Basic fibroblast growth factor destabilizes osteonectin mRNA in osteoblasts

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Delany, Anne M., and Ernesto Canalis. Basic fibroblast growth factor destabilizes osteonectin mRNA in osteoblasts. Am. J. Physiol. 274 (Cell Physiol. 43): C734–C740, 1998.—Osteonectin (secreted protein acidic and rich in cysteine, 40-kDa basement membrane) is a glycoprotein abundantly expressed in bone and in other tissues undergoing active remodeling. Fibroblast growth factors (FGFs) are important in skeletal development and fracture repair, events associated with extracellular matrix remodeling. We used the murine osteoblastic cell line MC3T3 to determine whether basic FGF (bFGF) regulates osteonectin expression in bone. Northern blot analysis showed that bFGF decreased osteonectin transcripts in a dose- and time-dependent manner. This regulation was independent of the mitogenic effect of bFGF but was dependent on new protein synthesis. Immunoprecipitation of [35S]methionine-cysteine osteoblast-conditioned medium and cell layer proteins showed that bFGF decreased osteonectin synthesis. Nuclear runoff assays failed to reveal regulation of osteonectin gene transcription by bFGF. However, bFGF dramatically decreased the stability of osteonectin mRNA in transcriptionally arrested osteoblasts. This destabilization of osteonectin mRNA may be one means by which bFGF regulates extracellular matrix remodeling.

OSTEONECTIN, or secreted protein acidic and rich in cysteine (SPARC), is a modular glycoprotein that is expressed in bone and in other tissues undergoing active remodeling. Although the precise role of osteonectin in bone and other tissues has not been defined, in vitro studies suggest that osteonectin has pleiotropic effects on gene expression (22). Osteonectin induces metalloproteinase expression in fibroblasts and stimulates angiogenesis in vitro and in vivo (17, 20, 41). In addition, osteonectin may localize the activities of growth factors and proteases within the matrix, because osteonectin binds type I collagen and platelet-derived growth factor (PDGF) B chains, thrombospondin, and plasminogen (19, 22, 32). Osteonectin limits the function of PDGF-BB and PDGF-AB by inhibiting their binding to fibroblasts (32). Similarly, osteonectin inhibits the basic fibroblast growth factor (bFGF)-induced migration and proliferation of endothelial cells, although not through a direct interaction with the growth factor (15).

Much of the work on the function of osteonectin has been done using nonosseous cells, and some of the effects of osteonectin are cell type specific. For example, osteonectin can disrupt cell spreading only in selected cell cultures (21). Similarly, in endothelial cells, osteonectin decreases DNA synthesis by delaying the onset of S phase, whereas data from osteoblastic cells suggest that osteonectin has a modest stimulatory effect on cell proliferation (10, 45).

In bone, osteonectin is among the most abundant noncollagenous extracellular matrix proteins, and the skeleton is one of the richest sources of the protein. Osteonectin is synthesized by cells of the osteoblastic lineage; binds hydroxyapatite, calcium, and type I collagen; and inhibits mineralization in vitro (7, 19, 34). Osteonectin expression is decreased in osteoblasts derived from patients with osteogenesis imperfecta, and osteonectin mRNA is decreased in osteoblasts derived from the frol/frol mouse, an animal model for some forms of bone fragility (8, 28). These data suggest that osteonectin modulates matrix organization and mineralization. This concept is further supported by the finding that, in rodent osteoblastic cells induced to form nodules in vitro, osteonectin expression increases as the matrix matures and mineralizes (3). In vivo studies suggest that osteonectin plays a role in development, because injection of anti-SPARC antibodies into Xenopus embryos results in developmental defects (30). Similarly, overexpression of osteonectin in transgenic nematodes causes developmental defects, suggesting that the appropriate and regulated expression of this matrix protein may be essential for normal development (36).

bFGF is a potent mitogen for cells of the osteoblastic lineage, and it represses the differentiated phenotype of mature osteoblasts (6). FGFs are important in skeletal development and fracture repair, events associated with active extracellular matrix remodeling (18, 43). Because osteonectin is expressed in areas of matrix remodeling, its coordinated and temporally appropriate expression may be important for the development of a transitional matrix and regulated angiogenesis, which are essential in development and fracture repair. This regulation may be mediated through the actions of growth factors and cytokines, and we postulated that bFGF regulates osteonectin expression in osteoblasts.

MC3T3-E1 is a clonal osteogenic cell line derived from neonatal mouse calvaria. These cells are well characterized and provide a homogeneous source of osteoblastic cells for study. They express high levels of alkaline phosphatase and differentiate into osteoblasts that can form calcified bone tissue in vitro (3, 39). The response of MC3T3-E1 cells to many growth factors and hormones mimics that of primary cultures of rodent osteoblastic cells. Therefore, using cultures of MC3T3-E1 cells, we examined the regulation of osteonectin by bFGF and initially characterized its mechanisms of action.
MATERIALS AND METHODS

Cell cultures. Early-passage MC3T3-E1 osteoblasts were cultured in α-minimum essential medium (GIBCO BRL, Grand Island, NY) containing 20 mM N-2-hydroxyethylpiperazine-N’-2-ethane sulfonic acid (HEPES) and 10% fetal bovine serum (Summit Biotechnologies, Ft. Collins, CO) (39). Cells were plated at a density of ~14,000 cells/cm² and were grown to confluence (~130,000 cells/cm²) after 4 days of culture. Cultures were then rinsed and transferred to serum-free medium containing 0.1% bovine serum albumin (Fluka Chemical, Ronkonkoma, NY) and 50 µg/ml ascorbic acid for 24 h. At the time of serum deprivation, the cells were considered to be in the matrix-deposition phase of the osteoblast phenotype. The cells were then exposed to test or control medium in the absence of serum for 2–48 h. After 48 h, cultures treated with bFGF had approximately twofold more cells than the untreated cultures.

Primary cultures of mouse osteoblastic cells were isolated from the parietal bones of neonatal mice (23). This procedure was approved by the Institutional Animal Care and Use Committee of Saint Francis Hospital and Medical Center. Parietal bones, dissected free of sutures, were subjected to five sequential 15-min digestions with bacterial collagenase (CLS II; Worthington Biochemical, Freehold, NJ). Cells harvested from digestions 3-5 were cultured as a pool at an initial plating density of ~10,000 cells/cm². These cells have been demonstrated to have osteoblastic characteristics (Ref. 23 and Delany, unpublished data). Cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with nonessential amino acids, 20 mM HEPES, 100 µg/ml ascorbic acid, and 10% fetal bovine serum. When the cells reached confluence (~1 wk after plating), they were rinsed and transferred to serum-free medium for 24 h and then exposed to test or control medium for 2–24 h.

Cycloheximide and 5,6-dichlorobenzimidazole riboside (DRB) (Sigma, St. Louis, MO) were dissolved in absolute ethanol, and, at dilutions of <1:10,000, an equal amount of ethanol was added to control cultures. Hydroxyurea (Sigma) and bFGF (Austral, San Ramon, CA) were dissolved in culture medium.

Northern blot analysis. Total cellular RNA was isolated with guanidine thiocyanate, at acid pH, followed by a phenol-chloroform extraction as described (4). Equal amounts of RNA (10 µg) were denatured and subjected to electrophoresis through formaldehyde-agarose gels, and the RNA was blotted onto GeneScreen Plus as directed by the manufacturer (NEN Life Sciences Products, Wilmington, DE). Restriction fragments containing a 1.5-kilobase (kb) bovine osteonectin cDNA (1) (provided by M. Young, Bethesda, MD) and a 750-base pair (bp) murine 18S rRNA cDNA (28) (American Type Culture Collection, Rockville, MD) were labeled with [α-32P]dCTP (specific activity 3,000 Ci/mmole; NEN Life Sciences Products) by random-primed second-strand synthesis (Prime-A-Gene kit, Promega, Madison, WI). Hybridizations were carried out at 42°C in 50% formamide, 750 mM sodium chloride-50 mM sodium phosphate-5 mM EDTA, 5× Denhardt’s solution, and 0.4% sodium dodecyl sulfate (SDS). Posthybridization washes were performed at 65°C in 150 mM sodium chloride-15 mM sodium citrate and 0.1% SDS. Appropriate exposures of the autoradiograms were analyzed by densitometry, and osteonectin RNA levels were normalized to those of 18S rRNA. The nuclear runoff assay shown is representative of two experiments.

Nuclear runoff assay. Nuclei were isolated from confluent MC3T3 cells by Dounce homogenization in a tris(hydroxymethyl)aminomethane (Tris)-HCl buffer containing 0.5% Nonidet P-40. Nascent transcripts were labeled by incubation of nuclei in a reaction buffer containing 500 µM each of ATP, GTP, CTP, and RNAsein (Promega) and 250 µCi [35S]UTP (800 Ci/mM, NEN Life Sciences Products) (modified from Ref. 13). RNA was isolated by treatment with deoxyribonuclease I and proteinase K followed by ethanol precipitation. Linearized plasmid DNA containing ~1 µg of DNA was immobilized onto GeneScreen Plus by slot blotting according to the manufacturer’s directions (NEN Life Sciences Products). A 2.6-kb cDNA for rat cyclophilin (provided by P. Danielson, La Jolla, CA) and 18S rRNA were used as controls for loading of the radiolabeled RNA (5). A 2.6-kb cDNA for rat collagenase 3 (provided by C. Quinn, St. Louis, MO) was used as a control for bFGF activity, and the plasmid vector pGEM5zf+ (Promega) was used as a control for nonspecific hybridization (31). Equal counts per minute of [35S]RNA from each sample were hybridized to cDNA, using the same conditions as for Northern blot analysis, and were visualized by autoradiography. Appropriate exposures of the autoradiograms were analyzed by densitometry, and osteonectin mRNA levels were normalized to those of 18S rRNA. The nuclear runoff assay shown is representative of two experiments.

MD-10 and 5-bromo-2′-deoxyuridine (BrdU) incorporation and immunoprecipitation. Confluent cultures of MC3T3 cells were cultured with or without bFGF for 24 or 48 h. For the last 5 h of culture, the cells were switched to methionine- and cysteine-free Dulbecco’s modified Eagle’s medium (GIBCO BRL) containing 20 µCi/ml [35S]methionine-[35S]cysteine (~1,000 Ci/mmole, NEN Life Sciences Products), with or without additional growth factor. Labeled condition medium samples were stored at −80°C after the addition of polyoxyethylene sorbitan monolaurate (Tween 20; Pierce, Rockford, IL) to a final concentration of 0.1%. The cell layer was washed with phosphate-buffered saline, scraped into lysis buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris-HCl at pH 8, 5 mM EDTA, and 1 µg/ml aprotonin) (Sigma), sonicated, and frozen at −80°C (14). The protein content of the cell layer samples was determined (DC protein assay, Bio-Rad, Hercules, CA), and equal amounts of cell protein were incubated with specific rabbit antiserum raised against mouse osteonectin (provided by Dr. H. Sage, Seattle, WA) or nonimmune rabbit immunoglobulin (Sigma) overnight at 4°C (34). In addition, samples of radiolabeled conditioned medium corresponding to equal amounts of cell protein were diluted with an equal volume of twofold-concentrated lysis buffer and incubated with the specific osteonectin antiserum overnight at 4°C (28). Immune complexes were removed from solution by incubation with protein A-Sepharose (CL-4B, Sigma) for 2 h at 4°C. Immunoprecipitates were washed three times with lysis buffer and eluted by boiling in reducing Laemmli sample buffer. Proteins were fractionated on a 10% polyacrylamide gel. The gel was fixed and incubated in Amplify (Amersham, Arlington Heights, IL) according to the manufacturer’s instructions. Immunoprecipitates were visualized by fluorography and analyzed by densitometry. This protocol appeared to quantitatively precipitate labeled osteonectin from the medium and cell layer preparations, because reprecipitation of the samples yielded very little radiolabeled protein. As a standard, osteonectin purified from bovine bone (Haematologic Technologies, Essex Junction, VT) was used and visualized by Coomassie blue staining. Immunoprecipitations are representative of three cultures.

Statistical analysis. Differences in the slopes of RNA decay curves were analyzed by the method of Sokal and Rohlf (38).

RESULTS

MC3T3 cells produce a single osteonectin transcript of ~2.2 kb. Treatment of serum-deprived MC3T3 cells
with 30 ng/ml bFGF caused a time-dependent decrease in osteonectin mRNA (Fig. 1). Northern blot analysis and densitometry showed that after 12 h of treatment with bFGF, osteonectin transcripts were decreased by \( \sim 50\% \), and after 24 h, bFGF decreased osteonectin transcripts by \( \sim 75\% \). The same level of inhibition was observed after 48 h of treatment. Similarly, bFGF decreases osteonectin mRNA in primary cultures of mouse osteoblastic cells, confirming that the effect is not a cell line-specific phenomenon (Fig. 2).

Downregulation of osteonectin mRNA by bFGF was dose dependent, because bFGF at 1–50 ng/ml decreased osteonectin transcripts by 30–90% after 24 h (Fig. 3). Immunoprecipitation of \(^{35}\)S-labeled osteoblast-conditioned medium and cell layer proteins showed that bFGF decreased the synthesis of osteonectin by 40–60% after 24 and 48 h of treatment (Fig. 4). In the conditioned medium, the primary osteonectin species detected by the anti-SPARC antiserum had an apparent molecular mass of \( \sim 38 \) kDa, and this protein had the same mobility as osteonectin purified from bovine bone. The cell layer contained multiple osteonectin species with apparent molecular masses of \( \sim 41, 38, \) and 29 kDa, and a less-abundant 34-kDa form. Nonimmune serum did not immunoprecipitate radiolabeled proteins (data not shown).

To determine whether the effect of bFGF on osteonectin mRNA expression was dependent on new protein synthesis, osteoblasts were treated with bFGF in the presence or absence of 2 \( \mu\)g/ml cycloheximide for 24 h. This dose of cycloheximide inhibited protein synthesis by 85–90% after 24 h of treatment, yet the cells were \( \sim 95\% \) viable as evaluated by trypan blue staining. Cycloheximide alone decreased osteonectin mRNA levels and prevented the repression of osteonectin transcripts by bFGF (Fig. 5). To determine whether downregulation of osteonectin expression by bFGF was dependent on cell replication, the DNA synthesis inhibitor hydroxyurea was used. In serum-starved MC3T3 cells, hydroxyurea at 1 mm abolished the proliferative effect of bFGF (data not shown), but hydroxyurea had no effect on the repression of osteonectin transcripts by bFGF (Fig. 6). These data indicate that the repression of osteonectin expression by bFGF is protein synthesis dependent and independent of the ability of bFGF to stimulate cell replication.

To determine whether bFGF modified the stability of osteonectin mRNA in osteoblasts, the RNA polymerase II-specific inhibitor DRB was used to arrest transcription, and the decay of osteonectin mRNA was monitored by Northern blot analysis (46). Serum-deprived cultures were treated with control medium or with 30 ng/ml bFGF for 6 h and then exposed to 72 \( \mu\)M DRB for up to 24 h. Although treatment of MC3T3 cells with DRB for 24 h decreased protein synthesis \( \sim 70\% \), the cells remained \( \sim 95\% \) viable as evaluated by trypan blue staining. In transcriptionally arrested osteoblasts, the half-life of osteonectin mRNA was \( \sim 24\) h, but in the presence of bFGF, the half-life of the transcript decreased to \( \sim 10\) h (Fig. 7). In contrast, bFGF increased the stability of glyceraldehyde-3-phosphate dehydrogenase mRNA in transcriptionally arrested cells (data not shown). To determine if there was a transcriptional component to the regulation of osteonectin by bFGF, nuclear run off assays were performed. bFGF did not alter the rate of osteonectin gene transcription at 2 (not shown), 6, 24, or 48 h of treatment (Fig. 8). However, bFGF did regulate collagenase 3 transcription, confirming growth factor activity (Fig. 8, Ref. 16, and Delany, 1995).

**Fig. 1.** Effect of basic fibroblast growth factor (bFGF) at 30 ng/ml on osteonectin mRNA expression in MC3T3 cells treated for 2, 6, 12, 18, 24, or 48 h. Total RNA from control (–) or bFGF-treated (+) cultures was subjected to Northern blot analysis and hybridized with a \(^{32}\)P-labeled bovine osteonectin cDNA (ON); blot was stripped and hybridized with a labeled rat 18S rRNA cDNA. Transcripts were visualized by autoradiography; osteonectin mRNA is shown at top, and 18S RNA is shown at bottom. These results are representative of 3 cultures.

**Fig. 2.** Effect of bFGF at 100 ng/ml on osteonectin mRNA expression in primary mouse osteoblastic cells treated for 8 or 24 h. Total RNA from control (–) or bFGF-treated (+) cultures was subjected to Northern blot analysis and hybridized with a \(^{32}\)P-labeled bovine osteonectin cDNA (ON); blot was stripped and hybridized with a labeled rat 18S rRNA cDNA. Transcripts were visualized by autoradiography; osteonectin mRNA is shown at top, and 18S RNA is shown at bottom. Similar results were obtained when cells were treated with 30 ng/ml bFGF. These results are representative of 3 cultures.

**Fig. 3.** Effect of bFGF at 1–50 ng/ml on osteonectin mRNA expression in MC3T3 cells treated for 24 h. Total RNA from MC3T3 cells was subjected to Northern blot analysis and hybridized with \(^{32}\)P-labeled bovine osteonectin cDNA (ON); blot was stripped and hybridized with a labeled rat 18S rRNA cDNA. Transcripts were visualized by autoradiography; osteonectin mRNA is shown at top, and 18S RNA is shown at bottom. These results are representative of 3 cultures.
unpublished data). After 6 h of treatment, bFGF caused a modest decrease in collagenase 3 transcription, followed by an approximately twofold increase in collagenase 3 transcription seen after 24 or 48 h. These data indicate that bFGF decreases osteonectin expression by decreasing transcript stability, and that changes in gene transcription do not play a role in the regulation of osteonectin by bFGF.

DISCUSSION

FGFs are potent regulators of gene expression in cells of the osteoblastic lineage, playing a role in skeletal development and fracture repair (18, 43). Osteoblasts synthesize FGFs, which can be stored in the extracellular matrix (11). bFGF is mitogenic for cells of the osteoblastic lineage and represses the differentiated function of mature osteoblasts (6). Our data demonstrate that bFGF downregulates osteonectin synthesis in cultured osteoblasts, further supporting this premise. bFGF was effective at a dose as low as 1 ng/ml, with a maximal effect at 50 ng/ml. These doses of bFGF also stimulate DNA synthesis and inhibit alkaline phosphatase activity and type I collagen expression in osteoblastic cells (6, 24). It is important to note that osteonectin itself can regulate the cell cycle (10, 45). However, experiments utilizing the DNA synthesis inhibitor hydroxyurea show that the downregulation of osteonectin expression by bFGF is independent of the mitogenic properties of the growth factor. Therefore, the inhibition of osteonectin is not simply due to stimulation of cell replication.

Osteoblast-conditioned medium and cell layer, in particular, contain multiple osteonectin species. The lower-molecular-weight species are most likely proteolytic fragments of osteonectin. Osteonectin can be cleaved by plasmin and a number of metalloproteinases, including collagenase 3,stromelysin 1, and gelatinases A and B; and osteoblasts synthesize and secrete collagenase 3, gelatinases A and B, and stromelysin 3.
late ribonucleases or RNA stabilizing factors. That downregulate osteonectin transcripts (26). Be-
overexpressing fibroblasts secrete a factor or factors 
action is indirect. Evidence for this include a late time 
have not been determined, studies suggest that its 
mRNA is downregulated (26). Although the mecha-
us mechanisms by which Jun decreases osteonectin expression 
mRNA by bFGF is mediated by c-Jun-regulated pro-
mRNA in the presence of cycloheximide. Such a factor may play a role in stabiliz-
protein is involved in the maintenance of osteonectin 
mRNA levels. Such a factor may play a role in stabiliz-
with the untreated control. This suggests that a labile 
consumption of osteonectin transcripts compared 
with the untreated control. This suggests that a labile 
attenuates in the maintenance of osteonectin 
mRNA levels. Such a factor may play a role in stabiliz-
om cell lines, because they are associated with different 
transcripts was shortened from 
Acetyl transferase or beta-galactosidase (Delany, unpub-
fragments linked to the reporter gene chloramphenicol 
transcript from nucleolytic cleavage.

It is possible that the repression of osteonectin 
mRNA by bFGF is mediated by c-j un-regulated pro-
induces c-jun expression, and in rat em-
the intercellular matrix protein in bone. Considering this, 
it is possible that the 38-kDa osteonectin species found 
in the cell layer could be different from those found in 
medium, because they are associated with different 
compartments (35).

Treatment with the protein synthesis inhibitor cyclo-
exhime decreases osteonectin transcripts compared 
with the untreated control. This suggests that a labile 
protein is involved in the maintenance of osteonectin 
mRNA levels. Such a factor may play a role in stabiliz-
ing osteonectin mRNA or in the transcription of the 
osteonectin gene. In cells cotreated with bFGF and 
cycloheximide, osteonectin RNA levels were modestly 
higher than those found in cells treated with cyclohexi-
mide alone. From these experiments, it is difficult to 
evaluate the significance of this effect. However, these 
data indicate that the downregulation of osteonectin by 
bFGF requires new protein synthesis, because the 
growth factor could not further repress osteonectin 
mRNA in the presence of cycloheximide.

It is possible that the repression of osteonectin 
mRNA by bFGF is mediated by c-j un-regulated pro-
ts. bFGF induces c-jun expression, and in rat em-
bro fibroblasts that overexpress c-jun, osteonectin 
mRNA is downregulated (26). Although the mecha-
isms by which J un decreases osteonectin expression 
have not been determined, studies suggest that its 
action is indirect. Evidence for this include a late time 
course of action and the finding that the c-jun-
overexpressing fibroblasts secrete a factor or factors 
that downregulate osteonectin transcripts (26). Be-
cause bFGF destabilizes osteonectin mRNA by an 
indirect mechanism, the effect may be mediated by a 
J un-regulated protein intermediate which could regu-
late ribonucleases or RNA stabilizing factors.

Nuclear runoff assays failed to demonstrate transcrip-
tional regulation of the osteonectin gene by bFGF. 
These findings were confirmed by transient transfec-
tion of MC3T3 cells with bovine osteonectin promoter 
fragments linked to the reporter gene chloramphenicol 
acetyl transferase or beta-galactosidase (Delany, unpub-
lished data). In contrast, the half-life of osteonectin 
transcripts was shortened from >24 h in control osteo-
blasts to ~10 h in osteoblasts treated with bFGF. The 
data on the half-life of osteonectin mRNA in control 
cells agree with those obtained by other investigators 
utilizing fibroblastic cells (42). However, the mecha-
nisms by which bFGF destabilizes osteonectin tran-
scripts remain uncharacterized. Although there has 
been great progress in understanding how gene tran-
scription is regulated, the mechanisms regulating eu-
karyotic mRNA stability are still largely unexplored. 
It is known that the 5'-cap structure and the poly-A tail 
play a role in protecting mRNA from exonucleolytic 
degradation; however, the sequences or structures that 
protect or target an RNA for endonucleolytic cleavage 
are less defined (12). RNA binding proteins may recog-
nize specific sequences or specific secondary structures, 
such as stem loops. Frequently, sequences that regulate 
transcript stability are found within the 3'-untrans-
lated region (UTR) (12). The osteonectin 3'-UTR is ~1 
kb long and is composed of most of exon 10 of the gene 
(25). The coding region of osteonectin is well conserved 
across species, and the 3'-UTR of osteonectin also 
appears to be well conserved. The 3'-UTR of the bovine, 
mouse, and human transcript has regions that share 
>80% homology (1, 25, 44). Two of these regions are 
>100 bases long, suggesting conservation of function-
ally relevant sequences. Modeling of the 3'-UTR of the 
mouse osteonectin transcript, using the Zucker-Stegler 
algorithm, shows potential for extensive secondary 
structure (47). The long half-life of the osteonectin 
mRNA may be mediated in part by secondary struc-
tures within the 3'-UTR, which could protect the 
transcript from nucleolytic cleavage.

In general, there is little information about the 
mechanisms by which growth factors regulate osteonec-
tin mRNA and peptide levels (2, 22, 29, 37, 42, 45). It is 
possible that other growth factors in addition to bFGF 
regulate osteonectin expression by posttranscriptional 
mechanisms. For example, PDGF-BB decreases osteo-
nectin mRNA in MC3T3 cells with a time course similar 
to that observed for bFGF; however, PDGF-BB is not as 
potent as bFGF in this regard. It is interesting to note 
that osteonectin can antagonize selected effects of 
PDGF-BB and bFGF in fibroblasts and endothelial 
cells, respectively (15, 32). The ability of osteonectin 
to modulate the activities of bFGF and PDGF-BB, coupled 
with the ability of these growth factors to downregulate 
osteonectin expression suggests a possible feedback 
mechanism of regulation in tissues and in remodeling 
events in which osteonectin and these growth factors 
are coexpressed.

Data on the effects of osteonectin on angiogenesis, 
metalloproteinase expression, cell proliferation, and 
cell matrix interactions support the concept that osteo-

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Fig. 8. Effect of bFGF at 30 ng/ml on osteonectin gene transcription in MC3T3 cells treated for 6, 24, or 48 h. Nuclei were isolated from control (-) or bFGF-treated (+) MC3T3 cells. Nascent transcripts were labeled in vitro with [32P]UTP, and labeled RNA was hybridized to immobilized cDNA for bovine osteonectin (ON), rat collagenase 3 (case), rat cyclophilin (cyclo), and rat 18S rRNA. pGEM5zf+ vector DNA (pGEM) was used as a control for nonspecific hybridization. Top row is a 16-h exposure, and bottom row, showing hybridization to 18S cDNA, is a 1-h exposure. These results are representative of 2 cultures.

(Refs. 6 and 35 and Delany, unpublished data). Metallo-
proteinases produce osteonectin species with apparent 
molecular masses ranging from 28 to 38 kDa and an 
additional 10-kDa fragment. Some of these metallopro-
tenase-derived osteonectin cleavage products have in-
creased affinity for type I collagen, the most prominent 
extracellular matrix protein in bone. Considering this, 
it is possible that the 38-kDa osteonectin species found 
in the cell layer could be different from those found in 
the medium, because they are associated with different 
compartments (35).
Osteonectin is important in development, wound healing, and matrix remodeling (25). Growth factors and cytokines are important mediators in these processes. The regulation of osteonectin by growth factors and cytokines would modulate the extracellular matrix composition and, in turn, modulate gene expression. In conclusion, BFGF decreases osteoblast osteonectin expression by decreasing the stability of its transcript. Further characterization of the mechanisms by which BFGF destabilizes osteonectin transcripts may help to define the role of BFGF and osteonectin in matrix remodeling.

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