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Regulatory Elements in the 5′-Flanking Region and the First Intron Contribute to Transcriptional Control of the Mouse Alpha 1 Type I Collagen Gene

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We have identified two blocks of regulatory sequences located in the 5′-flanking region and the first intron of the mouse alpha 1 type I collagen (COL1A1) gene. Both blocks were found to contain positive as well as negative regulatory elements. Sequences located within 222 base pairs upstream of the transcription start site showed a strong stimulatory effect on the COL1A1 promoter and were sufficient for tissue-specific regulation of the COL1A1 gene. The combined upstream and intron regulatory sequences showed a marked inhibition of COL1A1 promoter activity in fibroblasts. This finding suggests that additional, more remote regulatory sequences may be required for establishing the high level of activity of the endogenous COL1A1 gene in fibroblastoid cells.

Collagens are extracellular structural proteins that establish and maintain tissue architecture in vertebrates. There are at least 10 different types of collagens, all of which are synthesized in a tissue-, stage-, and cell-specific manner (17). Expression of the different procollagen genes in developing and adult organisms is regulated by a variety of growth factors, hormones, and other agents (5, 13, 19–21, 26, 27) and is affected by viral transformation (1, 12). A complex array of *cis*-acting regulatory DNA sequences and *trans*-acting factors involved in the stage- and tissue-specific regulation of a number of procollagen genes has been uncovered (2, 3, 10, 11, 14, 16, 18, 22–25). To identify sequences that may be important for transcriptional regulation of the mouse alpha 1 type I collagen (COL1A1) gene, we constructed a chimeric gene containing part of the first exon and the promoter region of the COL1A1 gene fused to the bacterial chloramphenicol acetyltransferase (CAT) gene (6). Sequences derived from the 5′-flanking region and the first intron of the COL1A1 gene were then tested for their effects on COL1A1 promoter activity by transfection and transient CAT expression studies in NIH 3T3 mouse fibroblasts. All experiments were performed by cotransfection with pCH110, a plasmid containing the bacterial β-galactosidase gene fused to the simian virus 40 promoter (8), and the results were normalized for transfection efficiencies.

A typical CAT assay is shown in Fig. 1, and a summary of our results is presented in Fig. 2. The highest transcriptional activity was consistently observed with construct ColCAT3, which contained part of the first exon and 222 base pairs of 5′-flanking sequences, including the COL1A1 promoter (*Xba*I-*Bgl*II fragment, nucleotides −222 to +116; Fig. 1 and 2). The level of CAT expression obtained with construct ColCAT3 was comparable to that obtained with pSV2CAT (6), which contains the simian virus 40 promoter and enhancer (Table 1), or pMoCAT, which contains the Moloney murine leukemia virus promoter and enhancer (data not shown). This finding shows that the sequences located immediately 5′ flanking of the COL1A1 promoter have a stimulatory effect on promoter activity similar to that of strong viral enhancers. Constructs containing increasingly longer 5′-flanking sequences showed reduced CAT activities of between 65% to less than 20% of that of ColCAT3 (Fig. 1 and 2).

The immediate 5′-flanking sequences appeared to be sufficient for tissue-specific regulation of the COL1A1 promoter. In NIH 3T3 fibroblasts, which synthesized large amounts of collagen (2.2% of total protein; Table 1), construct ColCAT3 showed high transcriptional activity comparable to that of pSV2CAT. In *src*-transformed NIH 3T3

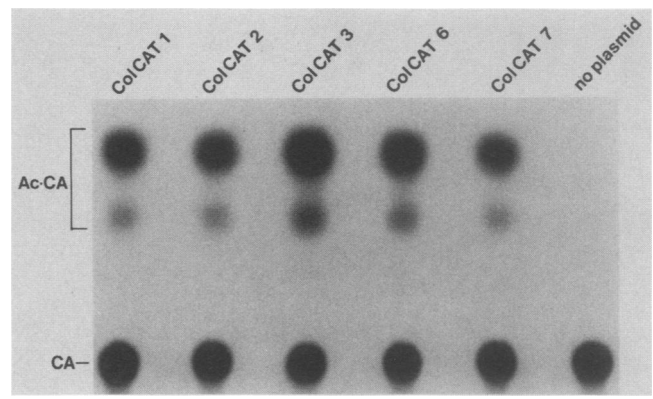

FIG. 1. CAT activity of ColCAT fusion gene constructs transfected into NIH 3T3 mouse fibroblasts. DNA fragments containing promoter and 5′-flanking sequences of the mouse COL1A1 gene were fused to the CAT gene (see Fig. 2). CAT plasmid (8 μg) and pCH110 (8) (2 μg) were cotransfected into NIH 3T3 mouse fibroblasts by calcium phosphate precipitation and assayed for CAT (6) and β-galactosidase (8) activities after 24 h by using cell extracts containing 200 μg of protein. The autoradiograph shows the chromatographic separation of radiolabeled chloramphenicol (CA) and its acetylated derivatives (Ac · CA). After autoradiography, radioactivity in each spot was determined by scintillation counting, and percent acetylation was calculated. In this experiment, the values for percent acetylation were as follows: ColCAT1, 26; ColCAT2, 31; ColCAT7, 26; ColCAT6, 44; and ColCAT3, 59.

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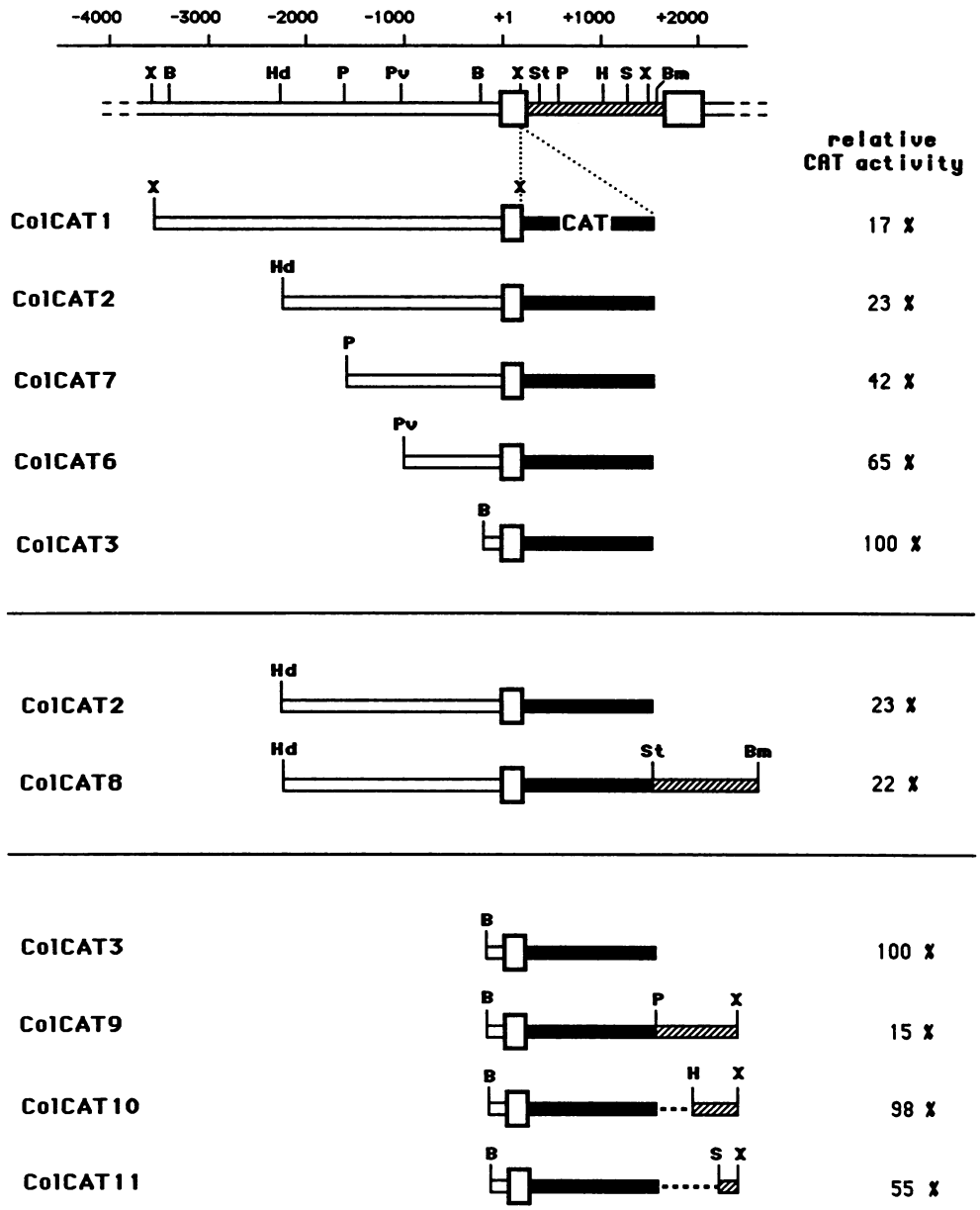

FIG. 2. Effects of 5'-flanking and intron sequences on mouse COL1A1 promoter activity. At the top is a map of the mouse COL1A1 gene (9). Symbols: ▭, first two exons; ▨, first intron; ▬, CAT gene. Positions of relevant restriction sites are indicated (X, *Xba*I; B, *Bgl*II; Hd, *Hin*dIII; P, *Pst*I, Pv, *Pvu*II; St, *Stu*I; H, *Hpa*I; S, *Sma*I; Bm, *Bam*HI). The DNA constructs were transfected into NIH 3T3 mouse fibroblasts and assayed for CAT activity as described in the legend to Fig. 1. CAT activity of the individual constructs was normalized for transfection efficiency as determined by cotransfection with pCH110 and assay of β-galactosidase activity (8). Each value represents the mean of at least three independent experiments and is expressed relative to the activity of construct ColCAT3 (100%), which corresponds to an average chloramphenicol acetylation of 50%.

fibroblasts (kindly provided by D. Jähner), in which expression of the endogenous COlA1 gene was downregulated approximately 10-fold (Table 1), transcriptional activity of ColCAT3 was similarly reduced, whereas no such effect was seen with pSV2CAT. Similarly, in MOPC315 cells, which did not synthesize detectable amounts of collagen, no CAT activity was detected with construct ColCAT3 (Table 1). We conclude that the sequences located immediately upstream of the mouse COL1A1 promoter exert a strong stimulatory effect on the COL1A1 promoter and are sufficient for its tissue-specific activity. An inspection of the DNA sequence of this region of the COL1A1 gene (9) shows no apparent sequence similarity with known enhancer core sequences. However, it contains a transcription-associated DNase-hypersensitive site (4) as well as several sequence elements which may be involved in transcriptional regulation. These are a polypyrimidine stretch at −129 to −169, two CCAAT boxes, at −96 to −100 and −126 to −130, the proximal of which has been shown to be involved in transcriptional activation by a CCAAT-binding factor (16), and two perfect 12-base-pair direct repeats TGGGGGCCGGGC, at −83 to −94 and −113 to −124, which resemble an Sp1-binding motif.

Our results demonstrate that sequences located further

TABLE 1. Relative collagen production and CAT activity

Cell type	Relative rate of collagen production (%)[a]	Relative CAT activity (%)[b]					
		ColCAT1	ColCAT2	ColCAT7	ColCAT6	ColCAT3	pSV2CAT
NIH 3T3	2.2	24	29	41	49	100	102
src-transformed NIH 3T3	0.2	5	5	8	14	29	134
MOPC315	0	0	0	0	0	0	17

[a] Collagen and noncollagen protein production were determined by the collagenase method as previously described (26). The radioactivity of collagenase-sensitive and collagenase-insensitive proteins was used to calculate the relative rate of collagen production.
[b] Determined as described for Fig. 1 and 2; 100% CAT activity of construct ColCAT3 corresponds to 59% chloramphenicol acetylation.

upstream (between −222 and −3700) of the mouse COL1A1 promoter contain one or several negative regulatory elements which can override the proximal positive element described above and strongly inhibit the COL1A1 promoter (compare ColCAT3 with ColCAT1, ColCAT2, ColCAT6, and ColCAT7; Fig. 1 and 2). To test whether these negative sequences are capable of reducing the activity of a heterologous promoter-enhancer, we cloned a 3.3-kilobase 5′-flanking *Bgl*II fragment (Fig. 2) in front of the Moloney murine leukemia virus promoter-enhancer of pMoCAT, but no inhibition of the promoter-enhancer was observed in this construct (not shown). Therefore, the negative regulatory element(s) in the 5′-flanking region of the COL1A1 gene appears to be promoter specific.

It has been shown that the first introns of the mouse COL1A2 and the rat COL2 genes contain transcriptional enhancers (11, 22). We therefore analyzed the effects of sequences derived from the first intron of the mouse COL1A1 gene on transcriptional activity of the COL1A1 promoter. We first tested whether the inhibitory effect of the distal 5′-flanking sequences on COL1A1 promoter activity could be overcome by sequences located in the first intron of the gene. When a 1.1-kb *Stu*I-*Bam*HI fragment containing most of the first intron was cloned downstream of the CAT gene into construct ColCAT2, no enhancing effect of the intron sequences was observed (construct ColCAT8, Fig. 2). Similarly, these sequences did not show transcriptional enhancer activity when cloned into pA10CAT2 (15) or pMoXCAT, both of which contain a viral promoter but no enhancer (not shown). We next tested various subfragments of the mouse COL1A1 first intron for their effects on transcriptional activity of construct ColCAT3. The fragments were cloned in their natural positions and orientations with respect to the promoter (constructs ColCAT9, ColCAT10, and ColCAT11, Fig. 2). Our results showed that the first intron of the mouse COL1A1 gene contains regulatory elements capable of exerting both negative and positive effects on transcriptional activity of the COL1A1 promoter. When a *Pst*I-*Xba*I fragment (nucleotides +550 to +1400) was cloned into ColCAT3, the high level of transcriptional activity was almost abolished (ColCAT9, Fig. 2). When a shorter fragment was used (*Hpa*I-*Xba*I, +950 to +1400), the original high transcriptional activity of ColCAT3 was restored (ColCAT10, Fig. 2). An even shorter fragment (*Sma*I-*Xba*I, +1200 to +1400) showed a slight negative effect on transcriptional activity (ColCAT11, Fig. 2). These results identify a strong negative regulatory element in the mouse COL1A1 first intron, located between nucleotides +550 and +950. In addition, our results suggest that a weak stimulatory element is located between +950 and +1200 and that there is a second weak negative element between +1200 and +1400. As described above for the 5′-flanking sequences, the negative elements appear to be dominant over the positive element. These findings are reminiscent of results reported recently for the human COL1A1 gene, which was shown to contain both positive and negative regulatory sequences in the first intron that, in combination, showed only a marginal effect on transcriptional activity (3). In another study, a strong transcriptional enhancer was identified in the first intron of the human COL1A1 gene (24). Further dissection of the intron regulatory elements in the murine COL1A1 gene will have to show whether this gene also contains a genuine enhancer.

The results reported here identify two blocks of regulatory elements located in the 5′-flanking region and in the first intron of the mouse COL1A1 gene which contribute to the transcriptional regulation of the gene. Each block contains a combination of both positive and negative regulatory elements. A similar array of regulatory elements has been found in other procollagen genes (2, 3, 24, 25) and probably reflects the complex mechanisms which regulate the tissue and stage-specific expression of the various procollagen genes in vertebrates. The combined 2.5-kb upstream and 1.1-kb intron sequences that surround the COL1A1 promoter and contain both blocks of regulatory elements have a strong inhibitory effect on the transcriptional activity of that promoter in fibroblasts (construct ColCAT8, Fig. 2). It has been reported that sequences located 50 kb upstream and 30 kb downstream of the human β-globin gene are necessary for completely regulated, position-independent high-level expression of the gene (7). Our results suggest that an analogous situation may exist for the mouse COL1A1 gene and that additional, more remote regulatory sequences may be required for establishing the high transcriptional activity of the endogenous COL1A1 gene in fibroblastoid cells.

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