Identification of the Key Regions within the Mouse Pro-α2(I) Collagen Gene Far-upstream Enhancer*

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Collagen type I is synthesized by fibroblasts, osteoblasts, odontoblasts, and other specialized cells in different organs. It is a fibrillar collagen, composed of two α1(I) chains and one α2(I) chain. These chains are encoded by the pro-α1(I) and pro-α2(I) collagen genes, which in mice are located on chromosomes 11 and 6 respectively (1, 2). Vuust et al. (3) demonstrated that the mRNA of the pro-α1(I) and pro-α2(I) collagen genes are only found in collagen-producing cells and are always present at the 2:1 ratio seen in the mature collagen molecule. This indicates that regulation of the collagen genes is primarily at the transcriptional level and that the pro-α1(I) and pro-α2(I) collagen genes are probably coordinately regulated. Given the complex expression pattern of collagen type I, which is produced in different tissues at different stages of development and in response to disease or injury, transcriptional control of the α1(I) and α2(I) collagen genes may involve a number of distinct elements, each directing expression in a separate tissue, cell type, or stage of development or disease. This hypothesis has been supported by studies of transgenic mice harboring various regions of the mouse, rat, and human pro-α1(I) collagen promoters that demonstrate a modular arrangement of distinct and separate tissue-specific elements within the first 3.2 kb of the proximal promoter (4). These elements were shown to be responsible for reporter gene expression in different subsets of type I collagen-producing cells, namely osteoblasts, odontoblasts, fascia, and tendon fibroblasts (4). More recently, an additional osteoblast element was characterized (5) as well as a uterine fibroblast element farther upstream between −7.0 and −8.0 kb from the transcription start site (6).

Although the mouse pro-α1(I) and pro-α2(I) collagen genes are coordinately expressed, the arrangement of the control elements appears to differ between the two genes. Unlike the mouse pro-α1(I) collagen gene, the pro-α2(I) collagen gene does not appear to contain discernable tissue-specific elements within the −3.2-kb proximal promoter, but the minimal promoter (−350 to +54 bp) alone is capable of promoting very limited levels of reporter gene expression in transgenic models (7), in contrast to the pro-α1(I) minimal promoter (8). A survey of the upstream region of the mouse pro-α2(I) gene promoter reported the presence of three DNase I hypersensitive sites (HS), HS5, HS4, and HS3, located approximately −17.1, −16.2, and −15.5 kb upstream of the transcription start site. These sites were present only in collagen type I-producing cells (9). Transgenic mice harboring a 6.0-kb region encompassing the three hypersensitive sites between −19.5 and −13.5 kb, linked to the minimal promoter (−350 to +54 bp), resulted in a high level of reporter gene expression in the majority of collagen-producing cells. However, unlike the mouse pro-α1(I) collagen gene upstream region, no reporter gene expression was detected in the osteoblasts of the endochondral bones or the odontoblasts, although some reporter gene expression could be detected in the osteoblasts of bones of intramembranous origin. We termed this region the far-upstream enhancer because of its ability to drive reporter gene expression independent of position and orientation and in the presence of nonspecific minimal promoters (9). These data suggested that this far-upstream enhancer contains a number of distinct cis-regulatory elements involved in the control of expression in different type I collagen-producing...
cells. In this study, we sought to identify the functional unit(s) of the far-upstream enhancer and to investigate the role of hypersensitive sites within the upstream enhancer. The results show that the minimal functional enhancer is 1.5 kb in length, and the sequences overlapping HS4 are probably the most critical for the functional activity of this enhancer.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs**—The 5′-flanking sequence of the mouse pro-α2(I) collagen gene was derived from a pFIX II library (Stratagene). The clone λ3-3 contained 5′-flanking sequences of the pro-α2(I) collagen gene from −53.3 to −11.0 kb relative to the transcriptional start site (9). Two reporter gene vectors were used in generating transgenic mice, pLacRM350 and pLacES350, which was adapted from pLacRM350, with the 3′ multiple cloning site in reverse orientation. These vectors were modified from pLacF (10), a pUC18-based plasmid containing a lacZ gene with a eukaryotic translation initiation codon and mammalian polyadenylation sequences. Both vectors also contain the mouse pro-α2(I) collagen gene minimal promoter (+54 to −350 bp) 5′ of the lacZ gene.

For constructs pGB(−17.0/−13.5) and pGB(−17.0/−15.45) del (0.25), two separate fragments were removed from λ3-3, blunt-ended, and cloned together into pBluescript, from where they were subcloned into pEFM350.

**Generation of Transgenic Mice**—Transgenic mice were generated using the mouse pro-α2(I) constructs shown in Fig. 1. Vector sequences were removed with NruI and SacII. The digested DNA constructs were gel-purified, suspended in 10 mM Tris/HCl (pH 7.4), 0.1 mM EDTA (pH 8) at a concentration of 2–3 μg/ml, and injected into the pronuclei of fertilized C57BI/10 × CBAC eggs as previously described (11). Undamaged eggs were transferred into pseudopregnant CD1 foster mothers. The foster mothers were sacrificed at 15.5 days of gestation, and the founder animals and used to genotype the animals by PCR analysis.

**Cell Culture**—Transient transfection assays were performed using the FuGENE 6® (Roche Diagnostics) transfection reagent according to the manufacturer’s instructions. Briefly, NIH3T3 and L929 cells were grown in 6-well plates in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. At log phase growth, the cells were transfected with 2 μg of total plasmid DNA and 6 μl of FuGENE 6® reagent (2 × 10⁶ cells per transfection). pRSVLuc was co-transfected (0.2 μg) to check the efficiency of transfection. The cells were harvested 72 h post-transfection using lysis buffer and activities of the reporter genes β-galactosidase and luciferase were measured using the Dual-Light® chemiluminescent reporter gene assay system (Applied Biosystems) according to the manufacturer’s instructions. Protein content was also measured using BCA reagents (Pierce). The specific activity was calculated by dividing the data from β-galactosidase assay by the luciferase data and then by the total protein content.

**DNAase I Footprinting and Hypersensitive Site Analysis**—The preparation of nuclei and DNase I digestion was carried out as previously described (9). Briefly, NIH3T3 and L929 cells were washed with ice-cold phosphate-buffered saline, scraped, pelleted, and resuspended in buffer A (15 mM Tris/HCl (pH 7.6), 15 mM NaCl, 60 mM KCl, 1 mM EDTA, 0.5 mM EGTA, 0.3 mM sucrose, 0.1% Triton X-100, 0.15 mM spermine, 0.5 mM spermidine, 1 μM phenylmethylsulfonyl fluoride, 1 μM dithiothreitol). Cells were mechanically disrupted, the resulting homogenate was diluted with an equal volume of buffer B (buffer A without Triton X-100, EDTA, EGTA), and DNA concentrations were estimated by UV absorption at 260 nm. 15 absorbance units were used for each DNase I digestion. These reactions were performed in 40 mM Tris/HCl (pH 7.6) and 6 mM MgCl₂ using 10 μl of DNase I (Amersham Biosciences) at concentrations between 0 and 10 units/μl. After a 15-min incubation at room temperature, the reaction was halted by adding 2 volumes of 50 mM Tris/HCl (pH 8.0), 100 mM NaCl, 100 mM EDTA, 1% SDS, and 40 μl of proteinase K (20 mg/ml). Purified DNA was then digested with appropriate restriction enzymes, and Southern analysis was performed as described in the standard protocol (12). To locate the specific restriction enzyme sites that lay 5′ and 3′ of the hypersensitive site cores, mouse genomic DNA was also digested with the same enzyme, in addition to one of nine specific enzymes located within the area of interest. These samples were run alongside the DNase I-treated extract. For DNase I footpointing, plasmid DNA was digested with the appropriate restriction enzyme and end-labeled by filling in 3′-recessed ends with Klenow enzyme (12). Gel purification of labeled DNA fragments, preparation of nuclear extracts from NIH3T3 cells, nuclear protein binding, and DNase I footprinting reactions were performed as previously described (13).

**RESULTS**

**Identification of the 5′ Boundary of the Far-upstream Enhancer**—Transgenic mice were generated containing the constructs pGB(−19.5/−13.5), pGB(−17.0/−13.5), and pGB(−16.6/−13.5). These constructs are depicted in Fig. 1A and consist of the far-upstream enhancer with progressive 5′ deletions fused to the -350 to +54 minimal promoter and lacZ gene. The number of embryos positive for the transgene are given in Table I.

DNase I hypersensitive site analysis using a genomic ladder digested with a series of known restriction enzymes refined the location of HS3, HS4, and HS5 reported by Bou-Gharios et al. (9) and showed the restriction enzyme sites between which the hypersensitive core sites were located. Construct pGB(−19.5/−13.5) contained DNase I hypersensitive sites HS3, HS4, and HS5, whereas constructs pGB(−17.0/−13.5) and pGB(−16.6/−13.5) contained only HS3 and HS4 because HS5 is located between the StyI site at −17.05 and the EcoRI site at −17.2 and, hence, beyond the 5′ boundary of the latter two constructs (Fig. 1).

Mice transgenic for constructs pGB(−19.5/−13.5) and pGB(−17.0/−13.5) showed a very similar staining pattern to that previously reported (9). No differences in expression pattern were noted with the smaller pGB(−17.0/−13.5) construct, and the percentage of transgenic mice expressing the construct was also similar to that of the larger construct (Table I). Because we have established that HS5 is beyond the 5′ boundary of pGB(−17.0/−13.5), these results show that the DNA sequence represented by HS5 is not required for the correct expression of the pro-α2(I) collagen gene during mouse development.

In contrast, the mice transgenic for and expressing the construct pGB(−16.6/−13.5) with a 400-bp deletion from the 5′ end showed a staining pattern different from that seen in mice expressing the pGB(−17.0/−13.5) construct. X-gal staining was only detected in 29% of the transgenic embryos and varied quite considerably in the pattern of expression between embryos (Fig. 2). Histological examination showed that the expression of the reporter gene could not be detected in the intramembranous bones of the calvarium, but the clavicle showed some limited staining. Expression of the reporter gene was also severely limited in the mesenchymal cells associated with skeletal muscle with only two embryos showing detectable expression in the body wall muscles along the back of the embryos. Reporter gene expression in the connective tissue of the fascia was also limited to certain regions of the back. X-gal staining in the internal organs was absent except for the connective tissue of the pancreatic primordia, and low level staining was seen in the fibrous and smooth muscle layers of the stomach.

These results show that the 5′ boundary of the functional far-upstream enhancer lies between the BamHI site at −17.0 and the AvoI site at −16.6 kb upstream of the transcriptional start site. This 400-bp region does not contain any detectable DNase I hypersensitive sites but has been shown here to have a significant impact on the functional expression of the far-upstream enhancer. Transgenic mice were also generated with the construct pGB(−17.0/−16.6), which contains the essential 400-bp region. Only 17% of the transgenic embryos expressed the transgene (Table I). Furthermore, in these mice the X-gal staining pattern was aberrant, with staining seen only in non-collagen-producing tissues (data not shown).

**Identification of the 3′ Boundary of the Far-upstream Enhancer**—
Fig. 1. Diagrams of plasmid DNA constructs. Several constructs were generated using upstream enhancer sequences of the mouse pro-o2(I) collagen linked to the proximal promoter (−350 to +54 bp) and to the β-galactosidase gene. The three hypersensitive sites are indicated by arrowheads (open, HS3; filled/bisected, HS4; and filled, HS5). A, constructs with deletion of 5’ sequences. B, 3’ deletions. C, two constructs with different size deletions of HS4.

### TABLE I

| Construct | Embryos positive by Southern/PCR | Embryos expressing the transgene | Embryos expressing the transgene |
|-----------|---------------------------------|----------------------------------|----------------------------------|
| Number    | Number                          | %                                |
| −350lacZ  | 15                               | 7                                | 47                              |
| pGBI−19.5/−13.5 | 10                               | 7                                | 70                              |
| pGBI−17.0/−13.5 | 14                               | 9                                | 64                              |
| pGBI−16.6/−13.5 | 14                               | 4                                | 29                              |
| pGBI−17.0/−16.6 | 12                               | 2                                | 17                              |
| pGBI−17.0/−14.7 | 10                               | 8                                | 80                              |
| pGBI−17.0/−15.45 | 15                               | 11                               | 73                              |
| pGBI−17.0/−15.7 | 8                                | 4                                | 50                              |
| pGBI−17.0/−14.7 | 6                                | 0                                | 0                               |
| del 0.5   | pGBI−17.0/−15.45 del 0.25         | 9                                | 4                                | 44                              |

Transgenic mice harboring the constructs pGBI−19.5/−13.5 and pGBI−17.0/−15.45 with 3’ deletions of 2.2 and 1.95 kb, respectively (Fig. 1B) were very similar to those expressing pGBI−17.0/−13.5, with high levels of reporter gene expression detected in the intramembranous osteoblasts, mesenchymal cells of the muscle, tendon, and fascia, smooth muscle cells of the blood vessels and gut, and connective tissue of the majority of internal organs (Fig. 3). This indicated that the region located between −15.45 and −13.5 kb may not be essential to the expression of the pro-o2(I) collagen gene during development.

In contrast, transgenic mice expressing the construct pGBI−17.0/−15.7, in which a further 280 bp had been deleted from the 3’-end of the enhancer region, showed a significant reduction in the level and pattern of transgene expression. However, that pattern was consistent in all founder animals, which were expressing the transgene (Fig. 4). This deletion effectively removed the HS3 core site, located between the HindIII site at −15.45 and the Styl site at −15.7 (Fig. 1B). Reporter gene expression was detected in the intramembranous osteoblasts and in fibroblasts of the fascia, tendon, and muscle of certain defined regions, primarily around the nose and the limbs (Fig. 4). Apart from these regions, no expression was detected in skeletal or smooth muscle (with the exception of some staining in the stomach) or in the connective tissue of the internal organs. These results suggest that the sequence between the Styl site at −15.7 and HindIII at −15.45 is required for reporter gene expression in the mesenchymal cells of connective tissue of the internal organs and in most regions of the muscle, tendon, and fascia. These results also indicate that HS3 plays a role in directing tissue- or region-specific control of the mouse pro-o2(I) collagen gene.

Nuclear Protein Binding Sites around HS3—In an attempt to identify possible binding sites contributing to the tissue- and spatial-specific regulation of the mouse pro-o2(I) collagen gene, a DNase I footprinting assay was employed to map sites of nuclear protein interaction in the region of the mouse pro-o2(I) collagen gene upstream enhancer represented by HS3. The analysis identified two distinct areas of nuclear protein protection within the region between the Styl site at −15.7 and the HindIII site at −15.45 kb upstream of the transcriptional start site (Fig. 5). These footprints (FP1 and FP2) were detected with extracts from both L929 and NIH3T3 cells. Both footprints lie at the 5’ of the fragment. This analysis strongly suggests that the region represented by HS3 is responsible for binding several nuclear proteins that may have a role in tissue- or stage-specific expression of the mouse pro-o2(I) collagen gene in mesenchymal cells. Indeed, computer-based sequence analysis...
searches through MatInspector V2 (14) revealed a number of transcription factors with 100% core recognition. These are shown in Fig. 5.

The Role of HS4—The DNase I hypersensitive site, HS4, is located between the PvuII site at −16.2 and the BbsI site at −15.95 kb upstream of the pro-α2(I) collagen gene (data not shown). Two constructs were generated containing deletions within the HS4 region. Construct pGB(−17.0/−14.7 del 0.5) consisted of the construct pGB(−17.0/−14.7) with a deleted region between the PvuII site at −16.2 and the Styl site at −15.7 (Fig. 1C).

No mice transgenic for the construct pGB(−17.0/−14.7 del 0.5) showed any detectable reporter gene expression. In contrast, 44% in embryos transgenic for the construct pGB(−17.0/−15.45 del 0.25) showed detectable expression (Table I). In all but one embryo, reporter gene expression was restricted to the tail region, in tendons and the fascia (Fig. 6). This result demonstrates that the sequence representing the HS4 region is essential in maintaining the functional integrity of the enhancer.

To further examine the influence of the HS4 core region on gene transcription, transient transfections were carried out to establish the effect of HS4 deletion on the ability of the far-upstream enhancer to promote reporter gene expression in vitro (Fig. 7). Although both pGB(−17.0/−14.7) and pGB(−17.0/−15.45) promoted an increase in β-galactosidase-specific activity relative to the −350 minimal promoter alone, the two HS4-deletion constructs, pGB(−17.0/−14.7 del 0.5) and pGB(−17.0/−15.45 del 0.25), also showed similar transgene activity when compared with the intact HS4-containing enhancer constructs.

DISCUSSION

Several studies of transcriptional control of the genes encoding collagen type I have suggested the existence of a number of distinct tissue-specific elements directing expression of the pro-α1(I) and pro-α2(I) collagen genes. The work presented in this study using transgenic mice demonstrates that the upstream enhancer has a core functional unit of 1.5 kb between −17 and −15.45 kb from the transcriptional start site. The sequences within this region contain several cis-acting elements that work in concert to produce an enhancer that drives high levels of expression in a tissue-specific manner. One of these elements is represented by the sequences overlapping HS4, which we show to be essential for the functional integrity of this enhancer.

The 5′ boundary of the functional regions of the mouse far-upstream enhancer has been located between −17.0 and −16.6 kb upstream of the transcriptional start site. Deleting the 2.5-kb region between −19.5 and −17.0 kb of the 6-kb far-upstream enhancer had very little effect on the expression pattern of the reporter gene, even though this deletion effectively removed the DNase I hypersensitive site, HS5. This is demonstrated here by transgenic mice expressing the construct pGB(−17.0/−13.5), which showed X-gal staining in the same pattern and intensity as seen with the larger pGB(−19.5/
13.5) construct. It is worth noting that the human COL1A2 shares high homology with the mouse enhancer and also contains three DNase I hypersensitive sites. The human enhancer requires all three HS sites for correct functioning, and deletion of the most 5’ HS site, which shares high sequence similarity with mouse HS5, results in the loss of most of the expression (15). This suggests that subtle differences in sequence homology are important, and/or there is a rearrangement within the conserved human sequences that promote regulation via different combinatorial elements.

![Transgenic mice generated with HS3 sequence deletion.](image)

**FIG. 4.** Transgenic mice generated with HS3 sequence deletion. A, two representative whole-mount embryos at E15.5 expressing pGb(−17.0/−15.7). Sagittal sections of both embryos show limited X-gal staining around the ossification of the frontal bone (B) and the clavicle (C, arrows). Staining is detected in the connective tissue in tendon (D), fascia (f), and muscle (m) of the limbs (E). X-gal staining is also seen in the fascia around the nose (F). Black bar, 100 μm.

![Footprint of sequences overlapping HS3.](image)

**FIG. 5.** Footprint of sequences overlapping HS3. The 280-bp DNA sequence between the StyI site at −15.7 and the HindIII site at −15.45 kb from the transcription start site in the pro-α2(I) promoter was incubated with nuclear extract from L929 and NIH3T3 fibroblasts. In each test, DNA was incubated with increasing amounts of Dnase I in the presence (+) and absence (−) of cell nuclear extracts. G/A indicates the Maxam and Gilbert reaction as a marker. Two footprints were detected, (FP1) and (FP2), with sequence indicated vertically. The sequence of the positive strand is outlined with putative transcription factors generated with MatInspector computer analysis. Arrows denote the core sequence used by the program.

-13.5)
Further deletions of the −17.0-kb BamHI site resulted in the loss of most reporter gene expression, as seen in embryos expressing the construct pGB(−16.6/−13.5). It is therefore apparent that the deleted 400-bp region contained sequences essential for the full function of the far-upstream enhancer. Furthermore, the variegated expression seen in the founder animals, where no two embryos expressed the same pattern, unlike any other deletions, may indicate that this 5′-sequence protects the enhancer from position effect variegation. This protection has been shown in other genes, such as the hCD2 locus in which sequences in its HHS3 region protect against position effect (16).

The 3′ boundary of the functional regions of the far-upstream enhancer has been shown to be located near the HindIII restriction site −15.45 kb upstream of the transcriptional start site. Transgenic mice expressing both constructs pGB(−17.0/−14.7) and pGB(−17.0/−15.45) demonstrated high levels of reporter gene expression in a pattern very similar to that of the larger −19.5/−13.5 enhancer. However, removal of the 250-bp region between −15.7 and −15.45, which included the DNase I hypersensitive site HS3, had a deleterious but consistent effect on the function of the far-upstream enhancer. The generation of transgenic mice harboring an additional construct containing the missing 250 bp (HS3) did not result in any expression (data not shown). These deletion experiments have demonstrated that the minimal sequence necessary to confer the full effect of the far-upstream enhancer is located between −17.0 and −15.45 kb. This region alone is capable of promoting reporter gene expression in the fibroblastic cell lineages of most organs in which in situ hybridization has demonstrated expression of collagen type I (17).

The most significant result of this study is the demonstration that deletion of the sequences overlapping HS4 abolishes the function of the enhancer. Although there is some evidence that small deletions of the core of HS sites may result in artificially severe phenotypes (18, 19), it is unlikely to be the explanation here because differently sized HS4 deletions resulted in similar phenotypes, showing that in the absence of HS4 the far-upstream enhancer is unable to promote expression in vivo. Furthermore, the results of the transient transfection assay demonstrate that when the enhancer is not integrated into the genome the deletion of HS4 has no effect on the level of expression. Taken together these results imply that the region represented by HS4 is essential for the function of the far-upstream enhancer, probably by modifying the chromatin environment.

Although these deletion experiments have demonstrated that the minimal sequence necessary to confer the full effect of the far-upstream enhancer is located between −17.0 and −15.45 kb, this enhancer does not constitute a locus control region (LCR), nor do these regions represent all the regulatory elements that control mouse pro-α2(I) collagen gene expression in all tissues. No reporter gene expression was ever detected in the osteoblasts derived from endochondral ossification or in the cells of the liver. It is therefore likely that as yet unidentified DNA sequences elsewhere in the pro-α2(I) collagen gene domain are necessary to achieve complete control of gene expression and to make up a functioning LCR.

The notion of separate DNA elements or modules directing tissue-specific expression has already been documented in a number of collagen-encoding genes, including the mouse pro-α1(I)s collagen gene (4). It was thought that the mouse pro-α2(I) collagen gene far-upstream enhancer may actually consist of a number of distinct tissue-specific elements, similar to those found in other collagen genes. We investigated the presence of such tissue-specific cis-acting elements around sequences represented by DNase I hypersensitive sites because these regions represent areas of DNA in a distinct non-nucleosome conformation and are composed of clusters of individual protein binding sites (20, 21). The deletion of sequences overlapping HS3 resulted in a significant loss of expression in several tissues, most notably in the skeletal muscle of the body wall and attenuated expression of fascia staining within the skin layers of the embryos. This pattern was repeatable in all expressors and did not suffer from position effect variegation, as seen with the 5′-deletion. This suggested that HS3 sequences contain tissue-specific cis-acting elements responsible for certain mesenchymal lineage. The footprinting results indicate that this region does indeed bind transcription factors. Moreover, the size of the footprinted region, at least in fibro-

![Fig. 6. Transgenic mice generated with HS4 sequence deletions.](http://www.jbc.org/)

**Fig. 6. Transgenic mice generated with HS4 sequence deletions.** X-gal staining in two typical whole-mount embryos at E15.5 harboring pGB(−17.0/−15.45 del 0.25), showing staining only in the tail (A). A sagittal section of the tail (B) shows the staining in layers of muscle (m), tendons (t), and fascia (f). Black bar, 100 μm.

![Fig. 7. Transient transfection in fibroblastic cell line L929 showing β-galactosidase-specific activity of constructs with intact and different size deletions of HS4 following transient transfection.](http://www.jbc.org/)

**Fig. 7. Transient transfection in fibroblastic cell line L929 showing β-galactosidase-specific activity of constructs with intact and different size deletions of HS4 following transient transfection.** No difference in the activity of the transgene was measured in transient transfection.
blasts, suggests that a complex of factors may be involved. The computational search for transcription factor binding sites revealed a number of transcription factors, many of which were muscle-specific transcription factor binding sites including myogenic basic helix-loop-helix MyoD protein, the MEF2 protein, Mt-binding protein, and the muscle initiator sequence TATA box, as well as the transcriptional activators and regulators (AP1, CAAT boxes, and GATA3) known to be required for their function in vivo (22). Further experiments are underway to delineate the critical transcription factors in this region of the enhancer.

One barrier to identifying specific regulatory elements in fibroblasts is the nature of these cells. Unlike osteoblasts, fibroblasts do not form a homogeneous population nor are they easily divided into subpopulations (23). Considerable heterogeneity has also been reported within the fibroblast population of single tissues, although attempts to define the subpopulations have so far been less than successful (24, 25). Interestingly, one of the phenotypes used to identify different subpopulations within a tissue has been the level of collagen type I synthesis. In conclusion, this study demonstrated that there are three key regions to the upstream enhancer, each of which appears to affect gene expression via different mechanisms. Together they generate the enhancer of the mouse pro-α2(I) collagen promoter.

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