4-29-1994


Sergio A. Jimenez  
*Thomas Jefferson University*

John A. Varga  
*Thomas Jefferson University*

Anne Olsen  
*Thomas Jefferson University*

Liye Li  
*Thomas Jefferson University*

Arturo Diaz  
*Thomas Jefferson University*

Follow this and additional works at: https://jdc.jefferson.edu/medfp

Let us know how access to this document benefits you

**Recommended Citation**

https://jdc.jefferson.edu/medfp/186

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's Center for Teaching and Learning (CTL). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Medicine Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.
To gain a further understanding of the regulation of human type I collagen gene expression under physiologic and pathologic conditions, we characterized 5.3 kilobase pairs (kb) of the human α1(I) procollagen gene promoter. A series of deletion constructs containing portions of the α1(I) procollagen 5′-flanking region (with end points from -5.3 kb to -84 base pairs (bp)) ligated to the chloramphenicol acetyltransferase (CAT) reporter gene were transiently transfected into NIH/3T3 cells. Maximal CAT activity was observed with constructs having 5′ end points from -804 to -174 bp. A further 5′ deletion to -84 bp caused a marked reduction in CAT activity. Cells transfected with plasmids containing longer 5′-flanking fragments of the α1(I) procollagen gene (-2.3 or -5.3 kb) showed reduced CAT activity compared with the -804 bp construct. The activity of the α1(I) procollagen promoter was much lower in cells that do not normally express type I collagen (HeLa cells) compared with collagen-producing NIH/3T3 cells. The CAT activity of deletion constructs containing longer 5′ regions than -84 bp was increased by ~2-fold in NIH/3T3 cells treated with transforming growth factor β1 (TGFβ1). Electrophoretic mobility shift assays indicated that protein-DNA complex formation with a probe corresponding to the -170 to -80 bp fragment of the α1(I) procollagen promoter was markedly enhanced in nuclear extracts prepared from TGFβ1-treated fibroblasts as compared with untreated fibroblasts. The DNA binding activity stimulated by TGFβ1 was specific for an Sp1-like sequence at positions -164 to -142 bp in the promoter. These results demonstrate that 1) there are both positive and negative cis-acting regulatory elements in the human α1(I) procollagen promoter, 2) these regulatory regions function differently in collagen-producing and -nonproducing cells, 3) the α1(I) procollagen promoter contains TGFβ1-responsive sequences located between -174 and -84 bp from the transcription start site, and 4) TGFβ1 caused marked stimulation of the DNA binding activity of a nuclear factor interacting with an Sp1-like binding site located within a region encompassing -164 to -142 bp of the α1(I) procollagen promoter.

Normal fibroblasts modulate their biosynthetic activity to maintain a precise balance between the synthesis and degradation of their products during dynamic events of tissue remodeling such as development, differentiation, and repair. It has been suggested that abnormalities in these regulatory mechanisms may be responsible for the excessive extracellular matrix accumulation in a variety of fibrotic diseases such as systemic sclerosis and idiopathic pulmonary fibrosis.

The collagens comprise a large family of widely distributed proteins that play a crucial role in the maintenance of the structural properties of the extracellular matrix. Despite the important structural and functional roles that the collagens play in normal tissues, the mechanisms that regulate their production are not completely understood. Variations in net collagen production occurring during growth and differentiation (1–3), viral (4–6) and chemical (7–9) transformation, cytokine and growth factor modulation (10–14), and spontaneous (15–17) and experimentally induced (18, 19) fibrotic processes have been ascribed to fluctuations in the steady-state collagen mRNA levels. The regulatory mechanisms responsible for the maintenance of normal procollagen mRNA levels have not been completely elucidated. Most of the available evidence suggests that the principal mechanisms operate at the level of transcription, although translational control and changes in mRNA processing and stability may also play a role (reviewed in Refs. 20 and 21). The broad spectrum of regulatory signals that can influence collagen gene transcription suggests that the collagen gene promoters are responsive to various trans-acting pathways. Several putative regulatory elements that may determine the transcriptional efficiency of procollagen genes have been identified in their corresponding promoters. These include the consensus TATA and CCAAT motifs as well as additional regulatory elements (22–25), which are the potential targets for the action of promoter-specific transcription factors (26–31). Furthermore, the transcriptional activity of some procollagen gene promoters appears to be modulated by enhancer and silencer elements located 3′ from the transcription start site (32, 33).

Detailed characterization of the cis-acting elements involved in modulation of collagen gene expression is crucial for understanding the physiologic and pathologic regulation of tissue collagen deposition. The purpose of the work reported here was to analyze the human α1(I) procollagen gene promoter in order to identify regulatory regions of the gene that may play a role in the modulation of its expression under normal and pathologic conditions.

**MATERIALS AND METHODS**

**Construction of Plasmids**—Several preliminary steps were necessary to prepare fragments with the appropriate restriction sites for ligations. The HindIII site in pSV2 CAT was changed to a KpnI site, and a 1.6 kb

---

*This work was supported by Grants AM 19106 and AR 42309 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.*

---

The abbreviations used are: CAT, chloramphenicol acetyltrans-
Human α1(I) Procollagen Gene Promoter

KpnI-EamHI fragment was isolated. A 7 kb α1(I) procollagen gene KpnI fragment extending from −5.3 to +1.7 kb was isolated from a human cosmid clone3 and subcloned into pUC19, and the KpnI site at −5.3 kb was changed to a NotI site to give pJH49. A HindIII-Thal fragment, extending from −804 to +42 bp, was subcloned into the HindIII and Smal sites of pUC19, excised as a HindIII-Thal fragment, and ligated with the 1.6 kb KpnI-BamHI coding fragment into the NotI and BamHI sites of Bluescript KS+ to give p804BS. The 4.5 kb NotI-HindIII fragment from pJH49 (containing α1(I) procollagen gene sequences from −5.3 kb to −804 bp) was then subcloned into the NotI and HindIII sites of p804BS to give p5.3kBS.

For the −5.3 and −804 bp constructs, the KpnI-KpnI fragments from 5.3kBS and p804BS were ligated with the 1.6 kb KpnI-BamHI coding fragment into the NotI and BamHI sites of Bluescript KS+ to give p5.3kCAT and p804α1I CAT, respectively. For the 2.3 kb construct, a 1.5 kb BamHI-HindIII fragment containing sequences from −2.3 kb to −804 bp was converted to a NotI-HindIII fragment and inserted into the NotI and HindIII sites of p804α1I CAT to give p2.3kα1I CAT. A series of deletions (from the HindIII site at −804 bp in p804α1I CAT toward the start of transcription site) was made following the exonuclease III digestion procedure of Henikoff (34). Exact deletion end points were determined by sequencing, and clones ending at −675, −463, −369, −174, and −84 bp were selected for analysis. A promoterless CAT plasmid, p0CAT, was prepared by removing the 846 bp HindIII-HindIII α1(I) procollagen promoter fragment from p804α1I CAT and religating.

The sequence from −2.3 kb to −804 bp of the α1(I) procollagen gene was obtained from deletions made from the NotI site in p2.3kα1I CAT toward the initiation of transcription site (also following the exonuclease III digestion procedure). DNA sequencing of both strands was performed using the dideoxy chain termination procedure (35) with T7 polymerase (Sequenase, U. S. Biochemical Corp.) following the instructions provided by the supplier.

Cell Transfections—NIH/3T3 cells (obtained from the ATCC) from subconfluent cultures were plated at a density of 3 × 10⁴ cells/100-mm dish and cultured in Dulbecco’s modified Eagle’s medium containing 10% calf serum. Transfections were performed 24 h later employing the calcium phosphate co-precipitation method, as described (36). The NIH/3T3 cells were transfected with 4 µg of α1(I) procollagen promoter-CAT plasmid DNA and 0.2 µg of the p522AP plasmid DNA containing the SV40 enhancer (8). DNA binding reactions for both samples were performed using the dideoxy chain termination procedure (35) with T7 polymerase (Sequenase, U. S. Biochemical Corp.) following the instructions provided by the supplier.

RESULTS

Nucleotide Sequence of −2.3 kb to −804 bp Region of α1(I) Procollagen Gene—The nucleotide sequences of the human α1(I) procollagen promoter and 5′-flanking region were determined. The sequence of the human α1(I) procollagen gene fragment extends from the TATA box (−101) to the transcription start site (−1) and contains a 5′-flanking region of 2.3 kb. The nucleotide sequence of the 2.3 kb fragment was determined using the dideoxy chain termination procedure of Henikoff (34). Exact deletion end points were determined by sequencing, and clones ending at −675, −463, −369, −174, and −84 bp were selected for analysis. A promoterless CAT plasmid, p0CAT, was prepared by removing the 846 bp HindIII-HindIII α1(I) procollagen promoter fragment from p804α1I CAT and religating.

The sequence from −2.3 kb to −804 bp of the α1(I) procollagen gene was obtained from deletions made from the NotI site in p2.3kα1I CAT toward the initiation of transcription site (also following the exonuclease III digestion procedure). DNA sequencing of both strands was performed using the dideoxy chain termination procedure (35) with T7 polymerase (Sequenase, U. S. Biochemical Corp.) following the instructions provided by the supplier.

Cell Transfections—NIH/3T3 cells (obtained from the ATCC) from subconfluent cultures were plated at a density of 3 × 10⁴ cells/100-mm dish and cultured in Dulbecco’s modified Eagle’s medium containing 10% calf serum. Transfections were performed 24 h later employing the calcium phosphate co-precipitation method, as described (36). The NIH/3T3 cells were transfected with 4 µg of α1(I) procollagen promoter-CAT plasmid DNA and 0.2 µg of the p522AP plasmid DNA containing the SV40 enhancer (8). DNA binding reactions for both samples were performed using the dideoxy chain termination procedure (35) with T7 polymerase (Sequenase, U. S. Biochemical Corp.) following the instructions provided by the supplier.
A further 3' deletion to -84 bp caused a significant reduction in activity in HeLa cells than in NIW3T3 cells. In NIW3T3 line that does not normally exhibit high levels of type I collagen gene production, constructs with end points at -5.3 kb, -2.3 kb, and -804 bp were transfected into HeLa cells. CAT activity driven by the -804 bp construct was about 26-fold higher than the CAT activity observed with the promoterless construct pOCAT, whereas in HeLa cells the relative activity of the -804 bp construct was 10-fold lower. In contrast to NIW3T3 cells, transfection of constructs with longer 3' sequences into HeLa cells resulted in about 2-fold greater CAT activity than that obtained with the -804 bp plasmid. These results suggest that the positive and negative transcriptional regulatory regions of the α1(I) procollagen promoter function differently in collagen-producing and -nonproducing cells.

**Localization of α1(I) Procollagen Promoter Regions Responsive to TGFβ1**—In order to localize regions within the α1(I) procollagen promoter that are responsive to stimulation by TGFβ1 in the NIW3T3 cells, the effects of TGFβ1 on the CAT activity in cells transfected with promoter deletion constructs with 5' end points at -5.3 kb, -2.3 kb, and -804 bp were examined. Incubation with TGFβ1 for 48 h resulted in an approximately 2-fold stimulation of CAT activity in cells transfected with each of the three constructs (Fig. 4), suggesting that TGFβ1-responsive elements were located within the proximal region of the promoter 3' to the -804 bp end point. To further experiments is shown in Fig. 2. The results indicate that CAT expression was maximal and constant in cells transfected with promoter constructs having 5' end points from -804 to -174 bp. A further 3' deletion to -84 bp caused a significant reduction in CAT activity, although even this short promoter was able to drive 10-fold higher CAT activity than the promoterless CAT construct; CAT and alkaline phosphatase activities were determined as described under "Materials and Methods." The hatched bars on the right represent CAT activity driven by each construct corrected for alkaline phosphatase activity in the same extracts relative to the CAT activity of the -804 bp construct. Plasmids were assayed in at least two separate transfection experiments with duplicate or triplicate determinations in each, except for the -675 bp construct, which was used in duplicate in a single experiment. Values shown represent the average ± S.E.

**Comparison of Activity of α1(I) Procollagen Promoter in Collagen-producing and -nonproducing Cells**—In order to assess the activity of α1(I) procollagen promoter sequences in a cell line that does not normally exhibit high levels of type I collagen gene production, constructs with end points at -5.3 kb, -2.3 kb, and -804 bp were transfected into HeLa cells. As shown in Fig. 3, the CAT activity driven by these three constructs was markedly lower in HeLa cells than in NIW3T3 cells. In NIW3T3 cells, CAT activity driven by the -804 bp construct was about 26-fold higher than the CAT activity observed with the promoterless construct pOCAT, whereas in HeLa cells the relative activity of the -804 bp construct was 10-fold lower. In contrast to NIW3T3 cells, transfection of constructs with longer 3' sequences into HeLa cells resulted in about 2-fold greater CAT activity than that obtained with the -804 bp plasmid. These results suggest that the positive and negative transcriptional regulatory regions of the α1(I) procollagen promoter function differently in collagen-producing and -nonproducing cells.

**Fig. 1.** Nucleotide sequence of the 5'-flanking region of the human α1(I) procollagen gene. The sequence of the BamHI-HindIII fragment encompassing positions -2392 to -798 bp is shown. Regions with greater than 90% identity to the corresponding rat sequences (43) are underlined.

**Fig. 2.** Summary of CAT activity driven by various lengths of the 5'-flanking sequence of the human α1(I) procollagen gene. A schematic linear map of p8.3K01 CAT and 5' end points of the deletion constructs are shown on the left. NIW3T3 cells were transiently co-transfected with the α1(I) procollagen-CAT constructs containing various lengths of promoter sequences and the alkaline phosphatase-CAT construct; CAT and alkaline phosphatase activities were determined as described under "Materials and Methods." The hatched bars on the right represent CAT activity driven by each construct corrected for alkaline phosphatase activity in the same extracts relative to the CAT activity of the -804 bp construct. Plasmids were assayed in at least two separate transfection experiments with duplicate or triplicate determinations in each, except for the -675 bp construct, which was used in duplicate in a single experiment. Values shown represent the average ± S.E.
identify TGFβ1-responsive sequences within the proximal region of the α(I) procollagen promoter. NIH/3T3 cells were transfected with constructs with 5' end points at −675, −463, −369, −174, and −84 bp or with the promoterless construct pO/CAT and incubated with TGFβ1. The results indicate that TGFβ1 caused a greater than 2-fold increase in CAT activity driven by the constructs with 5'-flanking regions longer than −84 bp. In contrast, TGFβ1 did not stimulate the CAT activity driven by the −84 bp promoter. A representative transfection experiment is shown in Fig. 5. These results indicated that TGFβ1-responsive sequences are located between −174 and −84 bp of the α(I) procollagen promoter.

Nuclear Protein Binding to α(I) Procollagen Promoter in Control and TGFβ1-treated Fibroblasts—In order to elucidate changes in trans-acting protein-DNA interactions involving the α(I) procollagen promoter that were associated with TGFβ1-induced stimulation of collagen production, electrophoretic mobility shift assays were performed. For this purpose, nuclear extracts were prepared from untreated and TGFβ1-treated fibroblasts. Two fragments (200-2 and 200-3) of the α(I) procollagen promoter region spanning the sequences from −389 to −80 bp relative to the transcription start site were used as probes (Fig. 6A). Incubation of the 200-2 probe (−170 to −80 bp) with nuclear extracts from untreated or TGFβ1-treated cells resulted in the formation of two complexes with retarded electrophoretic mobility (labeled R1 and R2), indicating the presence of nuclear DNA binding factor(s) recognizing sequences within the probe (Fig. 7). The intensity of the R1 and R2 complexes determined by laser densitometry of the autoradiograms was increased 18- and 8-fold, respectively, when nuclear extracts from untreated or TGFβ1-treated cells were used (Fig. 7). Competition experiments indicated that a 4-fold molar excess of unlabeled 200-2 probe completely prevented the formation of the R1 complex and re-
Human \( \alpha(1) \) Procollagen Gene Promoter

**A**

\[ \text{NcoI} \quad 200-3 \quad \text{HindIII} \quad 200-2 \quad \text{NaeI} \]

\[ 5' \quad \text{SmI} \quad 200-3 \quad \text{SmI} \quad 200-2 \quad 3' \]

**B**

- \( \text{Sp1.1} \quad 5'-\text{CTCCCTCCTCCTCCCCCTCCT} \quad -164/-142 \)
- \( \text{Sp1.2} \quad 5'-\text{GGCCGGGCGGCGGC} \quad -93/-77 \)
- \( \text{AP-1.1} \quad 5'-\text{GCCACCTGGAGGGAAGG} \quad -175/-160 \) (reverse)
- \( \text{NF-1.1} \quad 5'-\text{TAACCGACCCTGGCGGCC} \quad -102/-92 \) (reverse)
- \( \text{GC} \quad 5'-\text{ATTCGATCGGGCGGGCGAGC} \quad -1641/-142 \)

*Fig. 6. Locations and sequences of the probes used in electrophoretic mobility shift assays. A, 200-2 and 200-3 fragments and the Sp1, AP-1, and NF-1-like elements in the proximal region of the \( \alpha(1) \) procollagen promoter are shown. The arrow indicates the transcription start site. B, nucleotide sequences of the sense (Sp1.1 and Sp1.2) or antisense (AP-1.1 and NF-1.1) strands of the binding site oligonucleotides used. The core motifs are shown in boldface. The GC-box oligonucleotide contains the Sp1 consensus binding sequence.*

**Fig. 7. Electrophoretic mobility shift assay with competing unlabeled DNA.** A 90 bp double-stranded fragment of the human \( \alpha(1) \) procollagen gene promoter encompassing the sequence from positions \(-170 \) to \(-80 \) bp \((200-2)\) was end-labeled and used as a probe in electrophoretic mobility shift assays. Labeled probes were incubated with 1 \( \mu \)g of poly(dI-C)) and 5 \( \mu \)g of nuclear extracts prepared from normal human dermal fibroblasts that had been treated with TGF\( \beta \)1 (1 or 10 ng/ml) or left untreated for 48 h. Reactions were carried out in the absence or presence of 4- and 25-fold molar excesses of unlabeled 200-2 DNA probe, and the formation of DNA-protein complexes was analyzed by electrophoresis in 5% polyacrylamide gels under nondenaturing conditions. The two slowly migrating DNA-protein complexes are labeled R1 and R2. Lane 1, control containing the labeled probe but no protein. The increase in the intensity of the R1 complex is significantly increased by TGF\( \beta \)1.

**DISCUSSION**

The mechanisms involved in the regulation of collagen production under normal or pathologic conditions are not completely understood (reviewed in Refs. 20 and 21). Although the synthesis of most proteins in eukaryotic cells appears to be regulated at a transcriptional level, post-transcriptional events, such as the regulation of the stability of newly synthesized mRNA, may play an important role under certain conditions (46, 47). Studies of the transcriptional regulation of various collagen genes in human and rodent cells *in vitro* have demonstrated the presence of regulatory elements located immediately 5’ upstream of the transcription initiation site (22–26). In addition, an enhancer element located within the first intron has been identified in the type I collagen genes (32–34). Sequences located far upstream of the initiation of the transcription site may also be involved in the regulation of expres-


sion of the collagen genes (48, 49). Rippe et al. (49) analyzed murine α1(I) procollagen gene sequences encompassing −3700 to +1400 bp and found that a short segment of the promoter (220 bp upstream from the start of the transcription site) was sufficient for expression of the gene, whereas further upstream flanking sequences had a negative effect on transcription.

In order to identify upstream elements that may be involved in the regulation of transcription of the human α1(I) procollagen gene, we determined the nucleotide sequence of the promoter region encompassing from −804 to −2292 bp. Comparison of the newly obtained sequence with that of the promoter region in the rat α1(I) procollagen gene (43) indicated less than 65% overall nucleotide identity. However, several regions with highly conserved sequences in the two species were found between −1900 and −1540 bp (underlined in Fig. 1). The high degree of nucleotide sequence conservation between the two species in this region suggests that these sequences may have important regulatory functions. In the rat gene, this region contains two repeats that are variations of the palindromic sequence CCCTCCC. Analysis of the newly obtained sequences of the human gene reported here demonstrated two identical palindromes localized at −1687 and −1063 bp. Other putative regulatory sequences were also identified in the newly obtained sequence. These included two Sp1 binding sites (GGGCGG) at positions −2168 and −1614 bp and one NF-1 (half-site) binding sequence (GCCAA) at position −830 bp (reverse strand).

To perform a functional analysis of the upstream sequences, several chimeric constructs containing DNA fragments spanning up to −5.3 kb of the α1(I) procollagen promoter ligated to the CAT gene were prepared, and their expression was examined following transient transfection into NIH/3T3 cells. Maximal transcriptional activity was noted when promoter sequences with 5' end points from −176 to −804 bp were included. Constructs containing further upstream sequences showed progressively decreasing activity (Fig. 2). These results are similar to those reported by Rippe et al. (49) for the murine α1(I) procollagen gene.

In order to examine if there were differences in the regulation of α1(I) procollagen gene transcription in cells that constitutively produce high levels of collagen and cells that normally do not display expression of interstitial collagen genes, we compared the expression of the promoter-CAT constructs following their transfection into NIH/3T3 cells or into HeLa cells. Marked differences in the expression of the promoter were observed between these two types of cells. Transfection of non-collagen-producing HeLa cells resulted in relatively low levels of CAT activity, which, in marked contrast to NIH/3T3 cells, was increased when promoter constructs containing −2.3 and −5.3 kb upstream sequences were examined (Fig. 3). The dif-

![Fig. 8. Electrophoretic mobility shift assay with Sp1.1 and Sp1.2 probes and competitors. Panel A, the 23 bp Sp1.1 and the 17 bp Sp1.2 double-stranded oligonucleotides encompassing the human α1(I) procollagen promoter sequences −164 to −142 bp or −93 to −77 bp, respectively, were end-labeled and used as probes in electrophoretic mobility shift assays. Nuclear extracts were prepared from normal human skin fibroblasts that had been treated with TGFβ1 (10 ng/ml) or left untreated for 48 h. Labeled probes were incubated with nuclear extracts under identical conditions, as described in the legend to Fig. 7. The positions of the shifted complexes (R1 and R2) are indicated, along with the position of the unbound DNA. Lane 1, control containing labeled Sp1.1 probe but no protein. C, nuclear extract prepared from untreated fibroblasts; T, nuclear extract prepared from TGFβ1-treated fibroblasts. Panel B, same as panel A, except that only the Sp1.1 oligonucleotide probe and increasing concentrations (2.5-, 100-, and 250-fold excesses) of unlabeled double-stranded competitor oligonucleotides were used. Nuclear extracts were prepared from TGFβ1 (10 ng/ml)-treated fibroblasts, except in lane 2 (C, untreated fibroblasts). Panel C, same as panel A, except the AP-1.1 and NF-1.1 oligonucleotides, corresponding to the α1(I) procollagen gene sequences from positions −175 to −160 bp and −102 to −85 bp, respectively, were used. The arrowhead denotes the retarded DNA-protein complex (R1).]
Human α1(I) Procollagen Gene Promoter

12690

In the rat α1(I) and the mouse α2(I) procollagen genes, TGFβ-responsive elements resembling the NF-1 consensus sequence have been described (64, 65). In the rat α1(I) procollagen gene, the putative "TGFβ activating element" was reported to be located 1.6 kb upstream from the transcription start site (65). The element contains the 3' portion of the canonical NF-1 sequence GCCAAG also found in the mouse α2(I) promoter. However, in contrast to the mouse α2(I) procollagen gene, stimulation of rat α1(I) procollagen gene expression by TGFβ1 does not appear to involve NF-1 binding to its cognate DNA element (66). Analysis of the nucleotide sequence of the human α1(I) procollagen gene indicates that a NF-1-like sequence similar to the rat TGFβ activating element is located at -1718 bp (5'TGCCCCAGGCCAGC). However, our results indicate that deletion of a 1.5 kb fragment including this element did not prevent stimulation of promoter activity by TGFβ1 (Fig. 4), suggesting that in NIH3T3 cells this NF-1-like element is not involved in TGFβ1-induced transcriptional activation of the α1(I) procollagen gene. The nature of trans-acting factors, which are implicated in the modulation of the expression of diverse TGFβ-responsive genes, is not completely understood. Recent studies suggest that, depending on the cell type and the gene that is regulated, a variety of distinct nuclear proteins may be involved (67). Moreover, transcriptional regulation by TGFβ is likely to be a complex process, associated with the combinatorial interactions of ubiquitous and inducible trans-acting factors. The identification of cell-specific DNA binding factors and of the stimulatory and inhibitory cis-acting elements in the promoter of the human α1(I) procollagen gene that are targets for these factors will permit a better understanding of the complex mechanisms that modulate the transcriptional activity of collagen genes during physiologic processes of development and repair as well as in a variety of diseases characterized by excessive collagen production.

Acknowledgments—We thank Dr. Kyong Yoon for providing the pSV2AP plasmid and Dr. David Hall for thoughtful suggestions. The skilled secretarial assistance of O. Ma in the preparation of this manuscript is gratefully acknowledged.

REFERENCES
