Oncostatin M Stimulates Transcription of the Human α2(I) Collagen Gene via the Sp1/Sp3-binding Site*

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Oncostatin M (OSM), a member of the hematopoietic cytokine family, has been implicated in excessive bone growth and in the process of fibrosis. As part of an ongoing study of the molecular mechanisms of fibrosis, we have investigated the transcriptional regulation of the α2(I) collagen gene by OSM in human fibroblasts. An OSM response element was mapped by deletional analysis between base pairs (bp) −148 and −108 in the α2(I) collagen promoter. Further functional analysis of the α2(I) collagen promoter containing various substitution mutations revealed that both the basal activity and OSM stimulation of this promoter are mediated by a TCCCTCC motif located between bp −128 and −123. Furthermore, three copies of the 12-bp synthetic α2(I) collagen promoter fragment containing the “TCC” motif conferred OSM inducibility to the otherwise unresponsive thymidine kinase promoter. Electrophoretic mobility shift assays demonstrated that the TCCCTCC motif constitutes a novel binding site for the transcription factors Sp1 and Sp3. No differences have been observed in in vitro gel shift binding assays between unstimulated and OSM-stimulated fibroblasts. However, subtle conformational changes were detected in the region of the promoter surrounding TCC repeats after OSM stimulation using in vivo footprint analysis. In conclusion, this study characterized a dual-function response element that mediates the basal activity and OSM stimulation of the human α2(I) collagen promoter.

Type I collagen, the most abundant mammalian collagen, consists of two α1(I) chains and one α2(I) chain that are coordinately expressed (1–3). Excessive deposition of type I collagen, characteristic of many fibrotic disorders (4), most likely results from transcriptional activation of collagen genes in response to cytokines and other factors present in prefibrotic/inflammatory lesions. The most widely studied cytokine involved in collagen deposition is TGF-β1; nonetheless, other cytokines such as IL-4, IL-1, or OSM share many biological effects of TGF-β including stimulation of collagen synthesis and may play important roles in extracellular matrix accumulation during fibrotic process, especially immune-mediated fibrosis (5–7).

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The abbreviations used are: TGF-β, transforming growth factor-β; IL, interleukin; OSM, oncostatin M; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; BSA, bovine serum albumin; DMS, dimethyl sulfate; bp, base pair(s); TNF-α, tumor necrosis factor-α.

OSM is produced by activated T cells (8) and monocytes (9) and belongs to a subfamily of hematopoietic cytokines that also includes IL-6, IL-11, LIF (leukemia inhibitory factor), and CNTF (ciliary neurotrophic factor). Members of this family bind receptor complexes containing a signal-transducing subunit termed gp130 (10–12). Interestingly, OSM utilizes a dual-receptor system (13). First, a heterodimeric receptor complex consisting of gp130 and LIF receptor-β can be used by both OSM and LIF. A second heterodimeric receptor complex consisting of gp130 and OSM receptor-β is activated by OSM only. As a result, some of the biological effects are shared by OSM and LIF, whereas others are OSM-specific.

In fibroblasts, OSM stimulates both collagen and glycosaminoglycan production (7). Moreover, OSM has been reported to stimulate the synthesis of TIMP-1 (tissue inhibitor of metalloproteinases) and plasminogen activator (14, 15). OSM is a mitogen for murine NIH 3T3 cells and human foreskin and synovial fibroblasts (9). Recent studies with transgenic mice overexpressing OSM in a tissue-specific manner demonstrated its pleiotropic nature in vivo and its association with visceral fibrosis (16).

Our laboratory is involved in studies of the molecular mechanisms underlying the regulation of expression of the human α2(I) collagen gene in healthy and fibrotic human fibroblasts (17–19). The mechanism of modulation of type I collagen by OSM is of special interest because of a previous report that indicated a differential response of systemic sclerosis scleroderma and healthy skin fibroblasts to OSM. Unlike healthy skin fibroblasts, fibroblasts isolated from scleroderma lesions fail to respond to the stimulatory effect of OSM with regard to collagen and glycosaminoglycan synthesis (7). In this study, we have investigated the mechanism of the α2(I) collagen stimulation by OSM in healthy dermal fibroblasts. We show that OSM stimulates α2(I) collagen gene transcription through a constitutive positive cis-response element in the collagen promoter. We also characterize transcription factors interacting with this promoter element: two members of the Sp family of transcription factors, Sp1 and Sp3.

EXPERIMENTAL PROCEDURES

Cell Culture—Human dermal fibroblasts derived from a 2-month-old child (GMO5756A) were obtained from Coriell Cell Repositories (Camden, NJ) and propagated in DMEM supplemented with 10% FCS. Human foreskin fibroblasts were obtained from foreskins of healthy newborns (following institutional approval and informed consent). Primary explant cultures were established in 25-cm² culture flasks in DMEM supplemented with 10% FCS, 2 mM L-glutamine, and 50 μg/ml amphotericin. Fibroblast cultures independently isolated from different individuals were maintained as monolayers at 37 °C in 10% CO₂ in air and studied between the third and sixth subpassages. Both fibroblast types produced similar results in all assays.

RNA Preparation and Northern Blot Analysis—Fibroblasts were grown to confluence in DMEM supplemented with 10% FCS and then incubated for 24 h in serum-free medium (DMEM plus 0.1% BSA) before addition of cytokines and/or actinomycin D. Total RNA was
OSM Response Element in the α2(I) Collagen Promoter

and extracted by Northern blotting as described previously (20). Filters were sequentially hybridized with radioactive probes for α2(I) procollagen and glyceraldehyde-3-phosphate dehydrogenase. The filters were scanned with a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA).

Plasmid Constructions—The deletion and substitution mutant plasmids (with the exception of the −148 end point deletion construct) have been previously described (17, 18). The −148 α2(I) collagen promoter deletion construct was generated using polymerase chain reaction technology. Plasmids used in transient transfection assays were twice purified on CsCl gradients. At least two different plasmid preparations were used for each experiment.

Transient Transfections and Chlornephenicol Acetyltransferase Assays—Human fibroblasts were grown to 90% confluence in 100-mm dishes in DMEM with 10% FCS. Monolayers were washed, and cells were transfected by the calcium phosphate technique (17) with 20 μg of various deletion or mutant promoter-chloramphenicol acetyltransferase constructs. αSV-β-galactosidase control vector (Promega) was cotransfected to normalize for transfection efficiency. After incubation overnight, the medium was replaced with DMEM and 0.1% BSA containing 5 ng/ml OSM, and incubation was continued for 48 h. Cells were harvested in 0.25 M tris-HCl (pH 8) and fractured by freeze-thawing. Extracts, normalized for protein content as measured by Bio-Rad reagents, were incubated with butyryl-CoA. [14C]Chloramphenicol was extracted using an organic solvent (2:1 mixture of tetramethylpentadecane and xylene) and quantified by scintillation counting. Each experiment was performed in duplicate. The Mann-Whitney U test was used to determine statistical significance.

Preparation of Nuclear Extracts—For the preparation of nuclear extracts from untreated and OSM-treated fibroblasts, cells were plated in DMEM and 0.1% BSA for 24 h prior to OSM treatment. After incubation with 5 ng/ml OSM for the indicated length of time, cells were harvested. Nuclear extracts were prepared according to Andrews and Faller (21) with minor modifications. Briefly, confluent cells from five 150-mm dishes were washed with phosphate-buffered saline and resuspended in 1 ml of cold buffer containing 10 mM HEPES-KOH (pH 7.9) at 4°C, 1.5 mM MgCl₂, 10 mM KCl, 1 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride. The cells were allowed to swell on ice for 10 min and then vortexed for 10 s. The tube was centrifuged for 2 min, and the supernatant was discarded. The pellet was resuspended in 80 μl of cold containing 20 mM HEPES-KOH (pH 7.9), 25% glycerol, 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 1 mM dithiothreitol, and 0.7 mM phenylmethylsulfonyl fluoride and incubated on ice for 20 min for high-salt extraction. Cellular debris was removed by centrifugation for 2 min at 4 °C, and the supernatant fraction was stored at −80 °C until use. The protein concentration of the extracts was determined using the Bio-Rad reagent. In some experiments (e.g. OSM stimulation; see Fig. 5), nuclear extracts contained 1 mM sodium orthovanadate to ensure the phosphorylated status of STAT proteins. Addition of sodium orthovanadate had no noticeable effect on protein binding to the α2(I) collagen promoter probe used in this study.

Oligonucleotide Assays—Oligonucleotides used as probes, competitors, or polymerase chain reaction primers were synthesized using the Applied Biosystems nucleic acid synthesizer and were purchased from the Medical University of South Carolina Core Facility, except for the Oct-1 and NF1 consensus oligonucleotides, which were purchased from the Medical University of South Carolina Core Facility, except for the Applied Biosystems nucleic acid synthesizer and were purchased from the Medical University of South Carolina Core Facility.

DNA mobility shift assay was performed as described previously (17). Briefly, the binding reaction was performed on ice for 30 min in binding buffer (10 mM HEPES (pH 7.9), 50 mM NaCl, 1 mM dithiothreitol, 1 mM MgCl₂, 4% glycerol, 0.5 mM EDTA, 0.7 mM phenylmethylsulfonyl fluoride, 10 μg/ml apronin, 2 μg/ml leupeptin, and 2 μg/ml pepstatin containing 50,000 cpm labeled probe, 2 μg of poly(dI-dC)-poly(dI-dC), and nuclear extracts containing 5 μg of protein. In some assays, double-stranded competitors (200-fold molar excess) or antibodies (2 μg) (purchased from Santa Cruz Biotechnology) were added. Separation of free radiolabeled DNA from DNA-protein complexes was carried out on a 5% nondenaturing polyacrylamide gel. Electrophoresis was carried out in 0.5 × Tris borate electrophoresis buffer at 200 V at 4°C.

Plasmid Promoters—Human fibroblasts were grown to confluence in DMEM and 10% FCS and then incubated in DMEM and 0.1% BSA for 24 h. OSM (5 ng/ml) was added, and the culture was incubated for periods ranging from 30 min to 4 h. The medium was then replaced with DMEM and 0.1% BSA containing 0.1% dimethyl sulfate (DMS) and incubated for 2 min. Cells were rinsed three times with phosphate-buffered saline at 37°C, lysed on the plates using 1.5 ml of lysis buffer (300 mM NaCl, 50 mM Tris-Cl (pH 8.0), 25 mM EDTA (pH 8.0), 200 μg/ml proteinase K, and 0.2% SDS), and after scraping, incubated for 4 h at 37°C. DNA was extracted twice with phenol, twice with phenol/chloroform/isomyl alcohol (25:24:1), once with a mixture of chloroform/isomyl alcohol (24:1), and once with ether. DNA was precipitated once each with isopropanol and ethanol and redissolved in TE buffer (10 mM Tris-Cl (pH 7.5) and 1 mM EDTA). DNA samples were resuspended in TE buffer, precipitated with isopropanol and ethanol, and washed with 75% ethanol. The DNA was dissolved, and the concentration was adjusted to 0.4 μg/ml.

Ligation-mediated polymerase chain reaction was then used to detect DNA-protein interactions according to Mueller and Wold (22). All oligonucleotides were purified using denaturing polyacrylamide electrophoresis. Two oligonucleotides for staggered linker have been described (22). The following oligonucleotides were used to detect DNA-protein interactions on the coding strand of the human α2(I) collagen promoter: primer 1, TGCAGAGCACTCCGACGTGT; primer 2, ACCCTCAACCTAGCCGAAAACCT; primer 3, ACCCTCAACTTAGCCGAAAACCT; and primer 4, CATGTCGGGGCT-GCAGAGCACTCCGACGTGT. Ligation-mediated poly-
2-fold compared with the deletion to 2186. These data further corroborate the location of the previously mapped repressor site between bp 2164 and 2159 (18). Subsequent deletion to bp 2108 caused a decrease in promoter activity to 10% of the activity of the wild-type promoter. This dramatic effect on basal promoter activity most likely results from the removal of the previously identified positive constitutive cis-element containing a TCCTCC motif located between bp 2128 and 2123 in this promoter region (18). Since deleting the 2148 to 2108 promoter fragment also abolished the OSM response, we tested whether the same response element mediates the basal activity and OSM stimulation of collagen promoter activity using previously generated substitution mutants in the TCCTCC motif. As shown in Fig. 3, collagen promoter constructs carrying substitution mutations in this cis-regulatory element were unresponsive to OSM stimulation. On the other hand, stimulation of collagen promoter activity by TGF-β was not affected by the mutations in the TCCTCC motif, consistent with previous observations that other response elements in the collagen promoter mediate TGF-β stimulation (25, 32). Furthermore, none of the substitution mutations in other response elements previously identified in this promoter affected OSM stimulation (Fig. 3).

To further elucidate whether the regulatory sequences from the collagen promoter can mediate OSM stimulation of a heterologous promoter, three copies of the 12-bp oligonucleotide (from bp 2131 to 2120) containing the TCCTCC motif were cloned in both orientations into the pBL-CAT5 vector carrying thymidine kinase gene promoter elements. The tk promoter alone was not stimulated by OSM, whereas insertion of the mRNA expression in response to OSM and TGF-β were made between untreated and untreated cells (*, p < 0.01). D, effects of actinomycin D treatment on constitutive and OSM-stimulated mRNA levels. Actinomycin D (ActD; 400 ng/ml) was added alone or together with OSM (5 ng/ml) for 12 h to cells incubated in serum-free medium. Values from the actinomycin D- and OSM-treated cells indicate band density relative to untreated cells (NA), which was set at 1. Comparisons of a2(I) collagen mRNA expression levels were made between untreated and untreated cells (*, p < 0.01) and between cells treated with OSM alone and OSM together with actinomycin D (○, p < 0.01). All values were corrected for the loading difference, as determined by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA intensity.
TCCTCC motif mediates OSM stimulation of the human α2(I) collagen promoter. Plasmids carrying substitution mutations (18) were used in transient transfections of cells stimulated with OSM or TGF-β as described under “Experimental Procedures.” Sequences with the mutated nucleotides indicated in boldface are shown on the left. The stimulation index for OSM and TGF-β is shown on the right. The means ± S.E. for separate experiments are shown. The number of experiments used to calculate the mean is shown in parentheses. Comparisons were made between untreated and cytokine (OSM or TGF-β)–treated cells. Asterisks indicate statistically significant results (p < 0.001; N/T, not tested.

“TCCTCC” oligonucleotides consistently resulted in modest (2-fold) stimulation of the chimeric constructs independent of orientation (Table I). Interestingly, insertion of the TCCTCC-containing oligonucleotide in the correct orientation did not affect tk promoter activity, but insertion in the opposite orientation decreased significantly the basal promoter activity of the construct. It is possible that, in reverse orientation, binding of the nuclear proteins to the TCCTCC motif causes unfavorable conformational changes that negatively affect the activity of the tk promoter. A similar phenomenon has been observed with other heterologous promoter systems (24, 25).

Sp1 and Sp3 Bind to the Promoter Region Containing the TCCTCC Motif—Our previous analyses of the α2(I) collagen promoter indicated the presence of three specific DNA-protein complexes formed with the promoter fragment from bp −135 to −116 and nuclear extract from human dermal fibroblasts (18). To further characterize the nature of the nuclear proteins interacting with this promoter region, we performed gel shift analyses with a series of competitor oligonucleotides. As shown in Fig. 4A (lane 2), six DNA-protein complexes were formed. Complexes 1–4 and 6 were competed off with an excess of either unlabeled probe (lane 3) or the Sp1 oligonucleotide (lane 4). This suggests that complexes 1–4 and 6 are specific and that proteins with binding specificities similar to that of Sp1 are involved in formation of these DNA-protein complexes. However, complex 4 was also competed off with an excess of NF1, Oct1, and SIE (α-globin-inducible element present in the c-fos promoter known to bind STAT1 and STAT3 (26, 27)) oligonucleotides (lanes 5–7). Moreover, this complex appeared variably with the nuclear extract preparations (e.g. compare Fig. 5 in Ref. 18), suggesting that this complex may represent nonspecific binding of the proteins to this promoter region. The nature of the protein(s) involved in formation of complex 6 is presently unknown. We have previously shown that DNA sequences involved in binding of the nuclear factors to this promoter region correspond to the TCCTCC motif located between bp −128 and −123 (see Fig. 5 in Ref. 18). To test directly whether the TCCTCC c motif is involved in formation of the observed DNA-protein complexes, we performed gel shift analysis with an oligonucleotide probe containing a substitution mutation in the TCCTCC motif (18). Complexes 1–3 were not formed with this mutant probe (lane 8). This result provides additional evidence that the TCCTCC motif is responsible for binding of the nuclear proteins to this promoter region.

To further characterize the nature of the nuclear proteins interacting with this motif, we employed antibodies against four members of the Sp family (Sp1, Sp2, Sp3, and Sp4). As shown in Fig. 4B, addition of the anti-Sp1 antibody caused a supershift of complex 1 (lane 3), whereas addition of the anti-Sp3 antibody abolished formation of complex 3, with the appearance of a weak supershifted band (lane 5). Simultaneous addition of the anti-Sp1 and anti-Sp3 antibodies abolished formation of complexes 1–3 (lane 7). Addition of the anti-Sp2 and anti-Sp4 antibodies did not affect formation of the DNA-protein complexes (lanes 4 and 6), suggesting that Sp2 and Sp4 do not bind to this promoter region. Addition of the anti-STAT1 and anti-STAT3 antibodies did not affect formation of the DNA-protein complexes (lanes 8 and 9). In conclusion, the data obtained by gel shift analyses are consistent with the notion that two members of the Sp family of proteins, Sp1 and Sp3, interact with the TCCTCC motif in the α2(I) collagen promoter. Thus, the TCCTCC motif represents a novel binding site for Sp1 and Sp3.

OSM Treatment Does Not Affect Nuclear Protein Binding to the −135 to −116 Promoter Region—To determine whether DNA-protein interactions in this region are regulated by OSM, we performed DNA mobility shift assays using the promoter fragment from bp −135 to −116 and nuclear extracts from human fibroblasts treated with OSM for different intervals (15 min to 8 h). As shown in Fig. 5, no changes in the DNA-protein complexes were observed. Furthermore, OSM stimulation did not affect protein binding to the other regions of the collagen promoter: a −235 to −34 collagen promoter segment was used in DNase I protection and DNA mobility shift assays, and there was no difference in binding patterns between OSM-induced and control extracts (data not shown). However, consistent with previous reports (26, 27), we observed that OSM was capable of inducing STAT1 and STAT3 binding to the SIE response element in human fibroblasts used in our study (Fig. 6). These experiments demonstrate that OSM is capable of activating STAT1 and STAT3 proteins in human fibroblasts, but stimulation of the collagen promoter by OSM does not involve recruitment of STAT proteins.

OSM Alters DNA-Protein Interactions in the Human α2(I) Collagen Promoter in Intact Cells—To better understand the nature of the regulation of the human type I collagen gene expression by OSM in intact cells, we used in vivo DMS footprinting. Protein binding to the −198 to −74 region in the α2(I) collagen promoter in unstimulated and OSM-stimulated human fibroblasts was analyzed. As previously reported (18), differences in the methylation protection patterns on the mRNA noncoding strand between naked DNA and chromosomal DNA in vivo were observed (Fig. 7A). The positions of the protected G residues (bp −127/−126 and −124/−123), compressed doublet in the gel) correspond to the TCCTCC motifs (Fig. 7C). However, there were no significant changes in the protection patterns between serum-starved and OSM-stimulated fibroblasts. The other set of primers was used to visualize the coding strand (Fig. 7B). There was no visible protection on this strand in unstimulated cells, but protection of the G residues at positions −111, −117, and, to a lesser extent, −134 was

| Table I | OSM Response Element in the α2(I) Collagen Promoter |
|---------|------------------------------------------------------|
| RATIO | OSM+/OSM− | TGFβ+/TGFβ− |
| −302 | CTCGG | −271 | 2.9±0.3* (5) | N/T |
| −164 | TCCAA | −159 | 3.1±0.3* (6) | N/T |
| −128 | GAACTC | −132 | 1.1±0.2 (12) | 3.5±0.5* (5) |
| −128 | TCCGAT | −132 | 1.2±0.3 (12) | 3.8±0.7* (5) |
| −84 | TTCTGG | −80 | 3.0±0.4* (6) | N/T |

Values are expressed as percentage (mean ± S.E.) of the activity of pBL-CAT, which was arbitrarily set at 100%. The number of experiments is shown in parentheses. *Statistically significant results (p < 0.001).
observed in OSM-treated cells (Fig. 7, B and C). These changes in DNA-protein complexes were observed at 30 min and persisted for at least 4 h after the treatment of cells with OSM.

**DISCUSSION**

Several cytokines have been characterized as important modulators of collagen production in human dermal fibroblasts. For example, TGF-β (23), IL-4 (5), and OSM (7) upregulate collagen synthesis, whereas TNF-α (28), interferon-γ (28), basic fibroblast growth factor (29), and IL-10 (30) downregulate collagen production. Previous studies have characterized a TGF-β response element in the human α2(I) collagen promoter. In one study, a TGF-β response element (TbRE) was mapped to a −131-bp promoter region between bp −378 and −255, which contains three Sp1-binding sites (31), whereas in a different study, a TGF-β response element was mapped to a −265 to −241 region that contains an AP-1-binding site (32). The reason for the discrepancy between these two studies is presently not clear. Furthermore, TNF-α has been shown to inhibit α2(I) collagen transcription via the same response element that mediates TGF-β stimulation (32, 33). The response elements mediating modulation of α2(I) collagen promoter activity by other cytokines have not been reported.

In this study, we have characterized an OSM response element in the human α2(I) collagen promoter, which differs from the TGF-β/TNF-α response elements. We show that the OSM response element maps to the same promoter region as the previously characterized constitutive response element (18). This dual-function cis-regulatory element is located between bp −128 and −123 and contains a TCCTCC motif. We have previously demonstrated that substitution mutations in the TCC motif result in an −10-fold decrease in constitutive promoter activity (18). In this study, we also characterized transcription factors that interact with this motif, which are two members of the Sp family, Sp1 and Sp3. Our previous study has shown that the constitutive activity of the human α2(I) collagen promoter is regulated equivalently by the three positive cis-acting elements at bp −300, −125, and −80 (18). Transcription factors that interact with the −300 and −80 response elements have been characterized as Sp1 and CBF, respectively (17, 18). The results of this study suggest that the third positive response element at bp −125 also binds Sp1. Thus, Sp1 may contribute to the constitutive activity of the human α2(I) collagen promoter by interacting with several binding sites: the three GC boxes located around bp −300 and the TCCTCC motif located at bp −125. Previous studies have demonstrated that binding of Sp1 to multiple sites results in synergistic activation of transcription via formation of multimeric complexes (34). This type of interaction may facilitate high levels of expression of the type I collagen genes in fibroblasts.

OSM belongs to a family of cytokines that utilize the JAK-STAT signaling pathway (10), but OSM activation of other signaling pathways such as mitogen-activated protein kinase has also been observed (35). Indeed, it has been reported recently that OSM stimulates the MMP-1 (interstitial collagen-
ase) gene through AP-1 and STAT response elements that act synergistically (27). We have considered the possibility that OSM stimulation of the collagen promoter may also involve member(s) of the STAT family. However, our results are not consistent with this notion. Binding of the STAT transcription factors to the respective promoters is known to be rapidly and transiently induced after stimulation of cells with cytokines (36). We have also observed rapid and transient induction of STAT1 and STAT3 binding to the SIE response element (Fig. 6) in our cells. However, there is no detectable addition of proteins to or deletion from the collagen promoter after OSM stimulation at the various time points in in vitro assays (Fig. 5). In addition, antibodies to STAT1 and STAT3 (see Fig. 4B) were not associated with a supershift or decreased binding of nuclear proteins to the OSM response element in the collagen promoter. Finally, STAT proteins bind to DNA as homo- or heterodimers, and their binding sites are similar symmetrical dyad sequences (10). The OSM response element identified in this study, although sharing partial sequence similarity (TCC motif) with the STAT-binding sites, lacks dyad symmetry. These data suggest that, whereas the Jak-STAT signaling pathway is induced by OSM in human fibroblasts, this pathway is not directly involved in collagen stimulation.

This study indicates that OSM stimulates the \( \alpha_2(1) \) collagen promoter through the TCCTCC response element. This response element interacts with Sp1 and Sp3 in in vitro binding assays. Sp1 is a ubiquitous transcription factor that regulates the constitutive activity of many genes studied to date. Whereas our study does not provide direct evidence that Sp1 and Sp3 mediate the response to OSM, several recent reports suggest that Sp1 may be involved in mediating responses to various environmental stimuli, including responses to 12-O-tetradecanoylphorbol-13-acetate stimulation of the platelet thromboxane receptor gene (37), epidermal growth factor stimulation of the gastrin gene (38), and cAMP-dependent stimulation of the CYP11A gene (39). How Sp1 mediates such responses is presently unknown. Since no changes are observed after OSM treatment in the DNA-protein binding patterns in in vitro binding assays (Fig. 5), it is possible that OSM may affect the interaction of Sp1 with histones or other chromatin components. Such a mechanism has recently been described in the case of TGF-\( \beta \)/TNF-\( \alpha \) regulation of the mouse \( \alpha_2(1) \) collagen promoter, in which cytokine treatment affected the interaction of CTF/NF1 with histone H3 (40, 41). Our studies in intact cells using in vivo genomic footprinting suggest that OSM may induce conformational changes in DNA. OSM stimulation resulted in a different binding pattern visualized by protection of several G residues on the coding strand (Fig. 7B). Whether differences in the methylation pattern after OSM stimulation reflect conformational changes in the preexisting DNA-protein
complexes or whether additional factors bind to this region remains to be established.

In conclusion, this study characterizes the cis-regulatory element in the α2(I) collagen promoter that mediates activation of this gene in response to OSM. Similar to the previously characterized constitutive response element at bp −300 (17, 18), this element binds transcription factor Sp1. Interestingly, both response elements contribute equivalently to constitutive expression of this gene, but they appear to mediate distinct responses to exogenous stimuli. The Sp1-binding site at bp −24672 has been implicated in mediating TGF-β stimulation (31), whereas the response element at bp −125 characterized in this study mediates stimulation by OSM. Taken together with other recent reports (37–39), the results of this study further strengthen the role of Sp1 not only as a critical regulator of basal transcription, but also as a possible mediator of gene expression in response to various environmental factors.

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