Experimental

# Strain-Dependent Control of Transforming Growth Factor-β Function in Osteoblasts in an In Vitro Model: Biochemical Events Associated with Distraction Osteogenesis

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**Background:** Distraction osteogenesis is an important clinical method for increasing bone mass, but its effects on bone-forming cells are not well understood. In this study, the authors asked how the mechanical forces that occur during this procedure alter specific osteoblast activities such as matrix synthesis, the rate of cell replication, and enzyme activities. The authors further asked whether these changes relate to differences in the biochemical response of osteoblasts to transforming growth factor-β (TGF-β), a potent regulator of bone formation.

**Methods:** Osteoblasts were plated on flexible, collagen-coated membranes. One group was unstrained, a second group experienced a single maximum strain load once every 6 hours to simulate intermittent force associated with a distraction protocol of four screw turns per day, and a third group was strained continuously for 24 hours. In the third group, some cell cultures were allowed to recover from strain before analysis. Subsequently, each group was treated with vehicle or TGF-β at 12 pM (0.3 ng/ml) or 120 pM (3 ng/ml). Data were collected from a minimum of 15 replicate cell culture wells obtained from at least three separate primary culture preparations. Results were assessed with statistical software. Differences were considered significant with values of $p < 0.05$.

**Results:** Both strain protocols increased basal osteoblast DNA synthesis but suppressed the relative stimulatory effect of TGF-β on this event. However, neither intermittent nor continuous strain significantly altered collagen or noncollagen protein synthesis or the relative effect of TGF-β on these processes in osteoblasts. Basal alkaline phosphatase activity, an intermediate marker of osteoblast differentiation and an early marker of matrix mineralization, decreased significantly in response to continuous strain or to TGF-β treatment, and even more so in response to both conditions. In addition, TGF-β binding to the type III TGF-β receptor was increased in proportion to strain intensity.

**Conclusions:** This study shows that cyclic strain can alter osteoblast activity in multiple ways and predicts that TGF-β has different effects during the distraction process on osteoblasts and therefore on their ability to effect bone formation. They further indicate that mechanical load permits early aspects of osteoblast activation but delays in part later biochemical parameters associated with mineralization to allow new bone growth before consolidation. (*Plast. Reconstr. Surg.* 116: 224, 2005.)

First introduced by Codivilla in 1905,[1] distraction osteogenesis experienced a renais-

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sance as a result of the work of Ilizarov,[2–4] and has been used successfully to enhance bone growth in the limb and craniofacial regions.[5–10] Despite clinical experience and refinement of surgical distraction techniques, however, very little is understood about mechanical strain on bone formation and, in particular, how it translates into a biological response in osteoblasts. In addition to mechanical force, bone growth is strongly influenced by systemic and local growth factors.[11–15] One principal local regulator of bone formation is transforming growth factor-β (TGF-β), which potently enhances osteoblast replication and bone matrix synthesis while suppressing later aspects of bone development. In general, therefore, TGF-β is thought to enhance early events in osteogenesis to maintain high levels of bone growth or repair. The effective amount of TGF-β can vary in part through changes in its synthesis and its release from inactive complexes. All three known isoforms of TGF-β act by binding to the same panel of cell surface receptors on osteoblasts, although in some instances binding occurs with somewhat different affinities. Nonetheless, in the end, all known TGF-β isoforms induce the same panel of biochemical responses in osteoblasts.[16–18]

Earlier studies revealed that a variation in individual TGF-β receptors on osteoblasts alters the extent and the nature of its effects. This is most obvious with the so-called type III TGF-β receptor, a cell surface proteoglycan. Under some conditions, the type III TGF-β receptor appears to accumulate and present TGF-β to the type II receptor and therefore more efficiently activates the type I signal-transducing receptor. The expression of the type III TGF-β receptor significantly decreases in parallel with native or growth factor-induced osteoblast differentiation. Importantly, when type III TGF-β receptor levels are in excess, either through hormone treatment or gene transfection, they appear to limit type II receptor binding and some downstream TGF-β–dependent effects.[19–23]

To understand how distraction osteogenesis is achieved, we asked whether mechanical strain alters several basic features of early osteoblast function and whether it modifies aspects of the TGF-β system in osteoblast-enriched cell cultures in vitro. In this study, we used an in vitro device that applies graded and carefully controlled cell surface strain on primary cultures of osteoblasts that were grown on flexible surfaces coated with type I collagen, the major organic component of the bone matrix.[24–26] The cells experienced two strain regimens. The first was designed to simulate intermittent strain on osteoblasts as it might be experienced with a clinical situation of periodic application of force with the mechanical distraction device. This was compared with continuous cyclic strain and with no strain. A final set of cultures were strained and then allowed to recover, to assess whether strain applied in this way induced transient or irreversible effects.

## MATERIALS AND METHODS

### *Cell Cultures*

Primary cell cultures were chosen, because their biochemical characteristics are more reliable than those of many osteoblast-like cell lines currently available. Significant phenotypic drift is common in many of these cell lines, such as MC3T3-E1 cells, which occurs during subpassage over many years, and many investigators familiar with the field are well aware of this problem. These differences cause variations in cell character and often make it difficult to interpret results.[11] Biochemical characteristics associated with the primary cell population that was used in our studies have been established in our laboratory for over 16 years.[17,23,24] Primary osteoblast-enriched cell cultures were prepared from parietal bones of 22-day-old rat fetuses [stock designation Crl: CD(SD)BR, originally derived from Sprague-Dawley rats; Charles River Breeding Laboratories, Wilmington, Mass.]. Pregnant rats were killed under guidelines approved by the institutional animal care and use committee, and in agreement with National Institutes of Health recommendations. The explants were dissected free of sutures and digested five times with collagenase. Cells released at each digestion interval appear to represent populations at various stages of differentiation, and those from the last three digestions (populations 3 through 5) are enriched with cells exhibiting biochemical characteristics associated with differentiated osteoblasts.[24,25] Cells pooled from populations 3 through 5 were plated on type I collagen-coated flexible membranes (Bioflex, Flexercell Corp., Mc-Keesport, Pa.) in 9.6-cm$^2$ cultures. They were grown in Dulbecco's modified Eagle's medium containing 20 mM HEPES buffer (pH 7.2), 100 mg/ml ascorbic

acid, penicillin, streptomycin, and 10% fetal bovine serum (all from InVitrogen, Grand Island, N.Y.). Culturing was performed at 37°C, 5 percent carbon dioxide, and 88 percent humidity. At confluence, they were refed with identical medium lacking only fetal bovine serum for 20 hours before further perturbation.

*Strain Protocols*

Strain was induced with a Flexercell unit (Flexercell Corp.), an in vitro apparatus first introduced by Banes et al.,[26] which applies computer-controlled, vacuum-induced cyclic strain on the cells. In response to a strain of 20 kPa (−150 mmHg) applied in this way, cells throughout the culture experience somewhat different degrees of elongation, from 24 percent at the periphery to as little as 1 percent in the center of the culture well, resulting in an average of 10 to 12 percent elongation overall.[27–29] The average maximal force associated with strain was therefore approximately 100,000 to 120,000 microstrain units, and 0.1 Hz, or six cycles of strain per minute. Control cells were held static. A second group experienced a single rapid maximum strain load once every 6 of 24 hours to simulate intermittent forces associated with a clinical distraction protocol of four screw turns per day. A third group was strained continuously for 24 hours. In the third group, some of the cells were allowed to recover from strain before analysis. Portions of the cell cultures from each condition were incubated with vehicle (4 mg/ml bovine serum albumin in 0.1 M HCl) or recombinant TGF-$\beta$1 (obtained in collaboration from Bristol-Myers/Squibb, Seattle, Wash.). The protocols are shown in Figure 1 and the treatment protocols are depicted in Table I.

*DNA Synthesis*

DNA synthesis rate was measured by incubating the cell cultures with [methyl-$^3$H]thymidine (5 $\mu$Ci/ml, 80 Ci/mmol; New England Nuclear, Boston, Mass.) for the last 2 hours of culturing with vehicle or 12 pM TGF-$\beta$1. Radioisotope incorporation was terminated by medium aspiration. Cells were lysed in 0.1% (w/v) sodium dodecylsulfate and 0.1 M NaOH. Radiolabeled DNA was collected by precipitation with 5% trichloroacetic acid and centrifugation. The pellets were extracted with 95% ethanol, dried, and resolubilized in 350-Soluene (Packard, Meriden, Conn.). Radioisotope incorporation was measured in a scintillation counter.

*Collagen and Noncollagen Protein Synthesis*

Collagen and noncollagen protein synthesis were measured by incubating cell cultures with 12.5 $\mu$Ci/ml [$^3$H]proline (0.5 Ci/mmol) during the entire 24-hour period of exposure to vehicle or 120 pM TGF-$\beta$. Cells were lysed in 0.5% Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) in water. Lysates were diluted threefold, precipitated with 10% trichloroacetic acid, and chilled. Acid-precipitable material was collected by centrifugation. Pellets were extracted with 95% ethanol and acetone, dried, resolubilized in 0.5 M acetic acid, and neutralized with NaOH. The amount of [$^3$H]proline incorporated into the collagenase-digestible and noncollagen protein pools was measured as described by Peterkofsky and Di-

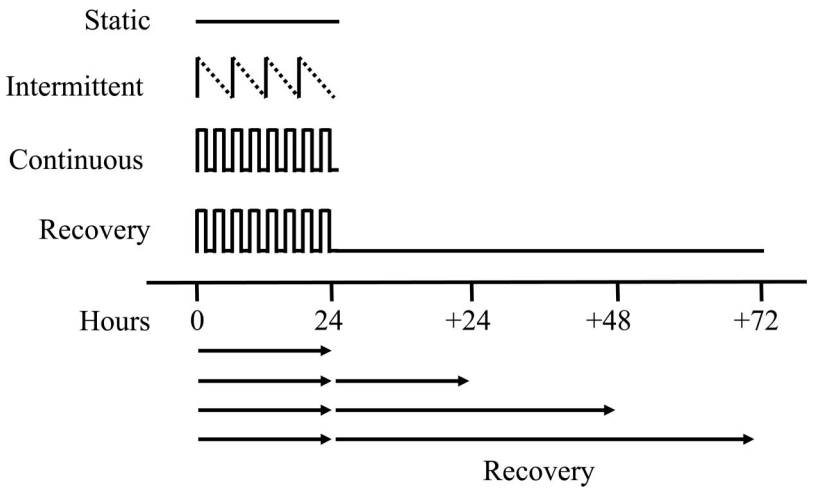


FIG. 1. Scheme of applied strain protocols.

TABLE I
Treatment Protocol

| | First Phase* | Second Phase† |
|---|---|---|
| DNA synthesis | Static or strain ± recovery | Vehicle or 12 pM TGF-β, then label with $^{3}$[H]thymidine during last 2 hr of culture |
| Collagen and noncollagen synthesis | Static or strain ± recovery | Vehicle or 120 pM TGF-β, then label with $^{3}$[H]proline during last 2 hr of culture |
| Alkaline phosphatase activity | Static or strain ± recovery | Vehicle or 120 pM TGF-β, then extract and assay enzyme |
| Radioligand binding | Static or strain | Bind and crosslink to $^{125}$I-TGF-β, then extract and SDS-PAGE |
| Western blot | Static or strain | Extraction and SDS-PAGE, then blot and stain with anti–type III TGF-β R antibody |

$^{125}$I, iodine-125; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; R, receptor.

* The first phase of the study consisted of three groups: (1) static, no strain; (2) intermittent, four cycles of strain, 0.1 Hz, once every 6 hr for 24 hr; (3) continuous, 24 hr of continuous strain, 0.1 Hz. For recovery studies, strained cells were reincubated under static conditions for an additional 24, 48, or 72 hr to assess the possible long-term effects of previous strain.

† The second phase of the study addressed the effect of strain, or strain and recovery, on TGF-β activity and binding. To assess aspects of TGF-β biochemical activity, static, strained, or strained and recovered cells were then incubated with vehicle or the amount of TGF-β indicated, as previously determined for its maximal effectiveness. To assess TGF-β receptors, static or strained cells were studied.

egelmann.[30] Amounts of collagen and noncollagen protein synthesis were calculated by correcting for the 5.4-fold greater abundance of proline in collagenase-digestible protein.

*Alkaline Phosphatase Assay*

Enzyme activity in cell extracts prepared in 0.5% Triton X-100 was assessed for the ability to hydrolyze *p*-nitrophenyl phosphate to *p*-nitrophenol. Substrate conversion was determined by spectrophotometry at 410 nm after 18 or more minutes of incubation at 37°C, according to the method of Lowry.[31] Data are expressed as nanomoles of *p*-nitrophenol released per minute per microgram of cell protein. Protein concentrations were determined by the method of Bradford.[32]

*Receptor Binding Studies*

TGF-β1 was radio-iodinated with chloramine T to a specific activity of 4000 to 4500 Ci/mmol. Iodine-125–TGF-β1 was separated from unincorporated iodine-125 by gel filtration on Sephadex G-50 in a solution of 0.1 M acetic acid and 4 mg/ml bovine serum albumin. Iodine-125–TGF-β1 binding to cell surface protein was examined by incubating the cell cultures for 3 hours at 4°C in serum-free medium that was supplemented with 4 mg/ml bovine serum albumin and 200 pM iodine-125–TGF-β1. Cultures were rinsed with cold isotonic buffer and proteins were cross-linked with 0.5 mM disuccinimidyl suberate by a 15-minute incubation at 4°C. Cells were extracted in reducing sample buffer and extracts were fractionated through a 5 to 15 percent gradient polyacrylamide gel. Bound iodine-125–TGF-β1 was visualized by autoradiography as reported previously.[23,33]

*Western Analysis*

Total cell lysate was fractionated through a 5 to 15 percent gradient polyacrylamide gel and transferred to nitrocellulose membranes (Millipore, Bedford, Mass.).[34] Membranes were blocked with 5% nonfat dry milk and then incubated with a biotinylated antihuman type III TGF-β receptor antibody (R&D Systems, Minneapolis, Minn.) diluted 1:1000 in blocking buffer. After several washings, a secondary antibody (1:2000 in blocking buffer) was added, and immunoreactive bands were visualized by enhanced chemiluminescence (ECL Western blotting system; Amersham, Piscataway, N.J.) and exposure to radiographic film.

*Statistical Analysis*

Biochemical data were analyzed from a minimum of 15 replicate cell culture wells obtained from at least three separate cell culture preparations and expressed as means ± SE. Statistical differences were determined by analysis of variance with commercial software from SigmaStat (San Rafael, Calif.). When multiple groups were compared, results were subjected to the Student-Newman-Keuls post hoc analysis test. By this method, differences were considered significant with values of $p < 0.05$.

## RESULTS

The biochemical effects of mechanical loading on osteoblasts, here induced by flex-associated strain, were studied in untreated and TGF-β1–treated osteoblast-enriched pri-

mary cell cultures. To do so, we examined differences in DNA and collagen and noncollagen protein synthesis, and in alkaline phosphatase activity, three processes associated with the activity of metabolically active but not terminally differentiated bone forming cells.

To assess effects by strain on the rate of DNA synthesis, cells were first serum-deprived to reduce the contribution of serum growth regulators and subjected to intermittent or continuous strain. The cells were then treated with diluent or TGF-β1 for 24 hours and supplemented with [$^3$H]thymidine during the last 2 hours of culture. By itself, each strain protocol produced an approximate twofold stimulatory effect on DNA synthesis rate by comparison with static, unstrained osteoblasts (left panel, Fig. 2, *left*). Treatment with TGF-β1 at a maximally effective mitogenic concentration of 12 pM (0.3 ng/ml)[17,23] increased the rate of DNA synthesis by approximately 40-fold in the static cultures. Nonetheless, the stimulatory effect of TGF-β1 was significantly decreased in cells that experienced either intermittent or continuous strain (Fig. 2, *right*). Although DNA synthesis returned to a baseline level when the osteoblasts were allowed to recover from strain for a period of 24 hours (Fig. 3, *left*), the suppressive effect of strain on TGF-β1–induced DNA synthesis persisted (Fig. 3, *right*). Therefore, the mitogenic effects of strain and TGF-β1 appear to occur through different and perhaps mutually exclusive pathways.

To examine effects on collagen and noncollagen protein synthesis, parallel serum-deprived, static, and strained cultures of osteoblasts were cultured without or with 120 pM (3 ng/ml) TGF-β1, which is a maximally effective concentration for this process.[24] Unlike its effect on DNA synthesis, strain did not alter basal or TGF-β1–induced collagen or noncollagen protein synthesis (Figs. 4, *left* and 5, *left*). After recovery periods of 24 to 72 hours, collagen and noncollagen synthesis remained similarly increased by twofold to threefold in the TGF-β-treated groups (Figs. 4, *right* and 5, *right*).

Strain by itself rapidly decreased alkaline phosphatase activity in differentiating osteoblasts. Consistent with earlier studies,[23] treatment with TGF-β1 also reduced alkaline phosphatase activity by itself. This effect was compounded by either strain protocol, with a greater suppression induced by continuous strain (Fig. 6, *left*). The suppressive effect of strain was lost by 48 hours of recovery. However, consistent with the effects seen on collagen and noncollagen protein synthesis, the sensitivity by osteoblasts to TGF-β1 also persisted on alkaline phosphatase activity during the entire recovery interval (Fig. 6, *right*).

Our results so far predicted that at least three aspects of osteoblast activity responded very differently to cell surface strain. Specifically, strain increased DNA synthesis, had no immediate effect on collagen or noncollagen protein synthesis, and suppressed alkaline

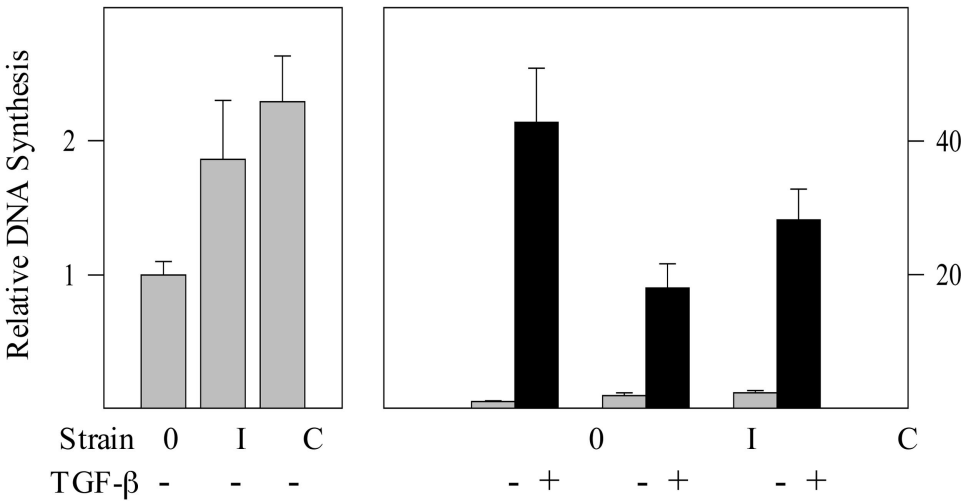


FIG. 2. Effect of mechanical strain on DNA synthesis in osteoblasts. Cells were held static or strained for 24 hours and then treated for the next 24 hours with diluent (−) or 12 pM TGF-β1 (+) as shown. DNA synthesis rates were measured by labeling with [methyl-$^3$H]thymidine for the last 2 hours of culture. Strain significantly increased DNA synthesis, whereas the relative stimulatory effect of TGF-β was significantly suppressed by both strain regimens ($p < 0.05$). Data are means ± SE of 15 cell cultures per condition. *0*, static; *I*, intermittent strain; *C*, continuous strain.

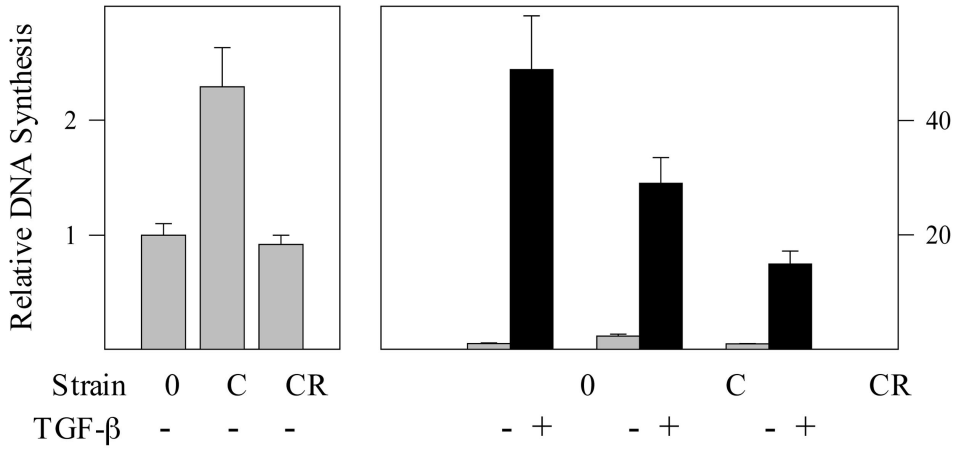


FIG. 3. Effect of mechanical strain and subsequent recovery on DNA synthesis in osteoblasts. Cells were held static or strained for 24 hours, held static for a 24-hour recovery period, and then treated for the next 24 hours with diluent (−) or 12 pM TGF-β1 (+) as shown. DNA synthesis rates were measured by labeling with [methyl-$^3$H]thymidine for the last 2 hours of culture. Strain significantly increased DNA synthesis by comparison with static or recovery cultures, whereas the relative stimulatory effect of TGF-β was significantly suppressed by strain and remained so during the recovery period ($p < 0.05$). Data are means ± SE of 15 cell cultures per condition. *0*, static; *C*, continuous strain; *CR*, continuous strain/recovery period.

phosphatase activity. Of these, strain mimicked the influence of TGF-β1 on DNA synthesis and alkaline phosphatase activity, but only seemed to alter the effect of this growth factor on DNA synthesis. Previous studies showed that variations in the levels of TGF-β receptors on osteoblasts could influence its activity in different ways, and that these invariably include changes in the type III TGF-β receptor.[21–23] Therefore, we then asked whether strain altered this TGF-β–binding component on osteoblasts. By iodine-125–TGF-β1 binding, type III TGF-β receptors increased in proportion to strain intensity (Fig. 7, *left*). However, by reactivity with anti-type III TGF-β receptor antibody, strain appeared to produce a more modest but similar increase in the total amount of type III TGF-β receptors under each condition of strain (Fig. 7, *right*). Therefore, strain appears to increase both type III receptor levels and binding affinity, which is more evident with continuous strain. We saw no similarly large or reproducible changes in type II or type I TGF-β receptor levels with continuous strain, and for unexplained reasons, these two classes of TGF-β receptors failed to be visualized under any circumstance with certain batches of Bioflex cell culture plates (data not shown).

## DISCUSSION

The applied forces and strains that occur during distraction osteogenesis are influenced by muscle activity and by the resistance of the soft-tissue envelope. As mechanical load increases, changes in mechanical strain occur directly on cells found in skeletal tissue and thereby influence bone formation. Despite a wealth of clinical experience and histological studies, there is minimal information about the underlying physiologic and biochemical events that occur during distraction osteogenesis. Moreover, little is known about how the mechanical forces associated with this procedure translate into a cellular response leading to new bone formation.

Mechanical strain has direct effects on the intracellular cytoskeleton, from which several intracellular second messengers activate a variety of downstream pathways.[12,36] The expression of local growth factors may also be increased in response to mechanical strain or distraction osteogenesis.[13,37–41] In this regard, Frost first proposed in his "mechanostat theory" that mechanotransduction pathways are modulated by systemic hormones and local growth factors.[35] In this study, we focused on TGF-β, a potent local bone growth factor the effects of which are modified by systemic and other local bone growth factors.[16] We asked how the function of TGF-β, as it specifically relates to differentiating osteoblasts, is influenced by mechanical strain. We applied a maximum of 10 percent strain to isolated osteoblasts in culture that, based on animal studies, represents a maximal mechanical load that occurs during distraction.[42–44] We found only spe-

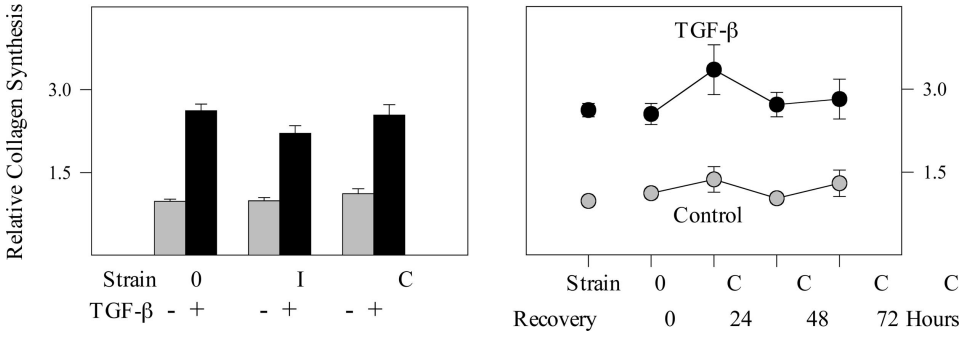


FIG. 4. Effect of mechanical strain and subsequent recovery on collagen synthesis in osteoblasts. Cells were held static or strained for 24 hours or strained and then held static for a 24-, 48-, or 72-hour recovery period and treated for the 24-hour period after strain or recovery with diluent (−) or 120 pM TGF-β1 (+) as shown. Collagen synthesis was measured by labeling with [$^3$H]proline for the last 24 hours of culture and assessing the amount of collagenase-digestible protein. Strain had no significant effect on collagen synthesis and did not modify the significant stimulatory effect of TGF-β treatment ($p < 0.05$). Data are means ± SE of 15 cell cultures per condition. *0*, static; *I*, intermittent strain; *C*, continuous strain.

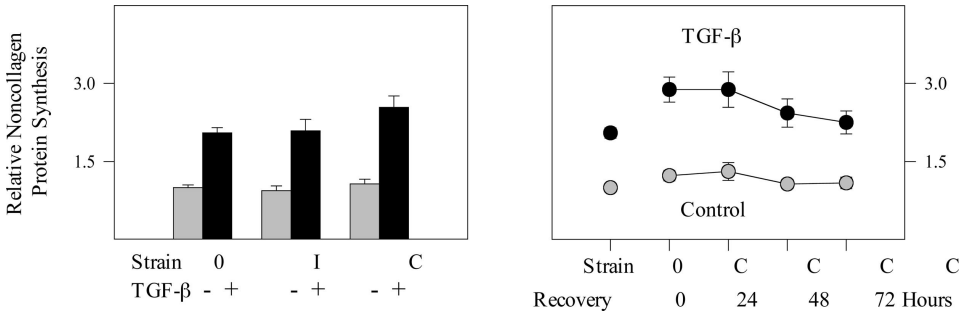


FIG. 5. Effect of mechanical strain and subsequent recovery on noncollagen protein synthesis in osteoblasts. Cells were held static or strained for 24 hours or strained and then held static for a 24-, 48-, or 72-hour recovery period and treated for the 24-hour period after strain or recovery with diluent (−) or 120 pM TGF-β1 (+) as shown. Noncollagen protein synthesis was measured by labeling with [$^3$H]proline for the last 24 hours of culture and assessing the amount of collagenase-resistant protein. Strain had no significant effect on noncollagen protein synthesis and did not modify the significant stimulatory effect of TGF-β treatment ($p < 0.05$). Data are means ± SE of 15 cell cultures per condition. *0*, static; *I*, intermittent strain; *C*, continuous strain.

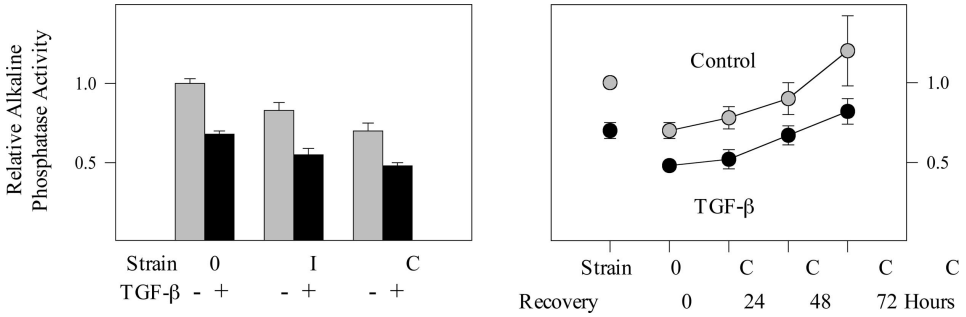


FIG. 6. Effect of mechanical strain and subsequent recovery on alkaline phosphatase activity in osteoblasts. Cells were held static or strained for 24 hours or strained and then held static for a 24-, 48-, or 72-hour recovery period and treated for the 24-hour period after strain or recovery with diluent (−) or 120 pM TGF-β1 (+) as shown. Alkaline phosphatase activity was measured in cell lysates by a colorimetric assay. Strain significantly reduced alkaline phosphatase activity in untreated and TGF-β–treated cells. The inhibitory effect of strain persisted during the initial 24-hour recovery period, but the cells remained sensitive to the suppressive effect of TGF-β at all time points ($p < 0.05$). Data are means ± SE of 15 or more cultures per condition. *0*, static; *I*, intermittent strain; *C*, continuous strain.

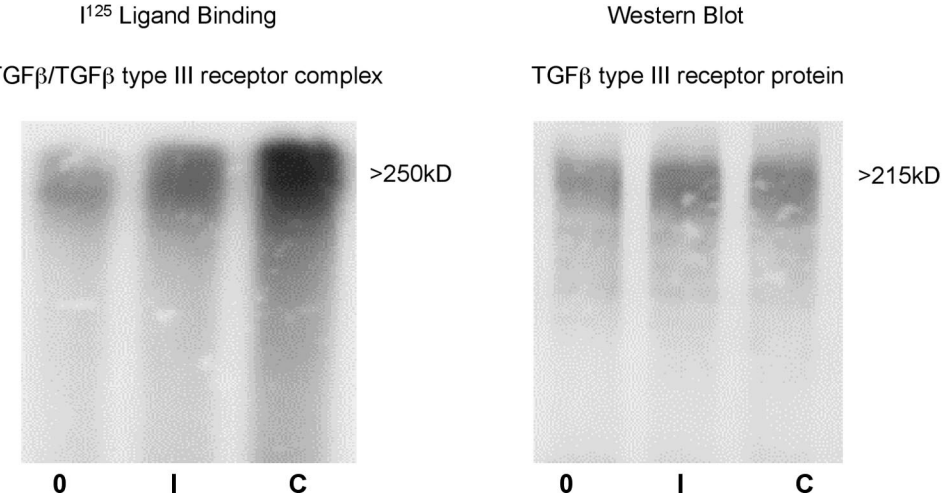


FIG. 7. Strain-dependent increases in TGF-β binding to the type III TGF-β receptor. Cells were held static or strained for 24 hours. A portion of the cultures were incubated with iodine-125–TGF-β, and binding was examined by chemical covalent cross-linking of ligand/receptor complexes, polyacrylamide gel electrophoresis, and autoradiography. Parallel cultures were extracted for Western blot analysis by reactivity with anti–type III TGF-β receptor–specific antiserum. Strain increased binding and total type III TGF-β receptor levels, although proportionally more TGF-β binding relative to total type III TGF-β receptor content occurred after continuous strain. Similar results were observed in at least two separate studies. *0*, static; *I*, intermittent strain; *C*, continuous strain.

cific aspects of TGF-β activity modified in mechanically strained osteoblasts, consistent with earlier results showing that the effects of TGF-β change during osteoblast differentiation.[16,23] However, effects induced by surrounding cells in intact bone through paracrine factors, or by signals generated through interactions with the skeletal inorganic matrix, are excluded.[15] Finally, fluid shear can also influence the complex process of bone cell activation.[45–47] Although we assume that our in vitro model specifically investigates the influence of mechanical strain on TGF-β function in osteoblasts, we cannot exclude that stresses produced by movement of the cell culture medium during the mechanical cycling event also contribute to the results we observed. Therefore, although our model may not fully represent the in vivo setting of intact, remodeling bone, it allows us to collect novel information regarding the physiology of mechanical strain on one key cell type in distraction osteogenesis, the bone-forming osteoblast.

Although strain by itself modestly increased cell proliferation, it suppressed the potent mitogenic effect of TGF-β treatment, and this change progressed for at least 24 hours after the strain was released. In contrast, strain had no effect on collagen or noncollagen protein synthesis or the stimulatory effect of TGF-β on these events. By contrast, alkaline phosphatase, an enzyme that is involved in the mineralization process and a later marker of osteoblast maturation, decreased in proportion to strain intensity. Osteocalcin is another important marker of late-stage osteoblast differentiation. However, as we previously reported, osteocalcin only achieves measurable levels of expression after 3 or more weeks in culture, in particular, after osteoblasts are treated with 1,25-dihydroxyvitamin $D_3$.[48] At this later point in culture, cell replication, matrix protein synthesis, and some molecular regulators of these events are reduced to nearly their basal rate,[49] making it difficult if not impossible to compare these events.

Our earlier studies showed that cell-surface TGF-β receptor levels vary in parallel with the function of TGF-β during osteoblast differentiation. Importantly, when TGF-β binding to the type III receptor is enhanced, the ability of TGF-β to activate osteoblasts is reduced.[21–23] We found a rapid increase in binding to the type III TGF-β receptor with strain, in parallel with changes in some aspects of TGF-β activity. During osteoblast differentiation, a reciprocal relationship between cell growth and matrix deposition has often been observed.[49] On the basis of these new results, strain appears to maintain cultured osteoblasts at an intermedi-

ate state of osteoblast-like activity, by both biochemical and phenotypic criteria, with regard to TGF-β activity or binding. Therefore, effects by strain on the reciprocal relationship between growth and differentiation might be critical for maintaining new bone matrix deposition during the active phase of distraction osteogenesis. Alkaline phosphatase activity, and presumably later aspects of matrix mineralization, is reduced with mechanical strain and during distraction, and does not resume until the strain is released. Allowing for a period of recovery after strain, osteoblasts appear to have the opportunity to reachieve a basal status and reestablish their sensitivity to TGF-β. Thus, osteoblast activity may rebound in part during the consolidation period, as depicted in our in vitro results. Other events unrelated to the TGF-β system must also occur during the distraction process.[45–47,50–52] Nevertheless, the changes that we observed in TGF-β activity and its binding to the type III TGF-β receptor might represent an important aspect of the ability of this process to modify and maintain active bone matrix protein synthesis before bone consolidation occurs.

## Conclusions

The changes that we found in static and strained cells, and the events that followed release from strain, have begun to clarify some of the biochemical mechanisms that may define more precisely the timing and duration of the clinical distraction process. We are now pursuing further molecular studies to establish how changes in type III TGF-β receptor levels occur in this culture model. In the end, these may provide new targets for molecular intervention that, in combination with distraction itself, may offer better postsurgical care and improve the physical outcome for the patient.

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