promote cellular activation of GL-A (5, 9). MT-MMP-1, the best-characterized MT-MMP, is known to be up-regulated in cultured cells by agents which promote cellular activation of GL-A, including concanavalin A, phorbol esters, and cyto D, suggesting that MT-MMP-1 is involved in the cellular activation of GL-A (6, 8).

It has previously been demonstrated that the three-dimensional organization of collagen fibers influences the organization of the actin cytoskeleton (10–15). Fibroblasts cultured in mechanically relaxed floating collagen lattices lack bundles of actin; rather, the actin is organized into a cortical meshwork (10, 11, 14, 15). In contrast, fibroblasts cultured on a thin coating of type I collagen or within mechanically stressed, stabilized collagen lattices organize actin into bundles, referred to as stress fibers, terminating at the cell surface into focal adhesions or fibronexus structures (12–15). In this study, using collagen lattice models and cyto D treatment, we demonstrate an inter-relationship between the mechanical properties of the ECM and actin cytoskeleton organization in the regulation of GL-A activation and expression of MT-MMP-1. The mechanical forces which develop in the collagen lattice are demonstrated to be critical regulators of GL-A activation and MT-MMP-1 expression. These results suggest that the mechanical properties...
of the ECM and the actin cytoskeleton transduce the signals that regulate MT-MMP-1 expression and thereby the activation of GL-A.

EXPERIMENTAL PROCEDURES

Cell Culture—Human palmar fascia fibroblasts were obtained and cultured, as described previously (13, 14, 16), in complete medium M-199 (Life Technologies, Inc.) supplemented with 10% fetal bovine serum ( Irvine Scientific, Santa Ana, CA), 2 mM glutamine, and 1% antibiotic-antimycotic solution (Life Technologies, Inc.). WI-38 fibroblasts were obtained from the American Type Culture Collection (Rockville, MD) and cultured as recommended. For experiments on planar substrata, fibroblasts were plated on 35-mm tissue culture dishes (Falcon, Lincoln Park, NJ) at 50% confluence and grown to 90% confluence. Cultures were briefly washed with serum-free medium and treated with 8 μg/mI cyto D (Sigma), 2 μg/ml nucodazole (Sigma), or 25 nm GM6001 for 24 h, after which conditioned medium or total RNA was collected.

For experiments in collagen lattices, stabilized collagen lattices were cultured for 5 days, washed briefly with serum-free medium, and treated as described above. GM6001, a peptide hydroxamic acid-based inhibitor of MMPs (17), was a generous gift of Dr. Richard Galaray.

Preparation of Collagen Lattices—Fibroblasts were cultured within type I collagen (Upstate Biotechnology Inc., Lake Placid, NY) lattices, as described previously (13, 14, 16), such that the final collagen concentration was 0.65 mg/ml and the cell concentration 1.25 × 10^5 cells/ml. Floating collagen lattices were prepared by placing a 250-μl drop of the collagen-cell suspension on 35-mm Petri dishes (Falcon). After a 1-h incubation at 37°C to allow polymerization of the collagen, 1.5 ml of complete medium was placed over the collagen lattice, which did not adhere to the Petri dishes, but floated freely in the medium. Stabilized collagen lattices were prepared by the identical protocol with the exception that the collagen/seed suspension was placed on a 35-mm tissue culture dish (Falcon). The collagen lattices remained attached to the underlying plastic substratum throughout the culture period. Released collagen lattices were obtained by mechanically releasing the stabilized collagen lattice from the underlying plastic substratum after 5 days in culture (13, 16). Thin layer collagen substrata were prepared by incubating 50 μg of collagen/0.25 ml per 24-well culture dish well for 1 h at 37°C, followed by a wash with phosphate-buffered saline.

Fluorescence Staining for Actin Filaments—Fibroblasts were cultured within floating, stabilized, or released collagen lattices, as described above. Collagen lattices were fixed with 4% paraformaldehyde and stained with the F-actin probe, BODIPY™ phallacidin (Molecular Probes, Eugene, OR), as described previously (13, 14). Briefly, lattices were permeabilized with ice-cold acetone, washed three times with phosphate-buffered saline, incubated with BODIPY™ phallacidin for 30 min at room temperature, and viewed and photographed as whole mounts using an Olympus Vanox microscope equipped with epifluorescence optics.

Electrophoresis and Blotting—Serum-free medium conditioned by cells was analyzed by gelatin zymography, as described previously (18). Gels were poured with 1 mg/ml gelatin, and non-reduced samples were electrophoresed as in standard SDS-polyacrylamide gel electrophoresis. Following electrophoretic separation, gels were incubated with 2.5% Triton X-100 to remove SDS, then incubated with substrate buffer. After incubation, gels were stained with Coomassie Brilliant Blue R-250. Areas of non-staining correspond to gelatinolytic activity.

Northern Blots—Total RNA was isolated from cells according to published methods (19). Samples representing equivalent amounts of total RNA, as determined by spectrophotometry, were separated by denaturing gels, and then RNA was transferred to MSI-NT nylon membranes by capillary action and cross-linked to membranes with UV light. Membranes were incubated for 1 h at 60°C with prehybridization buffer (500 mM NaPO4, pH 7.2, 5% SDS, 1 mM EDTA, 0.5% bovine serum albumin, 200 mM NaCl at room temperature) and then hybridized overnight in prehybridization buffer plus labeled cDNA probe at 60°C. Probes were 32P-labeled by random priming using a Prime-It II kit (Stratagene, La Jolla, CA), then separated from unincorporated label using ProbeQuant G-50 Micro columns (Pharmacia Biotech, Uppsala, Sweden). Following three low stringency washes (10 min in 40 mM NaPO4, pH 7.2, 5% SDS, 1 mM EDTA, 0.5% bovine serum albumin, 200 mM NaCl at room temperature) and two high stringency washes (10 min with 40 mM NaPO4, pH 7.2, 1% SDS, 1 mM EDTA) at room temperature and one 30-min high stringency wash at 60°C, membranes were exposed to x-ray film adjacent to an enhancing screen. Blots were scanned on a densitometer to detect relative changes in mRNA expression. Human GL-A probe was a 913-base pair PstI-EcoRI fragment derived from the full-length cDNA (p16SPT19–1) from W. Stetler-Stevenson, NCI). Human MT-MMP-1 probe was a 3.3-kilobase full-length cDNA isolated from a placental library (provided by S. Fisher, UCSF) by means of a polymerase chain reaction-derived cDNA fragment spanning the propeptide domain to the zinc-binding site. GAPDH cDNA was from the American Type Culture Collection.

RESULTS

Activation of GL-A by Fibroblasts in Collagen Lattices with Different Mechanical Properties—Fibroblasts were cultured within three-dimensional collagen lattices for 5 days in complete medium, after which they were allowed to condition serum-free medium for 24 h. Mechanically relaxed collagen lattices floated in the culture medium and maximally contracted over the initial 5-day period. Mechanically stressed collagen lattices remained attached to underlying culture dishes and developed maximum stress over this time period (12, 13, 15). Parallel cultures of these mechanically stressed collagen lattices were released from the underlying substratum, leading to the rapid contraction of the collagen matrix and the dissipation of mechanical stress, a process termed stress-relaxation (12, 13, 15). Stress-relaxed collagen lattices were cultured for 24 h after release in complete medium to allow for lattice contraction, after which lattices were cultured for an additional 24 h in serum-free medium.

Fig. 1 shows the levels of latent and activated GL-A after culture of human palmar fibroblasts or WI-38 fibroblasts within three-dimensional collagen lattices. Regardless of the type of collagen lattice, pro-GL-A was secreted by the fibroblasts, and a portion of this enzyme was activated, as detected by the appearance of a 60-kDa band in the zymogram (Fig. 1, Act GL-A). The only major bands observed by zymography of the conditioned medium were the latent and activated forms of GL-A. The zymographic ratio of active to latent GL-A in conditioned media from mechanically relaxed collagen lattices were higher than from mechanically stressed collagen lattices (Fig. 1). Stress-relaxation of collagen lattices resulted in a reversal of the levels of latent and activated GL-A from those observed in the mechanically stressed collagen lattice. This reversal resulted in a ratio of activated to latent GL-A similar to that observed in mechanically relaxed floating collagen lattices (Fig. 1).

Similar changes in levels of latent and activated GL-A in response to the mechanical properties of the collagen lattices were observed in adult human palmar fibroblasts, as well as in human fetal lung fibroblasts. In all the following experiments, both types of fibroblasts were examined.

Organization of the Actin Cytoskeleton Is Correlated with GL-A Activation and Expression—The organization of the actin cytoskeleton of fibroblasts cultured within the different three-dimensional collagen lattices was examined by staining with the F-actin probe BODIPY™ phallacidin. Palmar fibroblasts cultured for 5 days within mechanically relaxed collagen lat-
tices had very few stress fibers. Instead, F-actin was found predominantly in the cortical regions of the cells (Fig. 2 A). In contrast, palmar fibroblasts cultured for 5 days within mechanically stressed collagen lattices had prominent stress fibers present throughout the cell (Fig. 2B). Release of the mechanically stressed collagen lattices resulted in a loss of stress fibers within the first 10 min after release (not shown). By 24 h after release, the actin became organized into a F-actin cortex similar to that observed in the mechanically relaxed collagen lattice (Fig. 2C). Similar changes in the organization of the actin cytoskeleton were observed in WI-38 fibroblasts cultured in the different collagen lattices (not shown).

To test the possible role of the organization of the actin cytoskeleton in GL-A activation, fibroblasts in mechanically stressed collagen lattices were treated with either cyto D or nocodazole. Both of these treatments radically changed the shape of the fibroblasts within mechanically stressed collagen lattices. Treatment of human palmar fibroblasts with nocodazole resulted in a shortening of the cell processes and rounding of the cell body (Fig. 3B), in contrast to cyto D, which left the cells dendritic and which resulted in a rapid and persistent alteration in the actin cytoskeleton (Fig. 3A) (20). Cyto D caused the loss of stress fibers, and the actin filaments appeared to collapse into aggregates near the cell periphery. In contrast, nocodazole promoted the loss of microtubules (not shown), but with no loss of stress fibers (Fig. 3B). Alteration of the actin cytoskeleton with cyto D resulted in a dramatic increase in the level of activated GL-A compared with control mechanically stressed collagen lattices. The levels of latent and activated GL-A in cyto D-treated lattices were similar to those observed in mechanically relaxed or stress-relaxed collagen lattices (compare Figs. 4 and 1). In contrast, nocodazole treatment did not alter GL-A activation levels from control lattices. A similar activation of gelatinase A in response to cyto D treatment was observed in WI-38 fibroblasts cultured with mechanically stressed collagen lattices (not shown).

GL-A Activation Is MMP-dependent—Because the activation of GL-A has been linked to its enzymatic cleavage by MMPs, we evaluated whether inhibiting the enzymatic activity of MMPs in the fibroblast culture would inhibit activation of GL-A. Fibroblasts were cultured within mechanically stressed collagen lattices or in monolayer, then treated for 18 h with cyto D, both in the presence and absence of the MMP inhibitor GM6001. In both culture conditions, GM6001 completely blocked cyto D-promoted activation of GL-A (Figs. 4 and 5). To control for the possibility that GM6001 present in the culture medium remained bound to GL-A during electrophoresis and subsequently blocked its enzymatic activity in zymographs, conditioned medium from cyto D-treated fibroblasts cultured in monolayer was incubated overnight with GM6001 and subsequently analyzed by gelatin zymography. No obvious reduction

FIG. 2. Actin organization in fibroblasts cultured within collagen lattices. Human palmar fibroblasts were cultured in mechanically relaxed (A), mechanically stressed (B), and stress-relaxed (C) collagen lattices. Actin organization was observed by staining the cells with the F-actin probe BODIPY™ phallacidin. Large bundles of actin microfilaments, stress fibers, are present in fibroblasts within mechanically stressed collagen lattices (B). In contrast, the actin staining in fibroblasts within mechanically relaxed or stress-relaxed collagen lattices is predominantly at the cell cortex (A, C). Magnification × 525.

FIG. 3. Actin organization in fibroblasts treated with cyto D or nocodazole. Human palmar fibroblasts were cultured within mechanically stressed collagen lattices, then treated in serum-free medium with 8 μM cyto D (A) or 2 μg/ml nocodazole (B) for 24 h. Actin organization was observed by staining the cells with the F-actin probe BODIPY™ phallacidin. Cyto D treatment resulted in a loss of normal actin organization (A). In contrast, stress fibers remained in nocodazole-treated fibroblasts (B). Magnification × 350.
in the activated GL-A band occurred after incubation of conditioned medium with GM6001 (not shown).

Binding of Fibroblasts to Collagen Is Not Sufficient for Activation of GL-A—Activated GL-A was not observed in medium conditioned by fibroblasts cultured in a monolayer, similar to other reports (5, 8). In contrast, activated GL-A was observed in medium conditioned by fibroblasts cultured within the different collagen lattices. Because the activation of GL-A observed in the conditioned medium from fibroblasts cultured within the collagen lattices could be the result of integrin-mediated signaling through cell binding to the surrounding collagen matrix (5, 9), we tested whether binding to, and spreading on, collagen was sufficient to promote activation of GL-A. WI-38 fibroblasts cultured on type I collagen-coated tissue culture plates formed large stress fibers (not shown), but did not activate GL-A (Fig. 6). A similar response was observed in human palmar fibroblasts (not shown). These results suggest that the mechanical properties of the collagen are important in the promotion of gelatinase A activation.

The Mechanical Properties of Collagen Lattices Regulate MT-MMP-1 and GL-A Expression—Recent studies have suggested that a new family of MMPs, the MT-MMPs, are responsible for activation of GL-A (2–4). Northern blot analysis was performed to determine whether steady state levels of GL-A mRNA had increased under conditions which promoted its activation. GL-A mRNA in human palmar fibroblasts increased in mechanically relaxed and stress-relaxed collagen lattices compared with mechanically stressed collagen lattices (Fig. 8). The GL-A:GAPDH ratio was increased 1.3-fold in mechanically relaxed and 1.5-fold in stress-relaxed collagen lattices compared with mechanically stressed collagen lattices. Similar changes in MT-MMP-1 mRNA and GL-A mRNA levels were observed in WI-38 fibroblasts (not shown).

Changes in the Organization of the Actin Cytoskeleton Regulate GL-A and MT-MMP-1 Expression—Next we evaluated whether the steady state levels of MT-MMP-1 and GL-A mRNA increased after cyto D treatment, conditions which disrupt the actin cytoskeleton. The MT-MMP-1:GAPDH mRNA ratio increased 2.0-fold in cyto D-treated human palmar fibroblasts cultured in monolayer compared with untreated control cells (Fig. 9). Similarly, the GL-A:GAPDH mRNA ratio increased 1.6-fold by cyto D-treatment and 3.5-fold in stress-relaxed collagen lattices cultured in monolayer compared with untreated control cells (Fig. 9). Similar changes in MT-MMP-1 and GL-A mRNA levels were observed in WI-38 fibroblasts (not shown).

DISCUSSION

Using three-dimensional collagen lattices that differ only in their mechanical properties, we examined the effects of mechanical forces on the cellular activation of GL-A, which is thought to occur through its interaction with MT-MMPs (1–7). Fibroblasts in mechanically relaxed or stress-relaxed collagen lattices had high levels of GL-A activation and MT-MMP-1 mRNA levels compared to fibroblasts in mechanically stressed collagen lattices. Treatment of mechanically stressed fibro-
Cytoskeletal Regulation of Gelatinase A Activation

Adult palmar fibroblasts were cultured within mechanically relaxed (Floating), mechanically stressed (Stabilized), and stress-relaxed (Released) collagen lattices. Equal amounts of total RNA were analyzed by Northern blots. After probing for GL-A message, blots were stripped, then reprobed for GAPDH expression. GL-A mRNA level was decreased in stabilized collagen lattices compared with floating or released collagen lattices.

Fibroblasts with cyto D, which causes the disruption of stress fibers and which models mechanical relaxation, also resulted in increased levels of GL-A activation and MT-MMP-1 mRNA. GL-A activation could be blocked by the peptide hydroxamic acid-based inhibitor GM6001 (17), consistent with the proposal that GL-A activation is MMP-mediated. In addition, levels of GL-A mRNA increased in mechanically relaxed compared to mechanically stressed collagen lattices. These results suggest that as cells lose mechanical stress they regulate GL-A activation by increasing the expression of MT-MMP-1, as well as increasing the expression of GL-A.

While it has been proposed that the three-dimensional organization of the collagen matrix, as well as cell binding to collagen, regulates GL-A activation (5, 9), we found that mechanical stress, and not matrix organization, may regulate this activation. Mechanically stressed collagen lattices, when compared with mechanically relaxed lattices, showed a marked reduction in GL-A activation and MT-MMP-1 expression, even though fibroblasts were surrounded by a three-dimensional collagen matrix in both cases. Fibroblasts cultured on a thin layer of collagen or in a monolayer activate little or no GL-A. All cells in such cultures contain stress fibers. That virtually no activation of GL-A occurs on a thin layer of collagen suggests that more than just binding of collagen is required for GL-A activation. Fibroblasts in mechanically stressed collagen lattices always activate some GL-A, which may be accounted for by the presence of some cells which lack stress fibers (13). This suggests that it is not just the three-dimensional organization of the collagen lattice, but rather the mechanical forces present in the lattice, that regulate cellular activation of GL-A, presumably by regulating the expression of MT-MMP-1.

The changes in activity and expression of GL-A and MT-MMP-1 observed in collagen lattices, while significant, are not as dramatic as those observed after treatment with cyto D. Fibroblasts in floating and released collagen lattices are heterogeneous with respect to the amount of mechanical stress they experience. For example, some of the fibroblasts at the periphery of floating or stress-relaxed collagen lattices contain stress fibers (14) and would be expected to express less GL-A and MT-MMP-1 than cells in the center of the lattice. Treatment of cultures with cyto D models loss of mechanical stress and involves the entire cell population. Not surprisingly, cyto D treatment yields larger changes in GL-A and MT-MMP-1 levels (Fig. 9). Thus, while cyto D is not as relevant physiologically as are collagen lattices, it does provide an experimental model that may be useful for studying the role of the actin cytoskeleton in gene regulation.

The mechanical forces in the ECM may transduce intracellular signals through integrin-mediated focal adhesion complexes (21). It is well known that integrins induce formation of a specialized focal adhesion complex at the site of cell-ECM binding due to specific binding interactions between integrins, actin-associated molecules, and actin (22). The assembly of actin stress fibers and focal adhesion complexes leads to the activation of signal transduction mediators such as pp125 focal adhesion kinase (23). Fibroblasts in mechanically stressed collagen lattices form fibronexus-type focal adhesion complexes at their surfaces; in contrast, fibroblasts in mechanically relaxed or stress-relaxed collagen lattices lack these cell surface complexes (13–15). Although the mechanism remains to be elucidated, such matrix-mediated signaling could lead to regulation of MT-MMP-1 and GL-A expression observed in this study. In this study we have demonstrated that the lack or loss of stress fibers in response to altered mechanical forces in collagen lattices or to cyto D treatment is associated with increased activation of GL-A. Recently, cyto D treatment of rat mesangial cells cultured on a planar substratum was shown to increase GL-A activation and MT-MMP-1 mRNA (8). These results suggest that signals generated by mechanical stress in the ECM are transduced by the actin cytoskeleton and play an important role in regulating GL-A activation. Interstitial collagenase levels have also been demonstrated to be regulated by the organization of the actin cytoskeleton (24). Reorganization of the polymerized actin cytoskeleton, either by release of collagen lattices or by cytochalasin B treatment, promoted increased levels of secreted interstitial collagenase in rabbit synovial fibroblasts. These increased levels of collagenase were not due to cell shape changes, since treatment with colchicine, which disrupted the microtubule organization and changed the cell shape but not the actin cytoskeleton, had no effect on collagenase levels (24). In the present study, we found that disruption of microtubules and changes in cell shape by treatment with nocodazole had no effect on GL-A activation.

Previous studies have demonstrated that both urokinase and its inhibitor, plasminogen activator inhibitor-1, are induced by cyto D promoted cytoskeletal reorganization (25–27). It has been speculated that urokinase induction follows the activation of c-Jun, which then participates in transcriptional activation through interactions with the AP-1 site within the urokinase promoter (25). MT-MMP-1, which is induced by phorbol esters, may have an AP-1 site within its promoter, although no sequence data have been published (6). Similarly, the collagenase
gene has an AP-1 site within its promoter (1). These MMPs could be regulated by similar transcription factors responsive to mechanical stress and the organization of the actin cytoskeleton. The GL-A promoter has no AP-1 site within the published 1.6-kilobase sequence and is not responsive to phorbol esters or most cytokines or growth factors (28–30). Indeed, GL-A appears to be constitutively expressed in most tissues, and its promoter is TATA-less, similar to many so-called “housekeeping” genes. Thus, the mechanism by which mechanical stress and actin organization regulates transcription of GL-A may be different from that proposed for MT-MMP-1.

Mechanical forces may play an important role in regulating the expression and assembly of ECM macromolecules (15). Mechanical stress will promote the assembly of fibronectin into fibrils at the cell surface (14), as well as an increase in the expression of type I collagen (31). These results have led to the proposal that mechanical stress induces a matrix-depositing fibroblast phenotype (15). Our results demonstrating that mechanical stress results in decreased activation of GL-A are consistent with this proposal. Interstitial collagenase, as well as plasminogen activators, may be similarly regulated by mechanical stress (25–27, 31). These results suggest that, opposite to the matrix-depositing phenotype promoted by mechanical stress, a mechanically relaxed environment may promote a “proteolytic” phenotype favoring matrix turnover. Thus, the mechanical forces described here in the in vitro collagen lattice models may also play an important role in vivo during wound healing and pathological contractures. Fibroblasts in granulation tissue exert tension on the ECM, develop stress, and contract the matrix, thereby bringing the wound margins closer together (15, 32, 33). Once the wound defect is closed by a combination of contraction and new matrix synthesis, the fibroblast population regresses and ECM remodeling begins. Our studies suggest that, while the wound is under tension and depositing a new ECM, little MMP activity would be present. Once the new matrix replaces the wound and the tissue becomes mechanically relaxed, MMP expression and activation would occur, promoting ECM remodeling. Consistent with this proposal is the observation that increased skin tension contributes to increased scarring after surgery (34), as well as the presence of the contractile myofibroblast in many fibrotic conditions (15).

Acknowledgments—We thank Ben Han for help in the preparation of the figures and Elizabeth Bullen and Shelley Pierson for technical assistance.

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